NUCLEIC ACID-PROTEIN INTERACTIONS

Organizers: Jeremy Bruenn and Bik-Kwoon Tye

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Poster Sessions

Protein/DNA Interactions at Replication Origins

MZ 001 T-ANTIGEN INTERACTIONS AT THE SV40 AND POLYOMA ORIGINS OF DNA REPLICATION, Heinz-Peter Nasheuer, Christine Schneider, Linda Guarino, Andrea Brückner, Christoph Rehfuess, Franz Stadlbauer, Klaus Weißhart, Irene Boche, Ellen Fanning, Institute for Biochemistry, Karlstr. 23, 80333 Munich, Germany.

Specific interaction of the viral initiator protein T antigen with its cognate origin of viral DNA replication sets in motion a sequence of events that culminates in the initiation of DNA replication. The initial binding and opening of the origin, as well as bidirectional origin DNA unwinding, are regulated by the phosphorylation state of T antigen and its interactions with ATP (1, 2). Subsequent synthesis of primers at the viral origin requires physical and functional interactions between T antigen and the cellular replication proteins DNA polymerase α–primase and RP-A (3, 4, 5). Interactions of SV40 and polyoma (Py) large T antigens were analyzed to elucidate the molecular basis for the species specificity of polymerase α-primase in viral DNA replication. Purified polymerase-primase of human, mouse and calf origin bound to both SV40 and Py T antigens without significant species specificity. SV40 T antigen stimulated primer synthesis and primer elongation on M13 DNA by both human and mouse polymerase-primase, both in the presence and absence of RP-A. However, primer synthesis on SV40 origin DNA in the presence of SV40 T antigen and RP-A was catalyzed only by human polymerase-primase. Conversely, primer synthesis on Py origin DNA in the presence of Py T antigen and RP-A was carried out only by the mouse enzyme. A series of cDNAs encoding each of the multiple subunits of human and mouse polymerase-primase and human RP-A has been expressed using baculovirus vectors. The purified subunits and enzyme complexes have been tested for their protein-protein interactions, activity and species specificity in primer synthesis at the viral origin and in viral DNA replication. The results will be discussed.

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MZ 002 MULTIPLE FUNCTIONS OF THE BACTERIOPHAGE λ O INITIATOR PROTEIN IN THE INITIATION OF VIRAL DNA REPLICATION, Roger McMacken, Soo-jong Um, Li Huang, D.S. Sampath, and Brian Learn, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205

Ten purified λ and *Escherichia coli* proteins are required for the initiation of phage λ DNA replication *in vitro*. The λ O and P replication proteins direct the assembly of an ordered series of nucleoprotein preinitiation structures at the phage origin (*ori*λ). Partial disassembly of the complete preinitiation complex, through the action of the *E. coli* DnaJ, DnaK and GrpE heat shock proteins, results in unwinding of the chromosome, followed by priming and DNA synthesis. We have carried out a comprehensive examination of the functional roles of the λ O replication initiator in the initiation process. The λ O protein binds as a dimer to four, 19 base pair recognition sites (iterons) in *ori*λ and self-assembles into a nucleoprotein structure, termed the O-some, in which the origin DNA appears to be wrapped in a specific configuration. Each DNA recognition site is bent approximately 85° by the binding of O and the λ origin acquires 0.7 turns of left-handed writhe when it is present in an O-some. Additionally, formation of an O-some on negatively supercoiled DNA causes an A+T-rich segment of *ori*λ, adjacent to the O recognition sequences, to adopt a non-B DNA configuration. This alteration, which does not, as commonly believed, represent complete strand separation, may be required for initiation of chromosome unwinding by DnaB helicase. Biochemical, genetic, and physical studies indicate that O protein is composed of two independent structural and functional domains. Analysis of O deletion mutant proteins indicates that (i) an amino-terminal region encompassing amino acids 19 - 110 contains all the information necessary for specific recognition and bending of *ori*λ DNA as well as for protein dimerization; and (ii) the C-terminal domain of O (amino acids 183-299) functions in the recruitment of a λ P· *E. coli* DnaB helicase complex to the O-some. We have recently discovered that the C-terminal domain also has an intrinsic single-stranded-DNA binding activity. We find, further, that the affinity of this domain for random single-stranded DNA is strengthened by more than 100-fold following its interaction with the P· DnaB complex. Moreover, we have found that the second-stage *ori*λ·O·P·DnaB nucleoprotein structure has acquired special properties. Once formed on a supercoiled DNA template, this nucleoprotein structure, but not the O-some, retains its replication capacity for an extended period after DNA superhelicity is removed by linearization. Formation of this activated intermediate does not require the presence of ATP. We tentatively conclude that supercoiling is required for initiation of λ DNA replication to permit an allosterically-activated C-terminal domain of O to bind stably to a conformationally-distorted A+T-rich region of *ori*λ.

Sequence and Structure Specific Interactions with RNA

MZ 003THE MEANING OF RNA TETRALOOPS, Fiona Jucker & Arthur Pardi, Department of Chemistry & Biochemistry, University of Colorado at Boulder, Boulder, CO 80309.

RNAs are complex biomolecules with diverse biological functions. In our laboratory we are investigating key RNA structural elements by means of heteronuclear multidimensional NMR spectroscopy. These studies help us understand the role of these structural elements in larger biomolecular complexes such as the ribosome or the signal recognition particle. Correlating both mutational analysis and phylogenetic data with the structure, helps define the functional roles of these RNA structural motifs. RNA molecules are exceptionally suited for a "dissective" approach because the secondary structure elements (such as the helices and the hairpins) are thermodynamically very stabile. Additional interactions are thought to form that stabilize the tertiary structure, generally without major rearrangement or disruption of the secondary structure. Thus we can study isolated structural elements and extrapolate our knowledge of the structure of such a RNA structural motif to its structure and function within the context of a larger RNA. Excellent candidates for such an approach are the so called tetraloops, small RNA hairpins with four nucleotides in the loop. These tetraloops have (i) high thermal stability compared to standard four nucleotide loops (i.e. AAAA), (ii) appear very frequently in ribosomal RNAs and group I introns and (iii) are often highly conserved protein recognition sites. Tetraloops have been grouped into three major classes based on sequence analysis of biologically active RNAs. These classes are GNRA, UNCG and CUUG (N= any nucleotide, R= purine). The structures of these tetraloops have been investigated in our laboratory (GNRA (1), CUUG) and in Tinoco's laboratory (UUCG (2)). Although there are key structural features that are common to all three tetraloops, their detailed structures are quite different. Correlating the three dimensional structure with phylogenetic data and mutational data allows us to recognize the functional requirements of these tetraloops in different biological contexts.

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Prot./RNA Interactions at Promoter

MZ 004 PEPTIDE MODELS OF RNA-PROTEIN RECOGNITION, Lily Chen, Ruoying Tan, Jianshi Tao, and Alan D. Frankel, Department of Biochemistry and Biophysics, and Gladstone Institute of Virology and Immunology, University of California, San Francisco, CA 94143

Understanding the basis of specificity in an RNA-protein interaction requires detailed information about both protein structure and RNA structure. Because the folding of these molecules can be complex, it seems desirable to reduce each component to the smallest possible unit of recognition. Fortunately, one class of RNA-binding proteins, that have in common an "arginine-rich" motif, appears particularly well-suited to this approach. This set of proteins includes bacterial transcriptional antiterminators, ribosomal proteins, viral coat proteins, and the Tat and Rev proteins from HIV. In several cases, it has been shown that peptides spanning just the arginine-rich motif, as short as nine amino acids, bind specifically and with high affinity to their respective RNA sites. In HIV Tat, a single arginine residue on an unstructured peptide mediates specific binding to a bulge region in TAR RNA, while in a related Tat protein from bovine immunodeficiency virus, several amino acids, also on an unstructured peptide, are needed for binding. The structures of these two TAR RNA sites show interesting sequence similarities but appear to adopt very different conformations. In HIV Rev, several amino acids on an arginine-rich peptide are needed for binding to RRE RNA, but the peptide must be in an α-helical conformation to bind specifically. In at least two of these three examples, the RNAs undergo significant conformational changes upon peptide binding, rearranging groups in the RNA structure to help stabilize the bound RNA conformation. One feature common to all three RNAs is that bulge or internal loop regions are critical for recognition. Studies of these relatively simple model systems are beginning to identify important structural determinants that contribute to RNA-binding specificity and will be discussed.

MZ 005 DETERMINING THE STRUCTURE OF RNA-PROTEIN COMPLEXES BY NMR - THE RNP DOMAIN, Peter Howe, David Neuhaus. Kiyoshi Nagai & Gabriele Varani*, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England.

The RNP domain is one of the most common RNA-binding motives, and is found in many RNA-binding proteins, including hn RNP and snRNP protein components. The RNP domain from the human U1A protein N-terminal domain consists of two α-helices packed against a four stranded antiparallel β–sheet, where the highly conserved RNP-1 and RNP-2 sequences are located[1]. This important structure has revealed precious little on RNA recognition, because the same RNP structure recognizes a stem-loop structure in U1 snRNA, a bulge loop in the U1A mRNA 3' UTR, and single stranded polypirimidine tract in the case of hn RNP C. How does the same protein structure recognize diverse RNA structural elements? How does sequence specific recognition of RNA takes place? The first 98 amino acid within N-terminal RNP domain of the human U1A protein are both necessary and sufficient for high affinity ($K_d = 10^{-11}$ M) binding to stem loop II of U1 snRNP RNA. Because of its high affinity and relatively small size, this complex represents an ideal system for NMR investigation of the structure of RNA-protein complexes. The U1A 102-amino acid N-terminal domain complexed with a 27 nucleotide RNA hairpin loop from the human U1 sn RNA gives NMR spectra of high quality, despite the high molecular weight of the complex (21,000 da). Several features of the interaction have been observed by NMR, and are generally consistent with available data from chemical and enzymatic mapping, as well as site-directed mutagenesis. Two-dimensional experiments have revealed intermolecular contacts between the nucleotides in the single stranded apical loop and protein side chains. Furthermore, intermolecular NCE's have also been observed between the protein and a GU - UU small internal loop at the base of the stem-loop. This contacts are fully consisent with phosphate ethylation experiments, that revealed protection at both the single stranded large apical loop and the small internal loop at the base of the stem. The large loop appears to be unstractured in the absence of the protein, but binding of U1A stabilizes the loop, perhaps by formation of non Watson-Crick pairs in addition to RNA-protein contacts. The large molecular weight of the complex prevents a more quantitative investigation by proton NMR alone. Isotopically labeleded protein samples have been used in conjunction with unlabeled RNA to selectively edit the protein spectrum in the complex. The data have been used to map the sites of contact of the RNA on the protein. Similarly, isotopically labeled RNA will be prepared to identify at a much more detailed level the footprint of the protein on the RNA hairpin. This strategy should also allow resonances assignments of the protein and RNA components in the complex, and subsequently the determination of the structure of the RNA-protein complex.

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Prot./DNA Interactions at Promoter

MZ 006PROMOTER-PROXIMAL PAUSING OF POLYMERASE IN THE HUMAN C-MYC GENE, Anton Krumm[1], Tea Meulia[1], Linda Madisen[1], and Mark Groudine[1,2], [1]Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98104, [2]Department of Radiation Oncology, University of Washington School of Medicine, Seattle, WA.

A conditional block to transcriptional elongation is an important mechanism for regulating c-myc gene expression. This elongation block within the first c-myc exon was defined originally in mammalian cells by nuclear run-on transcription analyses. Subsequent oocyte injection and in vitro transcription analyses suggested that sequences near the end of the first c-myc exon are sites of attenuation and/or premature termination. More recently, the mapping of single stranded DNA in vivo with potassium permanganate ($KMnO_4$) and nuclear run-on transcription assays have revealed that polymerase is paused near position +30 relative to the major c-myc transcription initiation site. In addition, sequences upstream of +50 are sufficient to confer the promoter-proximal pausing of polymerases and to generate the polarity of transcription further downstream. Thus, the promoter-proximal pausing of RNA polymerase II complexes accounts for the block to elongation within the c-myc gene in mammalian cells. Currently, we are attempting to determine the sequence elements within the human c-myc promoter that contribute to the promoter-proximal pausing of polymerase in mammalian cells.

MZ 007 LOADING AND FIRING OF RNA POLYMERASE II IN DROSOPHILA AND YEAST. J. Lis[1], C. Giardina[1], A. Greenleaf[2], S. Hardin[2], T. O' Brien[1], E. Rasmussen[1], L. Shopland[1], and H. Xiao[1]. [1] Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853; [2] Department of Biochemistry, Duke University Medical Center, Durham, NC 27710.
RNA polymerase II is transcriptionally paused at the 5' end of Drosophila heat shock genes. Characterizations of nuclear run-on transcripts and mapping of transcription bubbles on the *hsp70* gene in vivo with the single-stranded DNA probe KMn04 demonstrate that this paused RNA polymerase is distributed over a 20 bp interval (+17 to +37) with two preferred positions separated by one turn of the DNA helix (1, 2). Both *hsp26* and *hsp27* genes show a distribution of pause sites similar to that of *hsp70* but the pausing regions begin approximately 10 bp farther from the transcription start sites. The mechanism for restraining polymerase after the initial pulse of transcription is unknown; however, the bimodal distribution of the paused polymerase suggests that polymerase may be tethered by interaction with a specific factor positioned on the same side of the DNA helix. Such a tether would have to be somewhat flexible to account for the range of pause sites. We have examined the phosphorylation status of the largest subunit of RNA polymerase on genes in vivo by UV-crosslinking using antibodies raised to the hyperphosphorylated or to the nonphosphorylated C-terminal domain (CTD) of the largest subunit. The RNA polymerases that are in productive elongation complexes on the heat shock induced gene have a phosphorylated CTD, while the CTD of the paused polymerase is mainly unphosphorylated. Thus, phosphorylation of the CTD in vivo can occur after polymerase has already initiated transcription, and the phosphorylation may be linked to the escape of the paused polymerase into a productive elongation complex. Genetic tests (3) show that sequences within and upstream of the pause region are critical for creating this association of polymerase with the 5' end of the gene, and thus, components of the promoter and pause region may provide the interactions that both recruit polymerase and slow its escape from the promoter. Heat shock leads to a 100-fold increase in the rate of escape from the pause by inducing the binding and activation of heat shock factor. The binding of HSF requires "open" chromatin that is dependent on promoter elements that are also responsible for creating paused polymerase. These features of the promoter may be general, since RNA Polymerase pauses at the 5' end of a number of higher eucaryotic genes, and it also has a long dwell time on yeast promoters. Domains of RNA polymerase can bind in vitro to basal factors of the promoter and these interactions may slow polymerase escape. The fact that upstream activators bind to the same basal factors as does polymerase suggests a mechanism for transcriptional control that is applicable both at the level of loading RNA polymerase on the promoter and the firing of paused polymerase into a fast elongation mode.

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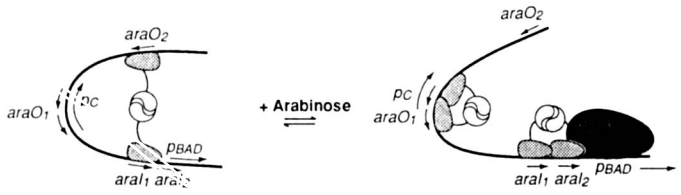
MZ 008ARAC DOMAIN STRUCTURE AND REORIENTATION IN REGULATION, Robert Schleif, Biology Department, Johns Hopkins University, 34th and Charles, Baltimore, MD 21218
A variety of data lead to the conclusion that the dimeric AraC protein contains a dimerization domain and a DNA-binding domain. The protein activates transcription when and only when its DNA-binding domain binds to a site partially overlapping the -35 region of the promoter. This occupancy is normally generated when arabinose induces the protein to relocate the relevant DNA binding-activation domain from the distal $araO_2$ site to the activating site at the -35 region. The minimal dimerization domain and minimal DNA-activation domain regions have been determined biochemically, thereby also locating the domain linker region. Genetic methods also identified the same linker region. RNA polymerase open and closed complexes can be formed at a derivative of the p_{BAD} promoter. AraC stimulates the rate of open complex formation, and its DNAse footprint is altered by RNA polymerase. The footprint of RNA polymerase changes upon formation of the open complex.



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AraC Protein Can Activate Transcription from Only One Position and When Pointed in Only One Direction, T. Reeder, R. Schleif, J. Mol. Biol. *231*, 205-213 (1993).

Structure Specific Interactions with DNA

MZ 009 *OXYTRICHA* TELOMERE-BINDING PROTEIN PROMOTES G-QUARTET FORMATION BY TELOMERIC DNA, Guowei Fang and Thomas R. Cech, Dept. of Chemistry and Biochemistry, Howard Hughes Medical Institute, University of Colorado, Boulder, Colorado 80309
Telomeres, the ends of linear chromosomes, typically consist of tandem repeats of a simple guanine-rich sequence. In several evolutionarily divergent organisms, the guanine-rich strand has two repeat units protruding as a 3' single-stranded overhang. At high DNA concentrations (micromolar), single-stranded telomeric DNA can form intermolecular complexes stabilized by G-quartets. A G-quartet consists of four guanine residues held together by G·G Hoogsteen base pairs in a square-planar unit. We show that under physiological conditions the β subunit of the *Oxytricha* telomere-binding protein promotes formation of these higher order complexes at nanomolar DNA concentrations. The reaction occurs with oligonucleotides ending in the *Oxytricha* ($T_4G_4T_4G_4$) or *Tetrahymena* ($T_2G_4T_2G_4$) telomeric sequences. The intrinsic ability of an oligonucleotide to form G-quartets is a prerequisite for the β-mediated reaction, and the β subunit enhances the rate of this thermodynamically favored transition. At lower protein concentrations, the β subunit acts without remaining stably bound to the DNA; at higher protein concentrations, which drive the formation of protein-DNA complexes, proteinase K treatment reveals that the same set of DNA structures are formed. Thus, the β subunit acts as a molecular chaperone to promote nucleic acid structure formation. At saturating protein concentrations (≥ 200 nM), β-mediated G-quartet formation is a first-order reaction with respect to DNA concentration, with $k \approx 1\ hr^{-1}$ at 37°C. In contrast, the protein-independent reaction is a second-order reaction. The β subunit enhances the rate of G-quartet formation by 10^5- to 10^6-fold at 20 nM telomeric DNA concentration. The β-mediated higher order complexes are identified as dimers and tetramers of telomeric DNA. Protein deletion analysis indicates that the C-terminal highly basic region, which bears a slight sequence similarity to histone H1, is important for the β-mediated reaction. Interestingly, the N-terminal two-thirds of β is sufficient for formation of the α·β·DNA ternary complex. Thus, the ability to promote G-quartet formation and the ability to form the ternary complex require different domains of the β subunit, and represent two different activities of the β subunit. Other basic proteins, such as histone H1 and cytochrome c, also promote formation of G-quartets; however, the rates of the reactions mediated by these proteins are much slower than that mediated by β. The finding that a telomere protein acts as molecular chaperone to promote G-quartet formation by telomeric DNA strongly suggests that the G-quartet structures are biologically relevant. This activity of the telomere protein may enable chromosome-chromosome association or the regulation of telomerase activity *in vivo*.

MZ 010 INTERACTIONS OF TBP WITH THE TATA BOX, Diane K. Hawley, D. Barry Starr, Stefan Hermann, Barbara C. Hoopes, James F. LeBlanc, Lara Baxley, and Charles Foulds, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

The TATA binding protein (TBP) is a DNA binding protein required by all three eukaryotic nuclear RNA polymerases for transcription initiation. We are interested in the role of TBP in transcription by RNA polymerase II and are using a variety of physical and functional techniques to characterize the interaction between TBP and the TATA box, a conserved DNA sequence element found in many class II promoters. TBP, which is functional *in vivo* as part of a larger protein complex called TFIID, binds the TATA box to initiate the assembly of a preinitiation complex. Base pair changes in the TATA box have been shown to alter transcription *in vivo* and *in vitro*, demonstrating that the TBP-TATA box interaction is an important determinant of promoter strength. Previous studies from this and other laboratories have revealed several unusual features of the mode of TBP binding to the TATA box. First, the rate of binding of TBP is highly concentration dependent, and binding is likely to occur in a multi-step pathway. Second, recognition of the TATA box does not require base-specific contacts within the major groove of the DNA helix and has been proposed to occur predominately through the minor groove. Third, formation of a TBP:TATA box complex results in a significant bending of the promoter DNA. All of these characteristics of TBP binding may be important to its role in transcription initiation and to the importance of the sequence of the TATA box to promoter activity. To begin to address those possibilities, we have analyzed the extent, location, and direction of the DNA bend induced by the binding of TBP to the consensus TATA box and a number of sites containing one or two base pair changes from the consensus. We found that TBP bends the DNA toward the major groove; and all of the TATA box mutants we have examined altered the magnitude of the bend but not the location or direction. Furthermore, we found a strong correlation between the degree to which the DNA is bent in the complex and the stability of the TBP-TATA box complex. This result suggests that the ability of TBP to deform a particular DNA sequence to the proper conformation is an important component of recognition and binding by TBP. This may explain how TBP can distinguish among different A-T-rich sequences, which do not display substantially different hydrogen bonding patterns within the minor groove. In a complementary approach, we have also begun to examine the binding properties of several TBP mutants that have strikingly altered DNA recognition properties *in vivo* and/or *in vitro*.

MZ 011 RECOGNITION, TRANSLOCATION AND RESOLUTION OF HOLLIDAY JUNCTIONS, Stephen C. West[1], David Adams, Richard Bennett[1], Fiona Benson[1], Adelina Davies[1], Hazel Dunderdale[1], Kevin Hiom[1], Alison Mitchell[1], Berndt Müller, Carol Parsons[1], Rajvee Shah[1], Irina Tsaneva[1], Andrzej Stasiak[2], Edward Egelman[3], [1]Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts EN6 3LD, U.K., [2]University of Lausanne, CH-1015 Lausanne, Switzerland, [3]University of Minnesota, Minneapolis MN55455.

The *ruv* locus of the *E. coli* chromosome encodes three proteins that interact with Holliday junctions during genetic recombination and the recombinational repair of DNA. The 22 kDa RuvA protein binds specifically to Holliday junctions and targets RuvB protein (37 kDa) to the site of the junction. In the presence of Mg/ATP, assembly of RuvB on DNA involves the formation of doublet ring-like structures which have been visualized by electron microscopy. Preliminary structural analysis indicates that there are 6-10 RuvB monomers per ring, indicating up to 20 subunits per protein structure. Images of the rings have been cylindrically averaged and 3-D reconstructions indicate that the RuvB rings are bipolar with a deep hollow core through which the DNA appears to lie. Using model Holliday junctions, we have shown that RuvAB catalyze Holliday junction movement in vitro. This process, known as branch migration, occurs at the expense of RuvB-mediated ATP-hydrolysis and leads to the formation of heteroduplex DNA. Given that RecA protein promotes Holliday junction formation and drives strand exchange in vitro, the apparent redundancy of function may indicate that RuvAB-mediated branch migration occurs at lower energy cost than similar reactions catalyzed by the RecA nucleoprotein filament.

Following branch migration, the production of mature recombinant DNA molecules requires resolution of Holliday junctions, and this can be carried out by the 19 kDa RuvC protein. This protein is a junction-specific endonuclease which catalyzes resolution via the introduction of nicks into two DNA strands of the same polarity. The cuts are placed with perfect symmetry in each duplex to produce nicked duplex products that are repaired by DNA ligase. The resolution reaction can be sub-divided into three distinct steps: (i) structure-specific recognition of the junction by RuvC, (ii) DNA distortion, and (iii) sequence-dependent cleavage. Although RuvC exhibits low activity in vitro, the rate of Holliday junction resolution is increased by reaction conditions that destabilise duplex DNA (high temp and pH), possibly through an increase in the rate of spontaneous branch migration. These results may indicate that the RuvC-Holliday junction complex needs to translocate along DNA until specific resolution sequences ('hotspots') are encountered. The possibility that RuvAB-mediated branch migration is required for efficient Holliday junction resolution by RuvC is being investigated.

Multiprotein Complex with RNA

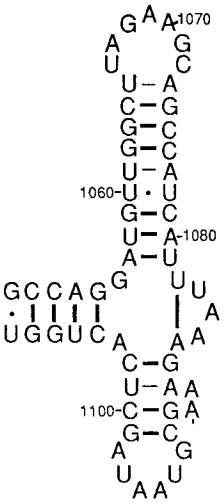
MZ 012 PROTEIN, ANTIBIOTIC, AND ION BINDING TO A CONSERVED RIBOSOMAL RNA JUNCTION, D. E. Draper, M. Lu, Y.-X. Wang, and Y. Xing, Department of Chemistry, Johns Hopkins University, Baltimore, MD 21218 USA

A 58 nt domain of large subunit ribosomal RNAs [see figure to right] has been highly conserved and probably functions as part of the binding site for elongation factors during protein synthesis. We find that the fragment contains significant tertiary interactions ($\Delta H \approx 40$ kcal/m), and in melting experiments have identified several conserved bases and base pairs as essential for their stabilization. All of the sites identified so far surround the junction of helices. Four different ligands stabilize tertiary structure:

- a single Mg^{2+} ion specifically binds to the RNA and stabilizes tertiary structure, probably by direct coordination; even Ca^{2+} is less effective.
- the same structure also binds a single NH_4^+ ion in preference to other monovalent ions.
- the conserved ribosomal protein L11 binds to the RNA fragment and requires both Mg^{2+} and NH_4^+ ions for full binding strength; mutations which destabilize the tertiary structure also weaken L11 recognition.
- the peptide antibiotic thiostrepton binds the RNA fragment directly, and the complex is further stabilized by L11.

All these ligands can bind simultaneously and cooperatively to the RNA structure; we are measuring the individual cooperativity parameters by a combination of melting and titration studies. Using L11 derived from a thermophile, the L11-thiostrepton-ion-RNA complex is stable to ~70°C.

Selection/amplification experiments have been used to explore the sequence requirements for L11 and thiostrepton binding further. Sets of sequence changes that weaken the NH_4^+ and thiostrepton affinities, but still allow L11 to bind, have been identified. None of the mutations changes a direct contact site for thiostrepton. It may be that L11 is tolerant of a greater range of conformations than is thiostrepton, and that thiostrepton inhibits protein synthesis by "freezing" the RNA in a single conformation.



MZ 013 PROTEIN-RNA INTERACTIONS IN THE RIBOSOME: CONSTRUCTION OF A SYNTHETIC RNP DOMAIN, AND REFINEMENT OF A MODEL FOR THE 30S RIBOSOMAL SUBUNIT. Harry F. Noller, Raymond R. Samaha, Bonnie O'Brien†, Thomas O'Brien† and Ted Powers. Sinsheimer Laboratories, UCSC, Santa Cruz, CA 95064, and †Dept. of Biochem.and Mol. Bio., Univ. of Florida, Gainesville, FL.

We have investigated the ability of the 3' domain of 16S rRNA to assemble autonomously in vitro. In vitro RNA transcripts corresponding to the 3' domain were reconstituted with total 30S ribosomal proteins (TP30).The RNA was converted quantitatively into an RNP particle containing only a specific subset of 9 of the 21 30S proteins. All of these proteins have previously been localized to the head of the 30S subunit by immuno-electron microscopy and by neutron diffraction. In the electron microscope, the synthetic RNP particles appear compact and globular, closely resembling disembodied heads of 30S subunits. Chemical probing shows that assembly is incomplete at the nucleotide level in certain local regions of the domain, suggesting that assembly steps involving some of the late-assembling proteins may be incomplete for at least a sub-set of the particles. Spectinomycin binds specifically to the RNP particles, with an affinity similar to that observed for complete 30S subunits. Since protein S5 is absent from the particles, we conclude that it cannot be essential for binding of this antibiotic, and that SpcR mutations in S5 must exert their effects by an indirect mechanism.

In a second effort, we have studied protein-RNA interactions in the 30S subunit using hydroxyl radical footprinting. Nearly all of the 30S proteins give specific footprints with this method, and these data more clearly define their contact sites on 16S rRNA. Using these data, alone, in conjunction with the neutron map of the positions of the 30S proteins, we have modeled the folding of the central domain of 16S rRNA. In general, it resembles our earlier model based on base-specific footprinting and crosslinking results, but with two significant differences. First, the platform of the 30S subunit is located further from the head, opening the cleft such that it bears striking resemblance to e.m. pictures. Second, the 620 stem, previously placed in the bottom of the subunit, now angles up into the center, giving the particle a more compact overall appearance. The refined model of the central domain is also in close accord with the base-specific and crosslinking data, even though neither they nor any e.m. information were used in the modeling procedure.

MZ 014 RNA-PROTEIN INTERACTIONS IN THE INITIATION OF TRANSLATION AND TRANSCRIPTION OF POLIOVIRUS RNA. Eckard Wimmer, Louis Alexander, Kevin S. Harris, Christopher U.T. Hellen, Akhteruzzaman Molla, Aniko V. Paul, Tatyana V. Pestova, Michael Schmid, Sang Hoon Shin, Gary W. Witherell, and Wenkai Xiang, State University of New York at Stony Brook, NY.

The poliovirus (PV) genome, after the virus' invasion into the host cell cytoplasm, serves first as the mRNA and subsequently as the template in genome replication. Initiation of translation follows a novel mechanism of "internal ribosomal entry" mediated by a complex genetic element called "internal ribosomal entry site" (IRES). IRES elements are common to all picornaviruses, a large family of pathogens consisting of 5 genera that can be broadly divided into two groups based on their genetic kinship. Accordingly, IRES elements can also be divided into two types: type 1 (example: PV), and type 2 (example: encephalomyocarditis virus, EMCV). IRES elements, located within the 5' non-translated region (5'NTR) of the uncapped mRNA, are highly structured RNA segments ca. 400 nt long . Interestingly, although type 1 and type 2 elements share little with respect to their sequence or their structure, they function in an identical manner. Thus, a viable poliovirus has been constructed in which the PV IRES has been replaced by the EMCV IRES. Tandemly arranged type 1 and type 2 IRES elements in the 5'NTR of the PV genome are genetically unstable, but a type 2 IRES, when inserted into a cleavage site of the viral polyprotein, yielded a unique and genetically stable dicistronic poliovirus. The only feature common to all picornavirus IRES elements is a YnXmAUG motif (Y, pyrimidine; n = 8 - 10; X, any nt; m = 20 - 25) whose integrity appears essential for function. We have discovered *trans*-acting factors distinct from conventional translation factors, whose interaction with the IRES was essential for function. We have studied, in detail, a predominantly nuclear protein called the pyrimidine tract-binding protein (PTB), or hnRNP I, and its specific binding characteristic to IRES elements. Specific point mutations in the IRES eliminate PTB binding and, concomitantly, initiation of translation. Several other parameters of IRES structure and function will also be discussed.

A clover leaf structure, that is formed by the 88 5'-terminal nt of the viral genome RNA (and proceeds the IRES element) has been shown by Andino et al. (EMBO J. **12**, 3587, 1993) to interact with the viral proteinase $3CD^{pro}$, the precursor polypeptide to the viral RNA polymerase $3D^{pol}$, in the presence of host cell proteins. Available evidence suggests that this interaction plays a role in viral transcription. We have identified two polypeptides that will facilitate the binding of the proteinase to the cloverleaf structure of the viral RNA. We will present a model explaining the initiation of transcription at the 3' end of minus RNA strands following the formation of the ribonucleoprotein complex at the 5' end of plus RNA strands.

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Processive Interactions with DNA

MZ 015DISSECTING THE MECHANISM OF *TETRAHYMENA* TELOMERASE. Carol W. Greider, Chantal Autexier, Kathleen Collins and Lea Harrington[1]. Cold Spring Harbor Laboratory Cold Spring Harbor NY and [1] Genetics Program SUNY, Stony Brook, NY

Telomerase is a ribonucleoprotein polymerase which synthesizes telomere repeat sequences *de novo* onto chromosome ends[1,2]. The ability of telomerase to extend chromosome ends suggest that it is involved in assuring telomere length maintenance. *Tetrahymena* telomerase contains an essential 159 nucleotide RNA with the sequence 5'-CAACCCCAA-3', that specifies the TTGGGG repeats synthesized. To understand the mechanism of telomerase, we have begun a detailed biochemical analysis. In analyzing the primer specificity of the reaction, we found that telomerase will processively add tandem repeats of TTGGGG onto primers greater that 10 nucleotides. However with shorter primers, repeat addition was non-processive[3,4]. This and other[5] data suggests that there are two distinct sites on telomerase for interacting with primer oligonucleotides. In addition to elongation of telomeric primers, *Tetrahymena* telomerase also catalyzed a primer cleavage activity. When telomeric oligonucleotides ending in the 3' sequence GGGGTTG were incubated with telomerase and 32P-dGTP, the 3' most dG was removed and a labeled dG was added. The cleavage activity was specific to telomeric primers with the appropriate 3' end sequence.[4] This nucleolytic cleavage activity is similar in many ways to the recently described cleavage activity associated with RNA polymerases. To understand the role of the telomerase RNA component and define the essential structural elements, we developed a functional reconstitution assay. We treated partially purified telomerase with micrococcal nuclease to remove endogenous RNA. After the addition of *in vitro* transcribed *Tetrahymena* telomerase RNA, fully functional telomerase was reconstituted. Reconstitution was specific for the addition of telomerase RNA and was not stimulated excess nonspecific RNA. A mutant in the template region, 5'-TAACCCCAA-3', specified the incorporation of dA into the sequence $(TTAGGG)_n$. Using this and other mutants, we are characterizing the function of the template and other regions of the *Tetrahymena* telomerase RNA. We are purifying telomerase to identify the polypeptide components. We have carried out UV crosslinking of protein bound to primers during the elongation reaction and identified a 100kDa polypeptide which specifically cross linked, copurified with telomerase activity and cosedimented in on a sucrose gradient. This protein is a candidate for a polypeptide component of telomerase.

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MZ 016 *E. coli* REP HELICASE-CATALYZED UNWINDING OF DNA, T. M. Lohman, M. Amaratunga, I. Wong, K. M. J. Moore, and K. Bjornson, Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, Box 8231, 660 S. Euclid Avenue, St. Louis, MO 63110.

DNA helicases unwind duplex DNA to form the single stranded (ss) DNA intermediates required for replication, recombination and repair in reactions that require nucleoside-5'-triphosphate hydrolysis. Most helicases require a ss-DNA flanking the duplex in order to initiate DNA unwinding *in vitro*; however, the precise function of the ss-DNA is not understood. Possible mechanisms for helicase-catalyzed DNA unwinding can be grouped into two general categories: "passive" *vs.* "active" mechanisms. If a helicase unwinds DNA by a "passive" mechanism, it would bind to and translocate unidirectionally along the ss-DNA and facilitate duplex unwinding by translocating onto the ss-DNA that is formed transiently by thermal fluctuations in the duplex. On the other hand, in an active mechanism, the helicase would participate directly in destabilizing the duplex, most likely by binding to and distorting the duplex DNA.

The active form of the *E. coli* Rep helicase (a 3' to 5' helicase) appears to be a dimer that forms upon binding DNA with both protomers of the dimer able to bind either single stranded (ss) or duplex (ds) DNA. Studies of the energetics of DNA binding and DNA-induced Rep dimerization in the presence of the nucleotide cofactors, ADP and the non-hydrolyzable ATP analog AMPP(NH)P indicate allosteric effects of nucleotide cofactors on DNA binding such that ADP binding favors Rep dimers in which both protomers bind ss-DNA (P_2S_2 complex), whereas AMPP(NH)P binding favors simultaneous binding of both ss- and ds-DNA to the Rep dimer (P_2SD complex). Based on these observations, a "rolling" model for the active unwinding of duplex DNA by the dimeric Rep helicase has been proposed in which a P_2SD complex is an essential intermediate. This model explains vectorial unwinding and predicts that Rep helicase translocation along DNA is coupled to ATP binding, whereas ATP hydrolysis drives unwinding of multiple DNA base pairs for each catalytic event.

Using rapid chemical quench-flow methods, we have examined the kinetics of Rep-catalyzed DNA unwinding using a series of non-natural DNA substrates possessing 3' flanking ss-DNA within which is embedded either a segment of ss-DNA possessing reversed backbone polarity or a non-DNA (poly-ethylene glycol) spacer, either of which should block unwinding by a strictly passive helicase. We observe that the *E. coli* Rep helicase effectively unwinds these DNA substrates ruling out a passive unwinding mechanism for Rep. Instead, the results are consistent with the proposed active "rolling" mechanism during which a P_2SD dimer intermediate forms.

MZ 017 AN INTERACTION REQUIRED FOR LAGGING-STRAND DNA SYNTHESIS AT A PROCESSIVE REPLICATION FORK, Killu Tougu, Hong Peng, and Kenneth J. Marians, Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York.

Using a specialized tailed form II DNA template, we have reconstituted the *E. coli* replication fork using highly purified ϕX-type primosomal proteins and the DNA polymerase III holoenzyme. These replication forks move at *in vivo* rates and are processive for at least 1 MB. Surprisingly, we showed that the primase, DnaG, does not remain associated continuously with the replication fork. Instead, once primer synthesis is completed primase dissociates from the replication fork to be replaced by another primase molecule for the next cycle of Okazaki synthesis. Proper association of primase with the replication fork is therefore essential. We have probed the interaction of primase with the replication fork. We find that primase can be divided into two domains, an N-terminal 49-kDa-domain that is sufficient for primer synthesis but is incapable of interacting with the replication fork, and a C-terminal 16-kDa-domain that possesses no enzymatic activity but is required for the interaction between primase and the replication fork. We also show that the interaction that localizes primase to the replication fork is with DnaB, the replication fork helicase.

MZ 018 INTRASUBUNIT DYNAMICS IN THE MULTIPROTEIN REPLICASE OF *ESCHERICHIA COLI*, DNA POLYMERASE III HOLOENZYME Mike O'Donnell*¶, Rene Onrust* and Todd Stukenberg*, *Microbiology Department and ¶Howard Hughes Medical Institute, Cornell University Medical College, 1300 York Avenue, New York, New York 10021.

The replicase of *Escherichia coli*, DNA polymerase III holoenzyme (Pol III), consists of 10 nonidentical subunits. Pol III holoenzyme is fast (750 nucleotides/second) and highly processive (over 50 kb) in DNA synthesis. Pol III owes its tight grip on DNA to the β subunit which is a ring shaped protein that encircles DNA and thereby anchors the Pol III machinery to its template. Pol III holoenzyme also contains within its structure one molecule of the γ complex which functions to load the β subunit onto DNA in a reaction that depends on ATP. The idea of a polymerase moving along with a sliding clamp fits nicely with the properties expected for a chromosomal replicase that must synthesize long chromosomes. In reality, this view is only favorable for the continuous extension of the leading strand. Due to the antiparallel orientation of strands in the DNA duplex, the lagging strand is synthesized discontinuously in many pieces known as Okazaki fragments. Thus the polymerase unit on the lagging strand must be capable of moving from one fragment to the next, a motion that would seem to be hindered by a tight grip to DNA via a clamp protein. One may consider that the 2-3 thousand Okazaki fragments are synthesized by an equal number of Pol III holoenzyme molecules, however the intracellular scarcity of the holoenzyme (10-20 molecules/cell) makes this impossible. The mechanism by which Pol III is tethered to DNA by a β sliding clamp yet is able to rapidly hop to another primed site has been investigated with the following result. Pol III is highly processive and remains firmly fixed to a β sliding clamp so long as there is still ssDNA template to be replicated. However, when a template is finished to the last nucleotide (ie. a nick), Pol III looses its grip to the β clamp and dissociates from it and the DNA; the β clamp simply remains behind on the product duplex. The Pol III is then free to rapidly reassociate with another primed site on a new template. This reassociation is rapid provided the new primed template contains a preassembled β clamp on it.

These dynamics fit nicely with the structural architecture of Pol III holoenzyme. The τ subunit of Pol III holds together two core polymerases presumably for the simultaneous synthesis of the leading and lagging strands of a chromosome. The molecule of the γ complex clamp loader is also held into the Pol III structure by the τ subunit. The γ complex is capable of rapidly loading multiple β clamps onto DNA and thus may repeatedly assemble β clamps on RNA primers on the lagging strand. Thus, at a replication fork, while the lagging polymerase is busy extending an Okazaki fragment the γ complex can position new β clamps on RNA primers. Then when the lagging polymerase finishes the Okazaki fragment it can release the DNA (and the β clamp), but due to the dipolymerase structure the lagging polymerase would not be released into solution. Instead it would be held at the fork by the leading polymerase. The lagging polymerase can then associate with the new β clamp on an RNA primer to initiate processive synthesis of the next Okazaki fragment.

Processive Interactions with RNA (Joint)

MZ 019 RECODING: INVOLVEMENT OF CIS ACTING SEQUENCE ELEMENTS, Raymond F. Gesteland[1,2], Norma M. Wills[1], and John F. Atkins[2], [1]Howard Hughes Medical Institute, Salt Lake City, UT, [2]University of Utah, Human Genetics Dept., Salt Lake City, UT 84112.

Programmed alteration of genetic decoding, or "recoding", involves a site on mRNA at which the genetic code is differently enabled and a stimulatory sequence that brings the recoding event up to its appropriate level of efficiency. Often these stimulatory signals are specific folded structures in mRNA including pseudoknots, simple and complex stem loops and mRNA:rRNA interactions. Various examples of recoding will be compared and contrasted. The case of stop codon readthrough used by Moloney Murine Leukemia Virus (MMTV) to express the gag-pol polyprotein will be discussed in detail. Here a nearby, downstream pseudoknot is the crucial stimulatory signal for decoding of a UAG stop codon. Not only are the two, presumably stacked, stems crucial, but specific bases spacing the pseudoknot and the first stem and bases in loop 2 must have the right identity. This suggests either a more complex structure or specific interaction of some components with the pseudoknot.

MZ 020 SPHERE ORGANELLES, Rabiya S. Tuma[1,2] and Mark B. Roth[2], [1]University of Washington, Seattle and [2]Fred Hutchinson Cancer Research Center, Seattle.

Sphere organelles are nuclear structures in amphibian oocytes that are easily visible by light microscopy. These structures are up to 10 mm in diameter and have been described morphologically for decades, yet their function remains obscure. We identified a protein component of the sphere organelle, named SPH-1, which is recognized by a monoclonal antibody raised against purified Xenopus laevis oocyte nucleoplasm. SPH-1 is an 80 kD protein which is localized specifically to spheres and is undetectable elsewhere on lampbrush chromosomes or in nucleoli. We have shown using confocal microscopy that SPH-1 is localized to the cortex of sphere organelles. Furthermore, we have isolated a cDNA that can encode SPH-1. When epitope-tagged forms of SPH-1 are expressed in X. laevis oocytes the protein specifically localizes to spheres, demonstrating that the cloned cDNA encodes the sphere antigen. Comparison of the predicted amino acid sequence with sequence databases shows SPH-1 is related to p80-coilin, a protein associated with coiled bodies; coiled bodies are nuclear structures found in plant and animal cells. The sphere-specific monoclonal antibody stains X. laevis tissue culture cells in a punctate nuclear pattern, showing that spheres or sphere antigens are present in somatic cells as well as germ cells and suggesting a general and essential function for spheres in all nuclei.

MZ 021 RHO PROTEIN AS A "STRUCTURE-FUNCTION" MODEL OF HEXAMERIC NUCLEIC ACID HELICASES, Peter H. von Hippel, Feng Dong, Johannes Geiselmann, Steven E. Seifried, Yan Wang, and Mark C. Young. Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, OR 97403.

The hexameric RNA-DNA helicase rho is required to bring about release of the nascent RNA at about one-half of the transcription termination sites of *E. coli*. Although the details of the molecular process catalyzed by rho to induce transcript release are far from clear, it is the nucleic acid helicase about which we have by far the most structural and enzymatic information. As a consequence it may serve as a paradigm for the study of the many related helicases that operate in DNA replication, recombination, and repair, and perhaps in RNA splicing. In this lecture we will review the molecular biological studies of rho that suggest that this protein functions at rho-dependent operons by initially binding to the nascent transcript at a largely unstructured and cytidine residue containing "rho loading site". This binding activates the RNA-dependent ATPase activity of rho, which, in turn, fuels the preferential 5' → 3' translocation of the rho hexamer along the nascent RNA. This translocation continues until rho "catches up" with the paused elongation complex at rho-dependent termination sites. By use of its 5' → 3' RNA-DNA helicase activity, rho then separates the RNA-DNA hybrid duplex within the elongation complex and brings about termination by releasing the nascent RNA into solution. Based on available and detailed knowledge of the enzymology and structure of rho we will put forward a plausible molecular model for this process and describe recent studies that we hope will test and refine the model further. The ability to translocate along single-stranded RNA or DNA with a preferred direction may be the central feature of the function of the hexameric helicases. Accordingly we will describe a recent steady-state kinetic analysis of the dependence on the length and properties of its single-stranded DNA cofactor of the DNA-activated ATPase of the DNA replication helicase of bacteriophage T4 (gene 41 protein). These studies show that this hexameric helicase, with a structure closely resembling that of rho, does appear to move along single-stranded DNA with a preferred direction. These results will be correlated with what is known about other hexameric helicases to come up with some general mechanistic suggestions for how these entities might work in discharging their physiological functions.

Tracking Mechanisms with DNA

MZ 022 NUCLEOTIDE EXCISION REPAIR: A TRACKING MECHANISM IN SEARCH OF DAMAGE[1]. L. Grossman, B. Ahn, L. Claassen, E. Hildebrand, S. Mazur, E. Oh, T. Seeley, S. Thiagalingam and J.T. Wang. Johns Hopkins University, School of Hygiene and Public Health, Baltimore.

The *E.coli* UvrABC endonuclease system is involved in the dual incision of DNAs damaged by a wide variety of chemically unrelated damage. The sites of breakage are essentially invariant regardless of the primary chemical nature of the genotoxic damage. In approaching the role(s) of ATP and the question of substrate specificity it became apparent that a complex consisting of $UvrA_2B_1$ required for tracking to damaged sites in DNA cannot locate such sites by passive diffusion because of the inadequate differences in association constants between undamaged and damaged sites in genomic DNA. Translocation to damaged sites is achieved as a consequence of multi-protein complexes which act as a helicase unwinding small DNA regions in a 5'->3' direction reforming hydrogen bonds behind it and generating, in the process, waves of supercoiling. This supercoiling probably qualifies the Uvr repair system to interact with those other biological processes which supercoil such as transcription and replication. To this end the T7 and *E. coli* holoRNA polymerases, in the presence of their specific promoters, interact with the Uvr A_2B complex in both supercoiling and transcription providing unique *start or landing sites* on the non-transcribed strand. ATP provides the energy for these preincision reactions by directing protein-protein and nucleoprotein associations generated by nucleotide binding whereas macromolecular dissociations are driven by ATP hydrolysis reactions. ATP is not required for the dual endonucleolytic reactions in incision once a damaged site is identified. The mechanisms of translocation are being studied by steady-state and time-resolved fluorescence spectroscopy.

[1]Grossman, L. and Thiagalingam, S. (1993) *J. Biol. Chem.* 268:16871-16874, Minireview.

MZ 023 The relationship between facilitated diffusion and catalytic parameters and the role of Z-DNA as a regulator of protein traffic on DNA.

Mark Surby and Norbert Reich*, Department of Chemistry and Interdepartmental Biochemistry Program, University of California, Santa Barbara, CA. 93106.

The *S*-adenosylmethionine-dependent *Eco*RI DNA methyltransferase efficiently ($k_{cat}/K_m^{DNA} > 10^8\ M^{-1}s^{-1}$) methylates the second adenine in GA**A**TTC. Placement of the canonical site within DNA fragments ranging from 14 to 4400 basepairs results in an 8-fold increase in k_{cat}/K_m respectively, largely due to decreases in K_m^{DNA}. After methylation the Enzyme•*S*-adenosylhomocysteine complex dissociates, thereby precluding any processive DNA methylation. The rate constant for site location (k_{on}) increases over 20-fold with DNA fragments ranging from 14 to 775 basepairs while k_{off} is independent of flanking DNA length. To account for our results, we have used the KINSIM modeling program and extended the theory for facilitated diffusion proposed by von Hippel and coworkers to include dissociation from the ends of DNA fragments.

*Eco*RI endonuclease was previously shown to use a facilitated search mechanism in binding its canonical site, GAATTC. The ability of the endonuclease to locate its site on linearized plasmids was shown to be sensitive to the presence of Z-DNA sequences. This effect does not derive from direct perturbation of the canonical site, but rather is a kinetic effect. Thus Z-DNA acts to slow down the enzyme's ability to locate its site.

MZ 024 SYMMETRY AND ASYMMETRY IN THE FUNCTION OF *E. coli* INTEGRATION HOST FACTOR: IMPLICATIONS FOR TARGET IDENTIFICATION BY DNA-BINDING PROTEINS, Milton H. Werner¥, G. Marius Clore¥, Angela M. Gronenborn¥ and Howard A. Nash†, ¥Laboratory of Chemical Physics, NIDDK and †Laboratory of Molecular Biology, NIMH, Bethesda, MD 20892

IHF has long been thought to be a paradigm of asymmetry in a DNA binding protein, comprised of two subunits (α and β) with only moderate amino-acid sequence similarity between them. We have challenged the notion that IHF is an obligate heterodimer by isolation and purification of the individual subunits. We show that each subunit is capable of specifically recognizing and binding to natural IHF binding sites and supporting site-specific recombination *in vitro*; mobility shift and footprinting data indicate that the isolated subunits interact with DNA as homodimers that assemble in the presence of a specific binding site. We have also investigated the asymmetry in natural IHF binding sites by design of symmetric oligonucleotides that are capable of specific binding to hetero- and homodimeric IHFs.

The available evidence provides little or no support that individual subunits of IHF can function *in vivo* without their counterparts. While a functional homodimer can replace the natural protein *in vitro*, it lacks internal stability. Closer examination of natural IHF binding sites indicates that there is a non-random distribution of nucleotides throughout the 30bp contacted by the protein, but there appear to be no internal symmetry elements that are consistently found in known sites. Our ability to symmetrize this system has forced us to consider the mechanistic basis for the prevalence of its asymmetry in nature. We have come to the conclusion that the clustering of recognition information is the selected feature, rather than the asymmetry. We propose that this reflects the need to focus the search for an IHF site to an acceptably small stretch of DNA--a single turn of the DNA helix. We emphasize that at equilibrium, a stable protein-DNA complex is expected to use all the information content of a full site. However, our analysis of IHF leads us to believe that the primary determinant in specific recognition is restricted to a single helical turn. Rather than reflecting an incidental detail of the protein/DNA interaction, we believe this insight reveals a fundamental limitation in the way proteins find their targets.

RNA Editing

MZ 025 THE DOUBLE-STRANDED RNA ADENOSINE DEAMINASE, Ron F. Hough, Andrew G. Polson and Brenda L. Bass, Department of Biochemistry, University of Utah, Salt Lake City, Utah 84132.

The double-stranded RNA (dsRNA) adenosine deaminase catalyzes the hydrolytic deamination of adenosines within dsRNA to inosines. The enzyme appears to be ubiquitous among organisms of the animal kingdom, although its biologic role is unclear. *Protein purification*: As a first step in understanding the in vivo role of the deaminase we have purified the protein 8000 fold from Xenopus laevis eggs. The purified protein migrates as a 120 kDa band as visualized by silver staining of an SDS-polyacrylamide gel and has a specific activity of ~2.0 nmol inosine min^{-1} mg^{-1} (25°C, pH 7.9 with a 794 base-pair dsRNA substrate). Work in progress is aimed at sequencing the protein and subsequently cloning the gene. An interesting feature of crude and pure protein preparations is the apparent inhibition of the enzyme at high concentrations of the dsRNA substrate. This characteristic is similar to another dsRNA binding protein, the dsRNA dependent protein kinase, suggesting an as yet undetermined mechanistic similarity between these proteins. *Substrate specificity:* After binding to dsRNA in a sequence independent manner, the deaminase selects adenosines for modification in a non-random fashion. In an attempt to understand the nature of endogenous substrates for the deaminase we have characterized the patterns of modification that occur on synthetic dsRNA substrates. We find that the deaminase has a preference for the 5' neighbor of adenosine targets according to the hierarchy A=U>C>G. The particular adenosines chosen for modification is also dependent on the proximity to the 5' or 3' termini: adenosines in close proximity to the 3' terminus of the dsRNA are modified less frequently than those close to the 5' terminus. Various models relating these observations to the structure of dsRNA, and possible endogenous substrates, are currently under consideration.

MZ 026IN VITRO RNA EDITING, Larry Simpson, Georges C. Frech, Agda M. Simpson, Marian Peris, Frederic Bringaud, and Elaine Byrne, Howard Hughes Medical Institute, UCLA, Los Angeles, CA., 90024, Department of Biology, UCLA, Los Angeles, CA., 90024.

To elucidate the mechanism of RNA editing in trypanosomatid mitochondria, it is necessary to develop an in vitro system where this can be studied biochemically. We have shown that a mitochondrial extract from Leishmania tarentolae is able to direct the incorporation of uridylate (U) residues within or close to the preedited regions of synthetic cytochrome b and NADH dehydrogenase subunit 7 mRNAs. The activity requires ATP, but non-hydrolyzable ATP analogues can substitute. Deletion of both cytochrome b anchor sequences or substitution with non-complementary nucleotides leads to a decrease of 55% and 80%, respectively, in the relative internal U-incorporation as compared to the 3' addition. Micrococcal nuclease digestion specifically inhibits internal U-incorporation, suggesting the involvement of endogenous RNA in this process. We have also shown that two classes of ribonucleoprotein complexes exist in the mitochondrial extract which appear to be involved in RNA editing. The 'G' class of RNP complexes consists of 170-300 Å particles which contain gRNAs and proteins, show little TUTase activity, and exhibit internal U-incorporation activity. The 'T' class consists of approximately six to eight RNP complexes, 80-140 Å in size, the endogenous RNAs (guide RNAs and mRNAs) of which can be self-labeled with [α-^{32}P]UTP. Complex T-IV exhibits TUTase activity in the native gel and contains bound guide RNAs. Both G-complexes and T-complexes are probably involved with RNA editing in vivo. These results represent a starting point for analysis of the biochemistry of RNA editing.

MZ 027 RNA-PROTEIN COMPLEXES AND RNA EDITING, Kenneth Stuart, Scott Seiwert, Laurie Read, Bob Correll, A.N.K. Jacob, Uli Göringer[1], Hsiao Hsueh Shu[1], George Riley, and Peter Myler. Seattle Biomedical Research Institute, Seattle, WA 98109-1651 and [1] Max Planck Institute for Biochemistry, Munich, Germany.

RNA editing in trypanosomes is a type of posttranscriptional RNA processing that adds and removes uridines to and from mitochondrial (mt) pre-mRNAs. Examination of all mt pre-mRNAs in *Trypanosoma brucei* reveals that five are not edited, three are edited by the addition and deletion of several uridines and nine are extensively edited by the addition of hundreds and removal of tens of uridines. The editing of some of the pre-mRNAs is also developmentally regulated. The edited sequence appears to be specified by small (~60 nt) guide RNAs (gRNAs). In *T. brucei*, most, if not all, of gRNAs are encoded in mt minicircles, DNAs characteristic of trypanosomatids. Several lines of evidence, including abnormal editing in mutants that lack most gRNA genes, support the role of gRNAs in specifying the edited sequences. The presence of chimeric gRNA/pre-mRNA molecules in cellular RNA suggest that chimeras are editing intermediates in reactions that may occur by transesterification or separate enzymatic steps. We are exploring the role of the gRNA and the significance of chimeras in editing using an *in vitro* editing system that we have recently developed. This system edits synthetic pre-mRNAs to sequences that are specified by exogenously added synthetic gRNAs. RNA complexes that occur *in vivo* and form *in vitro* are also being characterized as candidate complexes that catalyze editing. Several specific complexes, visualized by retardation in native gels, form *in vitro* with native or synthetic gRNAs and their formation involves 5' and 3' gRNA sequences. These *in vitro* complexes also fractionate to various positions in glycerol gradients. Gradient fractions of mt extracts that cosediment with the *in vitro* complexes form similar complexes *in vitro*. Formation of at least some complexes is stimulated by, but is not dependent on, the cognate mRNA. The protein composition of the *in vitro* complexes is being explored by RNA-protein crosslinking experiments which have identified several proteins that specifically crosslink to gRNA. These include a 90 kDa protein that appears specific to the 3' U tail of gRNA.

Protein/DNA Interactions at Replication Origins; Sequence and Structure Specific Interactions with RNA

MZ 100 *Abstract Withdrawn*

MZ 101 ANALYSIS OF ORIGIN-DEPENDENT DNA REPLICATION INITIATION IN BACTERIOPHAGE T4, Kelly Carles-Kinch and Kenneth N. Kreuzer, Department of Microbiology, Duke University Medical Center, Durham, NC 27710

Mutational analysis of two bacteriophage T4 origins of replication, ori*(34)* and ori*(uvsY)*, reveals that both origins contain two distinct elements: a T4 middle-mode promoter strictly required for replication, and a downstream region (approximately 50 bp) required for maximal levels of replication. We have been studying these components to determine their individual functions within the origin. Our results strongly suggest that the downstream region has a structural role in replication initiation, functioning as a DNA Unwinding Element (DUE) which perhaps provides replication-fork proteins access to the origin. First, an ori*(uvsY)*-containing plasmid is cleaved within the downstream region by single-strand specific mung bean nuclease, indicating that the origin contains a DUE. Second, a computer program designed to predict free energy within a DNA double-helix indicates that the downstream region has a propensity to unwind. We are currently analyzing the structure of ori*(uvsY)* at the nucleotide level both *in vitro* and *in vivo* with $KMnO_4$, a chemical probe sensitive to single-strand DNA.To clarify the role of the middle-mode promoter in replication initiation, we have substituted either a T7 class III promoter or the *E. coli* Tc^R gene promoter for the promoter within ori*(uvsY)*. T4-mediated replication initiates from the heterologous origin containing the T7 promoter when T7 RNA polymerase is provided in the host cell. However, the heterologous origin containing the *E. coli* promoter is not active. Transcription may be required from the origin promoter to initiate replication, perhaps because the transcript is needed to prime leading strand DNA synthesis. Since T4 infection greatly reduces the activity of *E. coli* promoters, current studies of these heterologous origins are examining whether a correlation exists between the timing of transcriptional activity and the ability to mediate replication.

MZ 102 PROPERTIES OF A 40 kD PROTEIN WHICH BINDS THE REPLICATION ORIGIN OF SEA URCHIN mtDNA.

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Earlier studies in *Strongylocentrotus purpuratus* (1) showed that in the mitochondrial (mt) DNA of sea urchin the non-coding region (NCR) contains the H-strand replication origin. In order to extend our knowledge on the replication process, we are investigating the interaction of protein(s) with relevant *Paracentrotus lividus* mtDNA sequences. Band-shift and DNase I protection experiments (2) allowed us to identify in a Heparin-Sepharose (H-S) fraction a protein that binds strongly and selectively two homologous mtDNA sequences, located one in the NCR, at the 3' end of the D-loop structure, and the other at the coinciding ends of ND5 and ND6 genes. The protein was purified from *P.lividus* mitochondria eggs at least 3000 fold by means of H-S and DNA-affinity cromatography. Southwestern blot experiments showed that the protein consists of a single polypeptide of about 40 kD. We identified the minimum sequence requirement for the binding as YCYYATCAN(A/T)RC. The protein shows also a single-stranded DNA binding activity, as it interacts with only the H-strand of the NCR binding site and with only the L-strand of the ND5/ND6 junction. The fact that the binding site in the NCR coincides with the 3' end of the D-loop and the higher level of the protein in mitochondria from eggs and blastulae (developmental stages where the DNA replication is blocked) suggest that the protein can modulate sea urchin mtDNA replication. To test this hypotesis we are currently assaying a possible antihelicase activity of the protein and its capacity to interact *in vivo* with the DNA. Meanwhile we are in the process of cloning the gene of this protein.

1) Jacobs H.T., Herbert E.R. and Rankine J. (1989) Nucleic Acids Res. **17**, 8949-8965
2) Roberti M., Mustich A., Gadaleta M.N. and Cantatore P. (1991) Nucleic Acids Res. **19**, 6249-6254.

MZ 103 THE SINGLE STRAND ARS CONSENSUS BINDING PROTEIN OF YEAST IS ENCODED BY *PUB1*, Moira Cockell[1], Severine Frutiger[2], Graham J. Hughes[2] and Susan M. Gasser[1], [1]Swiss Institute for Experimental Cancer Research (ISREC), Chemin des Boveresses 155, CH-1066 Epalinges s/Lausanne, Switzerland and [2]Centre Medical Universitaire, Université de Genève, Dept de Biochimie médicale, 9, avenue de Champel, CH-1211 Genève 4, Switzerland. We have characterised binding activities in yeast which recognise the T rich strand of the yeast ARS consensus element and have purified two of these to homogeneity. One (ACBP-60) is detectable in both nuclear and whole cell extracts, while the other (ACBP-67) is apparent only after fractionation of extracts by heparin-sepharose chromatography. The major binding activity detected in nuclear extracts was purified on a sequence-specific DNA affinity column as a single polypeptide with apparent mobility of 60kD (ACBP-60). It is predominantly nuclear, is present at several thousand copies per cell and has a K_d for the T rich single strand of the ARS consensus between 10^{-9} and 10^{-10} M. Competition studies with simple nucleic acid polymers show that ACBP-60 has marginally higher affinity for poly dT_{30} than for a 30 nt oligomer containing the T rich strand of ARS 307, and approximately 10 fold higher affinity for poly rU. Peptide sequencing of purified p60 identifies it as highly homologous to the open reading frames of genes *PUB1* and *RNP1* which encode polyuridylate binding protein(s).The second activity, ACBP-67, also binds specifically to the T rich single strand of the ARS consensus, but with several orders of magnitude lower affinity than ACBP-60. Peptide sequencing reveals that the 67kD protein is identical to the major poly A binding protein in yeast, PAB1.

MZ 104 CHARACTERIZATION OF THE BINDING SITE OF PROTEIN S7 ON 16S RIBOSOMAL RNA, François Dragon, Catherine Payant and Léa Brakier-Gingras, Département de Biochimie, Université de Montréal, Montréal, Québec, H3C 3J7, Canada

Ribosomal protein S7 is known to bind to the lower half of the 3′ major domain of Escherichia coli 16S rRNA and we have previously found that a fragment of about 100 nucleotides within this region contains the binding site of S7 [Dragon, F. and Brakier-Gingras, L. (1993) Nucleic Acids Research 21, 1199-1203]. This fragment contains two large loops connected via a six base-pair helix; an irregular hairpin with non-canonical base-pairs protrudes from each of these loops. In this study, we have attempted to define which parts of this fragment are important for S7 binding, using site-directed mutagenesis and molecular probing. Substitution of the non-canonical base-pairs with standard Watson-Crick base-pairs in the irregular hairpins did not affect S7 binding. However, deletion of either one of these hairpins strongly decreased S7 binding and this deletion perturbed the structure of the large loops, as shown by changes in their reactivity towards chemical probes. When the connecting helix was bent by inserting one nucleotide in either strand, the binding of S7 was decreased, but when this helix was lenghtened by addition of one base-pair, the binding of S7 was not affected. We propose from these observations that binding of S7 involves simultaneous contacts with the two large loops. Changes in the connecting helix would weaken the binding of S7 through an effect on the relative position of the two loops. The two irregular hairpins would impose constraints which contribute to maintain the conformation of S7 binding site within these loops (supported by the Medical Research Council of Canada).

MZ 105 *OXYTRICHA* TELOMERE-BINDING PROTEIN: DNA-DEPENDENT α/β SUBUNIT INTERACTION AND SEPARABLE DNA-BINDING AND DIMERIZATION DOMAINS OF THE α SUBUNIT, Guowei Fang, John T. Gray and Thomas R. Cech, Department of Chemistry and Biochemistry, Howard Hughes Medical Institute, University of Colorado, Boulder, CO 80309-0215

A telomere-binding protein consisting of 56 kD (α) and 41 kD (β) subunits binds specifically to the single-stranded T4G4T4G4 sequence at each terminus of macronuclear chromosomes in *Oxytricha nova*. By mixing wild-type subunits and electrophoretically distinct variants and by mixing telomeric DNAs of different length, we showed that the telomeric complex contains one α, one β and one DNA molecule. The subunit-subunit interactions were studied by glycerol gradient sedimentation and chemical cross-linking. When incubated together, both subunits exist predominantly as monomers in the absence of DNA. Upon cooperative binding to telomeric DNA, α and β subunits directly interact with each other to form a heterodimer. Libraries of plasmids with random deletions of the open reading frames were introduced into *E. coli*, and extracts were subsequently checked both for protein expression and for DNA-protein and protein-protein interactions. The α subunit was found to contain two structurally separable domains with distinct functions. The amino-terminal two-thirds is necessary and sufficient for sequence-specific DNA binding. The carboxyl-terminal one-third is responsible for α/β subunit interactions. When expressed separately in *E. coli*, purified, and then mixed together, these two domains reconstitute the activity of the wild-type α subunit (*trans*-complementation *in vitro*). The amino-terminal two-thirds of the β subunit is necessary and sufficient both for α/β subunit interactions and for ternary complex formation. We conclude that the α subunit of the telomere-binding protein, like many transcription factors, has separable DNA-binding and protein-protein interaction domains and that the DNA-dependent dimerization may allow each subunit to carry out distinct functions as a monomer, in addition to its participation in chromosome capping as part of the heterodimer.

MZ 106 THE USE OF RNA LIGASE TO PRODUCE LONG SYNTHETIC RNA, Mark A. Farrow, Andrew P. Walsh, Chris J. Adams, James B. Murray, Peter G. Stockley, Department of Genetics, University of Leeds, Leeds, United Kingdom, LS2 9JT.

Bases 1052-1112 of *E. coli* 23S rRNA comprise the highly conserved "GTPase centre" of the ribosome and are specifically recognised by the thiostrepton group of antibiotics and by the ribosomal protein L11. Attempts to synthesise the 61 nucleotides as full length RNA using 2'-silyl-5-dimethoxy-trityl phosphoramidite chemistry have given low yields of pure material. However, when the sequence was synthesised as two fragments of 24 and 37 nucleotides and then ligated together using RNA ligase, a much higher yield of full length, deprotected, material was obtained. Nitrocellulose filter binding assays indicate that the synthetic RNA binds to thiostrepton with an affinity comparable to that seen using *in vitro* transcribed RNA. This method provides a means of incorporating modified phosporamidites into the thiostrepton binding site. The results of which will be discussed.

MZ 107 INTERACTIONS OF EPSTEIN-BARR NUCLEAR ANTIGEN 1 WITH THE FUNCTIONAL ELEMENTS OF THE EBV LATENT ORIGIN OF REPLICATION, ORIP

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During latent infection of B lymphocytes, Epstein-Barr virus (EBV) initiates DNA replication from the dyad symmetry element (DS) of the EBV latent origin, *oriP*. Initiation of replication from DS is activated by a second *oriP* element, the family of repeats (FR), and requires Epstein-Barr nuclear antigen 1 (EBNA1). EBNA1 dimers assemble onto multiple binding sites present within both the FR and DS elements of *oriP*. We have previously demonstrated using electron microscopy that EBNA1 complexes formed on the FR and DS elements of *oriP* interact resulting in the formation of looped *oriP* molecules (Frappier and O'Donnell, Proc. Natl. Acad. Sci. 88, 10875-10879). Using a series of EBNA1 truncation mutants, overproduced using the baculovirus expression system and purified to apparent homogeneity, we have demonstrated that the DNA binding and dimerization domains of EBNA1 are not sufficient to mediate FR-DS interactions but that additional EBNA1 amino acids are required (Goldsmith et al., J. Virol. 67, 3418-3426). Using EBNA1 truncation mutants overproduced in *E. coli*, we have now mapped, within 12 amino acids, a region of EBNA1 containing sequences pertinent for the interaction of DNA-bound EBNA1 dimers at a distance. The results of DNA binding studies using these EBNA1 mutants indicate that EBNA1-mediated FR-DS interactions stabilize EBNA1 binding to the DS element. These results suggest that the stabilization of EBNA1 on the origin of replication by a DNA looping mechanism may be an important step in the activation of replication from *oriP*.

MZ 108 CRYSTALLIZATION OF POLIOVIRUS POLYMERASE
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RNA-dependent RNA polymerases are unique to RNA viruses and enable them to replicate their RNA genomes directly into RNA without DNA intermediates. We have obtained crystals of the RNA-dependent RNA polymerase of poliovirus and are solving its structure by x-ray crystallography. Our crystals of the poliovirus polymerase grow as hexagonal blocks up to 180 X 180 X 70 μm and diffract x-rays to at least 2.8 Å resolution. When stabilized in high salt, the crystals are space group $P3_{1,2}21$ with unit cell dimensions 88 X 88 X 159 Å. When stabilized in low salt the space group changes to $P6_722$ with unit cell dimensions 88 X 88 X 320 Å. Currently, our efforts are directed towards obtaining larger crystals for data collecting. We are also exploring additional stabilization conditions as well as quick freezing methods to optimize our data collection.

To date, high resolution structures of representatives for three of the four categories of nucleic acid polymerases have been published: a 2.6 Å structure for the DNA-dependent DNA polymerase, Klenow fragment of E. coli polymerase I; a 3.3 Å structure for the T7 DNA-dependent RNA polymerase; and a 3.0 Å structure for the HIV-1 RNA-dependent DNA polymerase (reverse transcriptase). These structures exhibit striking similarities and differences due to a common but distant evolutionary heritage and due to their related functions. A structure for poliovirus polymerase will provide a representative for the fourth class of polymerases, the RNA-dependent RNA polymerase.

MZ 109 IN VITRO SELECTION OF SINGLE STRANDED DNA'S THAT BIND SPECIFICALLY TO ARGININE,
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The ability of RNA to fold into fairly complex structures and to perform such tasks as binding to specific ligands and catalysis has been the subject of numerous studies. While it is quite likely that single stranded DNA (ssDNA) plays important biological roles, it's potential to fold into structures similar to RNA and to carry out various tasks has not been extensively studied.

Studies on the interaction between HIV Tat protein and it's RNA binding site TAR have shown that only a single arginine residue within the basic region of Tat makes sequence specific contacts with TAR RNA, and even free arginine has been shown to bind to TAR with specificities similar to Tat. This interaction between arginine and TAR RNA provides us with an excellent system to address the question of ssDNA structure and function because it is relatively simple, and the contacts important for binding have been well characterized. We have carried out in vitro selection of ssDNA molecules that bind to arginine from large random pools of DNA. ssDNA molecules that bind to arginine with an affinity similar to TAR RNA were selectively amplified.

Selection was carried out using a pool of DNA molecules containing a randomized region of 30 nucleotides flanked by defined regions necessary for PCR amplification. This random DNA was loaded onto an arginine agarose column and eluted using a salt gradient. DNA fractions with arginine binding affinities similar to TAR RNA were collected and PCR amplified to generate ssDNA for the next round of column selection. After 12 rounds of selection and amplification, the majority of the DNA molecules bound to the arginine agarose column as strongly as TAR RNA. Several sequences within this selected mixture were cloned. Interestingly, we have not been able to identify sequences that resemble TAR RNA, consistent with the observation that single stranded TAR DNA does not bind to arginine specifically. We are presently determining the arginine binding domains of these ssDNA's by modification interference experiments, and by chemical and enzymatic mapping. Elucidation of the structures of the arginine binding sites of these molecules should provides insights into some of the differences between DNA and RNA structure.

MZ 110 THE GLUCOCORTICOID RECEPTOR SPECIFICALLY BINDS DNA AT SITES OF CONSERVED GENETIC INFORMATION INDUCING DNA BENDING AND UNWINDING,
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Binding at specific sites on DNA by regulatory proteins is essential for genetic regulation. This suggests that a code for recognition between DNA regulatory proteins and cognate DNA binding sites exists. Recently, using the genomic structure of the GR gene as a guide, we reported that genetic information is conserved between the DNA sequence of a well characterized glucocorticoid response element (GRE) and its flanking nucleotides and the c-DNA encoding the glucocorticoid receptor (GR) DNA binding domain (DBD). The regions of nucleotide subsequence similarity to the GRE in the GR DBD occur specifically at nucleotide sequences on the ends of exons 3, 4 and 5 at their splice junction sites. These sequences encode the DNA recognition helix in exon 3, a beta strand in exon 4 and a putative alpha helix in exon 5. By model building, we observed that amino acids of the GR DBD located within the DNA recognition helix encoded in exon 3, the beta strand encoded in exon 4, and the putative DNA binding alpha helix encoded in exon 5 are spaced so that they align with trinucleotides identical to cognate codon/anticodon nucleotides within the GRE major groove halfsites and flanking regions. Using molecular dynamics simulations, we investigated protein/DNA interactions between the GR DBD amino acids and a GRE and its flanking nucleotides. Hydrogen bonding interactions were monitored. In addition, van der Waals and electrostatic interaction energies were calculated. The molecular dynamics show that the GR DBD amino acids of the DNA recognition helix interact at cognate codon/anticodon nucleotide base sites within the GRE right major groove halfsite. Amino acids of the beta strand and the predicted alpha helix, described above, interact with the DNA backbone at cognate codon/anticodon sites within the GRE major groove halfsites and GRE flanking regions, respectively. These interactions together induce breakage of Watson-Crick nucleotide base pairing hydrogen bonds, resulting in bending of the DNA, strand elongation and unwinding events similar to those described for helicases. We will show a videotape of molecular dynamics simulations of DNA regulatory protein/DNA interactions consisting of the GR DBD/GRE computed in vacuo and in solvent on CRAY-2 and CRAY-C90 supercomputers at the Minnesota Supercomputer Institute. The explicitly solvated model consists of approximately 25,000 atoms.

MZ 111 AN HIV-1 TAR RNA BINDING PROTEIN IN HUMAN CHROMOSOME 12-CONTAINING HUMAN-HAMSTER HYBRID CELLS IS TAR LOOP SPECIFIC.
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Human chromosome 12-encoded factors and the TAR RNA stem-loop in the HIV LTR are interdependent mechanisms that support viral *trans*-activation. The native loop sequences in wild type (wt) TAR RNA are critical for chromosome 12-supported *trans*-activation and binding of cellular proteins that increase LTR directed transcription *in vitro*. In this study we examined how human chromosome 12-encoded proteins effect the pattern of TAR RNA binding proteins. Nuclear extracts from a human cell line (HeLa), a human chromosome 12-containing human-hamster hybrid cell line (HHW271), and a hamster cell line (CHO) were examined for TAR RNA binding activity *in vitro*. RNA gel shifts showed a specific TAR RNA-nuclear protein interaction in all three nuclear extracts. Using a TAR RNA loop mutation (ΔTAR), the RNA-protein complexes had the same gel shift pattern as wt TAR complexes but were quantitatively less. UV crosslinking and SDS PAGE analysis of TAR RNA binding proteins showed a similar protein profile from the three cell types with the exception of a major TAR RNA binding protein in HHW271 and HeLa that was not found in the CHO. This protein was not found when UV crosslinking and SDS PAGE analysis was done using ΔTAR RNA. These results demonstrate that human chromosome 12-encoded factors in CHO cells (HHW271) provide a human TAR RNA-nuclear protein interaction that is dependent on the wt TAR RNA loop.

MZ 112 A NEW PROTEIN MOTIF FOR BINDING TO DNA MINOR GROOVE.

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Initiation of phage Ø29 DNA replication is activated by the viral protein p6 by forming a nucleoprotein complex at the replication origins, located at the linear genome ends. The complex consists in a DNA right-handed superhelix wrapped around a multimeric protein p6 core. Protein p6 binds to DNA through the minor groove. We have determined that the protein p6 N-terminal region, predicted to form an amphipathic α-helix, is involved in DNA binding. Protein p6 mutants in the putative polar side of the helix, have been obtained by site-directed mutagenesis, and assayed for replication activation and DNA binding, showing that both activities were affected. An *in vivo* functional assay, based on complementation with a Ø29 *sus* 6 mutant, has been set up, and used to test two of the *in vitro*-obtained mutants, carrying the amino acid substitutions K2A and R6A. In the mutant p6K2A the viral DNA synthesis was impaired, while in p6R6A, DNA synthesis was not detected, in agreement with the results obtained *in vitro*. Furthermore, DNA binding was detected with a 19 amino acids peptide corresponding to the protein p6 wild-type N-terminal region, while a 100-fold lower binding affinity was observed with the peptide corresponding to the mutant p6R6A. Wild-type peptide binds to DNA through the minor groove and, in a protein p6 high-affinity binding sequence, it recognizes the same signal as the intact protein. Circular dichroism data suggest that the α-helical content of the peptide increases in the presence of DNA. These results indicate that the N-terminal α-helix, and in particular the residue R6, plays a crucial role in protein p6 binding to DNA and constitutes a new protein motif for DNA minor groove binding .

MZ 113 THE ROLE OF LOOP E IN THE RECOGNITION OF 5S rRNA BY TFIIIA, Paul W. Huber, Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556.

Xenopus transcription factor IIIA (TFIIIA) binds to 5S rRNA genes and functions as a positive regulator of transcription. The factor also binds to 5S rRNA to form a storage particle for the nucleic acid. The protein makes extensive contact with both nucleic acids through nine cys_2-his_2 zinc fingers. Fingers 1-3 appear to be the most important for binding to the gene, while 4-6 are the principal determinants for RNA recognition.

5S rRNA was treated with the chemical nucleases Fe[EDTA]$^{2-}$ or OP-Cu to introduce random single-site nucleoside gaps into the substrate. The modified RNA was incubated with TFIIIA and bound nucleic acid was separated from unbound, and the two populations of rRNA were subsequently analyzed on sequencing gels. Missing nucleoside positions specifically enriched in the unbound fraction of the RNA are located in the two strands that comprise loop E. These are not necessarily sites of sequence-specific contacts, but rather may constitute a region of secondary structure essential to recognition.

The metal complex bis(phenanthroline)(9,10-phenanthrenequinone diimine)rhodium(III), $Rh(phen)_2(phi)^{3+}$, binds neither double-stranded RNA nor single-stranded regions that lack stacking interactions. However, the probe will target major groove segments that are opened as a consequence of tertiary interactions, noncanonical base pair appositions, or sites at stem-loop junctions. Pronounced cleavage by $Rh(phen)_2(phi)^{3+}$ occurs within loop E of 5S rRNA. Use of the probe in combination with site-directed mutagenesis revealed that single-nucleotide substitutions made on one side of the loop influence cleavage on the opposite strand. These results demonstrated that the two strands are intimately structured, either by stacking, unusual base pairing, or both. A subsequent NMR structure of a duplex that represents loop E is entirely consistent with the conclusions drawn from the experiments using $Rh(phen)_2(phi)^{3+}$. A reversed-Hoogsteen A:U pair and three other noncanonical appositions as well as a guanosine residue that bulges into the major groove make up the structure.

The exceptional structure of loop E created by the series of noncanonical base pairs may be the primary determinant for recognition. This is comparable to results obtained with other RNA binding proteins such as Rev and Tat. Currently, selection experiments are being carried out to analyze the structure of loop E and binding of TFIIIA. We hope to determine whether other noncanonical base pairings can engender the same necessary geometry as the wild type sequence.

MZ 114 CHARACTERIZATION OF A MOTIF NECESSARY FOR SPECIFIC BINDING TO DOUBLE-STRANDED RNA.

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The vaccinia virus E3L gene encodes the proteins p20 and p25. These proteins bind specifically to double stranded RNA: binding to dsRNA-agarose could be competed with an excess of soluble dsRNA but not ssRNA, dsDNA or ssDNA. A domain on p20/p25 necessary for binding to dsRNA has been mapped to the carboxy-terminal 100 amino acid residues. This region of p20/p25 shares homology with several other proteins known to bind to double-helical RNA, including the mouse and human dsRNA-dependent protein kinase, PKR; the rotavirus group C NSP3 gene products; a human protein that can bind to the HIV *tar* and *RRE* sequences; *E. coli* RNase III and the Drosophila *staufen* gene. Mutation of several conserved residues within this motif destroyed the ability of p20/p25 to bind to dsRNA. Vaccinia virus is resistant to the anti-viral effects of interferon, and can rescue other viruses, such as VSV and EMCV, from the effects of interferon. Vaccinia virus deleted for E3L (vp1080) was sensitive to the anti-viral effects of interferon, and could no longer rescue VSV from interferon treatment. However, vp1080 could still rescue VSV from the effects of interferon. Likewise, cloned E3L could rescue VSV but not EMCV from the effects of interferon. The ability to rescue VSV and vp1080 from the effects of interferon correlated with the ability of mutants of E3L to bind to dsRNA. Several other dsRNA-binding proteins, such as the rotavirus NSP3 proteins, the human *tar*/*RRE* binding protein, *RNase III* and the reovirus σ3 protein could replace E3L in promoting replication of vp1080. These results suggest that the ability of p20/p25 to bind specifically to dsRNA is necessary to promote replication of vaccinia virus and to rescue VSV from the anti-viral effects of interferon.

MZ 115 3′ END PROCESSING OF PRE-U2 RNA REQUIRES AN INTERACTION BETWEEN THE 3′ EXTENSION AND NUCLEOTIDES IMMEDIATELY PROXIMAL TO STEM-LOOP III.

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We have previously determined that accurate 3′ end formation of U2 small RNA is directed by a critical internal structure of pre-U2 RNA, distinct from the processing site (Jacobson et al., Mol. Cell. Biol. 13:1119-1129, 1993). It was further found that a mini-U2 RNA precursor lacking nucleotides 1-to-104 of U2 RNA was accurately and efficiently processed, demonstrating that all of the sequences necessary for 3′ processing reside within the 3′ half of the molecule. In the present investigation we have further examined the sequences/structures of this critical internal structure that are important for 3′ end processing, using mutant mini-U2 RNAs as substrates for the 3′ processing activity.

Maturation of the mini-U2 RNA (consisting of the 3′ half of U2 RNA) was previously shown to occur by an endonucleolytic-like process, similar to that observed for the full length, wild type pre-U2 RNA. Mutation of the extreme 5′ end nucleotides of this mini-U2 RNA (which correspond to nucleotides 105-to-110 of wild type U2 RNA) results in mutant RNAs that display an altered pattern of 3′ end shortening, characterized by the accumulation of intermediates. This pattern is reminiscent of the 3′ end shortening of pre-U1 and pre-U4 RNAs, and is suggestive of either an exonucleolytic-like reaction or a faulty endonucleolytic reaction at abnormal sites in the 3′ extension. These data suggest that normal 3′ end processing of U2 RNA requires not only the critical internal structure which we previously identified, but also an interaction between nucleotides of the 3′ extension and those immediately preceding stem-loop III of pre-U2 RNA, possibly including the Sm domain. We are currently examining additional mutant RNAs to determine the importance of these potential interactions in the 3′ processing of U2 RNA.

MZ 116 PURIFICATION AND CHARACTERIZATION OF *S. SHIBATAE* REVERSE GYRASE; ITS INTERACTIONS WITH SSV1 DNA. Christine Jaxel, Marc Nadal, Elisabeth Couderc, Danielle Thévenet and Michel Duguet. Laboratoire d'Enzymologie des Acides Nucléiques, Institut de Génétique et Microbiologie, Bat. 400, 91405 Orsay cedex, FRANCE.

Sulfolobus shibatae B12 is a thermophilic archaebacterium which contains an inducible virus named SSV1. The viral DNA has been shown to be positively supercoiled before encapsidation [1]. The archaebacterial DNA topoisomerase likely responsible for this positive supercoiling has been previously purified, cloned and sequenced from *S. acidocaldarius* DSM 639 [2,3]. In order to study an homogenous system containing both reverse gyrase and one of its preferential substrate, SSV1 DNA, we have purified this enzyme from *S. shibatae*. During the course of the purification, we have detected another topoisomerase activity. To separate and purify these two topoisomerases, we have devised a new purification procedure. Purified *S. shibatae* reverse gyrase is a 124 kDa monomer, which is able to perform a DNA reverse gyration per se at 10 mM NaCl in a Mg- and ATP-dependent manner. The other topoisomerase is a monomer of about 40 kDa. This additional topoisomerase activity is Mg-dependent and ATP-independent and catalyzes exclusively the relaxation of negatively supercoiled DNA at 150 mM NaCl. This new ATP-independent topoisomerase activity seems to be a proteolytic product of reverse gyrase. Indeed, reverse gyrase, treated with proteinase K, presents the same enzymatic characteristics as the ATP-independent activity. This result is also consistent with reverse gyrase sequence data exhibiting a clear similarity of its C-terminal part with eubacterial ATP-independent topoisomerase I (protein ω).

Now a homogenous system ("B12 system") is available in order to study both the topoisomerases activities *in vitro* and the role of these enzymes *in vivo*. Thus, the determination of catalytic sites of reverse gyrase on the genome of the SSV1 virus of *S. shibatae* is under investigation. For these experiments, cleavable complexes were obtained in the absence of ATP and in these conditions, the enzyme induced a specific cleavage pattern. The "B12 system" could be easily used in order to define the role of topoisomerases in the archaebacterial world.

[1] Nadal, M. et al. (1986) *Nature* **321**, 256-258.
[2] Nadal, M. et al. (1988) *Biochemistry* **27**, 9102-9108.
[3] Confalonieri, F. et al. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 4753-4757.

MZ 117 MAINTENANCE OF PLASMIDS CONTAINING DERIVATIVES OF EBV'S *ORIP*, Ann L. Kirchmaier and Bill Sugden, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

OriP is the origin of plasmid replication of Epstein-Barr virus, EBV, which, in the presence of viral origin-binding protein, EBNA-1, mediates plasmid DNA replication. *OriP* is composed of two *cis*-acting elements, the family of repeats and the dyad symmetry element, both of which are required for replication. The ability of plasmids containing *oriP* to be maintained stably in EBNA-1 positive cells reflects both the efficiency of their replication and segregation during each cell cycle. In order to determine whether plasmid maintenance could be affected by stalling of replication forks in the region of the family of repeats (Gahn and Schildkraut, Cell, 58, 1989), this composite value of maintenance was measured for five structurally distinct plasmids by measuring the rate at which they were lost from cells in the absence of selection (Figure 3). Derivatives of *oriP* (Figure 1) with either one copy of the dyad symmetry element and two copies of the family of repeats or two copies of the dyad symmetry element and one copy of the family of repeats were compared to wild type *oriP* in EBNA-1 positive cells. These measurements demonstrated that plasmids with derivatives of *oriP* with two copies of the family of repeats in one orientation are maintained less efficiently than is wild type *oriP* (Table 1). These measurements also showed that a derivative of *oriP* with two copies of the dyad symmetry element is maintained as efficiently as is wild type *oriP* and is not amplified relative to wild type *oriP* (Table 2).

MZ 118 THE INTERACTION OF YEAST RIBOSOMAL PROTEIN L25 WITH 26S rRNA

Engbert A. Kooi, Carla A. Rutgers, Jan van 't Riet, Jaap Venema and Hendrik A. Raué. Department of Biochemistry & Molecular Biology, Institute for Molecular Biological Sciences, BioCentrum Amsterdam, de Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.

The binding of *S. cerevisiae* r-protein L25 to domain III of 26S rRNA is one of the few examples of a protein-rRNA interaction that has been strongly conserved between pro- and eukaryotic cells. We have been studying the molecular details of this interaction by mutational analysis of both the L25 binding site on the rRNA and the rRNA-binding domain of the protein. The results show that long-distance secondary basepairing in helix 47 in the L25 rRNA-binding site is crucial for L25 binding *in vitro*. Disruption of potential tertiary interaction between $G_{1524}U_{1525}$ and $A_{1612}C_{1613}$ completely abolished binding of L25. Since the introduction of compensatory mutations restored binding to wild-type level, these data strongly support the existence of the proposed tertiary interaction. Current mutational analysis is aimed at further delineating the minimal structural features on the rRNA required for L25 binding. Mutational analysis of the rRNA-binding domain of L25 was first concentrated on an evolutionarily conserved sequence motif (aa 120-126). Mutations in this motif were analyzed both *in vitro* and *in vivo*. The latter system consists of a yeast strain (YCR-61) with a chromosomal L25 gene under control of the *GAL7* promoter. Mutant L25 genes, encoded on an episomal plasmid, were assessed for their ability to complement growth of transformants on glucose. The results provide evidence for the direct involvement of several amino acid residues of the motif in binding of L25 to 26S rRNA. A similar analysis of short C-terminal deletions showed five residues (134-138) to be essential for an as yet unidentified function *in vivo*. Further analysis strongly implicated one hydrophobic residue in this region (Ile^{135}). Not a single mutation in this region showed a strong effect on the binding to 26S rRNA *in vitro*. We are currently analyzing synthesis of both ribosomal subunits and 26S rRNA to elucidate the function of the L25 C-terminus *in vivo*.

MZ 119 MINUTE VIRUS OF MICE (MVM)INFECTION MODIFIES A GENERAL CELLULAR TRANSCRIPTION ELONGATION ACTIVITY, Anat Krauskopf and Yosef Aloni, Department of Molecular Genetics and Virology,Weizmann Institute of Science, Rehovot, Israel 76100

Our previous observations have indicated that upon infection with MVM , Ehrlich ascites (EA) cells lose a transcription elongation activity which is essential for the readthrough of the MVM attenuator. This was monitored by the ability of extracts from uninfected but not from infected cells to support readthrough of the MVM attenuator when added to partially purified transcription elongation complexes. We have set to investigate the nature of this change in transcription elongation following MVM infection. In this communication we report that infection of EA cells with MVM leads to a general shift in the length of nascent mRNA synthesized in isolated nuclei and separated by sucrose gradients. Furthermore, we report that infection specifically leads to attenuation of transcription of the cellular gene, c-fos but not c-myc as observed by hybridization of nascent RNA to filters containig 3' and 5' portions of these genes. Biochemical evidence, obtained from elongation of partially purified briefly initiated transcription complexes bearing the c-fos atteniuator, supports a model by which, following MVM infection there is a functional reduction in the activity of an TFIIS-like general transcriptional elongation activity.

MZ 120A PLANT CELL ENCODED DOUBLE-STRANDED RNA BINDING PROTEIN WITH PROPERTIES SIMILAR TO THE INTERFERON-INDUCED, DOUBLE-STRANDED RNA DEPENDENT PROTEIN KINASE, Langland, J.O.[1], Jacobs, B.L.[2], Roth, D.A.[1]. 1Department of Plant, Soil and Insect Sciences, University of Wyoming, Laramie, WY
[2]Department of Microbiology, Arizona State University, Tempe, AZ 85287

A plant cell encoded double-stranded RNA (dsRNA) dependent/binding protein kinase capable of phosphorylating an M_r = 68,000 product has recently been identified. This kinase is both cytosolic and ribosome associated and is capable of phosphorylating exogenous substrates including histone proteins. Endogenous phosphorylation levels of the M_r = 68,000 protein are induced upon viral infection with tobacco mosaic virus. These characteristics are similar to those described for the mammalian dsRNA-dependent protein kinase which is known to be involved in the establishment of an antiviral state and possibly involved in anti-tumorigenic activity.

MZ 121 MOLECULAR BASIS FOR DNA SEQUENCE DISCRIMINATION BY REL FAMILY PROTEINS, Christopher J. Larson, Alan P. Kaplan, Xiaoyan Zhang, and Gregory L. Verdine, Department of Chemistry, Harvard University, Cambridge, MA 02138

Transcription factors exercise control of genetic activity in all organisms, and comprehension of their role in normal and dysfunctional gene expression necessitates a greater understanding of the molecular recognition between these regulatory proteins and their target sites than is presently available. Our objective is the biochemical characterization of sequence discrimination by *rel* family proteins. Standard interference footprinting methods to detect DNA base contacts involve treating DNA with reagents that produce lesions and then testing for interference with protein binding. Unfortunately, existing chemical methods often produce only partially selective lesions, and can significantly alter DNA secondary structure. Template directed interference (TDI) footprinting yields site-specific chemical modifications that do not significantly alter DNA secondary structure by enzymatically incorporating nucleoside analogues into the base-contact surface of DNA. We have employed the analogues N7-methyl-2'-deoxyguanosine (m^7dG), 5-aza-2'-deoxycytidine (a^5dC), and 5-hydroxy-2'-deoxyuridine (h^5dU) to identify contact bases. In parallel experiments designed to verify base contacts identified by TDI, synthetic oligonucleotides containing the base analogues 8-oxo-2'-deoxyguanosine, 2'-deoxyuridine, N4-methyl-2'-deoxycytidine, and N6-methyl-2'-deoxyadenosine were constructed. Additionally, other synthetic oligonucleotides harboring abasic sites and phosphodiester nicks to determine the role of DNA deformability in protein binding were constructed, and all oligomers were employed in gel-shift binding assays to measure their effects on the protein-DNA dissociation constant. Finally, ethylation interference footprinting was performed to identify positions along the phosphodiester backbone of DNA that are critical for protein recognition. The data from this series of experiments permit us to describe sequence-specific DNA binding by *rel* family proteins in the language of molecular interactions between the contact surfaces of these macromolecules.

MZ 122 MCM PROTEINS ARE REPLICATION INITIATION FACTORS THAT MAY FORM A MULTISUBUNIT COMPLEX AND DIRECTLY ACT AT ARSs. Ming Lei and Bik Tye. Section of Biochemistry, Molecular and cell Biology, Cornell University, Ithaca, NY 14853

MCM1, MCM2, MCM3 and MCM5/CDC46 are essential proteins for the initiation of DNA syntheses at yeast replication origins. Mutations in *MCM* genes result in ARS-specific minichromosome maintenance defects and reduced frequency of replication initiation at chromosomal replication origins. MCM1 is a DNA binding protein which affects both DNA replication and mating type specific gene expression. MCM2, MCM3 and MCM5/CDC46 are homologous proteins which enter the nucleus at the end of mitosis, persist there throughout G1 phase, and disappear from it at the G1/S boundary. The cell cycle-dependent nuclear localization of MCM2, MCM3 and MCM5/CDC46 suggests that they may play regulatory roles in the control of replication initiation.

We are studying the interactions between MCM proteins and the replication origin sequences. *In vitro* DNA binding assays indicate that the MCM1 protein directly binds to ARSs. We are also studying the interactions between MCM proteins. Using the "two hybrid system" of Fields and Song (1989), we found that MCM1, MCM2 and MCM3 interact with each other. Moreover, MCM2 and MCM3 interacts with MCM5/CDC46. Thus, it appears that the MCM proteins may form a multisubunit complex that directly acts at ARSs to facilitate the initiation of DNA replication. Once DNA replication is initiated, this complex may be disrupted and removed from the nucleus so that reinitiation of DNA replication in the same cell cycle is prevented.

MZ 123 GENERATION OF A NEW TYPE I RESTRICTION MODIFICATION SPECIFICITY BY TRANSPOSITION, M. MacWilliams, J. Meister, H. Jutte, and T. Bickle. Department of Microbiology, Biozentrum, Basel University CH4056 Basel, Schweiz

We have characterised a novel mutant of *Eco*DXXI, a type IC DNA restriction and modification system, in which the specificity has been altered due to a Tn5 insertion into the middle of *hsdS*, the gene which codes the polypeptide that confers DNA sequence specificity to both the restriction and modification reactions. With the type I R-M systems, both the DNA restriction and modification functions are carried out by a single enzyme composed of 3 subunits: hsdS (DNA binding specificity), hsdM (modification/methylation), and hsdR (restriction). A complex of only hsdS and hsdM can catalyse methylation but not restriction. Type I restriction of unmodified DNA occurs a great distance from the enzyme's recognition site and is accompanied by large amounts of ATP hydrolysis. It is proposed that the ATP hydrolysis fuels the "pumping" of the DNA past the bound enzyme to reach the cleavage site.

Like other type I enzymes, the wild type *Eco*DXXI recognises a sequence composed of two asymmetrical half sites separated by a spacer region: TCA(N7)RTTC. Purification of the *Eco*DXXI mutant methylase and subsequent *in vitro* DNA methylation assays, identified the mutant recognition sequence as an interrupted palindrome, TCA(N8)TGA, in which the 5' half site of the wild type site is repeated in inverse orientation. The additional base pair in the non-specific spacer of the mutant recognition sequence maintains the proper spacing between the two methylatable adenine groups.

Sequencing of both the wild type and mutant *Eco*DXXI *hsdS* genes showed that the Tn5 insertion occurred at nucleotide 673 of the 1221 bp gene. This effectively deletes the entire carboxyl DNA binding domain which recognises the 3' half of the *Eco*DXXI binding site. Subsequent deletion analysis demonstrated that the Tn5 element as well as the distal *hsdS* 3' sequence are dispensible; an additional 65 bp of the *hsdS* sequence could also be removed without loss of methylation or restriction activity. The minimum characterised *hsdS* fragment encodes both the amino terminal DNA binding domain as well as the conserved repeated sequence that defines the length of the recognition site spacer region. The predicted 205 amino acid peptide must contain all the information necessary for DNA binding and subunit interactions. We propose that the *Eco*DXXI mutant methylase utilises two truncated hsdS subunits to recognise its binding site. The implications of this finding in terms of subunit interactions and the malleability of the type I R-M systems will be discussed.

MZ 124 CHARACTERIZATION OF A DOUBLE-STRANDED RNA ACTIVATED ENZYME: THE 2-5A SYNTHETASE INDUCED BY INTERFERON

Isabelle Marié, Josette Svab & Ara G. Hovanessian, Unité de virologie et immunologie cellulaire, Institut Pasteur, Paris, France.

The 2-5A synthetase is a key protein as mediator in the antiviral and perhaps anticellular action of interferon.This enzyme when activated by double-stranded (ds) RNA polymerizes ATP into 2'-5'-linked oligomers of adenosine referred to as 2-5A, with the general formula $pppA(2'p5'A)_n$, $n \geq 1$. The 2-5A molecules bind and activate a latent endonuclease responsible for the cleavage of cellular and viral RNAs. We studied the binding and the activation of 69 and 100 kDa isoforms of the 2-5A synthetase to single-stranded (ss) and dsRNA coupled to Sepharose. The synthetases bound to ssRNA-Sepharose manifested no activity but became activated in the presence of soluble dsRNA. This activation did not result in the dissociation of the enzyme from the ssRNA-Sepharose. These results indicate the presence of at least two classes of polynucleotide binding sites in the 2-5A synthetase.

The two isoforms have different dependence for the concentration of dsRNA required for maximal activation. The 100 kDa synthetase is completely activated at 1 μg/ml whereas the 69 kDa one needs at least 100 μg/ml for total activation. It is noteworthy that the concentration of dsRNA activator modifies the activity level of the enzyme and the nature of the synthesized products.

MZ 125 CHARACTERIZATION OF THE HELICASE I NICKING-RELIGATION REACTION AT THE F PLASMID ORIGIN OF TRANSFER, William C. Nelson, Michael T. Howard, Jonathan A. Sherman, and Steven W. Matson, Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3280

E. coli DNA helicase I, encoded on the F plasmid, has been shown to catalyze both a duplex DNA unwinding reaction and site- and strand-specific nicking at the F plasmid origin of transfer (*oriT*). The latter reaction is a key step in initiating the transfer of ssDNA from donor to recipient in the process of bacterial conjugation. We have reconstituted the nicking reaction using a linear dsDNA substrate. Consistent with previous genetic studies, the reaction is dependent on the addition of the F-encoded TraY protein (TraYp) and the host-encoded integration host factor (IHF). The addition of IHF and TraYp also enhances the nicking reaction observed using a supercoiled DNA substrate. We have also shown that helicase I remains covalently bound to the 5'-end of the nicked strand, suggesting that helicase I preserves the phosphodiester bond energy in the covalent protein-DNA complex. This energy may be used to catalyze the religation of the transferred strand. The ability of helicase I to catalyze a nicking-ligation reaction has been directly demonstrated using two different length oligonucleotides containing a portion of the *oriT* region. Intermolecular recombinant molecules were detected at a high frequency. A model for F plasmid-directed conjugative transfer will be presented that encompasses the nicking, helicase, and religation activities of helicase I and describes the role of TraYp and IHF in initiation of the process.

MZ 126 Modification of a Free Fe-S Cluster Cysteine Residue in the Active Iron-responsive Element-Binding Protein Prevents RNA Binding

Caroline C. Philpott, David Haile, Tracey A. Rouault, and Richard D. Klausner

The iron-responsive element binding protein (IRE-BP) binds to specific RNA stem-loop structures called iron-responsive elements (IREs) which mediate the post-transcriptional regulation of a variety of mRNAs involved in the uptake, sequestration and utilization of iron. Coordinate expression of IRE-containing mRNAs results from the ability of cells to reversibly regulate the affinity of the IRE-BP for IREs in response to changing levels of iron. The IRE-BP is identical to cytosolic aconitase and regulation involves the reciprocal switching between an active aconitase enzyme and an active RNA binding protein. Absence of an iron-containing cluster is associated with a loss of aconitase activity and high RNA binding activity. A [4Fe-4S] cubane cluster is required for aconitase activity and is associated with loss of IRE binding affinity. RNA binding ability is also abolished by chemical modification of one or more cysteine residues. The three cysteines predicted to coordinate the Fe-S cluster in the IRE-BP are likely targets for modification. We report the expression of recombinant IRE-BP in which the three putative cluster cysteines (C437, C503, and C506) have been mutated, singly and in combination, to serine residues. None of these mutants has aconitase activity. While all of the mutants bind RNA, substitution of C437 specifically renders the IRE-BP insensitive to RNA binding inactivation by low concentrations of N-ethylmaleimide or diamide. These results identify C437 as the target of *in vitro* regulation of RNA binding in the IRE-BP and suggest that, in the RNA binding form of the protein, C437 is free and therefore available for modifications which inhibit RNA binding. When stably expressed in mammalian cells, these mutants fail to demonstrate *in vivo* iron regulation and are active for RNA binding regardless of the iron status of the cell.

MZ 127 THE INTERACTIONS OF *E.COLI TRP* REPRESSOR WITH TRYTOPHAN AND WITH AN OPERATOR OLIGONUCLEOTIDE: NMR STUDIES USING SELECTIVELY ^{15}N-LABELLED PROTEIN,

Vasudevan Ramesh, Ronnie O. Frederick, Shabih E.H. Syed, Catherine F. Gibson, Ji-chun Yang and Gordon C.K. Roberts, Department of Biochemistry and Biological NMR centre, P.O. Box 138, Medical Sciences Building, University of Leicester, Leicester, LE1 9HN, U.K.

The effects of the binding of the corepressor L-tryptophan and an operator oligonucleotide to *E.coli trp* repressor have been studied, using selective ^{15}N labelling to permit observation of the backbone amide resonances of 48 of the 107 residues (per monomer) of the protein. Repressor molecules labelled in turn with ^{15}N-leucine, ^{15}N-alanine, ^{15}N-isoleucine and ^{15}N-glutamate were prepared by isolating them from prototrophic *E.coli* cells grown in media containing a mixture of unlabelled and the appropriate ^{15}N enriched amino acids. Analysis of the HSQC or HMQC spectra of the labelled repressors shows the value of selective labelling in resolving the crosspeaks of, for example 19 leucine and 12 glutamate residues. All forty-eight residues studied show significant changes in amide ^{1}H and/or ^{15}N chemical shift on the binding of tryptophan and/or the operator oligonucleotide, showing clearly that ligand binding has effects which are transmitted almost throughout the protein. The residues to be labelled were chosen in part to allow study of the helix-turn-helix DNA-binding domain. Tryptophan binding leads to significant shift changes in almost all the residues of this domain, reflecting the change in conformation leading to activation of the repressor. On operator binding, the changes are generally smaller than those produced by tryptophan, and there is selective broadening of the signals of residues in the N-terminal region of the protein and in the DNA-binding domain, perhaps reflecting a conformational equilibrium.

MZ 128 MEDIATION OF MOLECULAR RECOGNITION IN PROTEIN-DNA COMPLEXES BY BOUND WATER: A MECHANISM FOR "STAR ACTIVITY" OF RESTRICTION ENDONUCLEASES, Clifford R. Robinson and Stephen G. Sligar. Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

For many restriction endonucleases such as *Eco* RI, accurate protein-DNA recognition is disrupted by changes in buffer composition, leading to an unexplained loss of specificity termed "star activity." We have found that the extent of cleavage by *Eco* RI at non-canonical sites is strongly correlated with the osmotic pressure in the reaction. This relationship is unique to osmotic pressure, and is independent of other physical or chemical properties of the osmolyte. An analogous correlation between osmotic pressure and star activity is observed for other restriction enzymes including *Pvu* II and *Bam* HI. For *Eco* RI, specificity for cleavage at the canonical site is restored by the application of hydrostatic pressure to counteract the effects of osmotic pressure. Elevated osmotic pressures induce a fundamental change in the selectivity of *Eco* RI — at 100 atm osmotic pressure the rate of cleavage at the canonical site actually decreases, whereas the rate of cleavage at "star" sites increases. The alteration of specificity accompanying release of water clearly implicates one or more water molecules in mediating accurate recognition of specific sequences of DNA by the enzyme. The change in selectivity is manifested in both the association and catalytic steps of the reaction. Under standard conditions, water may participate as a general mediator for sequence specific recognition of DNA by restriction enzymes and other DNA-binding proteins.

MZ 129 Structure Determination of a Novel Double-Stranded RNA Binding Motif, Jodi Ryter, Steve Schultz, Department of Chemistry and Biochemistry, University of Colorado-Boulder, Boulder, CO 80309

Several diverse proteins that are known to bind double-stranded RNA were recently shown to contain discrete regions of amino acid homology 65-68 amino acids in length (St. Johnston et. al., 1992, and references therein). This observation lead to the characterization of a novel double-stranded RNA binding domain. Sequence comparisons showed that this novel domain apparently does not contain any of the previously defined RNA binding motifs. Our research is directed towards co-crystallizing this double-stranded RNA binding domain, as well as full-size proteins that contain one or more of these domains, with double-stranded RNA for structure determination by x-ray crystallography. High resolution structural information will enable us to understand at the molecular level how proteins interact with double-stranded RNA.

Currently we have developed purification procedures for both the full-length *Xenopus laevis* RNA Binding Protein A (Xlrbpa), which contains 2 1/2 of these double-stranded RNA binding domains, as well as a single domain of 68 amino acids derived from Xlrbpa. Crystallographic quantities have been prepared for both and crystallization trials are underway. We have also developed procedures for preparing large quantities of short RNA oligonucleotides. Co-crystallizations of Xlrbpa with short duplex RNAs as well as with other potential RNA substrates are being pursued.

St. Johnston, D., Brown, N.H., Gall, J.G., Jantsch, M. (1992). Proc. Natl. Acad. Sci. USA **89**, 10979-10983.

MZ 130 STRUCTURAL AND FUNCTIONAL ANALYSIS OF RNA-BINDING PROTEINS INVOLVED IN REPLICATION AND PATHOGENICITY OF THE PSTVd CLASS VIROIDS Rudolf Sägesser, Martin Tabler and Mina Tsagris, Institute of Molecular Biology and Biotechnology, P.O. Box 1527, Heraklion 711 10, Crete, Greece.

Viroids are subviral pathogens of several plants causing severe desease on, e.g., potatos, tomatos, and citrus. They consist as naked and noncoding RNA molecules of 246-386 nt., showing rod-like structures with extensive double-stranded regions separated by single-stranded 'loop-outs'. It is postulated that their genetic information (replicability and pathogenicity) is exerted by direct interaction of the viroid-RNA with pre-existing or induced host factors. We are currently optimizing a method for screening RNA-binding proteins, which are expressed from tomato cDNA libraries in lambda. This should allow the identification of cDNA clones due to either sequence- or structure-specific binding of viroid-RNA to the expressed protein. Potential clones, which we recently isolated from tomato cDNA libraries, are retested for their ability to react with various in vitro transcribed and structurally distinct PSTVd-RNAs. To confirm a specific viroid-protein interaction, positive clones will be expressed in E. coli and used for both gel retardation and filter binding assays. The functional and structural properties of selected PSTVd-binding proteins will then be studied in more details. Expression of PSTVd-binding proteins in E. coli will also be used for the production of polyclonal antibodies, which are essential for the intracellular in situ localization of viroid-binding proteins. One future goal of our work is to clarify the role of host proteins involved in viroid-replication and pathogenicity.

MZ 131 RECOGNITION OF SPECIFIC DNA SEQUENCES BY THE *c-myb* PROTO-ONCOGENE PRODUCT, Akinori Sarai[a], Jun Tanikawa[b], Takashi Yasukawa[a,c], Masato Enari[b], Kazuhiro Ogata[b], Yoshifumi Nishimura[b] and Shunsuke Ishii[a], [a]RIKEN Life Science Center, Tsukuba, Ibaraki, [b]Yokohama City University, Yokohama and [c]University of Tsukuba, Tsukuba, Japan.

The DNA-binding domain of c-Myb consists of three homologous tandem repeats of 52 amino acids (R1, R2 and R3 from N-terminal side). The solution structure of R3 has been obtained by NMR analysis[1]. The analysis showed that three α-helices fold into a conformation related to the helix-turn-helix motif. This domain is thought to form specific interaction with DNA. The role of R2 and R1 is not known. To identify the role of each repeat in the sequence recognition of DNA, we analyzed specific interactions between c-Myb and DNA by measuring binding affinities for systematic mutants of Myb-binding DNA sites and various truncated c-Myb mutants. We found that specific interactions are localized unevenly in the AACTGAC region in the consensus binding site of c-Myb: The first A, third C and fifth G are involved in very specific interactions, in which any base substitutions reduce the binding affinity by more than 500-fold. On the other hand, the interaction at the second A is less specific, with the affinity reduction in the range of 6 to 15-fold. The seventh C involves a rather peculiar interaction, in which only G substitution abolishes the specific binding. The binding analyses, together with the chemical protection analyses, showed that the c-Myb fragment containing R2 and R3 covers the AACTGAC region from the major groove of DNA in such an orientation that R3 covers the core AAC sequence. These results suggest that R3 recognizes the core AAC sequence very specifically, while R2 recognizes the GAC sequence in a more redundant manner. The stability analysis showed that the structure of R2 is much less stable than that of R3[2]. R1, which covers the major groove of DNA only partially, is not significant in the sequence recognition, but it contributes to increase the stability of the Myb-DNA complex. The presence of an N-terminal acidic region upstream of R1, which is important for the activation of *c-myb* proto-oncogene, was found to reduce the binding affinity by interfering with R1 in binding to DNA.

[1]Ogata, K., *et al.*, *Proc. Natl. Acad. Sci. USA 89*, 6428 (1992).
[2]Sarai, A., *et al.*, *Biochemistry 97*, 7759 (1993).

MZ 132 STRAIN VARIATION IN MAEDI VISNA VIRUS LTR SEQUENCE: EFFECTS ON TRANSCRIPTION FACTOR BINDING.

Keith A.Sutton, David R.Sargan and Gordon Harkiss, Department of Veterinary Pathology, Royal School of Veterinary Studies, Summerhall, Edinburgh, EH9 1QH, UK.

The ovine lentivirus Maedi-visna (MVV) causes chronic inflammatory and degenerative diseases in the lungs, joints and brain. In MVV strain 1514 the binding of transcription factor AP-1 to a known consensus sequence proximal to the TATA box has been proposed as a major regulator of viral replication.
Comparison of 1514 LTR sequence with that of strain EV-1, a MVV isolate cloned and sequenced at this laboratory, showed that EV-1 LTRs from tissue culture or ex-vivo lack a consensus AP-1 site. In strain EV-1 the TATA box proximal site is converted from TGAGTCA to TAAGTCA. The AP-1 sites and their flanking regions from these two strains have been compared using synthetic oligonucleotides in gel retardation assays. The results of these experiments suggest that these two strains differ in the transcription factors which bind in this region. The EV-1 site does not bind AP-1 under conditions where a shift is seen with the 1514 sequence suggesting that the EV-1 site is non-functional. These experiments have also demonstrated a previously unidentified transcription factor binding site overlapping the AP-1 site. This factor binds both the EV-1 and 1514 sequences. A series of vectors have been constructed to test the ability of these sites to drive transcription from a basal promoter in transfected cells. Data from gel retardation and transfection assays will be presented.

MZ 133 CHARACTERIZATION OF THE RNA BINDING PROPERTIES OF THE *XENOPUS* TRANSCRIPTION FACTOR, FRGY2. Sherrie R. Tafuri*, Donald Fink**, & Alan P. Wolffe***. *Signal Transduction, Warner-Lambert/Parke-Davis, Ann Arbor, MI 48105, **Division of Cell Biology, FDA, Bethesda, MD 20892, ***Laboratory of Molecular Embryology, NIH, Bethesda, MD 20892.

The Ybox transcription factor family is a highly conserved group of DNA binding proteins which positively activate transcription. Several of these proteins, including FRGY2, form RNP particles with mRNA in germ cells. When bound to mRNA, these proteins store the mRNA and prevent its translation. To determine which region of FRGY2 is required for RNA binding, mutants proteins were tested for binding capacity in a mobility shift assay. A C-terminal peptide containing alternating blocks of acidic and basic residues and lacking the DNA binding domain is sufficient to bind RNA. FRGY2 shows little RNA sequence specificity and although it binds most strongly to random sequence RNA, the protein will bind most single stranded(SS) homopolymeric RNAs. The only exception noted is that FRGY2 does not bind poly (A) suggesting that in the RNP particle, the poly (A) tail of the mRNA may be accessible to other regulatory proteins. FRGY2 cannot bind double stranded RNA. However, the size and number of complexes formed on SS RNA is dependent upon the length of the molecule. RNAs from 24-83 nucleotides form single complexes in a highly cooperative fashion. Above 83 nucleotides, the number of complexes per RNA increases incrementally approximately every 100 nucleotides. Moreover, RNA binding is solely dependent upon the FRGY2 concentration in the reaction. This data shows that RNP formation requires the assembly of protein complexes either on the RNA or in solution, and that multiple complexes bind a single RNA. Finally, evidence suggests that protein phosphorylation although present *in vivo* is not required for RNP formation nor does it prevent it. We hypothesize that these proteins store and package mRNA in a manner highly analogous to histones in the packaging of DNA in chromatin.

MZ 134 RNA STRUCTURE PREDICTION BY IN VITRO SELECTION OF ARGININE-BINDING RNAs, Jianshi Tao and Alan D. Frankel, Department of Biochemistry and Biophysics, and Gladstone Institute of Virology and Immunology, University of California at San Francisco, San Francisco, CA 94141

Many RNA molecules can fold into highly defined tertiary structures, unlike most DNA molecules that form relatively simple double helices. The tertiary structures of RNAs play key roles in their biological functions, yet prediction of RNA structure has been a challenging problem. Many attempts at structure prediction rely on rather large and complex RNAs. We have chosen to focus on a relatively small and well-defined system in attempting to predict RNA structure.

The transactivation response element (TAR) of HIV forms a stable stem-loop structure at the 5' end of the nascent viral transcripts and interacts with the arginine-rich RNA-binding domain of the Tat protein. The Tat binding site in TAR is composed of a bulged U and GC and AU base pairs above the bulge. Previously we have demonstrated that TAR RNA binds to the free amino acid arginine with similar specificity as the intact Tat protein or Tat RNA-binding peptides. Because of both the RNA and protein components involved in arginine recognition appear relatively simple, we reasoned that arginine-binding might provide a useful handle for studying RNA folding. To examine arginine-binding sites in RNAs in more detail, we have selected arginine-binding RNAs from random pools generated in vitro. We have found RNAs that resemble TAR but appear to have additional interactions in loops or other parts of the RNA structure that stabilize the arginine-binding site. Based on these results, we have used arginine-binding as tool to systematically examine nucleotides in loops that may form interesting tertiary interactions. The results suggest that, at least in this system, it may be possible to predict the structures of small domains of RNAs.

MZ 135 PEPTIDE ANTIBIOTICS OF THE TUBERACTINOMYCIN FAMILY AS MODULATORS OF GROUP I INTRON ACTIVITY, Herbert Wank, Jeff Rogers*, Julian Davies* and Renée Schroeder, Institute of Microbiology and Genetics, Vienna Biocenter, University of Vienna, Vienna, Austria and * Department of Microbiology, University of British Columbia, Vancouver, B.C. Canada V6T 1Z3

The tuberactinomycins are cyclic peptide antibiotics, which are potent inhibitors of prokaryotic protein synthesis and of the self-splicing reaction of group I introns. They are the strongest competitive inhibitors of splicing described so far, with micromolar concentrations being sufficient. They interact with the binding-site of the cofactor guanosine, but have much higher binding affinities than guanosine. In addition to forming H-bonds with a base of the RNA (G871 in the td intron), we suggest that the lysyl side chains of the antibiotics undergo electrostatic interactions with the RNA backbone. Mg^{2+} concentrations influence the inhibitory activity. Additionally to this inhibition, the tuberactinomycins induce oligomerization of the linear intron. Incubation of the excised linear intron in the presence of subinhibitory concentrations of antibiotic results in the formation of intron dimers, trimers and tetramers. The presence of tuberactinomycin shifts an intramolecular circularization reaction to an intermolecular ligation. These results indicate a potential role for antibiotics as modulators of RNA function in the RNA world.

MZ 136 IDENTIFICATION OF AMINO-ACIDS INVOLVED IN THE SEQUENCE SPECIFIC DNA BINDING OF THE TUMOUR SUPPRESSOR PROTEIN, p53

Timothy R. Waters and Peter F. Swann, Department of Biochemistry and Molecular Biology, University College London, Gower St., London, WC1E 6BT, UK.

Mutations of the tumour suppressor protein, p53, are among the most commonly observed genetic changes seen in human malignancies (Hollstein *et al.*, (1991) *Science* **253**, 49). Such mutations presumably lead to inactivation of p53 function, although its' normal biological role is not known. However, it is able to bind DNA in a sequence specific manner (El-Deiry *et al.*, (1992) *Nature Genetics* **1**, 45) and it can act as a transcriptional activator when such DNA sites are placed upstream of a minimal promotor (Funk *et al.*, (1992) *Mol. Cell. Biol.* **12**, 2866). A DNA binding domain has been mapped to the C-terminal (Foord *et al.*, (1991) *Nucleic Acids Res.* **19**, 5191) but the exact amino acids involved in sequence specific binding are not known. By using oligonucleotides containing a 4-thiothymidine base within a p53 DNA binding site, we have been able to irradiate p53/DNA complexes with 340nm light and form specific cross-links between the protein and DNA. After protein digestion and purification of the cross-linked peptide, the amino acid forming the bond with the oligonucleotide can then be identified by protein sequencing.

Prot./RNA Interactions at Promoter;
Prot./DNA Interactions at Promoter

MZ 200 BINDING OF MU REPRESSOR AND IHF TO THE EARLY OPERATOR REGION OF BACTERIOPHAGE MU IN VITRO. Robert J. Alazard, Philippe. Rousseau, Mireille. Bétermier and Michael. Chandler, Molecular Genetics and Microbiology, CNRS, 118 route de Narbonne. 31062 Toulouse Cedex. France

The integration host factor (IHF) from *E. coli* and the bacteriophage Mu repressor (c) regulate the transcriptional activity of the phage early operator region. This region, located at about 1kb from the left end of the phage genome, contains three operator sites (O1, O2 and O3) overlapping two divergent promoters, Pe and Pc. These control the early transposition functions and the synthesis of the repressor itself, respectively. A pre-requisite for the regulatory functions of c and IHF is their interaction with specific DNA sequences within the early promoter-operator region.

IHF stimulates transcrition from Pe over that from Pc on a supercoiled plasmid DNA *in vivo* and *in vitro*. Unexpectedly, it also stabilises the interaction between c and the strong operator sites O1 and O2, thus favouring lysogeny. Using a combination of gel retardation and DNAse I footprinting techniques, we have shown that c binds first to the operator site O2, then to O1 and finally to O3. Occupancy of O1 and O2 induces a conformational change in the DNA between these sites. Since we found that c oligomerises in solution and behaves mainly as a dimer, it could mediate long-range interactions leading to the formation of looped structures. Such structures might be stabilised by the interaction of IHF with its cognate binding site between O1 and O2. A restriction fragment containing the entire operator region is currently used in footprinting and cyclisation kinetics experiments to investigate c and IHF binding to the operator region.

MZ 201 STRUCTURE OF THE TELOMERASE RNA IN *TETRAHYMENA* AND RELATED CILIATES.

Anamitra Bhattacharyya and Elizabeth H Blackburn, Dept of Microbiology and Immunology, University of California, San Francisco, CA 94122.

The telomerase RNA genes from six different *Tetrahymena* species and the related ciliate, *Glaucoma chattoni*, have been cloned and sequenced [Romero & Blackburn (1991) *Cell* 67: 343-353]. Inspection of the cloned telomerase RNA sequences led to the proposal of a conserved secondary structure model for these RNAs. In order to clarify fully the RNA secondary structure, and subsequently to elucidate the biochemical properties of the holoenzyme, we have tested the predicted model by direct structure probing of the RNA *in vitro*.

The telomerase RNA from the related ciliate, *G. chattoni*, is the most divergent in sequence (35% variation) of this group of ciliate telomerase RNAs. However, the model predicts both the *T. thermophila* and *G. chattoni* RNAs fold into similar conformations. We performed a comparative secondary structure analysis of both these telomerase RNAs, using a variety of enzyme and chemical probes specific for single-stranded and double-stranded regions, to determine the presence of conserved structural motifs common to the two species of ciliate.

The results of these studies on the *Tetrahymena thermophila* and the related *Glaucoma chattoni* telomerase RNAs suggest:

1. There is general consistency with the predicted model proposed earlier [Romero & Blackburn, 1991] for the secondary structure of these RNAs.
2. However, there are indications of the presence of ordered structure in and around the templating region of the RNA. This region is responsible for binding telomeric primers, and was previously predicted to be single-stranded and unstructured.
3. There may exist an alternative structural motif in helix III (*e.g.* a pseudoknot).

The work presented here represents the first experimental determination of the structure of the telomerase RNA in solution.

MZ 202 Differential Expression of Jun and Fos Family Members in Young and Senescent Human Diploid Fibroblasts. Sharon C. Doll, Jeff Lickteig, and Terry C. Johnson, Division of Biology, Kansas State University, Manhattan, KS 66506

In cell culture, normal human diploid fibroblasts will divide for a programmed number of population doublings, proliferation then ceases, and the cells remained viable for months after entering senescence. Young diploid fibroblasts that stop proliferating either because of serum starvation, calcium depletion, growth to confluence, or inhibition by SGP (a cell proliferation inhibitor) are blocked in G1 of the cell cycle. Senescent diploid human fibroblasts are also stopped in G1. One of the hallmarks of senescent human fibroblasts is the inability of c-fos to be up regulated when the cells are serum stimulated, whereas young fibroblasts stimulated with serum do up regulate c-fos. In contrast, c-jun and jun B are expressed in both young and senescent fibroblasts stimulated with serum. The fos and jun family members form either homodimers (jun family members) or heterodimers and are responsible for binding the transcriptional regulatory AP-1 motifs. Since genes that contain an AP-1 site(s) in their promoters and enhancers are differentially regulated in senescent and young human diploid fibroblasts, it is important to characterize the fos and jun family members present in cells of both age types. For instance, transcription of collagenase is up regulated in senescent cells compared to young cells, while p53 transcription is down regulated. We have characterized the temporal regulation of the other jun and fos family members in young and senescent WI-38 cells, a diploid human lung fibroblast cell line. RT-PCR was used to investigate the expression patterns of fra-1, fra-2, Fos B and jun D in young and senescent WI-38 cells that had been stimulated for different times with serum. Using the human collagenase AP-1 site as a probe in EMSA, we have demonstrated different DNA-protein interactions when whole cell extracts of young WI-38 cells were compared to senescent cells after both had been stimulated for 1h with serum. This indicates that the different jun and fos family members, present in young and senescent human fibroblasts, may be important in the differential regulation of genes with AP-1 motifs in their enhancers and promoters.

MZ 203 MULTI-TRIMER COMPLEXES OF HEAT SHOCK TRANSCRIPTION FACTOR ON DNA, Becky L. Drees, Hays S. Rye, Alexander N. Glazer, and Hillary C.M. Nelson, from the Division of Biochemistry and Molecular Biology, Department of Molecular and Cell Biology, University of California, Berkeley, California 94720.

The heat shock transcription factor (HSF) is a trimeric DNA binding protein that is the transcriptional activator of the heat shock or stress response in eukaryotes. HSF binds as a trimer (1) to arrays of inverted repeats of a five base pair consensus sequence, nGAAn, found in heat shock promoters. At higher protein concentrations, HSF binds cooperatively to these sites, forming larger multimeric complexes. A truncation of HSF from the yeast *Kluyveromyces lactis* containing the trimerization and DNA-binding domains (HSF_{DT}) exhibits the same DNA-binding and oligomerization properties as the full length protein. We have used ethidium and thiazole orange homodimers in gel-mobility assays to detect complexes of HSF_{DT} with target DNA. These intercalating dyes form stable fluorescent complexes with double-stranded DNA that can be detected in gels by a laser-excited, confocal, fluorescence scanning system with a sensitivity higher than that attainable with radiolabeling (2). At a DNA/dye ratio of 40 bp per dye molecule, labeling a DNA fragment with dye does not affect HSF_{DT} binding. The spatial resolution of HSF_{DT}-DNA complexes detected with these fluorescent dyes is far superior to that of conventional autoradiography and permits us to analyze protein-DNA complexes that are not resolved by traditional detection methods. Using this technique, we have shown that HSF forms multimeric complexes on DNA by addition of trimers. We base this conclusion on analysis of the mobilities of the multiple HSF_{DT}-DNA complexes and on a two-color mobility-shift fluorescence assay using a mutant of HSF_{DT} engineered for site-specific labeling with fluorescein and target DNA labeled with an "energy transfer" dye, thiazole orange-thiazole blue heterodimer (3). We are continuing to study these multi-trimer complexes by this and other techniques in order to understand their spatial arrangement on DNA.

(1) Sorger, P.K. and Nelson, H.C.M.N. (1989) Cell 59: 807-813
(2) Glazer, A.N. and Rye, H.S. (1992) Nature 359: 859-861
(3) Benson, S.C., Singh, P., and Glazer,A.N. (1993) Abstracts, Human Genome Workshop, February 7-11, 1993, Santa Fe, NM.

MZ 204 INTERACTION OF HOST PROTEINS WITH THE 5' AND 3' TERMINI OF BROME MOSAIC VIRUS RNA, Rohit Duggal and Timothy C. Hall, Institute of Developmental and Molecular Biology, Texas A&M University, College Station, TX 77843-3155

Brome mosaic virus (BMV) is a tripartite positive-strand RNA virus that requires virally-encoded proteins 1a and 2a for genomic RNA replication. Proteins encoded by the host also participate in minus as well as positive-strand synthesis. In UV-cross linking experiments, using uninfected extracts from the leaves of the systemic host barley, we have identified the interaction of a 60 kDa protein with the 3' 200 nucletoides of the positive-strand of BMV RNA-3. This extract was also found to contain proteins that bound to the 5' terminus of the positive-strand and the 3' terminus of the negative-strand of RNA-2. These proteins interacted with the viral RNA specifically, since unlabeled specific competitors completely abolished radiolabeled ribonucleoprotein complex formation, while non-specific competitors were unable to do so. All of these proteins had a molecular weight less than 60 kDa, indicating that the proteins interacting with the 5' terminus of the positive-strand and the 3' terminus of the negative-strand of RNA-2 were different from the 60 kDa protein that recognized the 3' terminus of the positive-strand of RNA-3. The potential involvement of these RNA-protein interactions in negative- and positive-strand synthesis will be discussed.

MZ 205 TBP-TATA COMPLEXES ANALYZED BY FLUORESCENCE SPECTROSCOPY AND SCANNING FORCE MICROSCOPY, Stefan Hermann, Martin Guthold, Carlos Bustamante and Diane K. Hawley, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

We have followed the binding of yeast TATA Binding Protein (yTBP) to various DNA sequences by monitoring the changes in the intrinsic tryptophan fluorescence. Binding of yTBP to specific and nonspecific DNA induce distinct changes in the emission spectrum. Specific binding approximates a two-state process, thus equilibrium constants can be measured. We have measured the binding constant as a function of temperature. A van't Hoff analysis reveals that binding is entropy driven. Mutations in the TATA box affect both enthalpy and entropy of binding. At least four counterions are released from the DNA upon binding of TBP. Mutations in the TATA box can reduce the number of released ions. These and other findings will be discussed in the light of the recently solved structure of a TBP-TATA cocrystal. We have also been able to directly visualize TBP-TATA complexes by scanning force microscopy. TBP binding clearly induces a sharp bend into the DNA. The degree of bending seems to be a function of the DNA sequence, i.e., mutations in the TATA box do affect bending. The distribution of bend angles for various TATA sequences will be presented and implications for the energetics of binding and the binding of other transcription factors will be discussed.

MZ 206 SNAPSHOT BLOTTING: TRANSFER OF PROTEIN - NUCLEIC ACID COMPLEXES FROM ELECTROPHORESIS GELS TO GRIDS FOR ELECTRON MICROSCOPY, Stephen D. Jett and David G. Bear, Department of Cell Biology and the Cancer Center, University of New Mexico School of Medicine, Albuquerque, NM 87131

We have developed a novel technique, termed "snapshot blotting," for the transfer of nucleoprotein complexes in gel electrophoresis bands onto carbon film-coated grids for imaging by electron microscopy. The method permits structural analysis of DNA- and RNA- protein complexes resolved by gel mobility shift assays, and involves the following steps: (1) preparation of highly stable carbon film-coated hydrophilic grids, (2) pre-electrophoretic staining of the DNA using the fluorescent dye YOYO-1, permitting band localization without disruption of the complexes, (3) electrophoretic transfer of complexes onto the grids, (4) post-transfer fixation with glutaraldehyde, and (5) specimen dehydration and contrast enhancement by rotary metal shadowing. To demonstrate the efficiency and integrity of the transfer process for imaging a multiprotein-DNA assembly, we have used the technique to study a transcription complex formed between a DNA restriction fragment carrying the promoter of the E. coli rho gene and E. coli RNA polymerase. The assembly also contains two bound cleavage-defective EcoR1 protein dimers that indicate the orientation of the transcription reaction and also act as a barrier to generate transcription intermediates. Snapshot blotting should be of great utility in the structural characterization of nucleic acids and nucleoprotein assemblies generated from either purified components, or in cellular extracts.

MZ 207 ANALYSIS OF THE INTERACTIONS BETWEEN PapI AND LEUCINE-RESPONSIVE REGULATORY PROTEIN IN THE CONTROL OF PYELONEPHRITIS-ASSOCIATED PILI PHASE VARIATION, Linda S. Kaltenbach and David A. Low, Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT 84132

Expression of pyelonephritis-associated pili (Pap), which enable uropathogenic *Escherichia coli* to attach to epithelial cells, varies between ON and OFF phase states. Pap phase variation is controlled by interactions of two proteins, leucine-responsive regulatory protein (Lrp) and PapI, with *pap* regulatory DNA which contains two GATC sequences. Although most DNA GATC sites in *E. coli* are methylated by deoxyadenosine methylase (Dam), binding of Lrp to its target sequences in *pap* prevents Dam methylation of *pap* GATC sites. Control of pilin gene expression occurs by competition between Lrp and Dam for binding to *pap* regulatory sequences. If DNA is first methylated by Dam, Lrp cannot bind and transcription does not occur. In contrast, if Lrp binds first, DNA cannot be methylated. Lrp is necessary but not sufficient to activate *pap* transcription. PapI binds to Lrp/*pap* DNA complexes and is required for the phase OFF to phase ON transition. Interestingly, PapI alone does not bind to *pap* DNA. In order to study how PapI regulates phase variation, we chemically mutagenized a plasmid which contains the *papI* gene. The mutants are clustered in a 19 amino acid region that is highly conserved among PapI-like proteins encoded by other pilus operons. PapI mutants show decreased *pap* transcriptional activity and are unable to bind to Lrp/*pap* DNA complexes. These data suggest that this region of PapI may mediate interactions with Lrp. Protein crosslinking experiments using metabolically radiolabeled PapI cell extracts and purified Lrp show that PapI interacts weakly with Lrp in solution. Crosslinking was greatly increased when *pap* regulatory DNA, but not *ilvIH* (another Lrp controlled operon) regulatory DNA was added. This suggests that PapI/Lrp interactions are optimal in the presence of specific *pap* sequences. In agreement with this result, gel mobility shift assays show that Lrp binds to both *pap* and *ilv* labeled regulatory sequences as expected, whereas PapI binds Lrp/*pap*, but not Lrp/*ilv* complexes. These data indicate that efficient formation of Lrp/PapI complexes occurs only after Lrp binds to *pap* DNA sequences. Moreover, this requirement appears to be specific since binding of Lrp to *ilvIH* regulatory DNA sequences did not result in enhancement of Lrp/PapI formation. Taken together, these results suggest that PapI interacts directly with Lrp to control *pap* phase variation.

MZ 208 H1TF2a: A NOVEL HUMAN TRANSCRIPTION FACTOR INVOLVED IN HISTONE H1 CELL CYCLE REGULATION. Rosanna Martinelli and Nathaniel Heintz. Howard Hughes Medical Institute Laboratory of Molecular Biology Rockefeller University, New York N.Y. 10021

H1TF2 is a CCAAT binding transcription factor that binds to the histone H1 subtype specific consensus sequence, which has previously been shown to be necessary for temporal regulation of histone H1 transcription during the cell cycle. In this study, we report that H1TF2 is a heteromeric CCAAT binding protein composed of two polypeptide doublets of 33/34kd and 43/44kd that are not antigenically related. The cloning of H1TF2A, the large subunit of this factor, reveals it to be a glutamine rich protein with extremely limited similarity to previously cloned CCAAT binding proteins. Immunoprecipitation studies demonstrate that H1TF2 is heterodimeric in the absence of DNA in vivo, and identify several H1TF2 interacting proteins that may play a role in H1TF2 function in vivo. Further immunoprecipitation studis establish that HeLa cell H1TF2A is phosphorylated in vivo, but that no gross change in H1TF2A phosphorylation is evident during the cell cycle.

Examination of the phosphorylation state of H1TF2A related proteins during the HeLa cell cycle demonstrates that these proteins are mitotically regulated, suggesting that regulation of this factor may share features previously demonstrated for the histone H2b transcription factor Oct1

MZ 209 THE tRNA BINDING DOMAIN OF METHIONYL-tRNA SYNTHETASE: POSITIVE AND NEGATIVE ELEMENTS ARE CLUSTERED WITHIN A SHORT STRETCH OF THE PROTEIN, Thierry MEINNEL, Emmanuelle SCHMITT, Yves MECHULAM and Sylvain BLANQUET, Laboratoire de Biochimie, Ecole Polytechnique, F-91128 Palaiseau Cedex, France.

Methionyl-tRNA synthetase (MetRS) from *E. coli* binds and aminoacylates its tRNA substrates essentially according to the three bases (CAU) of the anticodon [1]. Moreover, a simplified domain composed of only the anticodon stem and loop binds MetRS provided it has the correct anticodon sequence [2]. In the course of mapping the anticodon-binding domain of the protein, genetic screening experiments have been carried out [3]. Random mutagenesis of the whole MetRS gene, followed by the selection of second-site mutants identify a single amino acid region (region 460) where most substitutions are found (residues 449-456). Region 460 is well conserved in the amino acid sequences of several MetRS originating from different organisms and is folded as an α-helix in the 3-D model of MetRS [4]. Site-directed mutagenesis confirms that two positive determinants are clustered within this region. Saturation mutagenesis of the residues belonging to this helix, followed by selection for enzymes with new anticodon specificities, as well as further point-mutagenesis, show that two aspartates allow the enzyme to reject tRNAs with wrong anticodons [5]. Specificity of the recognition of the three anticodon bases results therefore from a dual mechanism involving residues acting positively as well as residues acting negatively.

[1]. Schulman & Pelka (1983) PNAS 80:6755. [2]. Meinnel *et al.* (1991) JMB 220:205. [3]. Meinnel *et al.* (1991) PNAS 88:291. [4]. Brunie *et al.* (1990) JMB 216:411. [5]. Schmitt *et al.* (1993) JMB 233 in press.

MZ 210 FUNCTIONAL TOPOLOGY OF E. COLI RNA POLYMERASE REVEALED USING BIFUNCTIONAL RIFAMPICIN-NUCLEOTIDE COMPOUNDS AS A MOLECULAR RULER, Arkady Mustaev[1,2], Konstantin Severinov[1], Mikhail Kashlev[1], Evegeny Zaychikov[2], and Alex Goldfarb1[1], [1]Public Health Research Institute, New York, NY 10016, [2]Limnological Institute, Russian Academy of Science, Irkutsk, Russia.

Chimeric compounds in which rifampicin (Rif) is covalently attached to the 5' phosphate of a ribonucleotide can bind to RNA polymerase molecule in bifunctional manner and serve as primers for RNA chain initiation. It is demonstrated that the binding sites for the two ligands are connected flexibly permitting variation in the length of the linker with the optimum of 5-6 carbon atoms. A reactive side group introduced into the linker results in a crosslink with the -3 or -2 nucleotide in the template strand of DNA. The results indicate that bound Rif is juxtapposed to the DNA regon immediately upstream from the start site, in accord with the model that Rif binds in the "product channel" normally accomodating the nascent RNA chain.

MZ 211 MULTIPLE BINDING SITES WITH DIFFERENT BINDING AFFINITY FOR CELLULAR PROTEINS IN THE COMPLEMENT OF THE 5' TERMINUS OF SINDBIS ALPHAVIRUS RNA. Nathalie Pardigon and James H. Strauss, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Mutational analysis of the 5' nontranslated region (5' NTR) of the Sindbis alphavirus genome has demonstrated the deleterious effects for virus growth of deletions within the first 44 nucleotides. This sequence element, as well as 3 other conserved sequence elements (CSE) in the alphaviral genome, has been proposed to be a possible promoter for initiation of transcription/replication of the viral genome. The different effects of mutations within the CSEs in different cell lines have led to the hypothesis that their function is modulated by binding to host cell factors. In association with viral replicase components, these cellular proteins could promote viral transcription/replication by recognizing an RNA structure and/or sequence and interacting with it.

We previously demonstrated the ability of the 60 nucleotides representing the 3' end of the minus strand to interact with cellular proteins, using gel retardation analysis.We have now screened the 3' terminal 249 residues of the minus strand for protein binding sites and found that there are three high affinity and one low affinity binding sites for two mosquito cell proteins within this region. Deletion analysis of the 3' terminal binding element.showed that the 3' 10 bases are critical for the binding, while other deletions affect the binding differently.

We have begun the purification of the two cellular proteins by passing mosquito cell extracts on a cation exchange column and obtained a 10000 fold purification. After gel purification followed by electroelution, the proteins were submitted to endopeptidase digestion, and the resulting peptides, separated on a HPLC column, are currently being analyzed by microsequencing.

MZ 212 A POSSIBLE ROLE OF NF I AS REPRESSOR OF TRANSCRIPTION AND LACK OF OCCUPATION OF A HIGH AFFINITY NF-κB SITE REVEALED BY *IN VIVO* FOOT-PRINTING. Theo Rein, Ernst-L. Winnacker and Haralabos Zorbas. Institut für Biochemie im MPI für Biochemie, F3, Am Klopferspitz D-82152 Martinsried, FRG

NFI is a family of sequence-specific DNA binding proteins. We recently demonstrated that NF I interacts with an NF I-like sequence in the α-globin-promoter and not with the CCAAT-box, as claimed by others. Thus, the function of NF I as a transcriptional *activator* by binding to the CCAAT-box became questionable. We then performed *in vivo* footprints to obtain more information about possible cellular functions of NF I by correlating the α-globin transcription with the occupation of the NF I site. This site is occupied only in cells *not expressing* α-globin (HeLa), but not in those expressing it (K562). These results are consistent with a role of NF I as *repressor* of the human α-globin transcription. We have shown by immunoblot analysis, FACS, gel retention and proteolytic cleavage that K562 cells do produce NF I, but the species differ from those in HeLa cells. The different behaviour of NF I in the two cell lines may be achieved therefore by different usage of different species of NF I.

A conserved, high-affinity NF-κB site is present within the IRF-1 (interferon regulatory factor-1) promoter. CAT assay experiments aimed at elucidating the function of this site have not been conclusive. To clarify the role of this site we performed *in vivo* footprints after induction of NF-κB in mouse L929 cells. Neither in control nor in PMA-induced cells this site seems to be occupied *in vivo*. Instead, we do detect a footprint at a GC box and at the CCAAT box further upstream both before and after induction, additionally to strong deformations of the DNA structure. We conclude that the activation of this promoter must be achieved by a different site. Consequently, a classical NF-κB site is not active *in vivo*, and this, to our knowledge, is demonstrated here for the first time.

MZ 213 THE ENERGETICS OF PROTEIN-DNA INTERACTIONS: ANALYSIS OF THE BINDING ISOTHERMS, H. A. Saroff, Laboratory of Biochemical Pharmacology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892

Cooperative interactions on the binding of proteins to multiple sites on nucleic acids have usually been attributed to stabilizing forces between adjacently bound proteins (occupied-occupied interactions). This reasoning assumes that binding of a protein to a given site does not perturb a neighboring site (no occupied-unoccupied interactions).

A considerable body of data has accumulated indicating that, on binding, proteins distort the nucleic acid structure both in mononucleotides and polynucleotides. This distortion is probably transmitted to neighboring sites when proteins bind to multiple sites on polynucleotides. Thus it is reasonable to consider both occupied-unoccupied and occupied-occupied interactions in formulating equations for evaluating binding data. An analysis of this kind has already been presented for data on the binding of repressor to the right and left operators of bacteriophage λ [Saroff, H. A. (1993) Biopolymers,33,1327-1336].

When a ligand binds to a linear lattice of multiple sites, and the sites are non-specific and overlapping, the number of species becomes very large as the length of the lattice increases. Thus for a lattice of 21 sites in which the ligand occupies any 7 sites the number of species is 52. For 70 sites with the ligand occupying any 7 the number of species is 3,805,165. An exact solution for the binding equations which includes the occupied-unoccupied and occupied-occupied interactions has been developed. Evaluation of the theoretical expressions, although straightforward, requires a formidable amount of computation which is readily accomplished with the present very high-speed computers.

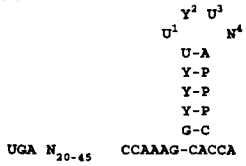
Some of the results of these calculations will be presented.

MZ 214 *Abstract Withdrawn*

MZ 215 SPECIFICITY OF BINDING OF THE SLBP TO THE 3' END OF HISTONE mRNA: ROLE IN NUCLEAR AND CYTOPLASMIC METABOLISM OF HISTONE mRNA. Anthony S. Williams, Roberta H. Hanson, Jian-Hua Sun and William F. Marzluff. Program in Molecular Biology, UNC, Chapel Hill, NC 27599

Histone mRNA ends in a stem-loop structure which has been conserved among metazoans (Fig. 1). The only processing reaction required for formation of histone mRNA is a cleavage reaction which occurs between the stem-loop sequence and a purine-rich sequence which binds U7 snRNP. A 45 kD protein (the SLBP) binds specifically to the stem-loop structure and present both in the nucleus and the cytoplasm, where it is a component of the polysomal histone mRNP. Binding of the SLBP to the histone stem-loop requires all the conserved regions of the sequence. Nucleotides 1 and 3 in the loop are required for binding of the SLBP as are the two GC base pairs at the base of the stem, the conserved adenosines 5' of the stem-loop and nucleotides 3' of the stem-loop, suggesting that the 3' end of histone mRNA assumes a unique tertiary structure. Footprinting of the binding site shows that 3 nucleotides 5' of the stem-loop and 2 nts 3' of the stem-loop are necessary for binding. Inhibition of DNA synthesis results in a rapid degradation of histone mRNA. Concomitant with the degradation of the histone mRNA, additional SLBP accumulates in the nucleus, suggesting that the SLBP shuttles between the nucleus and the cytoplasm. Mutations in the stem-loop sequence which reduce binding of the SLBP to the histone mRNA, reduce the efficiency of 3' end formation *in vivo* resulting in reduced expression of the histone mRNA. It is likely that the SLBP associates with the histone pre-mRNA, functions in the processing reaction and then accompanies the histone mRNA to the cytoplasm. Histone mRNAs with an adenosine in position 1 and 3 of the loop are transported inefficiently to the cytoplasm; 30% of the steady-state RNA remains in the nucleus. The mutant histone mRNAs are also not rapidly degraded when DNA synthesis is inhibited, nor are they stabilized by inhibition of protein synthesis. Thus the proper cytoplasmic metabolism of histone mRNA requires the binding of the SLBP.

Fig. 1



MZ 216 THE MINIMAL SELF-SUFFICIENT ELEMENT IN A MURINE G+C RICH PROMOTER IS A LARGE ELEMENT WITH IMPERFECT DYAD SYMMETRY, Cho-Yau Yeung, Susan L. Ackerman, and Audrey G. Minden, Department of Genetics (M/C 669), University of Illinois College of Medicine, 808 South Wood Street, Chicago, Illinois 60612.

The murine adenosine deaminase (ADA) gene has a structurally archetypal TATAA-box deficient G+C rich promoter. Although the promoter contains three Sp1 binding sites, these sites are neither necessary nor sufficient for promoter function. A 48 bp element downstream of the Sp1 binding sites was found to be both necessary and sufficient for promoter activity. This element shows an imperfect dyad symmetry with respect to the major transcriptional initiation site normally utilized by the wild-type promoter and contains two or more binding sites for murine nuclear proteins. The distinctive sequence characteristics and nuclear protein binding locations of this element led us to propose a novel operational model of promoter function which may be generally applicable to this class of promoters.

Structure Specific Interactions with DNA; Multiprotein Complex with RNA

MZ 300 RECA-ssDNA INTERACTION: RECOGNITION OF DNA STRUCTURE, Eiko Akaboshi, Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamadaoka, Suita 565, Japan

RecA protein of Escherichia coli promotes a variety of reactions of DNA in recombination, repair of DNA and induction of SOS functions. In vitro the recA protein can mediate homologous-pairing and strand-exchange reactions between suitable DNA molecules. The initial step in these reactions is polymerization of recA protein on ssDNA. However, relatively little is known about nature of this binding.

Interaction of recA protein with ssDNA was probed with hydroxyl radicals, which can cleave the DNA backbone. RecA protein induced two kinds of cleavage by hydroxyl radicals in 5'-end-labeled polynucleotides (41- to 60-mers). One kind of cleavage occurred at the 11th and 12th positions from the 5' end.

After this first site-specific cleavage, recA protein induced cleavages in a non-uniform manner. Each polynucleotide showed a unique pattern of cleavage. Substitution of a single nucleotide altered extent of cleavage to its 3' side. These results suggest that recA protein may recognize nucleotide sequence and/or local structure of a ssDNA molecule, and that change of conformation of the DNA backbone could be involved in the ssDNA-recA complexes. Since the ssDNA-dependent ATPase activity of recA protein depended on the length and sequence of the polynucleotide, ATP hydrolysis might be associated with conformational changes in DNA.

MZ 301 PRERIBOSOME ASSEMBLY : INTERACTION BETWEEN GAR PROTEINS AND NUCLEOLAR RNAs, Amalric F., Bagni C., Bugler B., Caizergues-Ferrer M., Créancier L., Erard M., Joseph G., Ghisolfi L., Kharrat H. and Lapeyre B. Institut de Biologie Cellulaire et de Genetique, 118 Route de Narbonne - 31062 Toulouse Cedex.

The main steps of ribosome biogenesis occurs in the nucleolus of eukaryotic cells, the site of rDNA transcription. Nascent transcripts are rapidly associated with ribosomal proteins and specific nucleolar proteins in preribosomes. A family of nucleolar proteins (GAR proteins) has recently been characterized. They share a repetitive domain (NOPI/fibrillarin, GAR1, NSR1 in yeast, nucleolin and fibrillarin in vertebrates) rich in glycine and arginine (more than 70% of total residues) and are implicated in pre-rRNA processing and ribosome assembly. Several proteins of splicosomes also possess a domain that presents analogies with the nucleolar GAR domain. (G and R are less than 50% of total residues and an increased variety in interspaced amino acids).

Nucleolin (rodents) and GAR1 (yeast) were used as models to study structure-function relationships of this protein family. Nucleolin (713 aa in rodents) is subdivided into three major domains. The N-terminal domain, that comprises long acidic stretches interspersed with basic repeats, is involved in modulation of chromatin condensation. The central part contains four RNA recognition motifs (RRM, RI to RIV) wich are involved in the recognition of a specific binding site in the 5' external transcribed spacer of pre-rRNA. RI alone confers this binding specificity while RIV does not. The GAR C-terminal domain (85 aa : p10) interacts in an efficient but non specific manner with RNA. Repeated ß-turns are the major structural components of this polypeptide which is consistent with the presence of the repeat motif RGGF. Interaction of p10 with RNA involves an unstacking of bases and an unfolding of the RNA. Spectroscopic data shows that the GAR domain unfolds parts of RNA to allow access of RNP domains to its specific binding site. However, RNA denaturation, prior to addition of nucleolin, decreases the binding *in vitro* by a factor of ten. Two binding sites were mapped in the 5' ETS upstream of the first processing site. These sites were conserved in human, mouse and rat. A fine mapping was carried out using directed mutagenesis and chemical or enzymatic probing.

In order to characterize the function of GAR proteins in ribosome biogenesis, three family members have been isolated in yeast (fibrillarin, GAR1 and GAR2). GAR1 is a 205 residues protein with two GAR domains at the N and C-terminus respectively. It is a non ribosomal protein localized in the yeast nucleolus and is essential for cell growth. Depletion of GAR1 results in an anormal processing of 35S pre-rRNA with a 18S synthesis inhibition. By immunoprecipitation GAR1 coprecipitates two snoRNAS (snR10 and snR30). Although GAR1 did not contain a classical RNP domain, it is able to bind snR10 and snR30 in vitro. It has been shown that the two GAR domains are not essential for cell growth and nucleolarisation.

Several sets of evidence suggest that the GAR protein, in association with nucleolar snoRNA, are involved in the assembly of the pre-rRNA processing complex. Some will interact directly with pre-rRNA and others with snRNA. The GAR domain could be implicated in RNA binding and in protein-protein recognition. Thus the deletion of one GAR domain in one protein could be complemented by the GAR domains of other proteins.

MZ 302 MAMMALIAN PROTEIN FACTORS THAT FUNCTION EARLY IN SPLICEOSOME ASSEMBLY, Silke Backes, Reto Brosi, Karsten Gröning, Patric Grüter and Angela Krämer, Département de Biologie Cellulaire, Université de Genève, 30, quai Ernest-Ansermet, CH-1211 Genève 4, Switzerland

Splicing of nuclear pre-mRNAs occurs within multicomponent complexes (spliceosomes) that are assembled in a stepwise fashion by interactions between pre-mRNA, snRNPs and non-snRNP protein factors. We obtained six chromatographic fractions (SF1, SF3a, SF3b, U2AF, U1 and U2 snRNPs) from HeLa cell nuclear extracts that function in the ATP-dependent formation of pre-splicing complex A.

SF1 is a heat stable protein of 75 kD; its exact function during pre-spliceosome assembly is unknown. The amino acid sequence derived from a cDNA encoding SF1 shows no significant homology to sequences in current data bases, and has revealed a putative leucine-zipper and several possible phosphorylation sites. SF1 is not tightly associated with the spliceosome and it could transiently contact the spliceosome or be required for modifications of other splicing components.

SF3a consists of three subunits of 60, 66 and 120 kD, which correspond to three proteins present in the active 17S form of U2 snRNP but not in the inactive 12S form. At least four proteins enriched in SF3b fractions appear to correspond to other 17S U2 snRNP-specific polypeptides. Our experiments suggest a two-step assembly pathway for the 17S U2 snRNP: binding of SF3b to the 12S U2 generates a 15S intermediate particle that is converted to the 17S form by the subsequent association of SF3a. Thus, both splicing factors function in the assembly of the active U2 snRNP and most likely mediate the interaction of U2 snRNP with the branch site during pre-splicing complex formation. cDNA cloning of the 60- and 120-kD subunits of SF3a has furthermore shown that these polypeptides represent human homologues of two yeast splicing factors that also function early during spliceosome assembly.

MZ 303 UNCOUPLING DNA BINDING AND DNA BENDING IN SRY PROTEINS FROM SEX-REVERSED PATIENTS

Marco E. Bianchi, Dept. Genetics and Microbiology, University of Milano, and DIBIT, San Raffaele Scientific Institute, Milano, Italy.

In mammals, sex determination is caused by the Y-chromosome gene *SRY*. The DNA binding domain of human SRY protein (which belongs to the HMG-box family) recognises kinked DNA structures, and produces a sharp bend of about 80° on binding sequence-specifically to linear DNA. DNA bending is required to attain the correct spatial conformation of nucleoprotein complexes in site-specific recombination, and is expected to play a similar role in transcription. We therefore tested whether mutations in *SRY* causing XY gonadal dysgenesis in humans affect the manipulation of DNA geometry. Three mutant SRY HMG-boxes, including one with a 100-fold reduced affinity for DNA, bend the DNA as the wild type. However, one mutant with slightly reduced binding affinity produces an anomalous DNA geometry, consistent with a role of DNA bending in the control of sexual differentiation. We also propose that the mechanical resistance to bending of localised DNA segments is a major determinant in binding site selection by SRY protein.

MZ 304 Co-Purification of E. coli RNase E and PNPase: Evidence for a Specific Association between Two Enzymes Important in RNA Processing and Degradation

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RNase E, an *E. coli* endo-ribonuclease, was purified as part of a large protein complex that also contains PNPase (polynucleotide phosphorylase). PNPase degrades RNA processively in the 3' to 5' direction. The products of the *E. coli rne* (RNase E) and *pnp* (PNPase) genes as well as two additional proteins of 50 and 48 kD co-purified and co-sedimented on glycerol gradients. Experiments aimed at identifying the 50 and 48 kD proteins are in progress. Further evidence for a protein complex comes from the immunoprecipitation of partially purified RNase E with an antiserum that was raised against the *rne* gene product. Highly purified RNase E correctly processed *E. coli* 5S rRNA and *ompA* mRNA as well as bacteriophage T4 *gene 32* mRNA at sites that are known to depend on the function of the *rne* gene for cleavage *in vivo*. Heat-treatment inactivated RNase E that was purified from the *rne* temperature-sensitive mutant strain. The same heat-treatment of the wild-type enzyme had no effect on RNase E activity. The difference between previous smaller estimates of the native size of RNase E and that reported here is apparently due the sensitivity of the enzyme to proteolysis during purification. The discovery of a specific association between RNase E and PNPase raises the intriguing possibility that these enzymes act cooperatively in the processing and degradation of *E. coli* RNA.

MZ 305 MAMMALIAN RIBONUCLEOTIDE REDUCTASE R1 mRNA-PROTEIN INTERACTION INVOLVES A PROTEIN KINASE C SIGNAL TRANSDUCTION PATHWAY: ROLE IN MESSAGE STABILITY, Frank Y. Chen, Francis M. Amara and Jim A. Wright, Manitoba Institute of Cell Biology, Winnipeg, Manitoba, Canada R3E 0V9

The signal transduction pathway mechanism(s) that regulates R1 message stability was investigated. We detected by cross-linking and band shift assays a similar 57 kDa R1 mRNA-binding protein (R1BP) complex in COS 7 cells we previously identified in BALB/c 3T3 cells, which bound specifically to a 49-nucleotide (nt) region of the mouse R1 mRNA 3' untranslated region (3'UTR). The R1BP RNA-binding activity was down-regulated by 12-0-tetradecanoylphorbol-13-acetate (TPA) and okadaic acid, and up-regulated by staurosporine in a dose-dependent manner. TPA inhibited the staurosporine effect and showed a synergistic effect with okadaic acid. Furthermore, the dose-dependent effects of the compounds on R1 and CAT/R1 mRNA stabilities were in contrast to their actions on R1BP-RNA binding, whereas forskolin did not show significant effects on both R1BP-RNA binding and mRNA stability. Interestingly, transfectants with R1 or CAT/R1 cDNAs with the 49-nt deletion failed to respond to the effects of these compounds on mRNA stability. The above observations have clearly demonstrated an integrated post-transcriptional gene expression regulation pathway involving a signal initiated by stimulation of protein kinase C, that regulates a *cis-trans* binding activity at R1 mRNA 3'UTR which, in turn, modulates the message stability.

MZ 306 RESOLUTION OF THE DNA FOUR-WAY HELICAL JUNCTION INVOLVES STRUCTURE-SPECIFIC RECOGNITION. Derek R Duckett and David M.J. Lilley.
CRC Nucleic Acid Structure Group, Biochemistry Dept., The University, Dundee. U.K.

Four-way DNA junctions generated during genetic recombination must ultimately be processed to recreate intact duplex molecules. Enzymes that catalyse such a reaction have been isolated from a number of sources, as evolutionary diverse as bacteriophages and mammals. It would appear that these enzymes interact with the junction in a manner that is primarily dependent on the structure formed.

We have shown that in the presence of cations the junction folds into a right-handed, antiparallel X-shaped structure (Duckett et al, 1988, Murchie et al, 1989), based on pairwise, coaxial stacking of helical arms. A major feature of the stacked X-structure is the presentation of two stereochemically distinct sides. The cleavage positions for resolving enzymes such as T4 endo-nuclease VII, on a DNA fourway junction have been investigated in detail. Single cleavage sites are introduced into two of the four strands, in keeping with the twofold structural symmetry of the junction. These cleavages occur on one side of the junction, the minor groove side, suggesting that such enzymes bind to this face of the junction.

In addition to four-way junctions, resolving enzymes cleave other branched DNA substrates, including three-way junctions and bulged molecules. A feature common amongst these species is a mutual inclination of DNA helices, and it seems probable that this aspect is critical in the recognition process (Bhattacharyya. et al, 1991).

We have generated a number of mutant enzymes that will allow us to investigate how this special class of enzymes recognise the molecular geometry of a folded DNA molecule.

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MZ 307 SELECTIVE INHIBITION OF PARASITE TOPOISOMERASES I AND II BY DICATIONIC MINOR GROOVE BINDING AGENTS, Christine C. Dykstra[1], Daniel R. McClernon[2], Lynn P. Elwell[2], W. David Wilson[3], David W. Boykin[3], and Richard R. Tidwell[1], [1]Department of Pathology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, [2]Department of Microbiology, Burroughs Welcome Co., Research Triangle Park, NC 27509, [3]Department of Chemistry, Georgia State University, Atlanta, GA 30303.

Both type I and II topoisomerases have been isolated from *Pneumocystis carinii*. Both enzyme activities were selectively inhibited by a series of dicationic-substituted bis-benzimidazoles when compared to topoisomerases from mammals. The most active compounds against the *P. carinii* topoisomerase were highly effective in an *in vivo* animal model of *P. carinii* pneumonia. An additional series, the dicationic furans, showed similar selectivity for the parasite enzymes when compared to those from mammalian cells. Both classes of molecules have been shown to bind in the minor groove of DNA with an AT preference and binding strength correlates with their activity against the parasite topoisomerases. Mechanistic studies are underway in order to determine their mechanism of inhibition. Selectivity against the parasite enzyme and relatively low toxicity to mammalian cells for both series of compounds suggests that they are promising therapeutic agents for treatment of the life-threatening AIDS-related disease, *Pneumocystis carinii* pneumonia. (supported by NIAID AI 33363).

MZ 308 USE OF SINGLE PHOSPHORODITHIOATE LINKAGES IN DUPLEX DNA TO STUDY DUPLEX STRUCTURE IN PROTEIN/DNA COMPLEXES, Mary J. Hall, Henry Sasmor, Marvin H. Caruthers, Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309

Proteins recognize and bind to specific sequences of DNA to perform many functions in the cell. Upon binding, it has been found that the DNA duplex is often distorted away from B form presumably to make additional contacts to the protein. It is not clear how important the distortion of the DNA is, if it is necessary for the protein to recognize the target sequence or simply a side effect of protein binding. To begin addressing this question we are using phosphorodithioate linkages in place of phosphodiester linkages to study the role of phosphate conformation in the deformability of duplex DNA. The proteins that we have been studying are *lac* repressor, lambda phage cro protein and *E. coli* CAP protein. We have found that at most positions in duplex oligodeoxynucleotide recognition sequences phosphodiester linkages can be substituted with phosphorodithioates without any effect on the binding of the protein. This is true even at sites known to be in contact with the protein. However, at certain sites the presence of the phosphorodithioate can reduce binding by 1000 fold or more. In most instances these large effects on binding are at sites where the phosphate has no contact with the protein. This suggests that there is a phosphate conformation at these positions that is necessary for proper binding, and that the phosphorodithioate is unable to adopt such a conformation. There are co-crystal structures of both cro and CAP complexes. The structures show the phosphates that have the binding effect to be at or near positions where the duplex is bent toward the minor groove. Phosphorothioates at these positions do not have the effect on binding. Further studies on these systems are in progress to determine the mode of the phosphoroditioate effect. Crystal structures of phosphoroditioate containing oligomers are also underway to determine if the linkage itself has any inherent structure. Phosphoroditioate replacement may be a useful method for studying DNA conformation in dynamic systems.

MZ 309 INTERACTION OF THE POLIOVIRAL POLYPEPTIDE $3CD^{PRO}$ WITH THE 5' TERMINAL 110 NUCLEOTIDES OF THE GENOMIC RNA: IDENTIFICATION OF VIRAL AND CELLULAR COFACTORS NECCESSARY FOR EFFICIENT BINDING, Kevin S. Harris, Wenkai Xiang, Louis Alexander, and Eckard Wimmer, Dept. of Microbiology SUNY Stony Brook, Stony Brook, N.Y. 11794-5222

A cellular protein has been purified and identified from HeLa cells which interacted with purified polioviral proteinase 3CD ($3CD^{pro}$) as well as the first 110 nucleotides of the viral RNA. A RNA gel shift assay was used to study the binding of the host factor-$3CD^{pro}$ complex to this RNA region whose secondary structure resembles a cloverleaf. The cellular protein recognized $3CD^{pro}$ as a precursor; neither purified $3C^{pro}$ nor $3D^{pol}$ alone nor the two proteins in combination at molar concentrations 10 times greater than that of $3CD^{pro}$ gave rise to a shift when incubated with purified host factor and labeled cloverleaf. The shift was specific for the full-length cloverleaf; deletion derivatives of the cloverleaf did not compete for the shift. The purified precursor to the poliovirus genome-linked protein (VPg), a viral polypeptide termed 3AB also interacted with $3CD^{pro}$. This complex of viral proteins was then able to bind the cloverleaf RNA. Unlike the host factor-$3CD^{pro}$ interaction, 3AB also interacted with $3C^{pro}$ and the cloverleaf although $3C^{pro}$ was required at a ten molar excess relative to $3CD^{pro}$. The 3AB-$3CD^{pro}$ interaction was also specific for the full-length clover leaf. UV cross-linking studies show that $3CD^{pro}$, 3AB, and the host factor are labeled by the cloverleaf. In addition RNA-footprinting experiments indicate that $3CD^{pro}$ binds to stem-loop D of the cloverleaf either in the presence of purified host factor or purified 3AB.

MZ 310 **A Specific DNA-binding Protein Activated by γ-Radiation, Abnormally Distributed in Ataxia-telangiectasia Cells.** KumKum Khanna,Brett Teale and M.F. Lavin. Cancer Unit, QIMR, Bancroft Centre, Australia.

We have recently described a DNA-binding protein activated by ionising radiation(1,2). This protein pre-exists in all normal human cells tested, in the cytoplasm in an active form(1). It responds to sub-lethal doses either by translocation or activation of a nuclear form of the protein within 15 min., and remains at a high level for up to 9 hrs. This protein exists in a constitutively active form in ataxia-telangiectasia(A-T) nuclei, with no additive effect from radiation exposure.

The protein can also be activated by neutrons, radiomimetic agents and phorbol esters(3), but not by UV-irradiation or heat shock. The DNA-binding protein, which recognises a sequence containing a central CAGTT motif, has sites in the SV40, c-*myc* and *kappa* light chain enhancer/promoter regions(1,2).

This protein may be regulated by dephosphorylation of an inhibitor or a site on the protein itself. Treatment of normal cells with kinase inhibitors have produced nuclear activity which is unchanged by γ-radiation.

Recent data by Kastan et al.(4) and Khanna and Lavin(5,6) show a decreased p53 response in A-T. This response to γ-radiation reflects an anomaly in cell cycle progression seen in A-T.

The abnormal distribution of this DNA-binding protein seen in cells from A-T patients, a cancer-prone, radiation sensitive group, and in several tumours tested (unpublished data) could indicate a potential loss of tumour suppression that may be directly linked to this protein's activity and function.

The activity of this DNA-binding protein and p53 may be linked through a defective kinase or phosphatase activity associated with the A-T defect.

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MZ 311 DIFFERENT DISTRIBUTION OF TWO RNA-BINDING PROTEINS ALONG TRANSCRIPTION UNITS, Elena Kiseleva, Tilmann Wurtz, Birgitta Ivarsson and Bertil Daneholt, Laboratory of Molecular Genetics, CMB, Karolinska Institutet S - 17177, Stockholm, Sweden

During transcription nascent hnRNA molecules form RNP particles by assembly with specific proteins, which probably play a role in the maturation, processing and transport of mRNA to the cytoplasm. Little is known about the distribution of the different proteins along the transcripts. We used the Balbiani ring (BR) system of *Chironomus* for the immuno-cytochemical localization of two hnRNP proteins: hrp36 and hrp45 (36 and 45 kD). In the salivary glands, the pre-mRNP particles of the BR genes (BR granules) can be morphologically identified both in the nascent state on the transcription units and during the transport through the nucleoplasm and the nuclear pores. The BR granules can also be isolated by sucrose gradient centrifugation.

In immunocytochemical experiments, antibodies against hrp36 and hrp45 bound to BR puffs and immunoprecipitated isolated BR RNP particles. Immuno-EM analysis of the hrp36 distribution on the transcription unit, showed a high intensity of labelling of the nascent RNP transcripts in the proximal and a lower intensity in the middle and distal parts of the BR gene. In contrast, the intensity of labelling of the hrp45 increased from the proximal to the distal region of the BR gene, coincident with the increase in RNA amount. However, both proteins were about evenly distributed along the entire transcripts, if isolated RNP particles were analysed after the unfolding of the elementary RNP fiber. We conclude that the difference in reactivity *in situ* depends on a different availability of the epitopes of these proteins during the assembly of the BR particles. The 4F9 epitope is being concealed during the higher order packaging of the particles, while the 2E4 epitope remains available. The significance of this observation for the RNP structure will be discussed.

The distribution of hrp36 and hrp45 on all the transcription units along the polytene chromosomes were different, although they overlap partly. It demonstrates that the protein composition of hnRNP particles depends on the nature of the transcript that they contain.

MZ 312 INTERACTION OF T4 ENDONUCLEASE V WITH PYRIMIDINE DIMER-CONTAINING DNA: FOOTPRINTING AND MOLECULAR MODELING STUDIES, Katherine Atkins Latham§, M. L. Dodson, and R. Stephen Lloyd, Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, TX 77555 and §Center in Molecular Toxicology and Department of Biochemistry, Vanderbilt University, Nashville, TN 37232.

T4 endonuclease V is a 16 kDa DNA repair enzyme that incises DNA at the site of ultraviolet light (UV) induced cyclobutane pyrimidine dimers. Three sequential activities have been identified that lead to DNA strand cleavage: salt-dependent linear diffusion on non-target DNA, pyrimidine dimer-specific DNA glycosylase activity, and abasic (AP) lyase activity. Studies have demonstrated the importance of the N-terminal primary NH_2 group for both catalytic activities. The N-terminus of endonuclease V attacks the 5' sugar of the dimer at the C1' position to form an imino intermediate, releasing the 5'-base. β-elimination then allows for phosphodiester bond cleavage. The imino intermediate can be reduced by $NaBH_4$ to produce a covalent dead-end DNA-enzyme product, a useful substrate for studying endonuclease V - DNA interactions.

Footprinting techniques, such as DNase I protection experiments, are being used to examine the interaction between endonuclease V and a 49 base pair oligonucleotide containing a site-specific *cis-syn* cyclobutane thymine dimer (courtesy of J.-S. Taylor, Department of Chemistry, Washington University). $NaBH_4$ is used in these experiments to trap the enzyme on DNA. Preliminary results suggest that endonuclease V protects ~9 bases of DNA on the strand opposite the dimer. Interestingly, only 1-2 DNA bases are protected by the enzyme on the dimer-containing strand. These and additional footprinting results are being used to model the docking of endonuclease V to pyrimidine dimer-containing DNA. Molecular modeling and simulated annealing calculations are being carried out using MidasPlus and Amber 4.0, respectively (both programs are from the University of California, San Francisco). Supported by ES04091.

MZ 313 HPB12, A B. subtilis BIFUNCTIONAL NUCLEOID PROTEIN IS INVOLVED IN RIBOSOME ASSEMBLY AND DNA COMPACTION, F. Le Hégarat, B. Arnold-Schulz-Gahmen and L. Hirschbein. Institute of Genetics and Microbiology, University Paris 11, 91405 Orsay, France and Department of Microbiology, Biozentrum, University of Basel, 402150 Basel, Switzerland

The HPB12 protein from the nucleoid of B. subtilis was previously described and its DNA binding properties have been reported (Salti et al, 1989). The DNA-HPB12 complexes were examined by electron microscopy. They appeared as short, slightly curved rods whereas naked DNA showed no compaction. Only a small number of complexes with an intermediate degree of folding were observed. Therefore, the nucleoid associated protein HPB12 binds cooperatively to DNA, confirming Salti et al., and gives rise to a tightly compacted DNA-protein complex.

N-terminal sequencing of purified HPB12 showed that the first 19 amino acids were identical to those of the L24 ribosomal protein, a major ribosome assembly protein. HPB12, the product of the rplX gene (Henkin et al., 1990) is the first bifunctional ribosomal protein described in Gram positive bacteria.

MZ 314 STRUCTURE-FUNCTION RELATIONSHIPS IN ESCHERICHIA COLI RIBONUCLEASE E, George A. Mackie, Robert S. Cormack, Julie L. Genereaux, Tannis J. Ellis, Xin Miao, and Ken Niguma, Department of Biochemistry, University of Western Ontario, London, ON, Canada.

RNase E is a major intracellular endoribonuclease in *E. coli* and is required for the processing of 5S rRNA and for the degradation of most cellular mRNAs. We have cloned the complete *rne/ams/hmp1* gene which is required for RNase E activity and have overexpressed the gene product (1061 aa residues). The Rne/Ams/Hmp1 polypeptide can be purified by preparative SDS-PAGE and can be renatured by dilution from 6 M guanidine-HCl. The purified polypeptide manifests authentic RNase E activity in the absence of other proteins as it performs site-specific cleavage of the 9S RNA precursor to 5S rRNA. The purified polypeptide also behaves as a sequence-independent RNA binding protein in the absence of cleavage. We have employed partial proteolysis, UV-crosslinking, deletion analysis, and RNA-protein (Northwestern) blotting to dissect potential functional domains of this enzyme. Up to 430 residues can be removed from the C-terminus of Rne/Ams/Hmp1 with no effect on RNA binding. Partial proteolytic fragments as small as 25 kDa also retain RNA binding activity. The amino terminal 300 residues (which cannot bind RNA) appear to be involved in multimerization as their overexpression leads to a dominant negative phenotype. Investigations of the substrate specificity of crude or partially purified RNase E show that it is essentially a single strand-specific endoribonuclease. Site-specific cleavage appears, however, to depend on adjacent stem-loop structures.

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MZ 315 PROTEINS THAT SPECIFICALLY BIND TO SINGLE-STRANDED DNA SEQUENCES WITH A HIGHER ORDER STRUCTURE: NOVEL DNA STRUCTURES RECOGNIZED BY E.COLI PROTEINS INCLUDING DNAA AND RNA POLYMERASE, Hisao Masai and Ken-ichi Arai, Department of Molecular and Developmental Biology, Institute of Medical Science, University of Tokyo, Tokyo 108 Japan

Melting or unwinding of duplex DNA is a process inherently necessary for varieties of biological reactions such as transcription, DNA replication and recombination. In search for proteins which specifically recognize DNA when it is melted, we screened libraries constructed on a derivative of single-stranded M13 phage DNA. We have isolated twelve novel sequences which can facilitate the phage proliferation presumably by providing efficient signals for initiation of DNA replication. Analyses of these single-strand *i*nitiation sequences (*ssi*) and proteins that recognize them lead to identification of four classes of single-stranded DNA sequences and the proteins that specifically bind to them and initiate DNA replication by promoting RNA synthesis. i) Primase (dnaG gene product) recognizes G sites and synthesizes RNAs of specific lengths. ii) PriA protein binds to n' sites or n'-*pas* (*p*rimosome *a*ssembly *s*ites) and triggers assembly of a protein complex (ϕX174-type primosome) which translocates on single-stranded DNA templates and synthesizes multiple RNAs . iii) DnaA protein binds to A site and directs assembly of an ABC-primosome, a protein complex functionally equivalent to the ϕX174-type primosome. iv) RNA polymerase binds to RPO site and synthesizes RNA transcripts of varied lengths at a specific site. All these *ssi* (G site, n' site, A site and RPO site) have potentials to form characteristic secondary structures and the presence of the predicted stem-loop structures for n' sites and A site has actually been shown. Precoating the *ssi* with single-stranded DNA binding protein (SSB) is essential for proper functioning of these proteins. Binding of SSB either creates or stabilize appropriate higher order structures of the *ssi* for recognition by the proteins. Striking conclusion from this study is that the proteins known to play central roles in DNA replication and transcription on duplex DNA templates can also recognize specific structures on single-stranded DNA templates for productive biological reactions. The predicted structures of the target sequences and the possible modes of their recognition by the respective binding proteins will be presented.

MZ 316 RNA DOMAINS IN MITOCHONDRIAL RIBOSOMES: DISPOSITION OF RNA IN THE SMALL SUBUNIT OF BOVINE MITOCHONDRIAL RIBOSOMES, Thomas W. O'Brien, Wesley H. Faunce and Christopher R. Cogle, Department of Biochemistry and Molecular Biology, University of Florida College of Medicine, Gainesville, FL 32610

Despite vast differences in their composition and physical properties, mammalian mitochondrial ribosomes and eubacterial ribosomes have similar ultrastructural features. While actually larger than E. coli 30S subunits, the small subunits of bovine mitochondrial ribosomes lack several stem-loop structural elements of the bacterial ribosome, containing instead several additional proteins. In order to understand the structure of mitoribosomal 12S rRNA in ribosomal subunits, to investigate differences between the small subunit RNAs of E. coli and mitochondrial ribosomes, and to provide a framework for localizing structural and functional domains in mitochondrial ribosomes, we probed the 12S rRNA in the 28S subunit with enzymes (RNases A, T1 and V1) and chemicals (dimethyl sulfate and 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-p-toluene sulfonate). Enzymatic probing of the 12S rRNA in 28S subunits has identified surface accessible nucleotides, including some in the central pseudoknot region and in conserved stem-loop structures implicated (E.coli) in the decoding site and in subunit association. Chemical probing of the RNA has identified bases in single stranded regions that are exposed to solvent. Chemically reactive bases in the 12S rRNA are clustered in discrete regions, and, for the most part, the analogous regions in 16S rRNA also contain bases that are chemically reactive in 30S subunits (Moazed, et al., J. Mol. Biol. (1986) 187:399-416), suggesting that these regions are similarly disposed in mitochondrial and bacterial ribosomal subunits. Some regions having conserved secondary structure in 12S and 16S rRNA have different chemical reactivities, probably reflecting a different environment imposed by the extra proteins in mitochondrial ribosomes. Supported by the USPHS, NIH Research Grant GM-15438.

MZ 317 CRYSTAL STRUCTURE OF THE BACTERIOPHAGE T4 GP32 DNA BINDING DOMAIN COMPLEXED TO ssDNA AS DETERMINED FROM MULTIWAVELENGTH ANOMALOUS DIFFRACTION (MAD). Yousif Shamoo, Alan M. Friedman, Mark R. Parsons, William H. Konigsberg and Thomas A. Steitz. Dept. of Molec. Biophys. and Biochem., Yale Univ. New Haven, CT 06520-8024. Gene 32 protein has served as a prototype for a class of proteins that bind preferentially to single-stranded nucleic acids in a non-sequence dependent fashion. Gp32 is an essential component of the T4 replisome *in vivo*, and is functionally analogous to *E. coli* SSB. We have cocrystallized the gp32 "core domain" (residues 17-254) with $p(dT)_6$, an oligonucleotide of about one site size in length as determined from flourescence quenching assays. After initial difficulties in obtaining suitable isomorphous heavy atom derivatives of the core gp32:ssDNA complex, selenomethionyl containing protein was produced to allow the use of MAD analysis for *de novo* phasing. Data was collected at the Cornell High Energy Synchrotron at 4 wavelengths and positions of the Se determined from difference fourier maps using phases from a poor lead derivative. The resulting set of MAD phases was combined with lead and selenium isomorphous replacement phases and electron density maps calculated. The resulting maps are at moderate resolution (3.1 A), but suitable for model building. We are currently in the process of tracing the polypeptide backbone of the protein. Given the extensive study of gp32:ssDNA interactions in the literature, a structure for the gp32:$p(dT)_6$ complex should finally provide a suitable context for the interpretation of this wealth of biochemical knowledge of single-stranded DNA binding proteins and their role in DNA replication.

MZ 318 INTERACTIONS OF CHLOROPLAST mRNA 3' UNTRANSLATED REGIONS WITH PROTEINS THAT PROMOTE RNA PROCESSING AND STABILITY, David B. Stern, Jianjun Yang, Rita Monde and Lynn Usack, Boyce Thompson Institute, Cornell University, Ithaca, NY 14853

In chloroplasts, the 3' untranslated regions of most mRNAs contain a stem/loop-forming inverted repeat (IR) sequence that is required for mRNA stability and correct 3' end formation. The IR regions of several mRNAs are also known to form stable complexes with chloroplast proteins *in vitro* (1). These RNA:protein interactions may be involved in the regulation of chloroplast mRNA maturation and in the modulation of RNA stabilities, which vary between RNAs and change during plant development. We have learned that for *petD* mRNA, the complex formed *in vitro* with wild-type RNA contains 55 Kd, 41 Kd and 29 Kd RNA-binding proteins (RBPs; 2). The 41 Kd RBP can bind RNA independently, and its binding site and also that of the 55 Kd RBP include a conserved AU-rich motif downstream of the IR. The 41 Kd RBP also requires most of the stem/loop in order to bind. The *petD* RNA:protein complex is reversibly destabilized by the sulfhydryl group inactivating agent diamide, suggesting a possible mode for regulation of its function *in vivo*. Furthermore, our evidence, based on the analysis of RNAs mutated in the conserved AU-rich motif, suggests that binding of the 41 Kd protein greatly enhances the correct processing and accumulation of mature RNA *in vitro* and in electroporated chloroplasts. Interestingly, *psbA* mRNA, which unlike that of *petD* does not accumulate in the dark, does not contain the sequence motifs recognized by 41 Kd RBP, and does not compete with *petD* RNA in binding reactions. We are currently attempting to reconstitute this RNA processing/decay system using the purified 41 Kd stem/loop binding protein and purified ribonuclease II from *E coli*, and to test the role of the AU-rich and stem/loop sequences *in vivo* using chloroplast transformation.

1. Chen and Stern (1991).*Molec. Cell. Biol* 11:4380-4388.
2. Chen *et al. Genes Develop.*, submitted.

MZ 319 DNA LOOPING BY THE HMG-BOX DOMAINS OF HMG1 AND MODULATION OF DNA BINDING BY THE ACIDIC C-TERMINAL DOMAIN, Michal Štros[1], Jitka Štokrová[2] and Jean O. Thomas[3], [1]Institute of Biophysics, Czech Academy of Sciences, 612 65 Brno, Czech Republic; [2]Institute of Molecular Genetics, Czech Academy of Sciences, Prague 166 37, Czech Republic; [3]Department of Biochemistry, University of Cambridge, Cambridge CB 1QW, England

We have compared HMG1 with the product of tryptic removal of its acidic C-terminal domain termed HMG3, which contains two "HMG-box" DNA-binding domains. (i) HMG3 has a higher affinity for DNA than HMG1. (ii) Both HMG1 and HMG3 supercoil circular DNA in the presence of topoisomerase I. Supercoiling by HMG3 is the same at ~50 mM and ~150 mM ionic strength, as is its affinity for DNA., whereas supercoiling by HMG1 is less at 150 mM than at 50 mM ionic strength although the affinity for DNA is unchanged, suggesting that the C-tail of HMG1 represses supercoiling at the higher ionic strength. (iii) Electron microscopy shows that HMG3 at a low protein:DNA input ratio (1:1 w/w; r=1), and HMG1 at a 6-fold higher ratio, cause looping of relaxed circular DNA at 150 mM ionic strength; with linear DNA only HMG3 causes looping. Oligomeric protein "beads" are apparent at the bases of the loops and at cross-overs of DNA duplexes. (iv) HMG3 at high input ratios (r=6), but not HMG1, causes DNA compaction without distortion of the B-form. The HMG-box domains of HMG1 are thus capable of manipulating DNA by looping, compaction and changes in DNA topology. The acidic C-tail in HMG1 down-regulates these effects by modulation of the DNA-binding properties.

MZ 320 CLONING A cDNA ENCODING A 127 kDa PROTEIN OF THE UV-DAMAGED DNA-BINDING COMPLEX, Masashi Takao, Vesna Rapic' Otrin, Marija Abramic', Malcolm Moos Jr.*, John C. Wooton+, Mary McLenigan, Arthur S. Levine and Miroslava Protic', National Institute of Child Health and Human Development, and +National Center for Biotechnology Infomation, National Institues of Health and *Laboratory of Developmental Biology, Division of Cellular and Gene Therapy, Food and Drug Administration, Bethesda, MD 20892

UV-light is a genotoxic factor for living organisms. Bacterial studies have demonstrated that subunits of the DNA excision repair complexes recognize and bind to UV-induced DNA lesions. In contrast, functionally similar eukaryotic DNA repair factors have not yet been fully characterized. A UV-damaged DNA-binding (UV-DDB) complex has been recently identified and purified from primate cells, and it is a likely candidate for a recognition factor involved in mammalian DNA repair[1,2]. The purified activity has a major polypeptide of ~127 kDa.The microsequence information on tryptic peptides of p127 was used to isolate a cDNA from monkey and human cDNA libraries[3]. The deduced sequences of monkey and human cDNAs consist of 1140 amino acid residues and are 100% identical. To confirm the presence of p127 in UV-DDB complexes, polyclonal antibodies were prepared against the recombinant protein overproduced in *E. coli*. On the Western blots of a band-shift gel, the p127 could be detected at the position of a UV-DDB complex, to which an unbound p127 does not migrate. The translated cDNA sequence does not show significant similarity to any known proteins in databanks nor recognizable protein motifs including a DNA-binding motif. However, database searches revealed striking similarity of p127 cDNA to sequences of uncharacterized proteins from *Dictyostelium discoideum* (44% identity over 529 aa) and *Oryza sativa* (54% identity over 74 aa). We also cloned a Drosophila UV-DDB homolog which shows ~60% identity to the primate p127. Therefore, the UV-DDB polypeptide is a member of a highly conserved family of proteins that may have an essential cellular function (e.g., UV-damage recognition).

[1]Hirschfeld et al., Mol. Cell. Biol. 10:2041, 1990; [2]Abramic' et al., J. Biol. Chem. 266:22493, 1991; [3]Takao et al., Nucl. Acids Res. 21:4111, 1993.

MZ 321 *TRANS*-ACTING FACTORS INVOLVED IN THE ALTERNATIVE TRANSLATION INITIATION PROCESS OF HUMAN BASIC FIBROBLAST GROWTH FACTOR mRNA. Stéphan Vagner[1], Francis Bayard[2], François Amalric[1] and Anne-Catherine Prats[1]. Laboratoire de Biologie Moléculaire des Eucaryotes, 118, route de Narbonne[1] and Laboratoire d'Endocrinologie Expérimentale, CHU Rangueil[2], Toulouse, France.

The basic fibroblast growth factor (bFGF) is a multifunctional cytokin able to stimulate cell proliferation, migration and differenciation, and which shows oncogenic features. Four forms of bFGF are synthesized from the same mRNA, using alternative translation initiation sites (1). Three CUG initiation codons are used for translation of the high molecular weight forms (22, 22.5 and 24 kDa, respectively) whereas an AUG codon allows translation initiation of the low molecular weight form of bFGF (18 kDa). It has been recently shown that the small AUG-initiated form localizes in the cell cytoplasm and is able to induce cell transformation, while the large CUG-initiated forms are localized in the cell nucleus and generate cell immortalization (2,3). These observations indicate that the process of alternative translation initiation can regulate the expression of proteins having not only different subcellular localizations but also distinct functions towards cell growth and differenciation.

We have investigated the roles of the 5' untranslated region and of the alternatively translated region (between the CUG and AUG codons) in the control of alternative translation, *in vitro* and *in vivo*. Site directed mutagenesis allowed us to identify in bFGF mRNA leader five *cis*-acting elements able to modulate alternative translation initiation (4) and one element that confer translation initiation by internal ribosome binding. These regulatory elements showed different behavior depending on the translation system used, suggesting the involvement of cell specific *trans*-acting factors. We used mobility-shift electrophoresis assay to show a specific RNA-protein complex between bFGF mRNA leader and components of different cellular extracts. Various polypeptides have been identified as part of the protein-RNA complex by use of an UV cross-linking assay. The identification and the roles of these factors in translation initiation are currently under investigation.

(1) Prats et al. (1989).*Proc. Natl. Acad. Sci. USA.* **86**: 1836-1840.
(2) Bugler et al. (1991).*Mol. Cell. Biol.* **11**: 573-577.
(3) Couderc et al. (1991).*Cell Regulation* **2**: 709-718.
(4) Prats et al. (1992).*Mol. Cell. Biol.*, **12**: 4796-4805.

MZ 322 DNA-PROTAMINE INTERACTIONS, Jean P. Wren and Pamela C. Stacks, Department of Chemistry, San Jose State University, San Jose, CA 95162

In mammals, there are essentially two types of protamine involved in spermatozoon DNA condensation: protamine P1 and protamine P2. This work is concerned with the kinetic aspects of the interaction of mammalian P1 (from bull) with short oligomers (10-14 base pairs) of DNA. The P1 is covalently attached to a Sepharose resin and the DNA is 5'-end labelled with [^{32}P] or [^{33}P]. A combination of centrifugation and filtration methods of analysis was used for determination of dissociation rate constants, k_2. The combination allowed independent quantitation of both free labelled DNA and the complexes. Two aspects of the interaction of DNA and protamine were investigated: determination of the dissociation rate constant, k_2, of a complex of P1 and a 12 base pair double stranded oligomer of DNA as a function of various physical and chemical parameters; and determination of the effect of different length oligomers on the kinetics of association and dissociation of the DNA-P1 complex.

The DNA-P1 complex is of high affinity. The extent of resin substitution affects the reversibility of DNA binding to P1. When 6300 nM P1, covalently attached to the resin, was incubated with a 12 base pair oligomer of [^{32}P]DNA (9.2-26.6 nM), the binding was only reversible if the NaCl concentration in the dissociation buffer was increased. Increasing the NaCl concentration in the dissociation buffer yielded a biphasic dissociation profile; the k_2 of both the slow and fast phases increased approximately linearly with increasing ionic strength. Thus, ionic interactions are important in the binding of DNA and P1. When 63 nM P1, covalently attached to the resin, was incubated with a 12 base pair oligomer of [^{32}P]DNA (9.26-26.6 nM), the observed k_2 was approximately 8×10^{-5} s^{-1}. With this system (63 nM P1), three different oligomers were used to evaluate the length of the DNA binding site. The 12 and 14 base pair oligomers showed similar rates of dissociation. In contrast, a significantly more rapid rate of dissociation was observed with the 10 base pair oligomer.

MZ 323 QUANTITATIVE ANALYSIS OF THE INTERACTION BETWEEN IHF AND DNA, Shu-Wei Yang, Andrew Granston, and Howard Nash, Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, MD 20892

Integration host factor of *E.coli* is a small, heterodimeric DNA binding protein involved in many DNA transactions such as recombination, replication, transcriptional regulation, and plasmid partition. A principal function of IHF is bending of specific target DNA sequences, thereby helping the formation of higher order DNA-protein complexes. An *E.coli* cell contains approximately 20,000 IHF molecules (Ditto and Weisberg, personal communication) and several hundred IHF binding sites in its chromosome.

By means of polyacrylamide gel retardation and competition assays, we have measured the binding affinity of IHF for several naturaly-occuring DNA sites and for various nonspecific nucleic acids. The apparent equilibrium constants for specific binding sites are 2-30 nM, as compared to 8,000-22,000 nM for non-specific binding sites. These data allow us to calculate the distribution of IHF within *E.coli*, a prediction we are testing by *in vivo* footprinting of a variety of IHF binding sites.

Using the same assays, we have also determined the change in free energy upon IHF binding to the H' site and derivatives in which an A:T base pair at a given position is replaced by either I:C or G:C base pair. At eight of nine positions tested, the penalty in binding free energy associated with replacement of a single A:T base pair by an I:C base pair is much smaller than that for replacement by a G:C base pair. The data provide an additional piece of evidence for the proposal (Yang and Nash, Cell, vol. 57, 869-880, 1989) that IHF interacts primarily with the minor groove of its DNA target.

Processive Interactions with DNA;
Processive Interactions with RNA (Joint)

MZ 400 TRANSCRIPT CLEAVAGE FACTORS GREA AND GREB UTILIZE INTRINSIC ACTIVITY OF RNA POLYMERASE, Sergei Borukhov[1], Marianna Orlova[1], Janet Newlands[2], Alex Goldfarb[1], Asis Das[2], [1]Public Health Research Institute, New York, NY 10016, [2]University of Connecticut Health Center, Fazrmington, CT 06030.

RNA polymerase isolated from a double *GreAGreB* mutant displays low-level transcript cleavage activity which cannot be due to contamination with GreA or GreB, or the presence of an unkllnown cleavage factor. Upon long incubation, the cleavage activity is stoichiometric to the numebr of ternary complexes. The rate of intrinsic cleavage is dramtically enhanced by the addition of the exogeneous factors. It is also demonstrated that GreA and GreB proteins bind to the elongation complex near the active center of RNA polymerase since they can be photocrosslinked to the nascent transcript via 3'-terminal 8-azido-ATP.

MZ 401 INTERACTIONS OF HIV-1 REVERSE TRANSCRIPTASE AND ITS "AZT-RESISTANT" MUTANTS WITH RNA AND DNA TEMPLATES AND AZT 5'-TRIPHOSPHATE. Sergei G. Sarafianos, Dmitry K.Pokholok, Vladimir O. Rechinsky and Sergei N. Kochetkov, V.A.Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia

The prolonged therapy of AIDS patients with 3'-azido-3'-deoxythymidine (AZT) was shown to result in the reduced HIV sensitivity to this drug. The analysis of virus isolates demonstrated multiple common mutations in the reverse transcriptase (RT) coding region conferring resistance to AZT.

To elucidate the mechanism of RT resistance to AZTppp 15 forms of HIV-1 RT bearing above-mentioned mutations have been obtained by means of site-directed mutagenesis and were used in the [illegible] experiments. The marked differences in interactions with RNA and DNA templates with identical nucleotide sequences were demonstrated both with w.t. and mutant RTs. For the "complete" mutant (containing four amino acid substitutions: D67•N, K70•R, T215•F and K219•Q) the affinity for RNA templates increased while for DNA templates it was close to that of the w.t. RT. However neither the mode nor the dNTP binding parameters have been affected by the mutations. AZTppp effectively terminated DNA chain elongation by "complete" mutant on RNA template. In the case of DNA template AZTppp did not terminate chain elongation but reflected the mixed inhibition mode. We suggest that the differences between RT complexes with RNA and DNA templates determine the incapability of mutant RT to utilize AZTppp in RNA directed (but not RNA-directed) DNA synthesis.

MZ 402 PROTEIN-DNA CONTACTS AND DNA STRUCTURAL TRANSITIONS DURING INITIATION OF ADENOVIRUS DNA REPLICATION, R.T. Hay, A. Freeman, I. Leith, A. Monaghan and A. Webster. School of Biological and Medical Sciences, University of St. Andrews, St. Andrews, Scotland.

Replication of adenovirus type 2 (Ad2) DNA represents an excellent system in which to study the basic mechanisms of viral DNA replication. The viral genome can be replicated *in vitro* by the action of three viral proteins (Ad pol, Ad pTP, Ad DBP) and two cellular transcription factors (NFI and NFIII), which we have expressed and purified. We have identified a number of protein-protein and protein-DNA interactions required for initiation of replication and developed a model for the assembly of a preinitiation complex at the origin of DNA replication. Our objective is to identify the protein surfaces and amino acid residues that participate in these interactions and to define the structural changes that take place in origin DNA during the initiation reaction.

To identify amino acids in the transcription factor NFI that make direct contacts with DNA photochemical crosslinking has been employed. Oligonucleotides containing the recognition site for NFI have been synthesised containing the highly photoactivatable analogues 4-thiothymidine and 6-thiodeoxyguanine. NFI bound to these substituted oligonucleotides has been crosslinked to the DNA by exposure to 340 nm light, trypsin digested and the peptide DNA adduct isolated and sequenced. A short region of the NFI protein that has the potential to form an amphipathic α-helix is crosslinked to DNA.

Once the preinitiation complex containing Ad pol, Ad pTP, Ad DBP and NFI has assembled on the DNA this nucleoprotein assembly must isomerise to expose single stranded DNA that can act as substrate for initiation of DNA synthesis. Bases within the origin that undergo these structural transitions have been modified by treatment of the nucleoprotein complexes with $KMnO_4$ or OsO_4 which both specifically oxidise thymidine bases that are not involved in Watson-Crick base pairs. Points of modification were identified by sequence determination of primer extension products. Both chemicals detected modified bases at two locations within the origin of DNA replication: at the terminus of the genome and within the highly conserved A/T rich core sequence. Structural changes within the origin could be detected in the presence of only Ad pTP and Ad pol but the extent of modification was increased by the addition of NFI or dCTP, the nucleotide utilised for initiation. The results suggest the structural changes within the core of the adenovirus origin of DNA replication leads to unwinding of the DNA duplex at the termini of the genome which creates the template for initiation.

MZ 403 MECHANISTIC STUDIES OF BACTERIOPHAGE T7 DNA HELICASE/PRIMASE PROTEIN.

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DNA helicases are ubiquitous proteins that unwind duplex DNAs into ss-DNAs, deriving energy from NTP hydrolysis. We are investigating the mechanism of DNA unwinding catalyzed by bacteriophage T7 helicase protein. Our studies have been carried out with a recombinant 4A' protein, an M64L mutant of 4A, whose biochemical properties are comparable to wild-type 4A and 4B proteins.

HPLC gel-filtration and chemical crosslinking studies have shown that both 4A' and 4B proteins form stable hexamers and dodecamers in the presence of MgNTP and ss-DNA. Analysis of 4A' by native PAGE indicate that the DNA binds predominantly to 4A' hexamer. In electron micrographs, 4A' hexamers appear ring-shaped with outer diameters between 100-125 A. Hence, if a ss-DNA wraps around the hexamer, 60-80 bases of ss-DNA would be protected. However, nuclease protection and quantitative equilibrium DNA binding studies indicate that each hexamer interacts with 25-30 bases of ss-DNA. Therefore, the DNA may not be wrapped completely around the hexamer, but may interact with one or two subunits of the hexamer. Protein-DNA interactions were studied by nitrocellulose binding experiments. The results indicate that 4A' binds DNA to completion only in the presence of MgdTMP-PCP. Also, 4A' has a higher affinity for ss-DNAs relative to ds-DNAs. Each hexamer binds two ss-DNAs, one with a high affinity and the other with a low affinity. Our studies suggest a rolling-type mechanism for translocation on DNA, which will be discussed.

MZ 404 A DNA HELICASE INDUCES TOPOISOMERASE II-ASSOCIATED DNA BREAKS

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The type II DNA topoisomerases are targets for a variety of chemotherapeutic agents, including the antibacterial quinolones and several families of antitumor drugs. These agents stabilize an enzyme-DNA cleavage complex, which consists of topoisomerase covalently linked to the 5' phosphates of a double-stranded DNA break. Although the drug-stabilized cleavage complex is readily reversible, it can result in cell death by a mechanism which remains uncertain. Using a cleavage reversal assay we have demonstrated that the action of DNA helicase II from *E. coli* can convert an *m*-AMSA induced T4 topoisomerase cleavage complex into a nonreversible DNA break. Helicase II disrupts the cleavage complex in a reaction that requires ATP hydrolysis and is independent of a protein denaturation step (e.g. SDS treatment). Formation of a nonreversible DNA break, induced by a DNA helicase, could explain the cytotoxicity of these topoisomerase poisons.

MZ 405 THE GEOMETRY OF DNA BINDING TO THE KLENOW FRAGMENT OF *E. COLI* DNA POLYMERASE I.

Catherine M. Joyce, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520.

The Klenow fragment of DNA polymerase I has two enzymatic activities, DNA polymerase and proofreading exonuclease, located on separate structural domains. The polymerase domain contains a large cleft at the base of which are located several amino acid side chains which play a role in catalysis of the polymerase reaction. The dimensions of the cleft indicated a likely role in DNA binding and early attempts at model building suggested that the duplex DNA upstream of the primer terminus could be accommodated in this cleft (1). More recent structural data for a Klenow fragment-DNA complex having the primer terminus at the exonuclease site suggests a different mode of binding. The duplex DNA upstream of the primer terminus is bound in a cleft formed between the polymerase and exonuclease domains as a result of movement of the so-called "thumb" subdomain of the polymerase region (2). If applicable to binding of a DNA substrate at the polymerase active site, this model suggests that the DNA approaches the active site from a direction opposite to that which was previously supposed. Although no structural data that address this question are available for Klenow fragment, the proposed geometry of DNA binding at the polymerase site agrees well with the crystallographic data for HIV-1 reverse transcriptase (3,4).

Comparative footprinting studies on whole Klenow fragment, the isolated polymerase domain, and a derivative having a deletion of part of the "thumb" subdomain are consistent with the more recent model. These and other experiments designed to constrain models for DNA binding will be presented.

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4. Jacobo-Molina, A. *et al.* (1993) *Proc. Natl. Acad. Sci. USA* **90,** 6320-24.

MZ 406 MOLECULAR DISSECTION OF THE RECBCD ENZYME FROM *ESCHERICHIA COLI.*

Douglas A. Julin, Mihaela Chamberlin, Dwight Randle, and Firouzeh Korangy, Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742.

In order to study the mechanism of ATP-dependent DNA unwinding and degradation catalyzed by the RecBCD enzyme from *E. coli*, we have sought to simplify these complex reactions by using small DNA oligomer substrates in the nuclease and ATP hydrolysis reactions. We have dissected the enzyme by using the isolated RecB and RecC subunits and the reconstituted RecBC enzyme, as well as the RecBCD holoenzyme.

The RecB, RecBC, and RecBCD enzymes hydrolyze ATP in the presence of oligo(dT) ranging in size from 4 to 16 nucleotides (nt), with those of 8-16 nt about equally effective. The dependence of the RecBCD-catalyzed ATPase rate on the oligomer concentration is as expected for a single DNA-dependent ATPase active site, which was unexpected since other evidence indicates that both RecB and RecD are ATPases, at least during DNA unwinding. The K_m's for oligo$(dT)_{12}$ are quite different for each enzyme: (K_m > 200 μM oligomer for RecB; K_m = 19 and 0.13 μM for RecBC and RecBCD, respectively). The additional subunits (RecC and RecD) apparently increase the affinity of RecB for the oligomer. The k_{cat} values for ATP hydrolysis with oligo$(dT)_{12}$ are 25,000 and 35,000 min^{-1} for RecBC and RecBCD, respectively, while the RecB-catalyzed reaction is about 10-fold slower than that of RecBC, at 100 μM oligo$(dT)_{12}$ and 250 μM ATP.

The RecBCD enzyme cleaves a 5'-end-labeled 15-mer in the absence of ATP. ATP (0.5 - 2 mM) stimulates the reaction by about 1000-fold. Neither the isolated RecB nor the RecC subunit has nuclease activity by itself in this assay, but the reconstituted RecBC enzyme has nuclease activity in the absence of ATP. The rate is about 5 times slower than that of the RecBCD enzyme, also without ATP. Unlike the RecBCD enzyme, this nuclease reaction is not significantly stimulated by ATP, although the 15-mer does stimulate ATP hydrolysis by RecBC.

MZ 407 COUPLING BETWEEN PRODUCT EXTRUSION AND TRANSLOCATION DURING ELONGATION.

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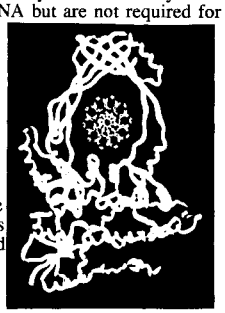
The relationship between parts of RNA polymerase during its inchworm-like propagation along DNA template was studied by footprinting of defined elongation complexes, using exonuclease III and transcript cleavage factor GreB as probes for DNA and RNA, respectively. Strict correlation was found between the leap-like translocation of the enzyme "front end" along DNA and the shielding of the nascent RNA from cleavage in the "tight product binding site". The front end of the enzyme and the front edge of RNA shielding are always separated by a constant distance of 18 nucleotides and are translocated synchronously. However, the length of each translocation leap is variable ranging from 1 to 12 nucleotides. The leaping pattern is determined by the nucleotide sequence reflecting the existence of specific "leap sites" in DNA. The unshielded 3'-proximal segment of RNA that is synthesized between the leaps varies in length and is equal to the span of the next leap; when the leap occurs this segment becomes shielded. The results suggest a model of elongation in which incremental RNA chain extension occurs internally in the non-moving complex and is followed by the extrusion of the nascent 3'-proximal segment though the product channel with concomitant leaping of the enzyme along DNA.

MZ 408 THE THREE DIMENSIONAL STRUCTURE OF THE 67-KDA AMINO-TERMINAL FRAGMENT OF *ESCHERICHIA COLI* TOPOISOMERASE I, Christopher D. Lima*, James C. Wang^ and Alfonso Mondragon*, ^Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138; *Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208

DNA topoisomerases are ubiquitous enzymes that alter the topology of DNA by transiently breaking one or two strands of DNA, passing either a single strand or duplex DNA through the break, and finally resealing the break. These enzymes are involved in nearly all cellular processes involving DNA including replication, transcription, and recombination. *E. coli* DNA topo I is a member of a subfamily of type I DNA topoisomerases including *E. coli* DNA topo III, *S. cerevisiae* topo III, and *S. acidocaldarius* "reverse gyrase". *E. coli* DNA topo I catalyzes the relaxation of negatively supercoiled DNA, knotting and unknotting of single stranded DNA rings, catenation and knotting of double-stranded DNA provided that a gap or nick is present in one of the rings, and linking of complementary single-stranded DNA rings into a double stranded ring. Intact *E. coli* DNA topo I is a 97 kDa metalloprotein containing three zinc (II) atoms in three carboxy-terminal tetracysteine motifs which are required for relaxing DNA but are not required for cleaving single-stranded DNA. We have overexpressed, purified, and crystallized a 67-kDa amino-terminal fragment of the *E. coli* enzyme that can cleave single-stranded DNA in a fashion similar to the intact and zinc-depleted protein. We have solved the crystal structure of the 67-kDa fragment to 2.2Å resolution. The structure shows a novel fold which includes a toroidal cavity large enough to accommodate B form duplex DNA. The structure includes an alpha/beta domain which may be involved in binding single-stranded DNA during catalysis. The active site has been located in the main body of the protein. A large conformational change must occur to allow DNA in and out of the large toroidal cavity as well as to allow DNA access to the active site residues. The structure of this fragment suggests *E. coli* DNA topoisomerase I mediates reactions with DNA through a strand passage mechanism.



MZ 409 ANALYSES OF THE PROCESSIVITY OF ENDONUCLEASE V OR UV-IRRADIATED PLASMID DNAs. R. Stephen Lloyd§, Mary Lou Augustine§, David Mitchell¥, Donald L. Robberson§, (§) Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, Texas 77555-0852, (¥) U.T. M.D. Anderson Science Park, Smithville, Texas 78957

T4 endonuclease V is a cyclobutane pyrimidine dimer-specific DNA glycosylase-AP lyase that has been previously demonstrated to locate its DNA substrate by a one-dimension diffusion along double-stranded DNA. Analyses of *in vitro* reactions of pure endonuclease V with UV-irradiated plasmid DNAs demonstrated a processive nicking activity that was sensitive to the salt concentration of the reaction, such that all pyrimidine dimers were incised in a subset of DNA molecules, prior to any incisions being made in other DNA molecules. The biological importance of this DNA scanning activity has been demonstrated by creating mutants of endonuclease V that retain full catalytic activity but have lost the scanning function. Cells expressing these mutant endonuclease V proteins failed to enhance the survival of UV-irradiated repair-deficient *E. coli*.

In order to visualize the frequency and distribution of pyrimidine dimers in plasmid DNAs, UV-irradiated plasmids were reacted with endonuclease V under conditions that would favor either a processive mode of incision or a random introduction of breaks within the population of DNAs. These DNAs were reacted with antibodies directed against cyclobutane pyrimidine dimers and the products visualized by electron microscopy.

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MZ 410 RANDOM AND TARGETED MUTAGENESIS OF THE GENE FOR T7 RNA POLYMERASE. Dmitry L.Lyakhov, Dmitry A.Kostyuk, Vera L.Tunitskaya, Sergey N.Kochetkov, and Vladimir O.Rechinsky V.A.Engelhardt Institute of Molecular Biology, Russ. Acad. Sci., Moscow, Russia

Random mutagenesis of the bacteriophage T7 gene 1 was used to delineate the functional amino acid residues of its product, RNA polymerase, using the genetic system for the phenotypic selection of inactive mutants. The sites of nine mutations determined were localyzed in two short regions (555-575 and 626-636) that are highly conservative in sequences of monomeric RNA polymerases. It was shown that substitutions P563→T (A), Y571→S, T636→P, Y639→D, and F646→C resulted in the enzyme inactivation, of the enzyme. Oligonucleotide-directed mutagenesis technique was applied to further investigate the functional role of the residues revealed by both affinity modification and random mutagenesis methods. A sizable set of T7 RNA polymerase point mutants was obtained as a result.

Mutant proteins were purified and characterized in respect of alterations in enzymatic properties resulting from the particular residues substitutions. It was shown that mutations at different positions have dissimilar effect on T7 RNA polymerase functioning. So, mutations within a region that spans amino acids 625 to 650 are expressed by a marked decrease in the catalytic activity, whereas the promoter binding of the enzyme is little affected. Recent data suggest that this region is a part of the elongating NTP binding site. By contrast, the Y571S mutant was shown to retain catalytic competence, but to lose the promoter binding capacity. Mutations of the Lys-172 residue do not affect significantly the capacity of T7 RNA polymerase for promoter-dependent transcription, whereas the specificity of the enzyme suffers minor distortion as far as its interaction with the template is concerned.

MZ 411 INTERACTIONS OF THE DNA HELICASE OF BACTERIOPHAGE T7 WITH DNA, Stephen M. Notarnicola and Charles C. Richardson, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, 02115.

Gene 4 of bacteriophage T7 is expressed as colinear proteins of 56- and 63–kDa that provide the helicase and primase activities, respectively, for T7 DNA replication. The 63–kDa gene 4 protein (primase) catalyzes both helicase and primase activities; whereas, the 56–kDa gene 4 protein (helicase), lacking the N-terminal 63 amino acids of the larger protein, catalyzes only helicase activity. The translocational and DNA unwinding activities of the gene 4 proteins require a hydrolyzable nucleotide substrate. A mutant gene 4 protein having an altered nucleotide binding site cannot hydrolyze NTP or bind ssDNA. In addition, the mutant gene 4 protein inhibits nucleotide hydrolysis catalyzed by the wild-type gene 4 proteins. These findings indicate that nucleotide hydrolysis, and thus translocation and DNA unwinding by the gene 4 proteins, is both cooperative and coordinated among the subunits of the complex. To further investigate the mechanism of unwinding and translocation by the gene 4 helicase we have used a DNA effector consisting of a small oligonucleotide annealed to the complementary sequence of the 3′-end of a larger, 75 base oligonucleotide. The rate of dTTP hydrolysis by gene 4 protein on this DNA molecule is significantly lower than the rate of hydrolysis on the 75mer alone; suggesting that the movement of the gene 4 protein is blocked when it encounters the duplex region. An oligonucleotide annealed to the 5′-end of the 75mer had no effect on the rate of hydrolysis. Challenge experiments indicate that the protein stalls at the block and then slowly dissociates. As expected from previous DNA unwinding experiments, the gene 4 helicase is not able to displace the oligonucleotide annealed to the 75mer. Interestingly, under the same reaction conditions an oligonucleotide block with 8 unpaired bases at its 3′-end also lowers the rate of nucleotide hydrolysis, however there is no displacement of this oligonucleotide until the helicase concentration is increased 4 to 8 fold. These results agree with the previously established direction of translocation and DNA unwinding conditions for the gene 4 proteins and indicate that there may be differences between translocating and actively unwinding gene 4 protein complexes.

MZ 412DNA Substrate Specificity of T4 dda Helicase. Kevin D. Raney and Stephen J. Benkovic. The Pennsylvania State University, Department of Chemistry, University Park, PA 16802.

DNA substrate specificity was investigated for the T4 dda helicase. Several substrates were utilized: 1) a DNA fork containing 5' and 3' single strand tails of 30 nucleotides and 30 base pairs, 2) a 5' tailed substrate containing a 30 nucleotide tail and 30 base pairs 3) a 3' tailed substrate containing a 30 nucleotide tail and 30 base pairs, 4) a blunt end duplex containing 30 base pairs, and 5) a 60-mer single strand oligonucleotide. Fluorescence quenching was used to study binding of the helicase to these substrates. The DNA fork substrate bound the helicase with equal affinity to that of the 5' tailed substrate as well as the 60-mer single strand substrate based on single strand DNA concentration. The 30-mer blunt end double strand substrate bound with ~10-fold lower affinity than the single strand substrate, even in the presence of AMP-PCP. Nuclease protection experiments utilizing the DNA fork substrate indicated that the 5' and 3' ss DNA regions were equally well protected upon binding of the helicase. No region along either ss DNA tail of the fork was preferentially protected, including the regions near the fork. These binding experiments illustrate that the dda helicase does not bind specifically to the DNA fork region, or to the duplex DNA region under a variety of conditions. DNA melting assays were performed with the helicase using the different substrates. Both the 5' tailed substrate and the DNA fork substrate were efficiently melted by dda helicase, while neither the 30 nucleotide blunt end substrate nor the 3' tailed substrate were melted. These results indicate that T4 dda helicase requires only a 5' ss DNA tail in its ATP dependent DNA melting reaction.

MZ 413 RESOLUTION OF HOLLIDAY JUNCTIONS BY RUVC PROTEIN: ACTIVATION OF RUVC *IN VITRO*.
Rajvee Shah, Richard J Bennett, Steve C West, Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts, UK.
The *E. coli* RuvC protein resolves Holliday junctions during genetic recombination. Resolution is structure-specific and occurs in a sequence-dependent manner by introducing nicks at the 3'-side of thymine residues. *In vitro*, resolution requires surprisingly large amounts of RuvC protein, however the reaction can be markedly stimulated by alteration of reaction conditions. For example, RuvC activity is stimulated 10-fold when Mg^{2+} is replaced by Mn^{2+}. In contrast, Ca^{2+}, Co^{2+}, Cu^{2+} and Zn^{2+} are unable to support cleavage. Activation by Mn^{2+} is optimal at physiological pH whereas the Mg^{2+}-dependent reaction is optimal at pH10. We also observe that RuvC activity is greatly stimulated by increasing the reaction temperature from 37°C to 55°C, and even 65°C. We have therefore investigated the effects of Mn^{2+}, alkaline pH and high temperature on *junction* stability. All three factors significantly lower the melting temperature of the junction. Since RuvC protein cleaves at specific sequences and can be activated by factors which destabilize the junction (possibly through an increase in spontaneous branch migration) we propose that the RuvC-Holliday junction complex needs to translocate along DNA until appropriate resolution sequences are encountered. The purified RuvA and RuvB proteins of *E. coli* have been shown to catalyse branch migration of Holliday junctions *in vitro*. We are therefore investigating the possibility that efficient resolution by RuvC is dependent on RuvAB-mediated branch migration .

MZ 414 DNA UNLINKING BY A THERMOSTABLE NICKING-CLOSING ENZYME, Alexei Slesarev†, Alexander Pushkin†, James Lake†, Martin Gellert*, Regis Krah* and Sergei Kozyavkin*, †Molecular Biology Institute, University of California, Los Angeles, CA 90024; *Laboratory of Molecular Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892.

DNA topoisomerase V is a novel prokaryotic enzyme related to eukaryotic topoisomerase I. It was found in *Methanopyrus kandleri*, a hyperthermophile which grows up to 113°C and contains 1.1 M tripotassium 2,3-cyclopyrophosphoglycerate. The enzyme remains active at 120°C and at optimal conditions can almost completely unlink complementary strands. The driving force for this reaction is DNA melting. The maximal activity is at 1.5 M K-glutamate and the effects of different ions follow the Hofmeister series. The selectivity of topoisomerase V interactions with DNA is greatly affected by the ionic conditions. At low ionic strength it binds double and single stranded DNA irreversibly. In concentrated K-glutamate the enzyme reversibly binds duplex DNA and does not interact with single stranded DNA . At moderate concentrations of Cl^- anion it loses selectivity and binds double and single stranded DNA equally well. The selective affinity to duplex DNA can be restored by the addition of osmoprotectants.

MZ 415 SMALL BASIC PROTEINS ARE ASSOCIATED WITH KDNA, C. Xu, J. C. Hines and D. S. Ray, Molecular Biology Institute and Department of Biology, University of California, Los Angeles, CA 90024
In eukaryotes, nuclear DNA is organized into nucleosomes and supra-nucleosomal structures mainly by histones. In striking contrast, the *in vivo* structure and organization of DNA-protein complexes in mitochondria are not well understood. Electron microscopic studies indicate that mitochondrial DNA of trypanosomes (kDNA) exist in the kinetoplast as a highly organized disc-like structure in which the DNA appears to be aligned as a regular stack of fibers. In contrast, kDNA networks purified and spread for electron microscopy are at least 10-fold larger in diameter (similar to that of the cell). In order to isolate protein components of condensed kDNA *in vivo*, we have crosslinked trypanosomatid *C. fasciculata* cells with formaldehyde. Crosslinked kDNA-protein networks purified by differential sedimentation retain a condensed structure similar to that observed in cells. Treatment with proteinase K causes the networks to expand to a size close to that of whole cells and suggests that the associated proteins may play a role in maintaining the condensed structure of the kDNA *in vivo*. Crosslinked proteins are released from the networks by reversal of the crosslinks by heating. Amino-terminal sequences were determined for five small proteins (p15, p16, p17, p18 and p21) released from crosslinked networks and the complete gene sequences encoding p16, p17 and p18 have been obtained. All three proteins contain very similar cleavable presequences nine amino acids in length. Calculated isoelectric points for the three proteins are 10.5, 11.1 and 11.4 respectively. The predicted amino acid sequences of p17 and p18 have 57% homology and 43% identity. The N-terminal half of p16 also shows significant homology to p17 and p18, suggesting that these proteins may represent members of a novel family of mitochondrial DNA binding proteins. Results on interaction of these proteins with DNA will be presented.

Tracking Mechanisms with DNA; RNA Editing

MZ 500 THE recA GENE FROM *THERMUS AQUATICUS*: CLONING, EXPRESSION AND PURIFICATION, Evelina Angov and R.D. Camerini-Otero, Genetics and Biochemistry Branch, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892

The *E. coli* RecA protein plays an essential role in genetic recombination via a multi-step pathway involving the formation of a nucleoprotein filament on ssDNA, DNA pairing and subsequent strand exchange forming a heteroduplex DNA. A large number of bacterial RecA proteins have been described, from closely related enteric bacteria to more distant genera. The high degree of similarity among these proteins suggests that structurally the RecA protein has been significantly conserved among eubacteria. We isolated a RecA analog from the thermophilic eubacteria *T. aquaticus* YT-1 which exhibits optimal growth at 70 °C. This is of particular interest because homologous recombination involves nucleic acid hybridization and exchange reactions, such as triplex DNA formation and branch migration, all of which show significant dependence on temperature. In the present study, we have cloned the recA gene from *T. aquaticus*, and overproduced the thermostable protein in *E. coli*. Analysis of the deduced amino acid sequence indicates that *T. aquaticus* RecA is highly conserved relative to the *E. coli* protein. *T. aquaticus* RecA has been purified and preliminary biochemical data indicate that the protein binds to ssDNA in an ATP-dependent manner and promotes pairing of homologous DNAs to form stable joint molecules at 65 °C.

MZ 501 USE OF LYSOZYME PREPARATION FROM STREPTOMYCES RECIFENSIS SUBSP.LYTICUS 2435 FOR ISOLATION OF DNA FROM STAPHYLOCOCCAL CELLS, Labenko Yu.O.,Oryabinskaya L.B.,Department of Microbiology,State University,Dniepropetrovsk, Ukraine, 320625

It was shown that the preparation G-10X from Streptomyces recifensis subsp.lyticus 2435, including a complex of bacteriolytic and concomitant enzymes provided lysis of thick staphylococcal suspensions within 15 to 20 minutes under optimal conditions after preliminary treatment of the cells with 0.1 M cysteine. A procedure was developed for isolating DNA from the cells of staphylococci and other microorganisms based on enzymatic lysis. In terms of major physicochemical properties,the preparations of DNA were not inferior to the preparations of DNA isolated by the classical Marmur technique with Kirbi's deproteinization and had transforming activity.The developed procedure for isolation of DNA with using the lysoenzyme preparation widened the possibilities of investigating the genetics of staphylococci and other microorganisms.

MZ 502 *Saccharomyces cerevisiae* RNase H1 HAS TWO DISTINCT DOMAINS, ONE WITH RNase H ACTIVITY AND ANOTHER THAT BINDS DOUBLE-STRANDED RNA, Susana M. Cerritelli and Robert J. Crouch, Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892

E. coli RNase HI, which has only 155 amino acids, specifically recognizes and cleaves RNA-DNA hybrids. Multiple ribonucleases H have been described in eukaryotic cells that appear to be larger and more complex than the bacterial enzymes. The larger size RNases H may contain in addition to the RNase H region, a distinct domain involved in the regulation of the protein activity, or in the interaction with other cellular components. *Saccharomyces cerevisiae* RNase H1 is being studied in our laboratory as one example from the eukaryotic class. In addition to a carboxyl-terminal region that possesses RNase H activity and exhibit similarity to other RNases H, the protein contains an amino-terminal domain that belongs to a completely different family of proteins: those that bind to double-stranded RNA. The dsRNA binding region of RNase H1 contains two copies of a motif that shares some similarities to other dsRNA binding proteins.

In vitro binding experiments indicated that yeast RNase H1 interacts with dsRNA forming high affinity complexes. It also binds to other nucleic acids with lower affinity. Two copies of the conserved motif are required for interaction with dsRNA. A proteolytic product of 30 kDa, in which the first 80 amino acids that includes one copy of the repeat unit have been deleted, loses the ability to bind tightly to nucleic acids. A mutation that selectively eliminates the first repeat sequence, or a change of two conserved amino acids within the first motif, entirely abolishes tight binding to dsRNA. Furthermore, the expression of the RNase H1 amino-terminal region containing the two repeat units, without the RNase H domain, results in a protein that interacts strongly with nucleic acids. However, this N-terminal peptide possesses a different cation requirement than the full length RNase H1, indicating that the C-terminal domain affects the binding properties of the N-terminal region. The interaction between the two domains, and its significance on the RNase H activity of the protein are presently under investigation.

MZ 503 TRANSPORT DNA OF STAPHYLOCOCCI AND THE ROLE OF MEMBRANE PROTEINS,Gavrilyuck V.G., Vinnikov A.I.,Department of Microbiology,State University,Dniepropetrovsk,Ukraine,320625

The sensitiveness of plasmid DNA conjugative transfer of staphylococci for an effect energetic metabolism uncouplers testifies the direct participation of transmembrane electrochemical potential in realization of this process.For definition of separate proton potential components role ionophore anthibiotics (valinomycin and nigericin),electoraly supressing the generation of membrane potential ($\Delta\Psi$) and gradient of protons ($-z\Delta pH$),were used.The conjugation in the medium with indifferent pH meanings was carried out and showed,that $\Delta\Psi$ and $-z\Delta pH$ are mutualy substitutive and equivalent in this process securing.At pH 6,0 meaning the valinomycin effect on the membrane potential leads to an inhibition of the formation transconjugants process,at pH 7,5 meaning $\Delta\Psi$ fall is compensated by an equivalent increase $-z\Delta pH$ that is why the conjugative DNA transfer is feebly suppressed. Nigericin treatment of cells in the incubate medium at pH 6,0 causes a decrease of the conjugation frequency.On the contrary,at pH 7,5 nigericin effect on conjugation is relaxing.The study of ATP intracellular concentration role in the DNA transport showed,that the presence of ATP in conjugative cells is necessary for conjugation initial stages only,while plasmid transfer takes place independently from ATP concentration.Thus,protonmotive force of conjugative DNA transport is a source of energy,and the ATP part consists in regulator function.Equivalent participation of the both components of protonmotive force in the conjugative process assumes the presence of chemiosmotic molecular organization,determinanting a specific interaction of DNA and membrane proteins necessary for DNA transport in symport with protons.

MZ 504 FUNCTIONAL CHARACTERIZATION OF RANDOMLY MUTAGENIZED VARIANTS OF VSR-MISMATCH ENDONUKLEASE

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The Vsr gene product is a DNA mismatch endonuclease that recognizes T/G mismatches in a special sequence context and initiates their strand-specific repair by setting an endonucleolytic cut on the 5'-side of the mismatched thymine residue (1). Action of the enzyme had a profound influence on the structure of the *E. coli* K-12 genome in that it catalyses events of mutation avoidance, active mutagenesis and recombination (2, 3). In order to obtain further structural and functional information about the endonuclease we mutagenized the *vsr* gene randomly by error-prone PCR. Individual clones were screened for loss of endonuclease activity by an assay that allows to investigate in parallel large numbers of samples of crude cell extracts (4). We identified 3 different single amino acid substitutions that resulted in loss of endonuclease activity with unimpaired production of a stable protein. These variants of the Vsr endonuclease are currently under further investigation.

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MZ 505 STRUCTURAL HETEROGENEITY OF TYPE I DNA TOPOISOMERASES, Sergei A. Kozyavkin*, Regis Krah*, Martin Gellert*, James A. Lake° and Alexei I. Slesarev°, *Laboratory of Molecular Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892; °Molecular Biology Institute, UCLA, Los Angeles, CA 90024

Type I topoisomerases are divided into two non-related groups, whose major difference is in the way they interact with the DNA substrate. Group A enzymes preferentially bind to single stranded regions of DNA and make a transient covalent complex with the 5' end of the broken strand. Group B enzymes act on duplex DNA and bind to the 3' end of the broken strand. Representatives of both groups have been found in pro- and eukaryotes. So far only monomeric forms of these enzymes are known. From a hyperthermophilic methanogen we purified a heterodimeric reverse gyrase, a type I-group A topoisomerase that positively supercoils DNA at the expence of ATP or dATP hydrolysis. In analogy with the DNA gyrase A subunit (DNA breakage and reunion activity) *and the* B subunit (ATPase), the 50- and 150-kDa components of *Methanopyrus* reverse gyrase have been designated the A and B subunits, respectively. The A subunit covalently binds to the 5' DNA phosphoryl group and nicks DNA at sites that predominantly have a cytosine at the -4 position. The B subunit binds ATP in South-Western blots. The ATPase or dATPase activity of the enzyme is DNA-dependent.

MZ 506 THE T4 DNA POLYMERASE ACCESSORY PROTEIN, GP45, IS A DNA TRACKING PROTEIN AND A COMPONENT OF THE T4 LATE PROMOTER COMPLEX, Rachel L. Tinker, George A. Kassavetis, E. P. Geiduschek, Department of Biology, UCSD, San Diego, CA 92093-0634

Expression of bacteriophage T4 late genes *in vivo* depends on DNA replication. In an *in vitro* transcription system reconstituting the connection between DNA replication proteins and T4 late gene expression, late promoter activation depends on: (i) two RNA polymerase-binding proteins, gp55 (the T4-encoded sigma factor) and gp33 (the coactivator protein); (ii) the three DNA polymerase accessory proteins, gp44/62 and gp45; (iii) ATP hydrolysis; and (iv) a cis-acting DNA site (the enhancer) that can be located at a great distance from, and either upstream or downstream of, the T4 late promoter. Three properties set the enhancer of T4 late transcription apart from other enhancers: (i) It is not a sequence, but an especially simple structure: a nick, or a single-stranded gap, serves as an enhancer. (ii) The enhancer and promoter must be connected by a continuous and clear path along DNA (i.e. activation involves a DNA tracking mechanism). (iii) Transcriptional enhancement is constrained with regard to polarity, in that the DNA nick must be located in the non-transcribed strand of the target transcription unit. The structure and composition of an enhanced transcription complex at a T4 late promoter has been analyzed by DNase I footprinting and site-specific photocrosslinking. We show that gp45 and its coactivator, gp33, are located in close proximity to each other upstream of the enhanced promoter complex and present evidence that gp44/62 complex serves as the accessory assembly factor of gp45. The mechanism of transcriptional activation and the polarity property of the enhancer of T4 late transcription can now be given a molecular interpretation. We postulate that: (i) the nick serves as the binding site for the assembly factors, gp44/62, and as the entry site for the transcription-enhancing protein, gp45. When gp45 is loaded onto *DNA*, its orientation is determined by the orientation of the gp44/62 complex at the nick. ATP hydrolysis releases gp45 from the gp44/62-DNA complex for tracking along DNA. The orientation of gp45 is compatible with transcriptional activation of only one orientation of RNA polymerase. By interacting with DNA and the upstream end of the RNA polymerase, perhaps directly through interaction with gp33, gp45 leashes RNA polymerase to the template, leading to enhanced promoter opening.

MZ 507 A MODEL DESCRIBING THE SEARCH FOR HOMOLOGY CARRIED OUT BY RECA PROTEIN, Janet E. Yancey-Wrona and R. Daniel Camerini-Otero, Genetics and Biochemistry Branch, NIDDK, NIH, Bethesda, MD 20892

RecA protein from *E. coli* is able to pair ssDNA oligonucleotides with homologous regions on duplex DNA to form synaptic complexes. These novel structures, consisting of three DNA strands and RecA protein, are formed in the absence of ATP hydrolysis and likely represent intermediates in the strand exchange reaction catalyzed by RecA protein. Synaptic complex formation is composed of multiple steps including presynaptic filament formation, the search for homology and final complex formation. Characterization of this reaction should help elucidate the mechanism of the homology search and final pairing reaction carried out by the RecA protein-ssDNA filament. The kinetics of synaptic complex formation were investigated using a variety of DNA substrates in order to determine the order of the reaction and to examine the effect of DNA complexity on complex formation. Synaptic complex formation follows apparent second-order reaction kinetics with an apparent rate constant on the order of $10^4\ M^{-1}s^{-1}$. Furthermore, the presence of excessive amounts of heterologous dsDNA exerts no effect on the rate of complex formation. In other words, the search for homology carried out by the RecA protein-ssDNA filament is not rate-limiting. A kinetic scheme is proposed to describe these observations in which the second-order rate constant is the product of the equilibrium constant for the fast reaction, including the search for homology, times the rate constant for the slow reaction, involving final complex formation. This scheme provides a model to define the kinetic parameters governing each step of the reaction. Experiments are underway to examine the effects of DNA structure and protein-DNA complexes on the rate and extent of synaptic complex formation.

Late Abstracts

STUDYING THE DIMERIZATION DOMAIN OF AN RNA-BINDING PROTEIN REQUIRED FOR TRANSCRIPTIONAL ANTITERMINATION, Amarel Boss, Andrew Wright* and Orna Amster-Choder, Hebrew Univ. Medical School, Jerusalem 91010 ISRAEL & *Tufts Medical School, Boston, MA 02111.

Expression of the *bgl* operon in *E. coli* is regulated by a mechanism involving antitermination of transcription at two termination sites. The ability of BglG to inhibit termination of transcription was shown to be modulated by BglF, a membrane bound BglG-kinase-phosphatase, that senses the presence of β-glucosides (1). BglG was shown to be an RNA binding protein that prevents transcription termination by blocking the formation of the terminator structure (2). It was further shown that BglG exists in two forms: a dimeric non-phosphorylated form that can bind RNA and is active as an antiterminator, and a monomeric phosphorylated form that cannot bind RNA and is inactive (3). Using the DNA binding domain of the bacteriophage λ repressor as a reporter for dimerization, we have now shown that the dimerization domain is located at the carboxy terminus half of the protein. The protein sequence contains a putative perfect leucine zipper domain. Based on the sequence of the four heptads of amino acids, an α-helix is predicted to exist so that the two monomers can form a coiled-coil. Unlike leucine zippers in eukaryotic DNA-binding proteins, this motif in BglG is not preceded by a basic region. Rather, another motif, suggested to be involved in RNA binding, resides close to it.

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SUGAR SUBSTITUTED DNA: EFFECTS ON DNA DUPLEX AND DNA/RNA HYBRID STABILITY, AND DNA & RNA POLYMERASE BINDING AND ACTIVITY, Thomas Mikita* & G. Peter Beardsley#, Department of Molecular Biophysics and Biochemistry*, and Pharmacology# and Pediatrics#, Yale University School of Medicine, New Haven, Ct 06510

As part of an ongoing investigation into the range of structural and conformational plasticity tolerated in the DNA backbone by DNA and RNA polymerase, we carried out site specific sugar substitutions to compare deoxyribose with ribose and arabinose. Our interest here being the C_2' position of the sugar ring where they differ. The order of base pair stability within duplex DNA is dC-dG >rC-dG >araC-dG >>dT-dG. The rate of DNA Polymerase(exo Klenow Fragment) catalyzed incorporation of dG opposite template sites of dC, rC, araC, and dT is consistent with the stability trend of these base pairs. For DNA Polymerase catalyzed addition to primer terminus dC, rC, araC or dT opposite template dG, addition to arabinose deviates strongly from the trend in base pair stability. Quantitative foot printing shows no difference in binding of Polymerase to these substrates. These and other findings map out a spacial volume around the terminus sugar in the Polymerase active site with regions that can and cannot tolerate steric bulk and/or electronegative substitution. Polymerase active site mutants are currently being screened on these substrates to identify amino acid side chains close to the sugar. We also made araC substitutions in a series of DNA duplex substrates containing a T7 RNA Polymerase promoter and a short coding strand. Promoter substitutions had no effect on T7 RNA Polymerase binding, initiation, or transcriptional output. Coding strand substitutions defined an area of high sensitivity in the initiation region where miss-starts, primer slippage, and an inability to escape from abortive cycling occured depending on the position substituted. Coding strand substitutions after position 10 had little if any effect. These highly variable and position dependent effects define a narrow window of vulnerability where transcriptional output is severely reduced (~100 fold) by a subtle DNA lesion that has little or no consequence when situated elswhere in these coding units. Stability data on sugar substituted DNA/RNA hybrids is compared to the substituted DNA duplex data in the context of A vs. B helix type.

P1, THE FIRST PROTEIN OF THE TURNIP MOSAIC VIRUS, IS A SINGLE STRANDED NUCLEIC ACID BINDING PROTEIN. Soumounou, Y and Laliberté, J.-F. Centre de recherche en virologie, Institut Armand-Frappier, 531 boul. des Prairies, Ville de Laval, Québec, Canada, H7V 1B7

The P1 protein of Turnip Mosaic Virus (TuMV) is thought to fonction as a protease to perform the first predicted cleavage in the potyvirus polyprotein. In addition to its proteolytic activity, the P1 protein of TuMV may be required for cell to cell movement of viral RNA, probably through plasmodesmata (plant cells natural interconnections). This protein is the N-terminal of TuMV-encoded polyprotein and has a predicted molecular weight of 40 kDa. We expressed the P1 protein, fused to an histidine tail, in *Escherichia coli*. The histidine tail, at the C-terminal of the protein, allowed its purification by metal chelation chromatography after solubilization of the inclusion bodies in guanidine-HCl. After purification and refolding by dialysis against water or Tris buffer, a very pure protein at a yield of approximately 10 mg per 100 ml of bacterial suspension was obtained. To explore the single-stranded nucleic acid binding properties of the P1 protein, we used polyacrylamide gel retardation electrophoresis using a ^{32}P-labeled RNA. We found that the P1 was able to bind RNA. The binding was P1 specific since BSA or heat-denatured P1 did not affect the migration of the RNA probe into the gel. The RNA-protein complex stability in high sodium chloride concentration was examined. The complexes were stable in sodium concentration of up to 600 mM, indicating that the association is quite specific. The competition between labeled RNA probe and single-stranded DNA of M13mp18, double stranded DNA and RNA of TuMV has been investigated. The P1 binds both single stranded RNA and DNA, but with different affinities.