EXTRACHROMOSOMAL DNA

Donald Cummings, Igor Dawid, Piet Borst and Sherman Weissman, Organizers

Plenary Sessions

Poster Sessions

Evolution and Protist Extrachoromosal DNA

308 ANALYSIS OF INTERSPECIES HOMOLOGY AND THE LOCATION OF RIBOSOMAL RNA GENES IN MITOCHONDRIAL DNA FROM PARAMECIUM, Donald J. Cummings and Richard A. Maki, University of Colorado Medical Center, Denver, Colorado 80262.

Unlike other animal mitochondrial (mt) DNA, mtDNA from Paramecium is a linear molecule, 14 μm in length (1). Replication is initiated by closure at one end, followed by unidirectional synthesis via a lariat intermediate, terminating in a linear dimer (2) which is processed to yield two semi-conservatively constituted monomer length molecules (3). Several species of Paramecium exist and Beale and Knowles (4) have utilized direct microinjection of isolated mitochondria to study the ability of mitochondria from one species to survive and reproduce in the nuclear environment of another. Interestingly, species 1, 5 or 7 can function, although to different extents, in the nuclear environment of the others but species 4 can neither support the growth of other species of mitochondria nor can its mitochondria survive when used as a donor in microinjection into other species.

Previous results from our laboratory have demonstrated that each species of Paramecium mitochondrial DNA has a characteristic EcoRI restriction enzyme pattern (5). We used this result to show that hybrid cells constructed by microinjection contain the mtDNA of the donor (5). In the present study, we have utilized these unique fragmentation patterns to determine the location of the ribosomal RNA genes in each species and the homology of these endonuclease generated DNA fragments with each other. We found that for species 1 and 5, both rRNA genes appear to be located on one fragment, of molecular weight 3.8 x 10^6 daltons or 5700 base pairs. The total number of bases in rRNA is about 4400 so these results suggest that each ribosomal gene is represented once and that the two genes are close together on the genome. For species 4 and 7, the ribosomal RNA hybridized to two DNA fragments, whose combined molecular weights amounted to 10,600 and 7,500 base pairs, respectively. Detailed analysis of these fragments is in progress but it is possible that the genes coding for the ribosomal RNA molecules are close together for some species but separated in others. DNA-DNA molecular hybridization studies using the Southern blotting technique showed as expected, that there is good homology between the rRNA gene-containing fragments in each species. Other DNA fragments were not all homologous however. Using ^{125}I-labeled species 4 mtDNA, we found homology to only one additional fragment from species 1, two from species 5 and one from species 7. Even assuming total homology to all the fragments detected, species 4 mtDNA was homologous to species 1 to only 41%, to species 5, 65%, and species 7, 71%.

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307 MITOCHONDRIAL BIOGENESIS: EVOLUTION AND REGULATION, Henry R. Mahler, Department of Chemistry, Indiana University, Bloomington, Indiana 47405

The polypeptide gene products expressed in and by the mitochondria of respiration-competent eukaryotic cells appear to be few in number and conserved in kind: they consist of three subunits of cytochrome oxidase and cytochrome *b* of the respiratory chain and a more variable number (≥2) of subunits of the membrane-integrated (F_o) portion of the ATP synthase (oligomycin-sensitive ATPase). In spite of this apparent invariance, the resident genophore and its means of expression appear to have been - and perhaps still are - subject to profound and relatively rapid evolutionary changes. This is the inference drawn from variations, in the first instance, in the size and base composition of mitochondrial (mt) DNA and its RNA gene products: rRNAs, tRNAs and mRNAs. Similar trends are also evident in the base sequences of these RNAs and, in the nature, expression and processing of the primary transcriptional units. A particularly instructive example is provided by various yeast strains of the species *Saccharomyces cerevisiae* and *carlsbergensis* in which the mtDNA, although topologically and functionally homologous, can vary in size by as much as 15 kbp (20% of the total). The consequences of the presence of such insertions/deletions - which vary from 25-50 bp, found throughout the genome, to 900-3000 bp in specialized regions (including at least two structural genes) - are generally not known. However, in two instances they have been shown to be expressed and correlated with specific alterations in the gene product: i) the conferral of polarity (factor ω) in the 21 S rRNA and ii) the variation in size of the gene product *var1* (see Butow, this symposium). In the case of the cytochrome *b* region - a gene intensively investigated by three groups (see Slonimski, this symposium) - the situation is more complex: three non-coding regions (introns) appear to be interspersed among six coding regions (exons), with the former accounting for ≥4 kbp of the total sequences in the gene. In this regard then the sequence organization resembles that found in nuclear and viral eukaryotic genes. Unlike these instances, however, mutations in introns can readily be isolated and have been shown to result in i) the appearance of novel polypeptides not present in the wild type and ii) regulation of the expression of a second, unlinked mt gene, i.e. the one responsible for the specification of the largest subunit of cytochrome oxidase. This gene as well may exhibit a complex sequence organization. However, other mt genes may not share this property. Thus the mt genophore in this organism may be unusual in several respects, among them i) an unusually large proportion and length of AT-rich sequences, ii) non-contiguity of genes for rRNA precursors, and iii) the simultaneous presence of genes exhibiting both a pro- and an eukaryotic pattern of sequence organization. (Experimental work in this laboratory by D. Miller and D. Hanson, supported by Research Grant GM 12228 from NIH-NIGMS; some investigations in collaboration with P.S. Perlman, Dept. Genetics, Ohio State University, Columbus.)

309 THE EXTRACHROMOSOMAL RIBOSOMAL RNA GENES IN TETRAHYMENA; STRUCTURE AND EVOLUTION. J. Engberg, Biochemical Institute B, University of Copenhagen, DK-2200 Copenhagen N, Denmark.

The macronuclear rDNA from a number of strains within several species of Tetrahymena have been characterized to provide insight in the degree of sequence divergence which exists in a specific gene among closely related lower eukaryotes.

Restriction enzyme analysis revealed that individual strains all contained entirely homogenous populations of extrachromosomal rDNA. The linear rDNA molecules were shown to be giant palindromes, varying in size from 18 kilobases (kb) to 21 kb in different strains, and to contain two genes for rRNA separated by a centrally located spacer. A detailed gene map of the regions coding for the different intermediates in rRNA maturation was deduced in some strains and it was further shown that the mature 17S, 5.8S and 26S rRNA species were transcribed in the listed order with the 17S closer to the 5' end of the primary transcript. The sizes of the centrally located spacer and of the genes were approximately the same in all strains investigated and altotether an extended structure homology was shown to be maintained throughout all the species. Nevertheless, differences both inside and outside the gene region could be detected when rDNA from different species were compared. The differences were most pronounced in the spacer regions. In one out of five different species (the T. pigmentosa group), interbreeding strains were found which exhibited different restriction pattern of their rDNA. These strains will be useful in investigating recombinational events in the rDNA during sexual crosses, but their demonstration questions the usefulness of comparing restriction patterns in estimating relatedness.
Detailed restriction mapping of rDNA from strains within the T. pigmentosa group gave evidence of an intervening non-ribosomal kNA sequence within the structural gene for 26S rRNA in some of the strains. The size of this intervening sequence is about 350 bp and it occurs at a point about three fourth of the distance of the intact 26S coding region closer to the 3' end of the 26S transcript.

Genetic Capacity and Structure of Chloroplast DNA

310 METHYLATION AND RESTRICTION OF CHLOROPLAST DNA, Ruth Sager, William G. Burton, Hiroshi Sano, Sidney Farber Cancer Institute, Boston, MA. 02115.

Some time ago we postulated that modification-restriction (M-R) of DNA may be a general mechanism for preferential "silencing" or regulation of gene expression in eukaryotes (1), as well as the specific mechanism underlying maternal inheritance of chloroplast DNA (chlDNA). In previous studies (2-3) we showed that 1) chlDNA of zygotes undergoes a bouyant density shift, becoming about 0.005 gm/cc lighter in CsCl than the chlDNA of parental cells; and 2) chlDNA of the mt- (male) parent is lost during zygote maturation as shown by prelabeling with ^{15}N. Here we report that the density shift is the result of methylation of cytosine to 5-methylcytosine. The pattern of methylation has been studied using ^{3}H-adenine, -thymidine, and -cytidine as a prelabel in parental cells. Only ^{3}H-cytidine contributed radioactivity to the 5-meC peak. After formic acid hydrolysis the bases were separated on an HPLC column by chromatography over Aminex A-6. A methylase has been isolated from vegetative cells of Chlamydomonas with site specificity that could determine the methylation seen in zygotes. Properties of the enzyme and its role in methylation will be discussed.

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311 ORGANIZATION AND EXPRESSION OF THE MAIZE CHLOROPLAST GENOME, Lawrence Bogorad, Department of Biology, Harvard University, Cambridge, Massachusetts 02138.

Recognition sites for several restriction endonucleases have been located on the 85 x 10^6 dalton circular chromosome of the Zea mays chloroplast (1). Genes for 16, 23 and 5S rRNAs have been located on the two inverted repeated segments of this chromosome by R-loop analysis with chloroplast DNA and by molecular hybridization of rRNAs with fragments of a chloroplast DNA sequence cloned in E.coli (2). Structural genes have also been located for the large subunit of ribulose bisphosphate carboxylase (3,4,5) and a photoregulated 32,000 dlaton photosynthetic membrane protein (6,7,8). The cloned DNA sequence for ribulose bisphosphate carboxylase has been used to study the regulation of expression of the gene(9).

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312 GENETIC ANALYSIS OF CHLOROPLAST DNA FUNCTION IN CHLAMYDOMONAS. N.W. Gillham, J.E. Boynton, H.S. Shepherd, E.H. Harris, Duke University, Durham, NC 27706

Chloroplast mutations of Chlamydomonas resistant to antibiotics which block protein synthesis on 70s ribosomes constitute a ribosomal region of 7 linked loci in the chloroplast genome based on mapping and allele testing experiments (1). As in bacteria, the chloroplast mutations confer streptomycin, spectinomycin and neamine resistance on the small subunit of the 70s ribosome, and erythromycin resistance on the large subunit (2). We have now isolated for the first time (3) chloroplast mutations with a spectrum of defects in photosynthesis, using the thymidine analog 5-fluorodeoxyuridine (FdUrd). Treatment with FdUrd causes a reduction in the amount of chloroplast DNA per cell (4) and increases the frequency of all known classes of chloroplast gene mutations with no concomitant increase in the frequency of nuclear mutations with similar phenotypes (5,6). Sixteen nonphotosynthetic chloroplast mutants characterized thus far comprise 9 recombinationally distinct loci (3,7). Mutants in 3 loci are missing chlorophyll-protein complex 1 (CF_1) from chloroplast membranes; mutants in 3 other loci have lost at least 3 of the polypeptides associated with chloroplast coupling factor (CF_1); while mutants in 2 loci are defective in assembly of the small subunit of the chloroplast ribosome. One CF_1 locus has been mapped some distance from the loci that define the ribosomal region of the chloroplast genome, and mapping of the other 8 loci is now in progress. Recently, Bennoun et al. (8) have used our FdUrd method to isolate additional chloroplast mutants with defects in photosynthetic electron transport. These mutants have not yet been allele tested, so the number of loci they represent is unknown, as is their relationship to the 9 chloroplast loci we have defined.

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313 CHLOROPLAST DNA OF *EUGLENA GRACILIS*: GENE MAPPING AND SELECTIVE *IN VITRO* TRANSCRIPTION OF THE RIBOSOMAL RNA REGION. Richard B. Hallick, Keith E. Rushlow, Emil M. Orozco, Jr., and Patrick W. Gray, Department of Chemistry, University of Colorado, Boulder, CO 80309.

A restriction nuclease map of the 140 kbp ctDNA, with the location of 31 cleavage sites of the enzymes BalI, BamHI, PstI, SalI, and XhoI, has been reported (1). The ctDNA contains three tandemly repeated 5.6 kbp segments, each coding for 16S and 23S rRNA. This map has been extended to include the 4 KpnI and 6 PvuII sites, and 16 of 28 EcoRI sites. Twenty EcoRI fragments have been cloned on the plasmid pMB9. Recombinant plasmids were used in detailed mapping studies of ct transcripts. Purified ct5S, 16S, and 23S rRNAs, and tRNAs were labeled in vitro and hybridized by the Southern method to DNA restriction fragments. Ct tRNAs hybridize to 7 EcoRI fragments, including EcoP in the rRNA coding region. EcoP contains the entire 16S gene, an intercistronic region, and the beginning of the 23S gene. TaqI cleaves EcoP into 4 fragments, in the order: a(320 bp)-b(1010 bp)-c(760 bp)-d(360 bp). 16S rRNA hybridizes to b and c, tRNA(s) to c, and 23S rRNA to d. 5S, 16S, and 23S rRNAs, and tRNA(s) all hybridize to the same DNA strand in the 5.6 kbp repeated DNA. Each repeated DNA may contain a single transcription unit, which is initially transcribed into a precursor RNA of ca. 5000 nt. This RNA would be subsequently matured to tRNA(s) and rRNAs. The RNA product transcribed in vitro (cRNA) by chloroplast RNA polymerase in a purified transcriptionally active chromosome (TAC; 2) has been characterized. [γ-^{32}P]ATP incorporated into cRNA is alkali labile, protease resistant, and migrates with [^{3}H]UTP labeled cRNA in denaturing sucrose gradients. Initiation of RNA synthesis is inhibited only 25% in 1 mg/ml heparin, and is not detectably inhibited in 100 µg/ml rifampicin. cRNA hybridizes predominately to DNA restriction fragments from the 5.6 kbp rRNA repeats. Hybridization is obtained only to the DNA strand coding for rRNA in vivo, and is completely inhibited by an excess of in vivo synthesized chloroplast RNA. Evidence for selectivity of initiation and for nuclease resistance of the 5'-end of the cRNA was obtained by the analysis of nuclease digestion products from [γ-^{32}P]ATP labeled cRNA on denaturing polyacrylamide gels. One major oligonucleotide was observed following a limit S1 nuclease digest. Exhaustive T1 or RNAse A digestion resulted in one or two oligonucleotides in each case. In summary the TAC carries out the selective initiation and transcription of rRNA from the ct rRNA transcription units. (Supported by NIH GM 21351 and 1-K04-00372).

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Models for Viral Replication and Function

314 REPLICATION AND INTEGRATION OF BACTERIOPHAGE MU DNA, Ahmad I. Bukhari, George C. Chaconas, Hajra Khatoon and Michael DuBow, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724

The temperate bacteriophage Mu presents a remarkable model of virus-host interaction. During its passive phase, Mu replicates as a part of the host chromosome, but during its active phage, it acts as an autonomously replicating transposable element. The replication of Mu DNA and its integration are at least superficially synonymous since the replication event normally cannot be separated from the integration event. No free replicating copies of Mu DNA have been clearly observed so far. We have earlier shown that upon induction prophage Mu replicates in situ and the products of this reaction are transposed to different locations (1). We know however that the X mutants of prophage Mu can be excised at a low frequency from the host DNA (2). A model for Mu transposition must resolve this apparent paradox. That is, although a mechanism for Mu excision exists, excision does not appear to be a normal course of events. We have used A, B and X mutants (which have insertions in the B gene) of Mu and small plasmids containing the Mu ends to study the process of Mu transposition. We will describe experiments that have led us to the following postulate. Recognition of the ends of Mu by the A gene product can lead to different consequences depending upon the presence or absence of the B gene function. If the B gene product is functional, the process initiated by the A gene culminates in the replication and transposition of Mu DNA. If the B gene is nonfunctional then the action of the A gene at the ends of Mu may leave the prophage in state susceptible to excision.

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315 INTEGRATIONS OF DNA INSERTION ELEMENTS IN BACTERIA, Nigel D. F. Grindley, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

DNA insertion elements are stretches of DNA that can transpose from one site to another in the genomes of bacteria, their plasmids and viruses. These elements include the small IS elements, the transposable drug-resistance elements (transposons) and some temperate bacteriophages that integrate into their host genome.

Integration of several of these elements has been analysed by sequencing the junctions between host DNA and the inserted element and also the corresponding target sites on the host genome before integration occurred (1-4). From these and other studies certain generalizations can be made.

1) The two ends of an individual IS element or transposon have identical or very similar sequences, suggesting that both ends may be recognized by a single element-specific protein.

2) The terminal sequences of an element are completely conserved during transposition and during the formation of element-associated deletions.

3) Integration of a transposable element into a new site results in duplication of a short (5 or 9 base pair) host sequence that pre-exists at the site. One copy of this sequence occurs at each end of the inserted element.

Target site Inserted element

No other alterations in host sequences are observed.

Based on these observations, models for the transposition process (5) will be presented and discussed.

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316 STRUCTURE AND REPLICATION OF PARVOVIRUS DNA. D.C. Ward,[1] M.B. Chow,[1] E. Faust,[1] C.R. Astell[2] and M. Smith.[2] Dept. of Human Genetics, Yale University, New Haven, Conn. 06510 and Dept. of Biochemistry, University of British Columbia, Vancouver, B.C., Canada V6T 1W5

Mammalian parvoviruses contain linear, single-stranded DNA genomes of 1.5 x 10^6 daltons. The adeno-associated virus (AAV) subgroup, which encapsidates both (+) and (-) strands in separate virions, are helper dependent and require a coinfecting Adenovirus to initiate AAV replication. In contrast, the autonomous parvoviruses (AP) replicate in the absence of helper virus (although only in host cells which are in S-phase) and encapsidate a unique (-) strand of DNA. The 3'- and 5'-termini of both types of genomes possess palendromic nucleotide sequences which exist as stable hairpin structures. The 3'-terminal hairpin [of both (+) and (-) strands] can be used as a primer to initiate DNA synthesis. This produces replicative DNA intermediates in which (+) and (-) strands are covalently linked. Progeny DNA is synthesized in a continuous fashion via strand displacement synthesis from both monomeric and concatemeric DNA duplexes. Site-specific nucleolytic cleavages, "hairpin rearrangement" and "hairpin transfer" processes are required to conserve and mature the genome termini during replication.

We have determined the sequence for the first 450 nucleotides from the 3'-termini of four serologically-distinct, autonomous, rodent virus genomes (KRV, MVM, H-1 and H-3). The terminus of each DNA exists as a Y-shaped hairpin structure involving 115 or 116 nucleotides. The sequence of this region of DNA is highly conserved and shows no evidence of sequence heterogeneity, a characteristic which is observed in the terminal nucleotide sequence of AAV DNA. In addition, we have characterized two classes of defective AP virions which contain less than 20% of the normal viral genome. One class appears to contain recombinant DNA molecules which have selectively retained both termini of the genome. These particles are selectively amplified when serially passaged in the presence of wild type virus. The second class, which contains only sequences from the extreme 5'-end of the genome, appears to arise as end products of replication errors. The implications of these results with respect to the models of DNA replication will be discussed.

317 REGULATORY MUTANTS OF SIMIAN VIRUS 40: CONSTRUCTED MUTANTS WITH BASE SUBSTITUTIONS AT THE ORIGIN OF DNA REPLICATION, David Shortle and Daniel Nathans, Department of Microbiology, Johns Hopkins University, School of Medicine, Baltimore, MD 21205.
Mutants of Simian Virus 40 (SV40) with base substitutions at or near the origin of replication of the viral genome have been constructed by bisulfite mutagenesis at the Bgl I restriction site of SV40 DNA, followed by transfection of cells with the Bgl I-resistant (Bgl I^r) DNA so generated. Based on plaque morphology at different temperatures, the resulting Bgl I^r mutants could be classified into four groups. Class I mutants (designated ar for "altered restriction") were indistinguishable from wild type SV40; class II mutants (designated shp for "sharp plaques") produced small, sharp-edged plaques; class III mutants (designated sp for "small plaque") produced small plaques at 32°C, 37°C and 40°C; and class IV mutants (designated cs for "cold sensitive") produced small plaques at 32°C and wild type plaques at 37°C and 40°C. That the altered plaque morphology of sp and cs mutants was related to mutation at the Bgl I restriction site was demonstrated by co-reversion to wild type of the plaque phenotype and Bgl I sensitivity. The nucleotide sequence around the original Bgl I site was determined in the DNA from one mutant of each class. In each case a different base pair substitution was found, at a site outside sequences coding for SV40 proteins. When rates of replication of mutant DNA's were measured during productive infection, ar mutant DNA was synthesized at a rate comparable to that of wild type SV40 DNA, shp mutant DNA was made at a rate exceeding that of wild type, sp mutant DNA was synthesized at a lower rate than that of wild type, and cs mutant DNA synthesis was reduced at 32°C, but about the same as the wild type rate at 40°C. These patterns of mutant DNA synthesis were unaltered in cells co-infected with mutant and wild type virus, i.e., the defects in DNA synthesis were not trans-complementable. We conclude that the defective mutants have single base pair changes in a cis element that determines the rate of viral DNA replication, presumably within the origin signal itself.

Sequence Analysis of Viral Nucleic Acids

318 COMPLEX STRUCTURES AND NEW SURPRISES IN SV40 mRNA. Sherman M. Weissman, V. Bhaskara Reddy, Prabat K. Ghosh, Michael Piatak, Department of Human Genetics, Paul Lebowitz, Department of Internal Medicine, Yale University School of Medicine, New Haven, Ct. 06510.
We have determined the nucleotide sequences of many of the SV40 mRNAs produced in transformed cells, early in the lytic cycle, and late in the lytic cycle. Late cytoplasmic mRNAs exhibit a large multiplicity of 5' ends, most of which have their templates lying within a region of approximately 10% of the viral genome. Some forms of SV40 16 S late mRNA encoding the major structural protein, VP1, may contain a second splice within the leader. None of the forms of 19 S mRNA (encoding the minor structural proteins VP2 and VP3) have this second splice, so that the splices are not randomly distributed with respect to one another. SV40 early mRNA made in lytically infected or transformed cells exhibits a limited degree of 5' end heterogeneity. Additional evidence has been obtained for 2 and probably 3 other splicing events that occur within SV40 early RNA derived from sequences near the 3' end of large T and small t mRNA. Some of these variant RNAs may be present in quantities approaching or even exceeding the abundance of the mRNA encoding the known early proteins, large T and small t antigen.

319 STRUCTURE AND FUNCTIONS OF ADENOVIRUS 5 TRANSFORMATION GENES, A.J. van der Eb, P. van den Elzen, H. Jochemsen, J.H. Lupker, J. Maat, H. van Ormondt,and P.I.Schrier, Sylvius Laboratories, University of Leiden, The Netherlands.

Transformation by human adenoviruses is a process in which only a small part of the genome (about 10%) is involved. The transforming segment is localized at the left-hand end of the viral DNA, between 1 and 11%, which corresponds to the early region #1. This was found to hold true for some representatives of subgroups A, B and C of the adenoviruses.

Transformation studies with DNA fragments have shown that in addition to fragments containing the entire left-hand early region, also smaller fragments representing various parts of this early region contain transforming activity. When the properties of baby rat kidney cells transformed by fragments of various sizes were compared, it was observed that the cells were not identical in their properties and could be divided into at least 3 categories:

1. Cells transformed by the entire left-hand early region (0-11%). These cells are similar in properties to cells transformed by intact Ad5 DNA or virus. The cells contain two major T antigens of 65 K and 19 K, and in addition, a number of minor species of T antigen ("Standard" transformed cells).
2. Cells transformed by HindIII (HsuI) G fragment (0-8%). These cells differ from the standard transformed cells in that they lack the major 65 K T antigen and are slightly more contact inhibited in their early passages, but otherwise are identical.
3. Cells transformed by the HpaI E fragment (0-4.5%). These cells lack both the 19 K and the 65 K major T antigen and clearly differ in their growth properties and cell morphology from the cells of the other two categories.

Our results obtained with *in vitro* protein synthesis, immuno precipitation and nucleotide sequence analysis will be discussed in their relationship to data on RNA splicing, as reported by Berk & Sharp and Chow et al. It will be shown that the left-hand 4.5% of the Ad5 genome codes for a series of (partially) overlapping proteins present at relatively low concentrations in transformed cells. These proteins apparently are able to transform a diploid cell with a limited life-span into a permanent cell line still lacking some of the properties found in other Ad5 transformed cells. The adjacent segment (4.5-9 or 10%) codes for at least 2 major T antigens of 19 K and 65 K which apparently are responsible for the induction of a number of additional changes in growth properties and cell morphology.

320 STRUCTURAL VARIATION IN HERPESVIRUS GENOMES, P. SHELDRICK, Institut de Recherches Scientifiques sur le Cancer, B. P. n° 8, 94800 Villejuif, France.

Virion morphology has been found to vary little, if at all, among the herpesviruses, and is one of several viral characters generally taken to illustrate an underlying unity in the group. Genome structure, on the other hand, exhibits an impressive variability among group members. Molecular weights of herpesvirus (duplex) DNAs range from 83 megadaltons (Md) for channel catfish virus (CCV) to 150 Md in the case of human cytomegalovirus (HCMV). All herpesvirus genomes studied thus far (thirteen) harbor repeated nucleotide sequences accounting for from 5 % to 30 % of the total genome mass. In terms of genome mass, and the way in which repeated sequences are organized in the genome, it is possible to recognize six distinct, but related, structural types. Of these, the structural type represented by CCV is simplest, being a unique sequence arrangement of 70 Md to which a direct terminal redundancy of 13 Md has been added ; a structural type akin to that of bacteriophage T5, for example. A second structural type is that of H.saimiri (1), in which a 70 Md unique sequence is bounded by multiple tandem (~40) repeats of 0.8 Md. The genome of Epstein-Barr virus (2) describes a third structural type with multiple (5-10) internal tandem repeats (2 Md) located between long (73-80 Md) and short (8 Md) unique sequence regions. A fourth type is seen in equine abortion virus and pseudorabies virus (3) ; both contain a long unique region (70-75 Md) and a short unique region (5-10 Md), only the latter of which is flanked by extensive inverted repeat sequences (8-12 Md). The prototype of the fifth structure is herpes simplex virus (4), wherein long unique (70-75 Md) and short unique (9-10 Md) regions are both flanked by inverted repeats (6 Md and 4 Md, respectively). In the sixth structural type, exemplified by HCMV and H.aotus 1, an owl monkey herpesvirus, the global arrangement of nucleotide sequences is essentially that of HSV, but the long and short unique regions are greatly expanded (114 Md and 21 Md, respectively). Five of the six structural types are formally related by the presence of a 70-80 Md unique sequence region, suggesting its possible role as a "fundamental" block of viral functions conserved in herpesvirus evolution.

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321 THE EPSTEIN-BARR VIRUS EPISOME, Joseph S. Pagano, M.D., Cancer Research Center, The University of North Carolina, Chapel Hill, North Carolina 27514.

The Epstein-Barr-Virus genome, the molecular weight of which is approximately 100 x 10^6 daltons, persists in non-virus-producing cells in a stable regulated state. In the Burkitt-Lymphoma-cell line, Raji, there are 50-60 copies of EBV genomes per cell. The EBV DNA is situated in the nucleus and it is chromosomally associated, but not covalently integrated into host-cell DNA. At least two-thirds of the viral DNA can be recovered in a supercoiled form with contour lengths approximating 100 x 10^6 daltons. Such molecular forms exist in Burkitt's lymphoma tissue and nasopharyngeal carcinomas in vivo.

Supercoiled forms of EBV DNA have also been recovered from the EBV-producing Burkitt's lymphoma cell line, P3HR-1, by suppression of virus production with the antiviral drug, acycloguanosine, and from the EBV-producing marmoset lymphocyte line, B-95-8, with the use of phosphonoacetic acid. The contour length of P3HR-1 supercoiled genomes appears to be less than the length of Raji EBV genomes. Restriction-endonuclease digestion analyses of these forms of the EBV genome are now becoming available.

This form of EBV DNA, which has been called the EBV plasmid or episome, appears to be replicated by host DNA polymerases rather than by virus-induced polymerases, can be induced to a limited extent with IUDR, but cannot be cured by any known treatment. The function of the stable EBV episome, whether it is expressed, particularly in relation to lymphocyte proliferation and transformation,and its relation to possible integrated viral DNA sequences are probably central issues in the cellular biology and pathobiology of EBV-associated diseases.

Mitochondrial DNA from Yeast

322 THE INFLUENCE OF THE NUCLEAR GENOME ON THE EXPRESSION OF MITOCHONDRIAL DNA IN *SACCHAROMYCES CEREVISIAE*. Anthony W. Linnane, Department of Biochemistry, Monash University, Clayton, Victoria,3168. Australia

The biosynthesis of the mitochondrial organelle involves the co-operative activities of both the nuclear and mitochondrial genetic systems (for review [1]). The present paper will be concerned with exploring this interaction.

From both genetic and physical studies of yeast mtDNA it has become apparent that there are significant differences (insertions, deletions) in the mitochondrial genomes of different strains of *Saccharomyces cerevisiae*. Sequence differences have been shown by Butow to be associated with the formation of variant polypeptides. It may be further asked whether sequence differences in the mtDNA in particular grande strains can lead to different gene products being produced, and concomitantly, does the appearance of potentially new mitochondrial gene products depend on particular nuclear genomic influences on the mitochondrial DNA?

We have addressed ourselves to these questions by constructing sets of new yeast strains containing specified mtDNAs and specified nuclear genomes. This has been achieved by constructing sets of isonuclear strains containing different mitochondrial genomes, making use of the nuclear mutation *kar1-1*. This mutation leads to defects in the fusion of haploid nuclei during zygote formation. Haploid cells containing the *kar1-1* mutation can mate with haploids of opposite mating type to produce heterokaryons, which subsequently segretate haploid cells containing one of the parental haploid nuclei together with the mixed cytoplasm from both parents. If one of the parents is a petite of the *rho*^{0} type, lacking mtDNA, and the other parent is a respiratory competent (grande of *rho*$^+$) strain, then it becomes possible to transfer the mtDNA of the *rho*$^+$ strain to haploid cells containing the nucleus of the *rho*^{0} strain.

The results of experiments which demonstrate that different nuclear genomes modulate mitochondrial gene expression will be discussed; specifically that certain products encoded in certain mitochondrial genomes are only synthesized in the presence of particular nuclear genomes.

It is also intended to discuss our recent result on the region of mtDNA coding for the OS-ATPase and cytochrome *b*.

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323 ORGANIZATION AND TRANSCRIPTION OF YEAST MITOCHONDRIAL DNA.

David Levens and Murray Rabinowitz, Departments of Medicine and Biochemistry, The University of Chicago, Chicago, Illinois 60637.

We have derived a detailed restriction map of the 70 to 76 kb yeast mitochondrial genome, and localized genetic loci and rRNA and tRNA genes on this map. The map provides a basis for the physical analysis of mitochondrial recombination and for detailed examination of transcription. Yeast mtDNA is extensively transcribed asymmetrically. RNA-DNA hybridization shows that at least 60% of a single strand equivalent is transcribed; less than half of this required to code for the known gene products. Asymmetrical transcription is suggested by complete transcription of one, and negligible transcription of the other separated strand of plasmid-Eco Rl fragment 7 recombinant DNA. High molecular weight transcripts are visualized on agarose-urea and agarose-methyl mercury gel electrophoresis, which may represent intermediates in the processing of large primary transcripts. Transcripts have been localized to genetic markers by analysis of their presence in genetically characterized petite strains and by hybridization of labeled DNA to electrophoretically separated RNA immobilized on diazobenzyloxymethyl paper.

Promoter function of yeast mtDNA has been examined by analysis of a mitochondrial transcription complex, and by the use of purified yeast mitochondrial RNA polymerase. The transcription complex primarily synthesises RNA from the same regions and same from the strand as code for the *in vivo* rRNA's. Since the 21S and 14S rRNA genes are widely separated, transcription occurs from at least two promoters. Template dependent RNA polymerase has been isolated from either the transcription complex, or from soluble protein fractions. Both enzyme fractions express similar properties. The soluble polymerase has been extensively purified by a variety of chromatographic techniques. The purified enzyme is associated with a 45,000 molecular weight peptide. Prior to a final glycerol gradient centrifugation a 65,000 molecular weight peptide is associated with the enzyme activity in molar ratio. The enzyme from the glycerol gradient is extremely labile. Antibodies to the 45,000 molecular weight band precipitate that peptide, and inhibit the RNA polymerase activity. Promoter function and mapping is being carried out using in vitro transcription of mtDNA, as well as binding of the purified RNA polymerase to restriction fragments.

324 TRANSCRIPTS OF YEAST MITOCHONDRIAL DNA AND THEIR PROCESSING.

L.A. Grivell, A.C. Arnberg*, P.H. de Boer, P. Borst, J.L. Bos, G.S.P. Groot, L.A.M. Hensgens, G.J.B. van Ommen and H.F. Tabak, Section for Medical Enzymology and Molecular Biology, University of Amsterdam and *Laboratory of Electron Microscopy, University of Groningen, The Netherlands.

Transcripts of the majority of mitochondrial genetic loci have been identified and mapped. In nearly all cases, these RNAs are much longer than minimally necessary to specify the known protein product of the locus concerned. In the oli-2 and oxi-3 regions, processing of transcripts is indicated by the presence of several RNAs displaying overlapping hybridization behaviour.

In two cases, electron microscopy has provided direct evidence for split genes in mtDNA. At least 4 inserts interrupt the sequences specifying an 18 S RNA (2200 nucl.), which contains mRNA sequences for cytochrome b. The gene for 21 S rRNA is interrupted close to its 3'-end by a 1160 bp insert, which may be identical with the ω^+ allele of the mitochondrial polarity locus.

Unusual features of mitochondrial gene organisation and transcription have also been revealed by DNA sequencing. The gene for ATPase subunit 9 is, in contrast to the neighbouring genes for cytochrome b and 21 S rRNA, continuous. It is flanked by sequences, which contain less than 4 mole percent G+C and which extend at least 250 bp in both directions. The AT-rich sequences upstream of the structural gene are transcribed and form part of a long leader sequence in a 12 S RNA (850 nucl.), which is the major transcript of this region of the genome.

325 ORGANIZATION AND EXPRESSION OF INTERSPERSED GENE SEGMENTS IN MITOCHONDRIAL DNA CODING FOR CYTOCHROME B AND CONTROLLING CYTOCHROME OXIDASE, Piotr P. Slonimski, Centre de Genetique Moleculaire du C.N.R.S. - 91190 Gif sur Yvette, France.

Yeast mitochrondrial DNA is 75,000 bp long. A region, called COB-BOX, situated in the sector S3 of the genetic map and between the 14,000 bp and 24,000 bp positions of the physical map has particulary interesting properties. This region, as shown by corsses and restriction analysis of cloned DNA segments, displays a mosaic organization of genetic information, i.e., coding and regulatory sequences controlling cytochrome b (of the cytochrome c reductase complex) and subunit I (of the cytochrome c oxidase complex) are interspersed (1,2,3). Three, at least, unlinked genetic loci represent cytochrome b exons. Deficient mutants (4,5,6) and drug resistant mutants (7) are located in them. They modify the structure, particularly by chain termination mutations, of a single cytochrome b polypeptide chain (2,8) and constitute a single unit of complementation (1). Within this cistron other mutations controlling cytochrome reductase and oxidase are located in introns. They constitute distinct units of complementation, present characteristic features of mitochondrially translated new polypeptides and a specific pattern of regulation (1,2,3). The COB-BOX region of mitochondrial DNA constitutes a particularly favorable model system for the analysis of function and genetic regulation in the intervening sequences of a split gene.

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Extrachromosomal DNA from Yeast

326 NUCLEOTIDE SEQUENCES OF MITOCHONDRIAL GENES IN SACCHAROMYCES CEREVISIAE. Alexander Tzagoloff, Francisco Nobrega and Giuseppe Macino, Department of Biological Sciences, Columbia University, New York, N.Y. 10027.

Mitochondrial DNA (mitDNA) of several cytoplasmic ρ^- mutants of Saccharomyces cerevisiae have been characterized by restriction endonuclease analysis and by DNA sequencing. One of the mitDNA's was obtained from a ρ^- clone (DS400/A3) whose only detectable mitochondrial genetic markers are the oli1 and pho2 loci of the ATPase proteolipid gene. The mit DNA of DS400/A3 has a unit repeat length of 1,800 base pairs with restriction sites for AluI, HpaII and HaeIII. The nucleotide sequence of the DS400 mitDNA has revealed the presence of the proteolipid gene plus two adjoining sequences rich in A/T. Even though yeast mitochondria utilize the expected codons of the universal code as evidenced by the excellent agreement between the primary structure and the nucleotide sequence of the ATPase proteolipid gene, there is an unusually marked preference for certain codons, indicating that the code is highly non-degenerate. A comparison of the restriction maps of mitDNA's from a series of oli1 containing ρ^- mutants has permitted the retained segment of DS400/A3 to be localized precisely on the restriction map of the parental wild type strain, D273-10B.

The second ρ^- mutant (DS400/M8) studied has mitDNA with a repeat length of 1,300 base pairs. This segment contains genetic markers in the cob2 locus which is believed to be included in the structural gene of cytochrome b. One of the strands of the DS400/M8 mitDNA has a reading frame with codons that are very similar to those used for the proteolipid gene and is presumably a region that codes for cytochrome b.

327 GENETIC AND BIOCHEMICAL ANALYSIS OF THE YEAST MITOCHONDRIAL GENE VAR1 AND ITS POLYPEPTIDE PRODUCT, R. A. Butow[1], R. D. Vincent[2], P. S. Perlman[2], P. Terpstra[1], and R. L. Strausberg[1], Department of Biochemistry, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235[1] and Dept. of Genetics, Ohio State University, Ohio 43210[2].
The protein product of the yeast mitochondrial gene var1 is found in all yeast strains examined by us and shows strain-dependent size polymorphism with molecular weights ranging from about 40,000 to 44,000. The var1 gene maps to a segment of the wild-type mitochondrial genome between the markers ery and oli1. Physical mapping studies have located var1 to a DNA fragment about 2 kb long in the ery oli1 span. The absolute size of this fragment varies directly with the size of var1 polypeptide.From these results and from additional biochemical data, we conclude that the size polymorphism of var1 polypeptide can be accounted for by variations in the size of its structural gene. We have analyzed the generation of different forms of the var1 gene in crosses. These forms arise by asymmetric gene conversion involving the insertion of discrete, genetically and physically defined DNA segments located within the structural gene. These DNA segments can insert independent of one another and may include as much as 15% of the entire structural gene (1). Biochemical studies have confirmed and extended the conclusion of Groot et al. (2) that var1 polypeptide is a protein associated with the 37S mitochondrial ribosomal subunit. It appears by Coomassie Blue staining to be roughly stoichiometric with the other ribosomal proteins of the small subunit. The bulk of var1 polypeptide labeled in vivo in the presence of cycloheximide (CHX) appears in a particle sedimenting between 15-20S. After a cold chase in the absence of CHX, var1 polypeptide sediments at 37S. Thus, the 15-20 particle may be a precursor to the mature 37S subunit. When wild-type cells are grown in the presence of 2 mg/ml erythromycin or when syn$^-$ strains are grown in the absence of antibiotics, the amount of 37S subunit recovered from mitochondrial lysates is reduced by at least 6-fold compared to the recovery of the large mitochondrial ribosomal subunit. From these data, we tentatively conclude that var1 polypeptide is required for the assembly of the small mitochondrial ribosomal subunit.

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328 IDENTIFICATION AND SEQUENCING OF YEAST MITOCHONDRIAL tRNA GENES. Nancy C. Martin, University of Minnesota, Minneapolis, MN 55455, Dennis Miller and John E. Donelson, University of Iowa, Iowa City, Iowa 52242.
We have cloned yeast mitochondrial DNA in the E. coli plasmid pBR322 by the poly dA:dT tailing method and identified fifty-eight clones that carry mitochondrial tRNA genes by hybridization with ^{32}P labeled tRNA. In order to correlate the cloned mitochondrial tRNA genes with their gene products, we screened the recombinants with nick translated DNA isolated from petites known to carry chloraphenicol (C), erythromycin (E), paramomycin (P) or oligomycin (O) markers as well as a limited subset of tRNA genes. In this way we were able to map the inserted sequences with respect to antibiotic resistance makers and predict which tRNA genes they were most likely to carry. Twelve clones that showed positive hybridization to petite DNA carrying the O_I marker have been analyzed in more detail. When ^{32}P tRNA was hybridized to Southern transfers of Hpa II restriction enzyme digests of DNA from some of these O_I positive clones we identified a 320 base pair fragment in some and a larger fragment in others that contained tRNA genes. Screening of the recombinant DNAs with ^{3}H seryl and ^{3}H glutamyl aminoacyl tRNA enabled us to correlate the presence of the 320 base pair fragments with the seryl tRNA gene and the larger fragment with the glutamyl tRNA gene. We have sequenced the 320 base pair Hpa II fragment and the corresponding mitochondrial seryl tRNA. This mitochondrial tRNA is 90 nucleotides long, has a G+C content of 38%, and the anticodon UGA. A comparison of the tRNA and DNA sequence show that they are colinear and that the CCA end is not encoded. The DNA sequence surrounding the structural gene is less than 10% GC.

329 STRUCTURE AND FUNCTION OF YEAST 2-μm DNA, Cornelis P. Hollenberg and Hans-Dieter Royer, Max-Planck-Institut für Biologie, Abt. Beermann, 7400 Tübingen 1, Federal Republic of Germany.

Yeast 2-μm DNA is a closed circular extra-chromosomal DNA element of which 50-100 copies are normally found in several strains of Saccharomyces cerevisiae. No gene products of this DNA element are known and no direct function has yet been established. Yeast 2-μm DNA contains a non-tandem inverted duplication (id), 600 base pairs long, which is involved in intramolecular recombination leading to the inversion of the enclosed unique DNA segment. Earlier analysis of the resulting two types of 2-μm DNA suggested that the recombinational event does not take place over the entire id sequence but is confined to its right arm, and probably to the border between the id sequence and the unique L-segment. Recently, we have started to analyze this recombinational process by using several recombinant 2-μm DNA plasmids introduced into yeast cells by transformation.

Progress in the analysis of the expression in Saccharomyces cerevisiae of foreign, pro- and eukaryotic, genes integrated in 2-μm DNA vectors, will also be discussed.

Genetics and Biogenesis of Mitochondrial DNA from Higher Eucaryotes

330 ORGANIZATION AND EVOLUTION OF ANIMAL MITOCHONDRIAL DNA. Igor B. Dawid and Eva Rastl, Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, Maryland 20014

Mitochondrial DNA in all metazoan animals is a circular molecule of 15 to 18 kilobase pairs. Earlier work has shown the location of the sites coding for the two mitochondrial rRNA molecules and for about 20 4S RNAs in Hela (1) and Xenopus (2) DNA. A comparison of these two maps shows that the overall arrangement of sites is quite similar in the two species even though the primary sequence of mitochondrial DNA has diverged extensively.

In addition to rRNA and tRNA the mitochondrial DNA codes for a set of poly(A)-containing RNA molecules that are probably mRNAs. A map for seven such RNAs in mouse L cells has been published (3). We have derived a map for mitochondrial RNAs in Xenopus and have localized on it at least nine poly(A)-containing and two nonribosomal poly(A)-lacking RNAs. These results show that a very large portion of the total DNA is transcribed into distinct RNA species. The map for Xenopus will be compared to the L cell map.

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331 QUANTITATION OF INTRAPOPULATION VARIATION BY RESTRICTION ENDONUCLEASE ANALYSIS OF HUMAN MITOCHONDRIAL DNA, Wesley M. Brown, University of California, Berkeley, CA 94720

In this study the technique of restriction endonuclease analysis of mitochondrial DNA has been applied to the field of population genetics. Mitochondrial DNA samples from 21 humans of diverse racial background were analysed by gel electrophoresis after digestion with eight restriction endonucleases. The mitochondrial DNA population within each individual appeared to be highly homogeneous. By contrast, mitochondrial DNA was observed to differ among the individuals. Thirteen of the 21 samples were identical to one another at the 64 restriction endonuclease recognition sites examined. The remaining eight samples differed from the wild type and from each other at one or more restriction sites. Calculations based on these data indicate that mitochondrial DNA from randomly chosen pairs of humans differs, on an average, at 32 base pairs per mitochondrial genome (16,500 base pairs), or at one base pair in 500. This degree of difference is consistent with other evidence indicating that the rate of evolution of mitochondrial DNA is very rapid. Restriction endonuclease analyses of mitochondrial DNA will thus be especially valuable for studies of genetic variability within and among populations and among closely related species.

332 ORGANIZATION AND INFORMATION CONTENT OF MITOCHONDRIAL DNA'S OF HIGHER PLANTS, C. S. Levings, III., Department of Genetics, North Carolina State University, Raleigh, NC 27650 and D. R. Pring, Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

The size and organization of the mitochondrial (mt) DNAs of higher plants vary widely among the different plant taxa. Plant mt DNAs with molecular weights ranging from 70-165 x 10^6 daltons have been observed; these are the largest mt DNAs found in nature. Although it differs in its degree, molecular heterogeneity is commonplace among plant mitochondrial genomes. For example, pea mitochondria contain a major class of circular DNA molecules which are 30μm in length and a minor class which are less than 5μm, minicircles (1). In contrast, maize mitochondria contain several classes of large circular molecules, 15, 21, and 30μm, as well as minicircles. Molecular heterogeneity has also been observed in mt DNAs from flax, soybean, and teosinte in our laboratory and reported by others in potato and Virginia creeper (2). Possible explanations for the molecular heterogeneity phenomenon are discussed. We have observed supercoiled molecules of mt DNA in maize, soybean, teosinte and flax. These results suggest that the native configuration for plant mt DNAs is that of a covalently closed circular molecule.

The large size of the mt DNAs suggests that additional information may be coded by these genomes which is unique to higher plants. Cytoplasmic male sterility appears to be an example of a trait unique to plants which is encoded by the mitochondrial genome. This view is supported by restriction endonuclease fragment analyses of mt DNAs from fertile and male-sterile cytoplasms, by studies of the effect of pathotoxin T on mitochondria, and by histological studies of anthers from fertile and sterile types.

The S cytoplasm of maize has associated with its mitochondria two plasmid-like DNAs which have molecular weights of 4.10 and 3.45x10^6 daltons and are in addition to the usual high molecular weight mt DNAs. Electron microscopy has revealed that these molecules exist in a linear configuration and contain terminal inverted repeats. A causal relationship between the plasmid-like DNAs and the S type of male sterility has been indicated by studies of stable and unstable sources of the S cytoplasm.

Diversity among mitochondrial genomes has been assayed by restriction endonuclease fragment analyses of their mt DNAs. Investigations with maize, sorghum, soybean and teosinte have detected substantial heterogeneity among mt DNAs from different cytoplasmic backgrounds. These studies have revealed that diversity among mt DNAs of a species complex is a common condition.

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Structure and Replication of Mitochondrial DNA from Higher Eucaryotes

333 STRUCTURE AND FUNCTION OF THE TWO MOUSE MITOCHONDRIAL DNA REPLICATION ORIGINS, David A. Clayton, Amanda M. Gillum and Phillip A. Martens, Department of Pathology, Stanford University, Stanford, California 94305

The major form of mouse L cell mitochondrial DNA (mtDNA) contains a small displacement loop at the heavy strand (H-strand) replication origin, created by synthesis of a 500-600 nucleotide portion of the H-strand. These species remain hydrogen-bonded to the parental light strand and are termed 7S mtDNA. The unique location of this 7S mtDNA suggests that it may function as a primer in the replication of full-length H-strands. Ribonucleotides have been detected at the 5'-end of some of these molecules. Using 5'-end in vitro labeling, we have determined that these ribonucleotides occur at several discrete positions along the nucleotide sequence of the origin region.

Replication of the L-strand origin does not begin until H-strand synthesis has proceeded past the L-strand origin. Because L-strand synthesis proceeds in the direction opposite that of the H-strand, molecules can be isolated in which there exist long single-stranded regions of parental H-strands separating the duplex region starting at the L-strand origin from the H-strand replication fork. Using a variety of techniques, it has been possible to locate the L-strand origin at a position 55 to 90 bases from a HpaI cleavage site 0.67 genome length from the H-strand origin. The nucleotide sequence of a 318 base region surrounding the L-strand origin has been determined using chemical sequencing techniques. It has also been demonstrated that a large number of mtDNA molecules contain alkali-labile sites near the origins of replication. Such sites are thought to be due to the presence of ribonucleotides incorporated into the DNA. A cluster of eight such sites, representing eight adjacent nucleotides, has been located in the L-strand at or near the L-strand origin.

The nucleotide sequence of the region containing the L-strand origin possesses the symmetry required for the formation of three hair-pin loops. The largest of these has twelve perfectly matched base pairs in the stem and a thirteen base loop. If formed by the H-strand template, this loop would contain eleven consecutive thymidine nucleotides. The sequence surrounding the L-strand origin has been found to possess a great deal of homology with several other origin sequences.

334 MAPPING OF TRANSCRIPTS AND GENES IN HeLa CELL MITOCHONDRIAL DNA, Giuseppe Attardi, Palmiro Cantatore, Stephen Crews, Christian Merkel and Deanna Ojala, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The position of the origin of replication in a physical map of HeLa cell mitochondrial DNA has been precisely localized and the nucleotide sequence of a segment that contains it, determined. The organization of the genes in the human mitochondrial genome and their mode of transcription are being investigated by a fine mapping analysis of the sequences coding for the various discrete poly(A)-containing and non-poly(A)-containing RNA species. Several approaches, involving different RNA-DNA hybridization techniques and sequencing methods, are being used in this work. Experiments of hybridization of nascent RNA chains with different restriction fragments of mitochondrial DNA have led to the identification in this DNA of a region containing a point of initiation of transcription.

335 CLONING OF HUMAN MITOCHONDRIAL DNA, Jacques Drouin and Robert H. Symons, MRC Laboratory of Molecular Biology, Cambridge, England.

In order to determine its nucleotide sequence, human mitochondrial DNA (mtDNA) purified from term placentae was cloned in E. coli using the vector pBR322. *mtDNA cleaved with HindIII,* HindIII + BamHI and EcoRI + BamHI was first ligated using T4 DNA ligase with pBR322 similarly restricted. The ligated DNA was used to transform E coli HB101 and $Amp^r tet^s$ clones were obtained. These were found to contain the 800 base pairs (bp) HindIII fragment, the 1,75kb BamHI-HindIII fragment, the 1,1kb EcoRI fragment and the 1,7kb BamHI-EcoRI fragment. MboI cuts mtDNA in 23 fragments of sizes of 2,800 to 25 bp and such a digest was ligated with BamHI-cut *pBR322.* $Amp^r tet^s$ clones obtained upon transformation were screened by agarose gel electrophoresis of colony lysates and further characterised by restriction analysis. Clones containing all but two fragments (#2 and 5) were obtained in this way but 750 $Amp^r tet^s$ clones had to be screened by colony hybridisation to find a clone containing fragment 5. None were found to contain fragment 2 and only one contained fragment 8. All other fragments were equally represented in the population of clones. Since MboI fragment 2 (2,4kb) overlaps with the previously cloned BamHI/HindIII fragments, it is not needed for complete sequencing of mtDNA. Clones representative of each Mbo fragment were shown to be complementary to mtDNA by hybridisation with Southern blots of mtDNA digests and were thereby partially mapped. Further mapping was obtained by restriction analysis of mtDNA sequentially degraded by exonuclease III. A collection of recombinant clones has thus been obtained using the mtDNA isolated from a single placenta and is now being used to obtain a complete DNA sequence of human mtDNA.

336 STRUCTURE AND REPLICATION OF MITOCHONDRIAL DNA FROM THE GENUS DROSOPHILA, David R. Wolstenholme, Judy M. Goddard and Christiane M.-R. Fauron, Department of Biology, *University of Utah, Salt Lake City, Utah 84112.*

Mitochondrial DNA (mtDNA) molecules from different species of the melanogaster group of the genus Drosophila differ in size from 15,000 to 18,700 base pairs (bp). These differences are almost completely accounted for by differences in size (940 to 5,150 bp) of a single region in each molecule which denatures at a lower specific temperature than the rest of the molecule due to an unusually high adenine + thymine (A+T) content. The sizes of the mtDNA molecules of other Drosophila species are within the narrow range 15,000 to 16,000 bp and contain an A+T-rich region of 750-950 bp. We have mapped the sites sensitive to cleavage by the restriction enzyme EcoRI relative to the A+T-rich region on the mtDNA molecules from D. virilis, D. takahashi, D. yakuba, and D. melanogaster. The A+T-rich regions of these mtDNA molecules are 950 bp, 2,100 bp, 1,400 bp and 5,150 bp respectively. All molecules examined have 3 EcoRI sites in common. Using this mapping information we have constructed a variety of heteroduplexes between mtDNA molecules of each of the four species. While extensive or complete pairing of regions of molecules outside the A+T-rich regions was found in all species combinations, A+T-rich regions from the different species completely failed to pair, suggesting that they differ extensively in their nucleotide sequences. Further, denaturation and renaturation of EcoRI and AluI restriction fragments of D. melanogaster mtDNA which contain the entire A+T-rich region, resulted mainly in perfect duplexes the length of the original restriction fragments. Molecular forms suggesting that the A+T-rich region contains *repeated sequences were not observed. We have studied the various structural forms of* partially replicated mtDNA molecules from D. melanogaster observed in the electron microscope and concluded that most molecules are replicated by a highly asymmetrical mode in which synthesis on one strand can be up to 99% complete before synthesis on the second strand is initiated. Replication of other molecules involves a more nearly symmetrical synthesis of the two complementary strands. Observations of partially replicated molecules of D. yakuba, D. takahashi and D. virilis mtDNA are consistent with there being an asymmetrical and symmetrical mode of synthesis employed in each case. Using the A+T-rich region and EcoRI cleavage sites as markers we have determined that in mtDNA of D. melanogaster, D. yakuba, D. takahashi and D. virilis replication is initiated in the A+T-rich region and proceeds unidirectionally around the molecule towards the nearest EcoRI site common to the mtDNA of all four species. In D. melanogaster mtDNA the origin of replication lies near the center of the A+T-rich region. However, in mtDNA molecules of the other three species studied the origin lies close to that end of the A+T-rich region distal to the nearest common EcoRI site.

337 ANALYSIS OF MITOCHONDRIAL DNA IN HUMAN-MOUSE HYBRID CELLS : CHARACTERISATION OF RESTRICTION DIGESTS WITH COMPLEMENTARY PROBES. S.E. Kearsey and I.W. Craig, Genetics Laboratory, Oxford.

Mitochondrial DNAs from selected human-mouse somatic cell hybrids have been examined by analysis of the DNA fragments produced by digestion with restriction enzymes. The fragments were detected, after their transfer to nitrocellulose filters, by hybridization to probes prepared by nick translation of purified, parental-type, mitochondrial DNA.

In two hybrids which retained mainly human chromosomes, sequences of human mitochondrial DNA were predominant, although some mouse sequences may have been present at low levels. Preliminary evidence suggests that both human and mouse sequences are present on the same restriction enzyme fragment of mitochondrial DNA from one of these hybrids.

Mitochondrial DNA from human and mouse cell lines carrying extrachromosomally inherited determinants conferring resistance to chloramphenicol have been examined by a similar approach.

Extrachromosomal DNA from Other Fungi and Trypanosomes

338 MITOCHONDRIAL DNA AND SENESCENCE IN PODOSPORA ANSERINA, Donald J. Cummings, University of Colorado Medical Center, Denver, Colorado 80262, and Leon Belcour and Claude Grandchamp, CNRS, Centre de Genetique Moleculaire, Gif sur Yvette, France.

Some twenty years ago, Rizet (1) and Marcou (2) reported that all races of Podospora anserina undergo vegetative death or senescence. Furthermore, this senescence is cytoplasmically inherited and appears to involve a transmissible cytoplasmic factor [Marcou and Schecroun (3); Smith and Rubenstein (4)]. More recent work with inhibitors of mitochondrial function and with cytoplasmic mutants implicate mitochondria in the senescent process [Tudzyniski and Esser (5); Belcour and Begel (6)]. Consequently, we set about to isolate mitochondrial DNA from wild-type, mutants and senescent mycelia and characterize it with respect to density in CsCl, contour length and restriction enzyme analysis.

We found that mitochondrial DNA from young wild-type mycelia had a density of 1.694 g/cm^3 and consisted of circular molecules 31 µm in length. Restriction enzyme analysis showed that each of the four races examined of Podospora anserina exhibited characteristic EcoRl fragment patterns. No apparent relationship of these patterns were noted with respect to the life span for each of the races. Five mitochondrial mutants from race s were studied and their mitochondrial DNA had the same density in CsCl as did wild-type. Electron microscopic examination showed that each of the mutants had a characteristic contour length DNA ranging from about 3 µm to 25 µm with no apparent monomer length unit. Restriction enzyme analysis with EcoRl showed that these mutants differed from wild-type in only 0 to 3 bands out of 16 fragments, suggesting that essentially the full complement of DNA was present. Analysis of the mitochondrial DNA from senescent mycelia showed dramatic differences with respect to wild-type and to mutants. First, two density species of DNA were noted, the majority of 1.694 g/cm^3 and a minority at 1.699 g/cm^3. The heavy density population consisted of a multimeric set of circular molecules ranging in size from the monomer 0.9 µm to about 15 µm. EcoRl digestion indicated that the heavy density DNA had no EcoRl sites. Digestion with HaeIII enzyme yielded one fragment of about 2600 base pairs, corresponding to the 0.9 µm contour length monomer circle described above.

These results will be discussed with respect to the rho-petite mutation in yeast (7).

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339 MITOCHONDRIAL tRNAs OF NEUROSPORA CRASSA: SEQUENCE STUDIES, GENE MAPPING AND CLONING.
U.L. RajBhandary, J. Heckman, S. Yin, B. Alzner-DeWeerd and E. Ackermann.
Dept. of Biology, M.I.T., Cambridge, MA 02139

Using simple two-step purification schemes, we have purified and sequenced four mitochondrial tRNAs: the initiator methionine, tyrosine, alanine and valine tRNAs. Interestingly, every one of these tRNAs contains unusual structural features which differ from normal tRNAs and each mitochondrial tRNA differs from the other in these features.

By Southern-hybridization of 5'-^{32}P-labeled initiator methionine tRNA to restriction digests, we have found that the mito DNA contains two gene copies for the initiator tRNA. DNA fragments corresponding to both these genes have been cloned in E. coli as PstI fragments 365 and 400 bp long and as HindIII fragments 2 and 3 Kbp long. The larger HindIII fragment also codes for alanine tRNA: the DNA corresponding to this tRNA has been separately cloned as a 195 bp long PstI fragment.

Using similar hybridizations, we have found that the gene for tyrosine tRNA maps between the small and large ribosomal RNAs. We have cloned DNA fragments from this region and have shown that both 17S rRNA and tyrosine tRNA hybridize to the same strand of the cloned DNA fragment. Thus, if both the rRNAs are synthesized as part of a single transcriptional unit, as proposed by Kuriyama and Luck, the tyrosine tRNA amy represent a transcribed spacer in the rRNA cistron.

340 STRUCTURE AND FUNCTION OF KINETOPLAST DNA, Piet Borst and Jan H.J.Hoeijmakers, Section for Medical Enzymology and Molecular Biology, Laboratory of Biochemistry, University of Amsterdam, Jan Swammerdam Institute, P.O.Box 60.000, 1005 GA Amsterdam

Functional kinetoplast DNA consists of networks of 10^4 catenated circles, mainly mini-circles varying in size between 1 kb in Trypanosoma brucei to 3 kb in Crithidia, the remainder maxi-circles varying in size from 20 kb in T. brucei to 34 kb in Crithidia. Mini-circles are micro-heterogeneous in sequence and undergo rapid sequence evolution, maxi-circles not (1).

We have hybridized total cell RNA from Crithidia with Southern blots of restriction digests of kDNA. At low input, only a 1500 bp segment of the maxi-circle binds RNA; at high input most of the maxi-circle but no mini-circle fragments hybridize, even though these are present at 100-fold excess over maxi-circle sequences (2).

The 20 kb maxi-circle of T. brucei bands at 1.682 g/cm^3 in CsCl, it contains a 6.5 kb segment only cut by AluI and MboII, but not by 20 other restriction endonucleases and this segment varies in size in different T. brucei strains. It may be AT-rich (cf. Drosophila mtDNA). Digestion of T. brucei mini-circles with TaqI gives a large number of discrete bands, which differ in different strains. This indicates that sequence heterogeneity is present but less extensive than inferred from renaturation studies (3). Electron microscopical spreads of T. brucei kDNA show doublet networks that appear to be segregating; spreading in the presence of ethidium shows that these networks contain only open circles. All forms observed fit a model (1) in which double-sized elongated networks are pinched in the middle and segregate by recombination involving open circles.

In African trypanosomes, unable to make functional mitochondria, we find all sorts of kDNA: 'normal' kDNA (1 T. brucei), 'normal' mini-circle networks without maxi-circles (1 T. equiperdum, 1 T. evansi), no kDNA at all (1 T. brucei, 2 T. evansi). Hajduk (personal communication) has found one T. equiperdum strain with 'normal' networks having maxi-circles with a 7 kb deletion, and one strain containing only maxi-circle-type molecules. In the two strains without maxi-circles we find no sequence heterogeneity in the mini-circles.

We conclude that the maxi-circles are the equivalent of mtDNA in other organisms, that the ribosomal RNAs of trypanosome mitochondria are very small, that mini-circles are not transcribed and that recombination between mini-circles and maxi-circles may contribute to mini-circle sequence heterogeneity.

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341 REPLICATION AND TRANSCRIPTION OF KINETOPLAST DNA, Larry Simpson, A.M. Simpson, H. Masuda and H. Rosenblatt, Biology Dept., University of California, Los Angeles, CA 90024.

The kinetoplast DNA (KDNA) of the hemoflagellate protozoa consists of a single large network of thousands of catenated minicircles and a smaller number of catenated larger maxicircles. Replication of the KDNA is limited to the nuclear S phase. Pulse-labeling of replicating network DNA showed that newly replicated molecules are localized at two peripheral loci and later somehow become dispersed throughout the network (1). Minicircles replicate once during the S phase (1). Replicated minicircles are nicked and covalent closure occurs at the end of S or in G2 (2). Completely replicated networks contain exclusively open circular molecules (3).

The maxicircle DNA seems to represent the analogue of the mitochondrial DNA found in other eukaryotes. Transcription of the two major stable kinetoplast RNA species (9 and 12 S RNAs) from the maxicircle DNA has been demonstrated (4). No minicircle transcript has yet been identified. Maxicircle DNA has been isolated by release from the network by cleavage with a restriction enzyme and separation on a buoyant density basis in the presence of the dye, Hoechst 33258. In general the maxicircle DNA is higher in %AT than the minicircle DNA. A restriction map of the maxicircle of Leishmania tarentolae has been constructed and the 9 and 12 S RNA genes localized within a small fragment bounded by a Hind III site and a Hpa II site. Several maxicircle fragments of L. tarentolae have been cloned into the bacterial plasmid, pBR322, including a 4.4 X 10 dalton Eco RI-Bam HI fragment containing the 9 and 12S RNA genes. An intramolecular base ratio heterogeneity was found, ranging from 74% AT to 85% AT. Unit length minicircles cleaved at the Hind III or Bam HI loci were also cloned in pBR322. A restriction map of one class of minicircles has been constructed. Mapping and sequencing of the several minicircle sequence classes is in progress.

Similar studies have been performed on the KDNA of the related species, Trypanosoma brucei, which undergoes a cyclical regression and biogenesis of the mitochondrion during the life cycle. Cloning of KDNA fragments in pBR322 has been carried out. The cloned fragments are being used as hybridization probes to examine the activation of the mitochondrial genome during the life cycle.

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Evolution and Protist and Chloroplast Extrachromosomal DNA

342 THE IDENTITY OF THE PROTEINS ASSOCIATED WITH CYTOCHROME b IN YEAST. Diana S. Beattie, Liviu Clejan, Yu-Shiaw Chen, Mount Sinai School of Medicine, New York, N.Y. 10029.

Two unidentified proteins with molecular weights ranging from 45,000 to 60,000 are present in immunoprecipitates formed when labeled yeast mitochondria are incubated with the specific antiserum against yeast cytochrome b (Lin,Clejan and Beattie, Eur.J.Biochem. 87, 171, 1978). These proteins are products of mitochondrial protein synthesis as they are labeled in the presence of cycloheximide but not chloramphenicol and are absent in petites. They are not identical with the core proteins of a purified complex III nor do they copurify with cytochrome b. Results obtained when the time course of labeling was varied from 3 to 30 min followed by a chase indicated that these proteins are not precursors of cytochrome b. By contrast, labeling of cytochrome b and a protein of 50,000 daltons reached a maximum after 3 min and then decreased. Furthermore, after translation of poly(A)-containing RNA from yeast mitochondria in a wheat germ system, a product of 31,000 daltons as well as a product of 50,000 daltons is present in the immunoprecipitates formed both directly and indirectly. Currently, peptide mapping after limited proteolysis is underway to determine whether these proteins represent cytochrome b migrating in gels as a dimer or as its true molecular weight as calculated from Ferguson plots or whether they are completely different proteins.

343 RANDOM DRIFT OF GENE FREQUENCIES IN INTRACELLULAR POPULATIONS OF ORGANELLE GENES C. William Birky, Jr., Dept. of Genetics, The Ohio State University, Columbus 43210

Crosses involving mitochondrial genes in yeast, or chloroplast genes in Chlamydomonas or the geranium, produce both uniparental and biparental zygotes (UPZ and BPZ). Different BPZ from a single cross produce very different proportions of progeny cells homozygous for alleles from the "paternal" parent. This can be seen by plotting frequencies of zygotes versus gene frequencies in their progeny. In yeast, such distributions are often U- or L-shaped, with nearly uniform distributions of gene frequencies among the BPZ and a single very broad mode or none at all (Birky 1975 Molec. Gen. Genet. 141:41). This observation has been extended to Chlamydomonas (Birky, Van Winkle-Swift, Sears, Shepherd, Boynton, & Gillham) and geraniums (Birky and Tilney-Bassett). Delaying cell division of the Chlamydomonas zygote causes some BPZ to become UPZ (Van Winkle-Swift 1978 Nature 275:749; Sears et al. 1977 Genetics 86:s57). Yeast zygotes held in water also show an increase in UPZ and in variance of gene frequencies. These observations strongly indicate that all three organelle gene systems are intracellular population systems, genetically as well as physically. They further suggest a strong role for repeated stochastic events which modify gene frequencies inside individual zygotes and their progeny, analogous to random drift in Mendelian population genetics. Such stochastic events might include gene conversion, which is repeated and random in yeast mitochondria; replication of DNA molecules, which is random in mouse cell mitochondria and some plasmids; or degradation of DNA molecules. (Supported by NIH GM19607)

344 CONSERVATION OF THE CHLOROPLAST GENOME: PRELIMINARY EVIDENCE FOR CONSERVED, REPEATED SEQUENCES, Gayle K. Lamppa, University of Washington, Seattle, Wa 98195

The extent of sequence conservation of the chloroplast genome was investigated. Reassociation of labeled pea chloroplast (ct) DNA with a 5800-fold excess of total corn DNA indicates that 30% (S1 assay) to 45% (HAP assay) of the pea chloroplast genome is conserved in corn. Similar analyses were performed for other higher plants including broad bean, watermelon and barley. Studies to locate the conserved sequences in pea and corn ct DNAs have been made using Southern blots of restriction enzyme fragments separated by agarose gel electrophoresis. 32P-pea ct DNA hybridizes to 12/25 Eco RI fragments of corn ct DNA. 32P-corn ct DNA is homologous to sequences in 13/28 pea ct DNA RI fragments. An estimate of the total amount of DNA homologous between genomes is 40 X 10^6 d, although this is considered an upper limit since the percentage of conserved sequences in each fragment is as yet unknown. The RI digestion pattern of Chlamydomonas ct DNA shows two major bands of homology with 32P-pea ct DNA. P22 DNA does not hybridize. Bam HI patterns were also analyzed. Individual corn fragments, Bam HI 4, 7 and 9 show preferential hybridization to different, single fragments of the pea genome and hybridize to as many as seven others. Bam HI 4, 7 and 9 also hybridize to other fragments of corn ct DNA although the degree of hybridization varies. Thermal denaturation of the hybrids formed between Bam HI 9 and other fragments from corn indicate sequence fidelity. This suggests there are repeated sequences in corn ct DNA. Work is being performed to determine their nature, location and conservation between species.

345 RESTRICTION ANALYSIS OF MT-DNA FROM OENOTHERA BERTERIANA
Axel Brennicke, Institut für Biologie I, D-7400 Tübingen

Oenothera berteriana is one of the few higher plant species, for which extrachromosomal inheritance has been shown through crossbreeding experiments and seems a likely object for extrachromosomal molecular biology in higher plants. To obtain homogenous material tissue cultures were established. Mitochondrial DNA was isolated from DNAse treated mitochondria by centrifuging into a CsCl cushion and further puriefied in a CsCl/EtBr gradient. Length of circular molecules was determined by electron microscopy to be $60\pm5 \times 10^6$ dalton. Restriction fragments were obtained with Eco RI, Bam HI and Sal I. Between 30 and 40 fragments could be separated and measured on agarose and acrylamide gels of various concentrations. The added lengths of these fragments amounted in all cases to $100\pm10 \times 10^6$ dalton. These data seem to indicate at least two partly different mt DNA populations in Oenothera. Current experiments are designed to study the distribution and inheritance mechanisms of these mt DNA's.

346 CHLOROPLAST 30S RIBOSOMAL PROTEINS ENCIDED IN CHLOROPLAST DNA OF C. REINHARDI, Stefan Surzycki and Francis T. Chandler, Indiana University, Bloomington, Indiana 47402

Informational content of chloroplast DNA of C. reinhardi was studied using antibodies against the small subunit proteins of chloroplast ribosomes and an in vitro wheat germ protein synthesis system. Two independent methods were used (1) the HART (hybrid-arrested cell-free translation; B.M. Peterson et al. PNAS 74:4370) and,(2) the cell-free linked transcription-translation method (S.Rozenblatt et al. PNAS 73:2747). In the Hart experiments, RNA from the cells was isolated and hybridized to chloroplast DNA in a concentration of formamide that favor formation of DNA.RNA hybrids but essentially prevents DNA.RNA reannealing. DNA.RNA hybrids are not translated in eucariotic cell-free system, while heat dissociation of the hybrids reinstates complete translational activity. The synthesized in vitro proteins were twice precipitated with antibody and pansorbin and analyzed on SDS gradient gels. Three proteins disappeared from the hybridized reactions which were not heated, and were present in heated reactions. This indicated that these proteins are encoded in chloroplast DNA. The same results were obtained in the cell-free linked transcription-translation system when E. coli RNA polymerase was used for transcription of chloroplast DNA, followed by translation using the wheat germ system. The experiments were carried out using HART and linked systems with separated EcoRl fragments of chloroplast DNA to establish the precise localization of these genes. Preliminary experiments indicate that the genes for chloroplast ribosomal proteins in question are not located on the fragments containing chloroplast ribosomal RNA genes.

347 CHLOROPLAST GENE EXPRESSION IN CHLAMYDOMONAS REINHARDI, Yoshihiro Matsuda and Stefan Surzycki, Indiana University, Bloomington, Indiana 47402

The transcription of chloroplast DNA during the cell cycle of synchronously growing Chlamydomonas strain CW15and regreening of yellow mutant Y-1 were examined. The cells were pulse labeled with ^{32}P-orthophosphate at different times during the cell cycle or development of chloroplast. A total RNA was isolated and hybridized to Southern blots of EcoRl or Bam fragments of chloroplast DNA. The results indicate that there are at least three classes of DNA fragments with respect to their transcription (1) a fragments which are transcribed continuously throughout the cell cycle and chloroplast development, (2) a fragments for which expression varies during cell growth and/or regreening, and (3) a fragments which are not transcribed at all at any stage of cell cycle or chloroplast development (EcoRl fragments of MW 2.14,1.9, 1.2×10^6). The physical map of EcoRl fragments of chloroplast DNA (Rochaix,1978), indicate that these untranscribed fragments are located in a cluster near the end of the one of two 16S rRNA genes. The rate of total RNA synthesis during the cell cycle was also measured. The results indicate that total RNA was synthesized at different rates during the cell cycle with maximum synthesis between 4 and 6 hr. of light. The exhaustion hybridization experiments with excess chloroplast DNA showed that chloroplast RNA synthesis was c.a 2.5 percent of total RNA synthesis in the cells. This indicates that chloroplast RNA synthesis is proportional to the total RNA synthesis. Therefore maximum rate of chloroplast RNA synthesis also occurred at 4 to 6 hr. of light. The synthesis of chloroplast ribosomal RNA however at this time is relatively decreased as indicated by Southern blot hybridization.

348 CHARACTERIZATION OF THE CHLOROPLAST GENOME OF THE MARINE ALGA, OLISTHODISCUS LUTEUS, Jane Aldrich and Rose Ann Cattolico, University of Washington, Seattle, WA. 98195. The chloroplast genomes of higher plants have been shown to be circular molecules of approximately 90 million daltons M. Wt. To date, only green algal chloroplast DNA (ctDNA) has been studied with regard to size and the M.Wt.'s range from 90-200 million daltons dependent upon the organism. Preliminary studies with the wall-less unicellular non-green alga , Olisthodiscus luteus, an organism whose chloroplast biogenesis is under intense investigation, indicate a chloroplast genome size smaller than any other so far examined. We have isolated chloroplast DNA from intact chloroplasts of O. luteus. Cell homogenates were DNAse treated to remove nuclear DNA (nDNA) and the DNAse resistant chloroplast pellets obtained following centrifugation of the homogenate were repeatedly washed to eliminate mitochondrial contamination. DNA was extracted from the chloroplasts and was fractionated by CsCl-ethidium bromide gradient centrifugation. The single band obtained characteristically contained 75% ctDNA (buoyant density 1.691) and 25% nDNA (buoyant density 1.702) and consisted of short linear DNA and open circular DNA. The open circles had avg. contour lengths of 11, 22 and 44 um corresponding to M. Wt. of 22, 44 and 88 million daltons with the 22 million dalton circle size being the largest size class. Recently, isolation of ctDNA free of nDNA contamination has been achieved by use of Hoechst dye - CsCl gradient centrifugation. Preliminary restriction enzyme analysis with this DNA indicates a genome size of at most 60 million daltons M. Wt. Denaturation mapping will be employed to assess relatedness among circles. The smaller chloroplast genome size of O. luteus may relate to the evolutionarily primitive position of this organism.

349 EVIDENCE FOR A DNA GYRASE IN RAT LIVER MITOCHONDRIA, Frank J. Castora and Melvin V. Simpson, S.U.N.Y. at Stony Brook, Stony Brook, N.Y. 11794
DNA gyrase is capable of introducing superhelical turns into DNA and is required in several microbial systems which replicate negatively supercoiled closed circular DNA. Since isolated mtDNA possesses these properties, we have sought evidence for the involvement of this enzyme in mtDNA replication employing an in vitro mtDNA replication system developed in our laboratory (J. Biol. Chem., 248,1912 (1973)). The incorporation of (^{3}H)dATP into mtDNA by isolated mitochondria was studied in the presence of known E. coli gyrase inhibitors, namely novobiocin, coumermycin, nalidixic acid and oxolinic acid. All showed concentration-dependent inhibition of incorporation, and 50% inhibition was attained at concentrations which give equivalent inhibition of DNA replication in the E. coli system. Analysis of the mtDNA species separated upon sucrose gradient centrifugation showed a greater inhibition of synthesis of closed than of open circular mtDNA and these results were supported by the analysis of upper and lower band mtDNA after CsCl-ethidium bromide centrifugation. These results are consistent with the involvement of the DNA gyrase in mtDNA replication. Sucrose gradient analysis of mtDNA labeled as above and subsequently denatured to liberate the 7S initiator strand shows that the above inhibitors depress dATP incorporation into high molecular weight mtDNA more than three times as strongly as into 7S DNA, suggesting that the site of action of the inhibitors, and therefore of the presumed gyrase, may be on the elongation of the 7S strand. Supported by NIH(GM22333), American Cancer Soc.(NP87J), and an NRSA (GM06873) to F.J.C.

350 CHROMATIN STRUCTURE OF AN EXTRACHROMOSOMAL GENE, Thomas Cech, University of Colorado, Boulder, Colo. 80309, and Kathleen Karrer, Indiana University, Bloomington, In. 47401.

The chromatin structure of the amplified, extrachromosomal rRNA genes of Tetrahymena thermophila has been studied by photochemical crosslinking of the rDNA in vivo with trimethylpsoralen. Living cells were treated with psoralen plus 360nm radiation for 40 sec. The DNA was then purified and the rDNA separated from the bulk DNA. The locations of psoralen crosslinks were determined by electron microscopy of the DNA under totally denaturing conditions. The non-ribosomal DNA showed crosslinks separated by intervals of 200 and 400 base pairs, a pattern that is consistent with a nucleosome structure for the chromatin. Each functionally-defined region of the rDNA showed a distinctive crosslinking pattern. The terminal spacer regions had crosslinks spaced at intervals of about 220 and 440 base pairs, which could represent a nucleosome structure. The central spacer region was highly protected from psoralen crosslinking in vivo. In the transcribed regions, the distribution of distances between adjacent crosslinks showed peaks at 210, 335, and 460 base pairs. The crosslinking patterns observed upon in vitro photoreaction of deproteinized rDNA eliminated the possibility that the in vivo crosslinking patterns were simply a reflection of the DNA base sequence. We conclude that the in vivo chromatin structure of the rDNA does not closely resemble that of the bulk chromatin, and that there are significant differences between the structures of transcribed and nontranscribed regions.

351 RESTRICTION ENDONUCLEASES USED TO MEASURE MITOCHONDRIAL DNA SEQUENCE DIVERSITY IN NATURAL POPULATIONS, Robert A. Lansman, John C. Avise and Rosemary Shade, University of Georgia, Athens, Ga., 30602.

Sequence heterogeneity in mitochondrial DNA (mtDNA) can be easily detected by examining the sizes of fragments produced by cleavage with restriction endonucleases. We have used six enzymes to digest a large number of mtDNA samples from natural populations of two rodent genera, Peromyscus and Geomys. Individuals collected within a single geographic locale show less than 0.5% sequence diversity while those collected from populations separated by 50-500 miles differ by approximately 1.5%. Two sibling species within the genus Peromyscus have a 13% sequence divergence while non-sibling species differ by more than 20%. These data suggest that the rate of mtDNA sequence divergence may be greater than that of nuclear DNA and that restriction analysis of mtDNA may become the most sensitive technique yet available for reconstructing evolutionary relationships between conspecific populations.

The usefulness of the technique is demonstrated by a comparison of mtDNA sequences in Geomys colonus and an "endangered species" Geomys pinetus. Since restriction analysis does not detect any differences between G. pinetus and G. colonus from the same locale and sequences differences between geographically-separate populations of G. colonus are easily detectable, we believe the species status of G. pinetus should be reconsidered. (Supported by N.I.H. Grant GM23246).

352 ORGANIZATION AND EVOLUTION OF RIBOSOMAL DNA IN PARAMECIUM, R. Craig Findly* and Joseph G. Gall, Department of Biology, Yale University, New Haven, Conn. and *Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

The genes coding for ribosomal RNA (rDNA) in the macronucleus of Paramecium tetraurelia exist as relatively small, extrachromosomal molecules with both linear and circular forms. Electron microscopy and restriction enzyme analysis revealed that the rDNA is arranged as nonpalindromic, tandem repeats with an average repeat size of 5.5×10^6 daltons (Findly & Gall, PNAS 75, 3312 [1978]). To study the evolution of rDNA in P. tetraurelia, we compared the organization of rDNA in seven allopatric stocks, which were originally collected on five continents. Several of the stocks have probably been genetically separated since the late Mesozoic. Whole cell DNA from each stock was digested with EcoRl or Hind III and after electrophoresis in agarose gells the DNA was eluted onto nitrocellulose filters and hybridized with ^{32}P-cRNA made from purified rDNA, or with ^{32}P-rRNA. The cRNA hybridizations revealed that the rDNA from these stocks are very similar in restriction pattern and possibly in sequence, although limited heterogeneity in the size of fragments containing spacer sequences was observed. These results indicate that the rDNA repeat, including spacer, has been conserved during the evolution of these stocks. It is suggested that in some species selection pressure may act to conserve the structure of spacers of tandemly repeated rDNA genes. The conservation may be related to the number of rDNA copies in the germinal nucleus.

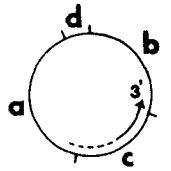
353 ORGANELLE BIOGENESIS IN A MARINE CHLOROMONAD, R.A. Cattolico, University of Washington, Seattle, WA. 98195.

The primary purpose of my work and that of my students is to analyze the plasticity, contribution and interaction of the "cell cycle" and "organelle cycle" during chloroplast biogenesis. Olisthodiscus luteus, a member of the Chloromonadaceae which is considered to be a primitive algal division, has been used in our studies. This multiplastidic organism has a number of unique experimental assets: both cell and chloroplast division occur as separate and discrete events during synchronous growth, chloroplast number per cell can be varied by manipulation of environmental conditions, the small discoidal chloroplasts can be counted by direct microscopic observations (thus allowing stringent statistical criteria to be applied to population analysis), and the cell is naturally wall-less, which facilitates organelle isolation. We have correlated genome and plastome ploidy levels using reassociation kinetic data, demonstrated a variability in ctDNA levels which is dependent upon organelle complement, and have shown that this organism contains a covalently closed circular ctDNA which is probably significantly different from that observed in all other plant species studied to date (see Aldrich and Cattolico abstract). Parasextual fusion experiments, ctDNA restriction mapping, cycle dependent induction of chloroplast division response, and cDNA replication are research areas in which we are actively engaged. I welcome the opportunity of this symposium to share our most recent studies with this specialized group of colleagues.

354 INHERITANCE OF CHLOROPLAST DNA SEQUENCES IN INTERSPECIFIC HYBRIDS OF CHLAMYDOMONAS, Laurens J. Mets, Department of Biology, Case Western Reserve University, Cleveland, Ohio, 44106.

Chloroplast DNA (cpDNA) from Chlamydomonas eugametos and C. moewusii are sufficiently different in sequence that they give distinguishable digestion patterns with a number of restriction endonucleases. These two species can mate to produce fertile hybrid zygotes, and the restriction pattern differences have been used as markers for following the inheritance of cpDNA sequences. In complete tetrads germinated from hybrid zygotes, cpDNA is inherited in a non-Mendelian pattern which correlates with the inheritance of a Uniparental genetic marker present in the same cross. These results demonstrate that cpDNA could carry the Uniparental genome of these species, but other extrachromosomal DNA's are not excluded as possible carriers.

355 EXPRESSION OF CAULIFLOWER MOSAIC VIRUS DNA IN PLANT CELLS, Joan T. Odell and Stephen H. Howell, Biology Department, Univ. Calif. San Diego, La Jolla, Calif. 92093.



We are studying the transcription and translation of the cauliflower mosaic virus (CaMV) genome which may be important to the use of this virus as a vehicle for carrying foreign DNA into plant cells. We are examining a region of the viral genome, apparently dispensible to the multiplication of the virus, into which foreign DNA might be inserted and extensively expressed in the infected plant cell. An abundant poly A+ RNA found in the CaMV (Cabb B-J.I.) infected plant is largely derived from the $EcoR_1$ fragment c of the CaMV genome. ^{32}P-labeled cDNA produced from infected cell poly A+ RNA hybridizes with CaMV R_1 fragments c and b. When the cDNA is size fractionated, the small cDNA pieces (<0.3 kb) hybridize almost exclusively to b, suggesting that poly A+ RNA extends from c to its 3' terminus in b.

A new protein, p66, produced in great abundance in infected leaves, but unrelated to the viral coat protein, may be encoded in the same region of the CaMV genome. We have shown this by hybrid-arrested translation (Paterson et al., PNAS 74, 4370, 1977) in which the in vitro translation of p66 from infected plant poly A+ RNA is suppressed by hybridization to DNA from plasmid pLW111, which contains fragment b, c and d inserted into pMB9. Further evidence suggesting that p66 is derived from fragment c was obtained by analyzing plants infected with CM4-184, a non aphid-transmitted isolate in which 5% of the genome is deleted in fragment c (Hull and Howell Virology 86, 482, 1978). Plants infected with CM4-184 do not produce p66.

356 IN VITRO SYNTHESIS AND PROCESSING OF RNA 'S SYNTHESIZED FROM A PLASMID CONTAINING THE CHLOROPLAST RIBOSOMAL DNA OF ZEA MAYS, Gerhard C. Gross and John J. Dunn; Brookhaven National Laboratory, Dept. of Biology, Upton N.Y. 11973

RNase III of E.Coli was originally described as an enzyme that nonspecifically degrades double-stranded RNA, but its physiological role appears to be as an RNA processing enzyme, which cuts specifically primary transcripts for ribosomal RNAs, some tRNAs, and perhaps other RNAs. Since it is widely believed that chloroplasts are of prokaryotic origin it was of interest wether chloroplast rRNAs are processed by an enzyme similar to RNase III. When the DNA of the plasmid pZm 134 which contains the chloroplast rDNA (Bedbrook et al. Cell 11, 739 (1977)) is transcribed in vitro with E.Coli RNA polymerase two major RNA products of about 45S and 50S are produced. The 50S RNA contains chloroplast rRNA sequences as judged by competition hybridisation with isolated chloroplast rRNA. When the 45S and 50S RNAs are incubated with RNase III under conditions known to promote a cleavage of high fidelity (Dunn J., J.Biol.Chem. 251, 3807 (1976)) the 45 S species is not cleaved but the 50S species is cut into three major fragments of approximately 23S, 17S and 14 S. Studies wether these cleavage products are related to in vivo chloroplast rRNA of Zea Mays are under way.

357 FRACTIONATION OF THE CHICKEN GENOME INTO DNA SEGMENTS DEPLETED AND ENRICHED IN GLOBIN GENE SEQUENCES, Carol Mirell and Isaac Bekhor, University of Southern California, Los Angeles, Ca. 90007

Chromatins from mouse Ehrlich ascites tumor cells, chicken reticulocytes, and chicken liver have been purified and subsequently extracted with 2 M NaCl. The pellets remaining after high-speed centrifugation consisted of essentially two components, protein-free DNA and protein-bound DNA. The protein-DNA complexes (DNA-P) were then separated from the protein-free DNA (DNA-S) by low-speed centrifugation. The DNA-P was determined to be free of histones but still showed some resistance to micrococcal nuclease digestion when compared to total DNA or DNA-S; however continued digestion of DNA-P did result in complete degradation. Digestion of the fractionated DNA's with restriction enzymes Eco RI and Hae III revealed some differences between electrophoretic migrations of repeating sequences in the DNA-S and DNA-P obtained from Ehrlich ascites cells. Eco RI digestion revealed no differences in the two fractions from chicken reticulocytes; digestion with other restriction enzymes resulted in the absence of detectable banding. Reticulocyte and liver chromatins were fractionated into DNA-S and DNA-P; these two fractions as well as whole chromatin were deproteinized, sheared, and hybridized to a globin cDNA probe. The $Cot_{\frac{1}{2}}$ values suggest that in reticulocytes virtually all of the globin gene sequences are present in DNA-P, while DNA-S shows negligible hybridization to globin cDNA. In liver the reassociation of the cDNA probe to DNA-S was essentially the same as for total DNA, while the DNA-P showed negligible reassociation. The data suggests that the proteins bound to DNA after 2 M NaCl extraction may act as regulatory molecules.

358 PURIFICATION OF PAL I AND ITS PROPERTIES, Krishna Baksi and George W. Rushizky, National Institutes of Health, Bethesda, MD 20014

Cibacron Blue F3GA, a polyaromatic dye covalently cross-linked to agarose, was recently shown to afford a rapid, single-step purification of four different restriction endonucleases (Baksi et al., Biochemistry 17, 4136, 1978). The enzymes so obtained were free of contaminating nucleic acids and other nucleases, and yielded fragments that could be ligated. Pal I was found in a very large amount, ranging from 128,000- 190,000 units per gram of frozen cells. Chromatography on Cibacron Blue, Heparin agarose and Sephacryl S-200 was employed to purify Pal I 1650-fold with a yield of 33%. The enzyme moved as a single band on polyacrylamide gel electrophoresis, and had a mol. wt. of 30,000 upon SDS-polyacrylamide gel electrophoresis and of 32,000 upon gel filtration on Sephacryl S-200. Other properties of Pal I and applications of the blue dye procedure to the purification of other restriction enzymes will be presented.

359 CLUSTERING OF TRANSFER RNA GENES IN TETRAHYMENA THERMOPHILA, Richard Jefferson, Eduardo Orias, and John Carbon, University of California, Santa Barbara, CA 93106.

A preparation of hybrid plasmids generated by insertion of Hind III digested Tetrahymena DNA into the Hind III site of pBR 322 was used to transform E. coli. Two thousand of the resulting clones were screened by colony hybridization for sequences complementary to Tetrahymena ^{32}P labeled 4S RNA; 12 positives were detected by autoradiography. Considering the complexity of the Tetrahymena genome (~1.5×10^{11}d) and the repetition frequency of tRNA genes (~1000/haploid genome) and assuming that the cloning process does indeed give a random sampling of the Tetrahymena genome, this proportion of positive clones is much lower than would be expected for sequences dispersed throughout the genome. Upon analysis of plasmid DNA isolated from each of the 12 candidates, it was found that 9 of the hybrid plasmids contained either one or both Hind III fragments of a doublet of 2300 and 2134 bp. Four of these clones contained the doublet together in the same absolute orientation, as determined by restriction endonuclease cleavage mapping. Seven of the 9 clones also contained additional Tetrahymena Hind III fragments. Further analysis of the clones by Southern gel hybridizations has shown a minimum of 8 tRNA genes included within the DNA of the Hind III doublet, and possibly many more. Thus far, every region of the doublet accessible to analysis by Southern hybridizations has been found to contain tRNA genes. Detailed restriction mapping with 12 restriction endonucleases has failed to show any regular spacing of the kind associated with the 5S RNA gene clusters of many organisms, including Tetrahymena. These results indicate that the tRNA genes in Tetrahymena are highly clustered within a discrete repeating unit of at least 4.1 kb, but with little, if any, of the lower order regularity often associated with gene clusters.

360 RAPID EVOLUTION OF MITOCHONDRIAL DNA, Matthew George, Jr., Wesley M. Brown, and Allan C. Wilson, University of California, Berkeley, CA 94720.

The mitochondrial DNA of five primate species (human, green monkey, rhesus, baboon, and langur) was digested with each of eleven restriction endonucleases. The digests were analyzed by agarose gel electrophoresis and electron microscopy, and cleavage maps were prepared for each of the five species. With a mathematical approach similar to Upholt's (1977, Nucleic Acids Research, 4, 1257), we calculated s, the fraction of shared restriction sites, and p, percent sequence difference among the five DNAs. The results indicate that mitochondrial DNA has been evolving about 10 times faster than single-copy nuclear DNA. Rate analysis indicates that about 80% of the sites evolve very rapidly, while approximately 20% of the sites evolve at a much slower rate. This is inferred from the non-linearity of the relationship between p and divergence time. Several of the conserved sites are located in a portion of the genome that does not code for ribosomal RNA, and their evolutionary persistence may indicate that a functionally important sequence is located in this region. The generally high rate of mitochondrial DNA evolution could be due either to a high rate of mutation or to a high rate of fixation. Regardless of the reason for its rapid evolution, mitochondrial DNA may prove to be an extremely useful tool for probing relationships among species that diverged within the past 10 million years.

361 EVIDENCE FOR A DISTINCT DNA TOPOISOMERASE IN MITOCHONDRIA. F.R. Fairfield, W.R. Bauer, and M.V. Simpson, SUNY, Stony Brook, New York 11794.

The replication and transcription of closed circular DNA's, such as mtDNA, requires a swivel which could be produced by a nicking-closing enzyme (topoisomerase). We have detected (Fed. Proc., 35, 1595 (1976)) and have now purified 3000-fold such an enzyme from rat liver mitochondria. The enzyme is not yet homogenous. However, its specific activity at this point is roughly 1/4 that reported for the most active nicking-closing enzyme,M.Luteus type I topoisomerase. It is, in common with several other such enzymes, sensitive to NEM. The principal evidence that the enzyme is mitochondrial and distinct from the rat liver nuclear enzyme, isolated by Champoux and in this laboratory, is as follows: (a) Well-washed inner membrane-matrix preparations rather than intact mitochondria have been used as the enzyme source, thus reducing the possibility of adventitious contamination. (b) The mitochondrial enzyme is at least 200-fold more sensitive to inhibition by ethidium bromide than is the nuclear enzyme, a situation which parallels the sensitivity to this agent of the mitochondrial and nuclear DNA polymerases. (c) The two enzymes show a marked differential sensitivity to the trypanosomal drug berenil, which is known to inhibit mtDNA replication in cultured human cells and to increase the proportion of the relaxed form of mtDNA. Thus far, berenil is the only specific inhibitor found for a nicking-closing enzyme and it may prove useful in elucidating the role of this enzyme in the intact cell. (Supported by NIH grants GM22333 (to MVS) and GM21175 (to WRB) and Amer. Cancer Soc. grant NP87J (to MVS)).

Sequence Analysis and Viral Replication and Function

362 STRUCTURE AND EXPRESSION OF MITOCHONDRIAL DNA IN RESPONSE TO ONCOGENIC VIRUS TRANSFORMATION AND DRUGS, Margit M. K. Nass, Univ. of Penna. Sch. of Med., Phila., Pa. 19104

The question of whether a gene alteration in mtDNA is essential for expressing malignancy has been tested using induction of malignancy by oncogenic viruses as a model. MtDNA was isolated from over 20 cell types of hamster, avian and reptilian origin. Cells were transformed either in vivo or in vitro by polyoma virus, SV40, adenovirus, RSV, or spontaneously. Also used were cells resistant to ethidium, harboring intramitochondrial virions, treated with N-methyl-N'nitro-N-nitrosoguanidine or with methylglyoxal-bis(guanylhydrazone), a potent antimitochondrial drug. Restriction cleavage patterns were compared using Hae III, Hpa I, Hpa II, Hind III, Hind II, Bam HI, Eco RI and Pst I. No differences were detected between any mtDNAs within the same animal type. Some control and transformed cells were further compared by analysis of alkaline denaturation and psoralen-crosslinking sites, S_1 endonuclease sensitive sites in heteroduplex molecules, and D-loop structure. Only minor differences were noted. The mobilities of mt 12S and 16S rRNA in native and denaturing gels did not differ in control and transformed cells. Differences were found with respect to mtDNA-protein association, mtDNA and RNA synthesis and methylation, properties of isolated mtDNA polymerase and methylase, and sensitivity to transcriptional and other inhibitors. It is concluded (1) that no major deletions, insertions or base substitutions are present in mtDNA of transformed cells under the conditions tested, (2) the results do not rule out the possible presence of a minor fraction of very small but potentially significant base changes, (3) the multiple alterations in mitochondrial biogenesis patterns observed here and earlier suggest complex interactions between nuclear and mitochondrial genetic systems in transformation. Supported by NIH and NSF.

363 CHROMOSOMAL LOCATION OF BACTERIOPHAGE MU DNA REPLICAS, Martin L. Pato, Nancy Tyler and Barbara T. Waggoner, National Jewish Hospital and Research Center, Denver, CO 80206

Bacteriophage Mu is novel in that its DNA can be integrated essentially at random into the host chromosome and integration events occur throughout the lytic cycle. We are trying to quantitate the extent of integration and to determine the location of Mu DNA replicas after induction of a lysogen. Most, if not all, of the replicas cosediment with isolated bacterial nucleoids for at least 20 min after induction. The Mu DNA replicas are not released from the nucleoids after treatment with SDS and heating to 70° C, suggesting that they are covalently integrated into the host chromosome from which they are packaged into mature virus particles.

364 THE METHYLATION OF HERPESVIRUS SAIMIRI DNA IN CULTURED TUMOR CELLS, R. Desrosiers, C. Mulder, C. Kaschka-Dierich and B. Fleckenstein, N.E. Regional Primate Research Ctr., Harvard Medical School, Southboro, MA. 01772 and Institut fur Klinische Virologie, Erlangen, West Germany.

Herpesvirus saimiri (H. saimiri) causes lymphoma and death in several species of New World monkeys and a number of continuous lymphoid cell lines have been established from H. saimiri induced tumors. The virion DNA of H. saimiri ($\sim$100 X 10^6 daltons) consists of a unique region of 71 X 10^6 daltons (L-DNA 36% G+C) positioned between a variable number of units of repetitive DNA at each end (H-DNA 71% G+C). At least a portion of the viral DNA in the non-producer cell line 1670 is present as covalently closed circular episomal DNA. The use of restriction enzyme digestion, Southern transfer and hybridization of the viral specific DNA has produced evidence that H-sequences present in total 1670 DNA and in isolated episomes are extensively methylated. MspI (recognition sequence CCGG) cleaves H-sequences in 1670 DNA identical to virion DNA. HpaII has the same recognition sequence as MspI but unlike MspI it fails to cleave when the C of the CG dinucleotide is methylated. H-sequences of 1670 DNA are refractory to cleavage by HpaII; less than 10% of the HpaII cleaved H-DNA is the same size as the MspI cleaved H-DNA. Similarly, H-sequences of 1670 DNA are refractory to cleavage by SmaI (CCCGGG) and SacII (CCGCGG) but not SacI, PvuII or PstI which lack the dinucleotide CG in their recognition sequence. Methylation of mammalian DNA is known to occur exclusively at C residues in the dinucleotide CG. Analysis of total cell DNA of two virus producing lymphoid lines revealed no evidence of methylation of viral H-sequences.

365 SEQUENCE AT 5'-TERMINUS OF SPRING VIREMIA OF CARP VIRUS MRNA SYNTHESIZED IN VITRO K.C. Gupta and Polly Roy, Department of Microbiology, University of Alabama Medical Center, Birmingham, Alabama 35294.

Sequence analyses of the 5'-termini of RNA species synthesized in vitro by spring viremia of carp (SVCV) virion associated trascriptase have been determined. The SVCV in vitro transcription process, unlike that of VSV is stimulated by the presence of S-adenosyl-L-methionine (SAM) in reaction mixture. In vitro transcripts were synthesized in the presence or absence SAM. 5'-Terminus structures were analyzed by DEAE cellulose column chromatography, paper electrophoresis and paper chromatography. Our results suggest that there is a basic capped structure at the 5'-terminus, GpppApAp. This structure can be modified by methylations at the 2-O-ribose of the penultimate adenosine and at the 7 position of guanosine. In contrast to many other viral and eukaryotic mRNAs, the penultimate adenosine is methylated prior to methylation of guanosine. Three 5'-termini have been recognized: GpppApAp..., GpppAmpAp..., and 7mGpppAmpAp. In the presence of SAM an increased amount of 7mGpppAmpAp is synthesized. No evidence for internal methylation was found. On comparing the results obtained with SVCV to those reported for VSV it is evident that the basic structure and the sequence of methylation in RNA transcripts is conserved for different rhabdoviruses.

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366 CLONING OF TOBACCO CLOROPLAST RIBOSOMAL RNA GENES. Masahiro Sugiura, Jun Kusuda and Kazuo Shinozaki, National Institute of Genetics, Mishima 411, Japan.

Tobacco chloroplast DNA was digested with EcoRI. The DNA fragments were fractionated by agarose gel electrophoresis and transferred to a millipore filter according to Southern. The (5'^{32}P)rRNAs hybridized to the two fragments of MW of 1.9×10^6 and 2.8×10^6 daltons. Total EcoRI fragments of chloroplast DNA and pMB9 digested with EcoRI followed by phosphatase treatment were joined using T4 DNA ligase, and the ligated DNA was used to transform E. coli HB101. 373 Tc^r transformants were screened for the presence of the rDNA using the colony hybridization. The recombinant plasmids containing a 1.9×10^6 fragment (TC1) and a 2.8×10^6 fragment (TC309) were selected by agarose gel electrophoresis followed by Southern image hybridization. The chloroplast DNA was digested partially with EcoRI in the presence of distamycin A. The partial fragments of MW of ca. 5×10^6 daltons were extracted and cloned. 12 clones containing the rDNA were screened from the 271 transformants. Ten out of the 12 clones contained the 1.9×10^6 and 2.8×10^6 fragments and an extra 0.46×10^6 fragment. One of the plasmids (TCP6) was purified and digested with EcoRI. The Southern image of the fragments hybridized separately to the ^{32}P-23s and 16s rRNA. The 23s rRNA hybridized to the 2.8×10^6 fragment and the 16s rRNA to the 1.9×10^6 fragment.

367 COPY NUMBER MUTATIONS OF THE COL E1-DERIVED VECTOR PLASMID, pBGP120, M. Shepard, B. Polisky, Indiana University, Bloomington, Ind. 47401, and D. Gelfand, Cetus Corp., Berkeley, Calif. 94710.

The ColE1-derived pBGP120 plasmid (11.5MD;15 copies per chromosome equivalent) has been employed as a model system for the study of plasmid replication control. Two copy number (COP) mutants have been isolated and studied. pOP1 (11.5MD; 74 copies per chromosome equivalent) is unstable in an E. coli (cya$^+$) background, and breaks down to an array of smaller plasmids which retain the COP phenotype. One of these is designated pOP1V6 (4.6MD; 210 copies per chromosome equivalent) and has been used in a cis-trans test with the pBGP120 plasmid. These experiments have shown that the COP mutation of pOP1V6 is complemented in trans by a product specified by the wild-type plasmid. The sequences encoding the negative control function, and the site at which it acts have been localized to a small region of DNA surrounding the Col E1 origin of replication. This plasmid replication control region is currently being sequenced, and experiments are underway to further characterize the putative repressor function.

368 RARE CYTOPLASMIC TRANSMISSION OF SV40 TRANSFORMATION IN MOUSE CYBRIDS, Neil Howell and Ruth Sager, Sidney Farber Cancer Institute, Boston, MA. 02115

Cybrids formed by fusion of SVT2 (SV40-transformed mouse cells) cytoplasts and 3T3 cells were analysed for cytoplasmic transmission of transformation phenotypes (T-antigen, growth in low serum, anchorage independence and tumorigenicity). To detect transient or rare cytoplasmic transmission, cybrids were selected in medium with 1% serum, a concentration in which only transformed cells can grow. Colonies were recovered at a frequency of about 10^{-6} in three independent experiments but were not observed in control platings. (In 10% serum, cybrid yield was about 10^{-3}.) Some of these rare isolates are cybrids in which there has been cytoplasmic transmission of SV40 genes. When first isolated, cells of these cybrid lines were heterogeneous for T-antigen expression. Cells of one cybrid subclone were 100% T-antigen positive when grown in 1% serum for 30 population doublings. However, when the culture was grown in 10% serum and the growth rate increased, the percent of T-antigen positive cells decreased. This and other evidence suggests an episomal location of the SV40 genome in these cybrids. Mouse cells are non-permissive for SV40 DNA replication but chromosomal integration leading to cell transformation occurs readily. Our studies reveal a new phenomenon in which SV40 replication and expression of transformation apparently occur in the absence of chromosomal integration. Supported by NIH research grant CA-21365.

369 CLONING OF MULTIPLE, TANDEM COPIES OF THE LACTOSE OPERATOR, Joan L. Betz, John R. Sadler and Marianne Tecklenburg, University of Colorado Medical Center, Denver, Colo. 80262.

A 40 base, mainly duplex DNA segment, with the following sequence

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pAATTCCACA TGTGGAATTGTGAGCCGATAACAATT TGTGG      (3')
    GGTGT ACACCTTAACACTCGCCTATTGTTAA ACACCTTAAp   (5')
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has been synthesized by chemical and enzymatic methods by Dr. Marvin Caruthers. It consists of a wild-type lactose operator sequence (boxed) bracketed by "linker" sequences which permit excision of the segment from plasmid vehicles by the EcoRI restriction endonuclease. This segment has been ligated into the pMB9 plasmid and the resulting operator plasmids used to transform E.coli K12 . Among the transformant products were strains carrying plasmids with one, two, three or four operator segments in tandem. Derepression of the lactose operon effected by these plasmids in vivo as well as the lifetimes of complexes formed between repressor and these plasmids in vitro increase with increasing numbers of operators per plasmid. Stability of oligo-operator plasmids appears to require that all of the operator segments have the same (direct repeat) orientation. Using 3 and 4 operator fragments obtained from stable oligo-operator plasmids, we have constructed plasmids carrying up to 12 operators in tandem. Our polyoperator plasmids permit the purification of ca. 500 ug of this 40 base operator from 13 liters of cells grown in a high density fermentor(10mg. of plasmid).

370 5'- AND 3'-TERMINAL NUCLEOTIDE SEQUENCES OF KILHAM RAT VIRUS DNA,[1] P. Jagadeeswaran[2] and Sankar Mitra, University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830

We have been interested in the replication of Kilham Rat Virus (KRV) because of the unique hairpin structures that apparently exist at both ends of the linear, single-stranded virion DNA. It is not clear whether these structures are linear or multiple ("rabbit-ear") hairpins, but the self-priming of KRV DNA in vitro with DNA polymerases indicates that the 3'-terminal nucleotide is in duplex form, and the ability of E. coli DNA ligase to join the 3'- and 5'-termini of the DNA replicated in vitro indicates that this is also true of the 5'-terminus. The obvious conclusion regarding the biological role of the 3'-hairpin is that it functions as a primer for DNA replication, but the biological role of the 5'-hairpin is not clear. To help elucidate the functions of the hairpin structures, we have begun studies to determine the primary sequence of both the 3'- and the 5'-terminus of KRV DNA. Because preliminary experiments showed that the first nucleotide added during replication of KRV DNA in vitro is dAMP, we used T5 phage DNA polymerase and [α-^{32}P]ATP in the absence of other deoxynucleotide triphosphates to label the 3'-end. The procedures of Maxam and Gilbert were used to label the 5'-end and to establish the sequence of a stretch of 70-odd nucleotides on both hairpins. We also determined the terminal nucleotide of each. ([1]Operated by Union Carbide Corporation under contract W-7405-eng-26 with the U. S. Department of Energy. [2]Postdoctoral Investigator supported by contract No. 3322 from the Biology Division of the Oak Ridge National Laboratory to the University of Tennessee.)

371 A NOVEL MECHANISM OF THE CONTROL OF GENE EXPRESSION BY ENDOGENOUS CHROMATIN AND POLY(A)-mRNA PEPTIDES, Marian Hillar and Gian L. Gianfranceschi, Texas Southern University, Houston, TX 77004

Two groups of low molecular weight peptides, one associated with chromatin and the other with poly(A)-mRNA, were isolated, purified and characterized with respect to their amino acid composition, molecular weight, migration on thin layer chromatography on silica gel and HPLC on μBondapak C_{18}. Isolated peptides control at very low concentrations transcription and translation in reconstituted peptide-free, cell-free systems, stabilize double stranded structure of DNA and protect it from DNase digestion. Transcription and translation are not affected in endogenous systems (without prior extraction of peptides). These peptides are decreased and their control is lost in neoplastic tissue preparations (Novikoff hepatoma, mouse fibrosarcoma, mouse lymphosarcoma). Therefore added exogenously they may exert the same effect as in reconstituted systems preventing tissue dedifferentiation and growth of tumor.

A postulated theory of the gene control of tissue differentiation and carcinogenesis is discussed.

Supported by a grant from National Cancer Institute No RR 08061

372 IDENTIFICATION AND IN VITRO TRANSLATION OF THE "SARC" mRNA OF MSV 124. Dino Dina, Edmund Benz and Bernardo Nadal-Ginard, Albert Einstein College of Medicine, New York, New York 10461.

Cell clones infected with Moloney MSV 124 have been shown to contain three main species of virus specific mRNA. Three RNA species of 30S, 22S and 15S have been identified. The 30S mRNA is identical to mature vRNA and codes in vitro for large polypeptides (72K, 62K) containing "gag" information. A second mRNA, sedimenting at 22S has been shown to contain "sarc" information and 3' common information. Several mRNA preparations contain a 15S mRNA containing both 5' and 3' genetic information. The role of these two messages in vivo has not been established. When virion RNA is fractionated on sucrose gradients and translated in vitro, the 22S fraction specifically codes for three polypeptides which cannot be immunoprecipitated with anti-MuLV antibodies. These polypeptides are not coded for by MuLV virion RNA and appear to be non-structural "MSV specific" proteins. Both the size and informational content of the 22S RNA and the lack of immunoprecipitation by anti-MuLV antisera suggest that these polypeptides may be related to the transforming gene product of Moloney MSV. The possibility that the virions may efficiently package mature "sarc" mRNA and the relationship of the MSV specific polypeptides to the src gene product made in vivo will be discussed.

373 RESTRICTION MAPS OF FELINE LEUKEMIA VIRUS FROM SUBGROUPS A, B, & C AND COMPARISON TO THE ENDOGENOUS FELV-RELATED SEQUENCES IN CATS. F. Wong-Staal, R. Koshy, K. Green and R.C. Gallo, Laboratory of Tumor Cell Biology, National Cancer Inst., Bethesda, Md. 20014

There are epidemiological and serological data indicating that the horizontally transmitted feline leukemia viruses (FeLV) are the causative agents in at least some leukemias and lymphomas of domestic cats. Three serotypically distinct groups of FeLV have been isolated, subgroups A, B & C, with FeLV-A being the most commonly occurring. Examination of DNA from cat tissues for FeLV proviral sequences has been hampered by the presence of endogenous DNA sequences (putative virogenes) bearing homology to the prototype FeLVs.

Here we attempt to construct restriction maps of FeLV-A, B, & C to evaluate regions of conservation and variability. For these experiments unintegrated proviruses of each subgroup were harvested from infected canine thymus cells six hours post-infection. The viral fragments after digestion with various restriction endonucleases were identified by blot hybridization to ^{125}I-FeLV RNA. Similar experiments were performed with cellular DNA from a large number of "virus-negative" cat tissues (normal or leukemic). Several conclusions can be drawn from these studies: (1) The endogenous FeLV-related sequences in normal cats have a complexity pattern consistent with a non-tandem arrangement of multiple copies of a virogene in cats. (2) The endogenous FeLV-related "virogene" is distinct from the prototype FeLVs. (3) Differences in the restriction patterns were observed with DNA from tissues of the same cat, or from different cats (healthy or leukemic), indicating that either rearrangement of the virogene sequence can occur in some tissues or that some of the tissues of virus negative cats have acquired FeLV sequences by infection.

374 ORGANIZATION OF THE ENDOGENOUS RD114 VIROGENES IN DOMESTIC CATS. R. Koshy, R.C. Gallo, and F. Wong-Staal, Laboratory of Tumor Cell Biology, National Cancer Institute, N.I.H. Bethesda, Maryland 20014

The domestic cat (Felis catus) is one of four closely related species of the genus Felis, which has multiple endogenous virogenes of the RD114 virus. The virus can be elicited from normal feline cells in culture. Hybridization and serological data have shown its partial relatedness to the baboon endogenous viruses (BaEV), but none to the feline Leukemia Viruses (FeLV) which have been shown to be the causative agents in at least some leukemias and lymphomas of cats. The possible role of RD114 virus in leukemias of cats has been postulated, but not substantiated. Nevertheless, the retention of these virogenes in the germ-line against selective pressures suggests that they may have a role in some normal cellular processes.

In this study, we examined the organization of the RD114 virogenes in cat tissues and the integration of RD114 provirus in RD cells (a human rhabdomyosarcoma cell line), by means of restriction endonucleases and "blot" hybridization. We also studied the restriction patterns of unintegrated RD114 provirus obtained from canine thymus cells (A7573) infected with RD114 virus.

The results indicate that the 8-10 copies of RD114 virogenes are not arranged in a tandem fashion in the cat genome. Also, there appears to be very little divergence among the different copies of RD114 virogenes, since a homogeneous pattern corresponding to that of the unintegrated provirus was observed. In the heterologously infected RD cells, some two to five sites exist for provirus integration and some of these sites may be preferred over others. In addition, in these cells, some unintegrated form of the provirus is present. No differences in the organization of RD114 genes were observed between different tissues of normal and leukemic cats. Thus leukemia in cats is not correlated with rearrangements of RD114 virogenes.

375 MUTATIONAL ALTERATIONS WITHIN THE SIMIAN VIRUS 40 LEADER SEGMENT GENERATE ALTERED 16S and 19S mRNA's, Luis P. Villarreal, R. White, and P. Berg, Stanford University, Stanford, California 94305

We have analyzed the structure of the late cytoplasmic RNAs made after infection with wild-type simian virus 40 and a set of viable mutants, four of which have deletions and one an insertion within the nucleotide sequence specifying the leader segment of the 16S and 19S mRNA's. The principal findings are: (i) simian virus 40 16S and 19S mRNA's made during infections with wild-type virus are heterogeneous with respect to the map location of their 5' ends (ii) "Spliced" 16S and 19S mRNA's are made during infections with each of the mutants although, in some cases, the ratio of 19S to 16S mRNA species is reduced. (iii) The deletion or insertion of nucleotides within the DNA segment defined by map position 0.70 to 0.75 causes striking alterations in the types of leader structures in the late mRNA's. (iv) Many of the late RNA leader segments produced after infection with the mutants appear to be multiply spliced.

376 ANOMALOUS BK PAPOVAVIRUS GENE EXPRESSION IN PERSISTENTLY INFECTED AND TRANSFORMED HUMAN FETAL BRAIN CELLS, Hawley K. Linke, Kenneth K. Takemoto, Tatsuo Miyamura and George C. Fareed, Molecular Biology Inst. and Dept. of Micro. and Immunol., UCLA, CA. 90024; and Lab. of Viral Diseases, Nat. Inst. of Allergy and Infect. Diseases, NIH, Bethesda, MD 20014.

The continuous cell line designated BKHFB was recovered subsequent to a lytic infection of primary human fetal brain cells with the papovavirus BKV. After three years in culture, these cells exhibit many transformed growth properties; are tumorigenic in athymic nude mice; and are persistently infected and shed BKV. Cloning experiments in media containing BKV antiserum established cloned lines which were all initially T-antigen negative and virus free. After removal of the antiserum some clones began to release BKV and again become persistently infected while others remained virus and T-antigen negative (by immunofluorescence) indefinitely. Analysis of all clones demonstrated existence of the BKV genome as episomal, prototype DNA and we were unable to detect evidence of viral integration into the host genome. Analyses of the polyadenylated RNA produced in the cloned lines indicate the production of early transcripts, even in the absence of detectable T-antigen. Analyses of apparent deviation from normal transcription and transcriptional modification will be presented, as well as investigations into the distribution of viral induced early proteins detectable by immunoprecipitation.

If the initial transforming events in the BKHFB cells were due to BKV or its DNA, then this represents the first time that this virus has transformed human cells into stable cell lines. Furthermore, the apparent absence of immunofluorescence-detectable T-antigen represents a unique, heretofore unrecognized type of transformation by papovaviruses.

377 MOSAIC ADENOVIRUS 2-SV40 RNA SPECIFIED BY THE NONDEFECTIVE AD2-SV40 HYBRID VIRUS $Ad2^{+}ND4$. Charles Lawrence, Tony Hunter and Gernot Walter. The Salk Institute, San Diego, Ca. 92112 and Heiner Westphal and Sing-Ping Lai, National Institutes of Health, Bethesda, Md. 20014.

SV40 T-antigen related proteins of 42K, 56K, 60K, 64K, 74K, and 95K are expressed in cells infected with the nondefective Ad2-SV40 hybrid virus $Ad2^{+}ND4$. We have previously shown that these proteins have overlapping amino acid sequences with their C-terminal sequences in common. We have isolated SV40 specific RNA from cells infected with $Ad2^{+}ND4$ and shown that each T-antigen related protein is coded by a specific mRNA species. These RNAs have been further analyzed by heteroduplex mapping in the EM and were found to be complex mosaics of Ad2 and SV40 RNA sequences. Each SV40 specific RNA has an Ad2 leader sequence consisting of from three to five non-contiguously coded parts. The SV40 sequences of these RNAs exhibited four different types of sequence arrangements.

378 VA RNA IN ADENOVIRUS TYPE 12 INFECTED KB CELLS , Bernd Föhring, Arnold Geis and Karel Raska, Jr., Dpt.of Pathology, CMDNJ-Rutgers Medical School, Piscataway, N. J. 08854

Synthesis of virus-associated (VA) RNA was studied in KB cells infected with adenovirus type 12 (Ad 12). Its synthesis begins early but markedly increases late after infection. The VA RNA isolated from nuclei and cytoplasm of the infected cells was analyzed by two-dimensional gel electrophoresis and by a sequence of three different gel electrophoresis separations. Each of these procedures resolves two VA RNA species in group C adenoviruses, but no minor species was resolved in Ad 12-infected KB cells. VA RNA synthesized by RNA polymerase III in isolated nuclei in vitro also showed only one distinct species upon electrophoretic analysis.

The gene for VA RNA was mapped on Ad 12 genome by blot hybridization experiments. It hybridizes to the following DNA fragments obtained by cleavage with the restriction endonucleases: Eco RI fragment B ; Sma I fragment D ; Bgl II fragment A ; Xho I fragment D. Hybridization was also detected with Bam H-I fragments G and H. These results indicate that the gene for Ad 12 VA RNA maps at position 0.304 of the unit map. The T1 ribonuclease oligonucleotide fingerprint analysis of RNA selected by hybridization to fragments G or H of the Bam H-I digest of Ad 12 DNA so far failed to provide evidence for two species of VA RNA of distinctly different structure in adenovirus type 12. Supported by PHS Grant CA-21196 from the National Cancer Institute.

379 INDUCTION OF ECOTROPIC ENDOGENOUS MURINE LEUKEMIA VIRUS IN BONE MARROW CULTURES FROM HIGH LEUKEMIA-INCIDENCE MOUSE STRAINS, Joel S. Greenberger, Diane Donahue, and Mary A. Sakakenny, *Joint Center for Radiation Therapy*, Department of Radiation Therapy, and Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA 02115

The cell-specific and virus-specific requirements for leukemogenesis by RNA type-C viruses (retroviruses) are unknown. Continuous in vitro hemopoiesis in corticosteroid-stimulated bone marrow cultures derived from mouse strains with a low incidence of lymphoid leukemia; produced rare preleukemic transformation morphology in 2-10% of granulocytic cells generated between weeks 1 and 12. In cultures from high-incidence strains, a 100% incidence of spontaneous generation of preleukemic cell morphology was detected in cells removed at week 12. In these cultures high titers of ecotropic endogenous retrovirus were detected as early as week 4, a shift to >30% dysplastic myeloid cells by week 6 and >80% dysplastic cells by week 12. There was no detectable lymphocyte differentiation and transformed cells released virus and constitutively expressed granulocyte marker enzymes lysozme and peroxidase. Both stromal macrophage, fibroblast, and endothelial cells as well as non-adherent hemopoietic cells released virus. These data indicate that hemopoietic stem cells differentiating in a granulopoietic microenvironment are targets for transformation by viruses which normally cause thymic lymphoma in vivo. This system provides a unique opportunity to analyse the virus-specific and eukaryotic cellular specific genes for leukemogenesis.

380 NUCLEOTIDE SEQUENCES FOR THE ORIGIN OF DNA REPLICATION AND THE RIBOSOMAL RNA GENE IN RAT MITOCHONDRIA GENOME. K. Koike, M. Kobayashi & *T. Sekiya. Cancer Institute, JFCR, Toshima-ku, Tokyo 170 & *National Cancer Center Research Institute, Chuo-ku, Tokyo 104.

The rat mtDNA has a molecular length of about 16 kilobase pairs containing genes for ribosomal, transfer and messenger RNAs as well as the specific nucleotide sequences for its replication. The restriction enzyme EcoRI cleaves mtDNA into seven distinct fragments, and all of the DNA fragments were cloned in E. coli by using the λ phage vector λgtWES·λB. Each recombinant DNA contains the outer left and right arms from the vector DNA fragmented by EcoRI and one or few DNA fragments separated from EcoRI-digested mtDNA.

DNA amplified in the recombinant phage λgt·mt was shown to contain the same restriction enzyme cleavage sites as in the EcoRI fragment previously determined by restriction mapping of the mtDNA. The results permitted us to determine the DNA sequence of any portion of the mitochondria genome for the study of replication and specific gene systems.

As a part of the nucleotide sequences surrounding the L-strand replication origin was previously presented, we would like to present further progress of DNA sequencing of the replication origin and the ribosomal gene.

381 STRUCTURE AND DISTRIBUTION OF INVERTED REPEATS (PALINDROMES) IN DNA OF DROSOPHILA MELANOGASTER AND MOUSE, Naomi Biezunski, Dept. of Anatomy, Hebrew University Medical School, Jerusalem, Israel, and Dept. of Biology, Case Western Reserve University, Cleveland, Ohio, 44106.

Studies on the structure and distribution of inverted repeats (palindromes) in DNA of Drosophila melanogaster and mouse revealed similarities in the size of inverted repeated segments but distinct and different patterns in their distribution. Inverted repeated sequences were analyzed by electron microscopy after the DNA was denatured and allowed to renature to a very low Cot. In DNA of mouse 54% of palindromes were looped, containing a renatured stem and a single stranded loop. About 45% of the palindromes appeared as renatured double stranded regions lacking a discernible single stranded loop. The palindromes were usually widely spaced and only rarely occurred in clusters. In contrast, DNA molecules of the same size (about 18kb) from Drosophila yielded palindromes of which about 79% were unlooped and about 20% were looped. Unlike inverted repeated sequences in mouse DNA, the inverted repeats in Drosophila were arranged in distinct clusters. The spacing of palindromes within clusters in Drosophila DNA and in mouse DNA, when observed, was often less than 1 kb. Although the distribution of inverted repeats in the 2 species is different, their length distributions are similar. The majority of renatured repeats in looped and unlooped structures of both species are about 100 b.p. in length. However longer inverted repeated sequences, up to 6 kb were seen more frequently in mouse than in Drosophila. From the above observations it is clear that although the organization of DNA with respect to palindromic sequences from mouse and Drosophila is quite similar, they are sufficiently different that they can be distinguished upon inspection by E.M.

Yeast and Protozoan

382 CHARACTERIZATION OF T. BRUCEI KINETOPLAST DNA USING RECOMBINANT DNA TECHNIQUES. John E. Donelson*+, Pheliz A. O. Majiwa*, and Richard O. Williams*. *I.L.R.A.D., Box 30709, Nairobi, Kenya and +Department of Biochemistry, University of Iowa, Iowa City, Iowa 52242 U.S.A.

Kinetoplast DNA (kDNA) of Trypanosoma brucei is composed of a network of maxi-circle DNA (about 24 kb) and mini-circle DNAs (1.1 kb) of different sequences. We have digested purified T. brucei kDNA with 12 different restriction enzymes and constructed a cleavage map of 4 of these enzymes on the maxi-circle sequence. Each of the 12 restriction enzymes makes a single cleavage in a different subset of the 1.1 kb mini-circle sequences. Individual mini-circle DNA molecules, cleaved once by either Hae III, Hpa II, Alu I, Hha I, Pst I or Hind III, were cloned in E. coli via the plasmid pBR322 and the poly(dG):(dC) tailing technique or the DNA ligase technique. The cloned mini-circle DNAs were compared by renaturation kinetics, filter hybridization, heteroduplex analysis and DNA sequence determination. The individual cloned molecules have a sequence complexity about 1/275 that of the total mini-circle sequence population. All of the cloned mini-circle molucles possess sequences in common with each other as determined by the filter hybridization procedures of Southern or Grunstein and Hogness. None possess sequences in common with maxi-circle DNA. Experiments are in progress to determine the complete nucleotide sequence of 2 of the cloned mini-circle sequences.

383 REPLICATION OF CIRCULAR DIMER MITOCHONDRIAL DNA, Clifford Lowell, Daniel Bogenhagen and David A. Clayton, Stanford University School of Medicine, Stanford, CA 94305.

We have compared aspects of the replication of unicircular dimer mitochondrial DNA (mtDNA) in mouse ID cells with previous studies of replication of monomeric mouse L cell mtDNA. Whereas monomeric mtDNA requires about one hour for a round of replication, the dimeric molecules require almost three hours. Both types of mtDNA replicate by an asynchronous displacement mechanism in which replication of the light strand of the DNA duplex lags significantly behind that of the heavy strand. Denaturing agarose gel electrophoresis of replication intermediates reveals that replication of unicircular dimers occurs with discontinuities in the rate of daughter strand synthesis. The results indicate that the same set of control sequences is functional in both dimer and monomer mtDNA replication. Immediately following a round of replication, the majority of dimer molecules contain displacement loops, as judged by their sensitivity to nicking by single-strand DNA specific S1 nuclease under conditions which leave supercoiled DNA intact (1). This result is in contrast to the conformation of newly-replicated monomeric mtDNA molecules, which lack both superhelical turns and displacement loops. We postulate that intermediates at late stages of dimer DNA replication contain displacement loops which remain intact following closure of the full-length daughter strands.

1. Lowell, C., Bogenhagen, D. and D.A. Clayton (1978), Anal. Biochem. 91, in press.

384 CYTOPLASMIC INHERITANCE OF ERYTHROMYCIN RESISTANCE IN HeLa CELLS. Claus-Jens Doersen and Eric J. Stanbridge, Dept. of Medical Microbiology, Stanford University School of Medicine, Palo Alto, CA 94305; Dept. of Medical Microbiology, University of California Irvine,College of Medicine, Irvine 92717

Erythromycin resistant mutants of lower eukaryotes have been described where the mutation is mitochondrially encoded. This report describes our isolation of an erythromycin resistant human cell population. HeLa cells were treated with low concentrations of ethidium bromide in order to substantially decrease the target size of functional mitochondria and then were mutagenized with ethyl methane sulfonate or nitrosoguanidine. Erythromycin resistant cells were selected in growth medium containing 300 µg/ml erythromycin. Several clones arose after incubation at 37°C for several weeks. One clone, HeLa 200, was examined in detail. In order to confirm that the site of drug resistance lay in the mitochondrial apparatus rather than being due to a permeability mutation cell-free mitochondrial protein synthesis assays were performed using a modified Attardi procedure. Protein synthesis was resistant to erythromycin and carbomycin, but retained sensitivity to chloramphenicol. In order to establish cytoplasmic inheritance HeLa 200 cells were enucleated and the anucleate cytoplasms fused with an $HGPRT^-$ variant of HeLa. Cybrids were then selected in 8 µg/ml 6-thioguanine and 300 µg/ml erythromycin. Surviving clones were analyzed for chromosomal content and were found to contain the same number of chromosomes as the $HGPRT^-$ parent. These results support the contention that HeLa 200 represents the first report of an erythromycin resistant phenotype in mammalian cells which is cytoplasmically inherited.

385 ANALYSIS OF MITOCHONDRIAL RNA FROM GRANDE AND PETITE YEAST, Joseph Locker, Richard Morimoto, Richard Synenki, and Murray Rabinowitz. The University of Chicago, Chicago Illinois 60637.

MtRNA has been analyzed using 6M urea-agarose gels, methyl mercuric hydroxide-agarose gels and transfer of RNA from gels to diazobenzyloxymethyl paper with subsequent hybridization of labelled mtRNA. 21S and 14S rRNA are calibrated to 3800 and 1800 nucleotides respectively. In the grande, over 40 different species of mtRNA molecules have been characterized, including at least 7 species larger than 21S RNA, and ranging from 4400-9500 nucleotides. Such high molecular weight molecules are presumed to be precursors of mature processed RNA. Many petites show fully processed RNA molecules and also a subset of the high molecular weight molecules present in the grande. Thus these RNA species could be mapped by petite deletion analysis. Petites also show new bands not present in the grande. Some new bands are found only in individual strains and probably result from the specific deletions of these strains. Other new bands are associated with specific regions of the petite genome, and as such, may represent intermediate molecules that are turned over more rapidly in the grande, or altered control of transcription in petites. tRNA's are frequently transcribed in petites, but processed mature tRNA is present only in petites that contain a specific region of the genome that maps near the P marker and 14S rRNA. How such a gene locus functions in petites in the absence of protein synthesis remains to be clarified. Thus, petite RNA can be used for mapping the transcripts of the grande genome, and for defining several different types of post-transcriptional RNA processing.

386 CHARACTERIZATION OF POLY(A)-CONTAINING RNA FROM *XENOPUS LAEVIS* OVARIAN MITOCHONDRIA, Andrew C. Webb and Josephine A. Wegert, Department of Biological Sciences, Wellesley College, Wellesley, MA 02181.

The *Xenopus* oocyte provides a model system for the study of mitochondrial biogenesis. The accumulation of mtDNA is completed half-way through oogenesis, apparently due to termination of DNA synthesis rather than acquisition of steady-state kinetics. It was therefore decided to examine the associated production of mitochondrial poly(A)-containing RNA during oocyte growth. Poly(A)-RNA was purified from ovarian mitochondria by oligo(dT)-cellulose affinity chromatography and was found to comprise 1.5% of the total mtRNA. PAGE of [^{3}H]$NaBH_4$-labeled, RNase resistant poly(A)-RNA revealed four discrete fractions. Comparison with poly(A) standards of known lengths indicated the predominant size class (50%) to be 50 NT long, with the remainder being equally distributed between fractions with poly(A) sequences of 30, 60 and 80 NT in length. A [^{3}H]cDNA copy of the poly(A)-RNA was synthesized using reverse transcriptase. The mean length of this cDNA in alkaline sucrose gradients was 900 NT (range 300-1400), using *Hpa* I restriction endonuclease fragments of ΦX174 RF-DNA as molecular weight markers. The [^{3}H]cDNA hybridized only to *Xenopus* mtDNA, with no detectable binding in the presence of a 50-fold excess of *Xenopus* nuclear DNA. These data suggest that our poly(A)-RNA preparation is (i) transcribed exclusively from mtDNA and (ii) does not contain any nuclear-derived transcripts (either contaminating or naturally occurring). The data also lend support to previous conclusions that *Xenopus* nuclear DNA does not harbour a complete or even partial copy of the mitochondrial genome. Studies utilizing the cDNA probe to analyze the temporal appearance of mitochondrial poly(A)-RNA during *Xenopus* oogenesis will also be reported.

387 FORMATION OF INTERSPECIFIC MITOCHONDRIAL HYBRIDS IN AMPHIBIAN OOCYTES, Andrew C. Webb, Department of Biological Sciences, Wellesley College, Wellesley, MA 02181.

The successful construction of viable interspecific populations of mitochondria within eukaryotic cells would provide an invaluable system for the study of the interactions between nuclear and mitochondrial genomes. Attempts have been made to form mitochondrial hybrids in amphibian oocytes by microinjecting mitochondria from one species of frog into the oocytes of a closely related species. Several *Rana* species were found to have readily distinguishable mt MDH isoenzyme profiles on isoelectric focusing gels, but this method of detecting microinjected mitochondria was too insensitive. Attention was directed towards utilizing a more sensitive hybridization assay between radioactive complementary RNA (cRNA) and its mtDNA template. Within the genus *Xenopus*, species differ sufficiently in their mtDNA sequences that species-specific mt-cRNAs can be used to distinguish them. With this methodology, it has been possible to detect *X.tropicalis* (*Xt*) mitochondria in the cytoplasm of full-grown *X.laevis* (*Xl*) oocytes up to 20h after their microinjection. By simultaneously hybridizing *Xt*[^{3}H]cRNA and *Xl*[^{14}C]cRNA to extracts of injected oocytes, the ratio of host (*Xl*) to donor (*Xt*) mtDNA can be monitored. It was found that this ratio remained essentially constant for about 5h following mitochondrial injection, whereupon the level of *Xt* mtDNA declined progressively over the next 12-15h. The question of whether this failure of oocytes to support a population of "alien" mitochondria over an extended period resides in some inherent incompatibility between the host cytoplasm and the microinjected mitochondria, or alternatively is merely an artefact of the experimental procedure is being investigated.

(supported by a grant from Research Corporation)

388 MITOCHONDRIAL DNA POLYMERASE FROM ANIMAL CELLS. INTERACTIONS WITH INTERCALATING DRUGS AND PROPERTIES OF THE ENZYME FROM ANUCLEATED CELLS (HUMAN PLATELETS). A.Araya, L. Tarrago-Litvak, C. Desgranges and S. Litvak. Dept.Biochem. University of Bordeaux II. 33405 Talence. France.

Studies with DNA polymerase γ purified from mice cells mitochondria showed that the dramatic inhibition of the enzyme by intercalating drugs, like ethidium bromide or acriflavine, is specific, since DNA polymerases α and β were much less affected under the same conditions of incubation . A study with different analogs of ethidium bromide has given some indications on the part of the intercalating molecule responsable of the inhibition. This inhibition is at the enzyme level, most probably at the deoxynucleoside triphosphate site of the protein.

Studies with anucleated cells has shown that human platelets posses two DNA polymerases. One is found in the mitochondria and has the same properties than DNA polymerase γ from nucleated cells, while the other,found in the extramitochondrial cytoplasm, is different to DNA polymerases α , β or γ from normal cells. The effect of inhibitors of mitochondrial or cytoplasmic protein synthesis has been used with nucleated cells and platelets, and their effect on mitochondrial DNA polymerase will be discussed.

Supported by CNRS, INSERM and the University of Bordeaux II.

389 CHARACTERIZATION OF YEAST MITOCHONDRIAL DNA DURING CELL AND PETITE MUTANT RECOVERY FROM ULTRAVIOLET IRRADIATION, Sharon C. Hixon, Ricky L. Irons and Harold L. Franks, Department of Biochemistry, University of Alabama in Birmingham, B'ham, Ala. 35294

Previous studies have verified a dose and time dependent degradation of tritium prelabelled yeast mitochondrial DNA (mDNA) following the ultraviolet irradiation of exponential phase cells. These observations were made during a liquid holding (LH) period in non-nutrient media following irradiation. Cells that were withdrawn at increasing time intervals during LH and spread onto complete media plates exhibited a recovery of irradiation induced petite mutation. It has been postulated that recovery of irradiation damage for mDNA requires those events which take place during LH followed by new DNA replication after a return to nutrient media. The S values of mDNA at different times during the LH period following irradiation have been determined on neutral sucrose gradients. Immediately following an irradiation dose of 1500 ergs UV (10% cell survival) the molecular weight distribution of recovered mDNA exhibited a minor population at a control value of 25S while most of the tritium prelabelled DNA had been reduced to an average value of 18S. Samples recovered at 24 and 40 hrs LH had stabilized to an average S value of 18. A quantitation of the number of nicks and gaps in control and irradiated mDNA during LH is currently underway employing the S1 nuclease from Aspergillus oryzae (Miles). Cells which had been LH for 2 days were placed in new growth media with ^{14}C-adenine. Mitochondrial DNA from previously irradiated cells gradually shifted from 18S toward a normal recovery value of 25S during the first cell doublings in complete media.

390 BIOCHEMICAL AND GENETIC ANALYSIS OF OLIGOMYCIN RESISTANT CHO CELLS. Gail A. M. Breen and Immo E. Scheffler, Dept. of Biology, B-022, University of California, San Diego, La Jolla, California 92093

We have isolated oligomycin-resistant clones from Chinese hamster ovary cells (CHO) after treatment with ethidium bromide and mutagenesis with ethylmethane sulfonate, followed by selection in oligomycin. Resistant clones grew up after 2-3 weeks in selective medium. The doubling time of several clones varied from 13 to 16 hours, with or without oligomycin, compared to 13 hours for the parental cells. The oligomycin-resistant cells are stable when grown for several generations in the absence of the drug. Studies on the mitochondrial ATPase indicated that the enzyme from the mutant cells is more resistant to inhibition by oligomycin than the enzyme from wild type cells, although both have similar total ATPase activities. In hybrid cells made by fusing resistant and wild type cells, oligomycin resistance behaves as a dominant trait. Experiments are in progress to determine whether oligomycin resistance is due to a mutation in a nuclear or cytoplasmic gene.

391 STRACTURE AND FUNCTION OF A+T RICH REGION IN DROSOPHILA MITOCHONDRIAL DNAs, Akio Sugino, Dilip M. Shah and Charles H. Langley, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, N.C. 27709

Mitochondrial DNAs from three species of the genus Drosophila (D. melanogaster, D. simulans, and D. virilis) were compared by restriction endonucleases digestion and electron microscope heteroduplex mapping. Analysis of heteroduplex molecules revealed that the A+T-rich region of these mtDNAs has undergone quite extensive base sequence divergene, whereas the remainder of the molecule was found to share apparently complete base sequence homology in all three species. The differences in the sizes of the A+T-rich regions completely account for the differences in the total sizes of these mtDNAs. In order to get more complete information about A+T-rich regions, we have cloned the restriction endonuclease segments containing A+T-rich region and DNA replication origin into E. coli plasmid pBR322DNA, and currently been determining the nucleotide sequence. We will present nucleotide sequence of A+T-rich region of these DNAs. Using this chimeric DNA, we have also been developing a soluble in vitro mtDNA replication system which is able to complement with exogenous protein factors.

392 MOLECULAR BASIS OF MITOCHONDRIAL TRANSMISSIONAL TYPES, Elissa P. Sena, Case Western Reserve University, Cleveland, Ohio 44106

Matings between Saccharomyces cerevisiae respiratory deficient strains (petites) and respiratory sufficient strains (grandes) have identified two basic mitochondrial phenotypic transmission types: 1- a neutral petite, and 2- a suppressive petite. When a neutral petite is mated to a grande (type 1 mating), the progeny cells are almost exclusively grande. However, a cross between a suppressive petite and a grande (type 2 mating)can yield varying percentages of grand and petite progeny, depending upon the degree of suppressiveness of the petite . Since the molecular behavior of mitochondrial DNA (mt-DNA) in such crosses was unknown, an investigation of the mt-DNA composition of zygotes and zygotic buds from type 1 and 2 crosses was undertaken. Large scale synchronous matings of both types were carried out. The parental strains in each cross contained mt-DNA of different intrinsic buoyant densities as well as different mitochondrial genetic markers. The molecular data showed that both types of zygotes contain mt-DNA from grande and petite parents during first zygotic bud initiation,however, first zygotic buds from type 1 crosses contained mostly grande mt-DNA, while early zygotic buds from a 55% suppressive type 2 cross contained mt-DNA predominantly from the petite parent. These data suggest that neutrality or suppressivity of certain mitochondrial genomes may be directly correlated with the subsequent transmission of these molecules into zygotic buds. The mitochondrial DNA composition of aging zygotes from both types of crosses is presently under investigation.

393 LACK OF DNASE I SENSITIVITY OF RIBOSOMAL DNA IN PHYSARUM, Volker M. Vogt and Linda Swofford, Section of Biochemistry, Cornell University, Ithaca, NY 14853.
The DNA coding for ribosomal RNA in Physarum polycephalum exists as extrachromosomal palindromic molecules (1) that replicate independently of chromosomal DNA. We have investigated the sensitivity of rDNA in purified nuclei to digestion by DNase I. Several transcribed genes in animal cell nuclei are known to be degraded selectively in this procedure (2,3). Restriction fragments corresponding to the transcribed and the large, presumably non-transcribed spacer portions of the rDNA were labeled by nick translation, and the reannealing of these fragments was driven by DNA isolated from DNase I-treated nuclei. The curves describing the reannealing kinetics, or "C_0t curves," for both fragments shifted no more than a factor of two when DNA from nuclei digested with the nuclease to the extent of 5% to 35% acid solubility of total DNA was compared with DNA from undigested nuclei. As controls for these experiments, we have used two radioactive probes from DNA sequences not present in the rDNA. One is a random clone of Physarum DNA that is present in the genome in few copies and appears not to be transcribed. The other is complementary DNA synthesized in vitro from total poly A-containing RNA. DNase I treatment of nuclei degrades these sequences equally, and the slight shifts of the reannealing curves are identical to those for rDNA. These results suggest that the features of chromatin structure that enhance susceptibility of transcribed sequences in animal cells may not be present in Physarum.
References: (1) Vogt, V.M. and R. Braun, J. Mol. Biol. 106, 567 (76); (2) Garel, A., M. Zolan and R. Axel, PNAS 74 4867 (77); (3) Weintraub, H. and M. Groudine, Science 193 848 (76).

394 ANALYSIS OF KINETOPLAST DNA OF TRYPANOSOMA BRUCEI 110 AND TRYPANOSOMA RHODESIENSE 1895. Gerald Keilman, Rance LeFebvre, E. Jay Bienen and George C. Hill, Colorado State University, Fort Collins, CO 80523
During the course of cellular transformation from the infective bloodstream trypomastigote form to the non-infective procyclic trypomastigote form, the African trypanosomes T. brucei and T. rhodesiense undergo biochemical changes. After two days of incubation in SM medium at 27 C, 100% of the bloodstream trypomastigotes had morphologically transformed into procyclic trypomastigotes. O_2 utilization changed from azide-insensitive to 18-20% azide-sensitive. Proline stimulated O_2 consumption after two days of incubation. The loss of surface coat integrity was measured by agglutination with concanavalin A. Reactivity to Con A was also observed after the 48 hours in SM medium. In our studies to determine the relationship of the kinetoplast DNA to these metabolic changes, characterization of these DNA's has been initiated. All of the purified networks of kDNA's showed an apparent bouyant density of 1.691 gm/cc equivalent to a G+C content of 33%. T_m determinations in 0.1xSSC were also similar between the different forms of these cells showing a value of about 70 C and a G+C content of 39%. Analysis of the intact networks by their sensitivities to various restriction endonucleases was made on 0.4% agarose gels. Pst I, Bam I, Sal I, and Pvu I nucleases were active and caused the release of a single polynucleotide approximately 18.7 kbp in length. Eco RI, Hind III, and Hae II enzymes yielded 3 high molecular weight fragments less than 18.7 kbp. Hha I yielded at least 5 polynucleotides. Currently, the positions of these cleavage sites are being determined to obtain a cleavage map of maxi-circles released by the restriction enzymes.

395 INTRASPECIFIC mtDNA POLYMORPHISM AND THE MECHANISM OF MATERNAL INHERITANCE. Gregory G. Brown and Melvin V. Simpson, SUNY, Stony Brook, N.Y. 11794
Two types of mtDNA, A and B, distinguishable by restriction enzyme analysis, and diverging in sequence by 0.9%, occur in the laboratory rat population, any individual possessing a single type (FEBS. LETT. 79, 291 (1977)). Our cleavage maps of type A and B DNA should dispel the prevailing confusion regarding published rat mtDNA maps. In addition, they show the absence of translocation or of intermediate forms whose presence would suggest genetic recombination. We and others have used these two DNA's to demonstrate maternal inheritance in mammals and we are now studying its mechanism. Since oocytes contain many more mitochondria than spermatozoa, passive dilution of male mtDNA could lead to maternal inheritance. However the presence of male parental mtDNA was not detected in any tissue of the offspring of heterologous crosses, nor in liver even when analytical sensitivity was increased to 1 molecule in 5000. This suggests the operation of some other mechanism. Experiments at higher sensitivity to confirm this are in progress. Why have we uncovered the same two mtDNA types in all rat strains examined? As explanation,considerations of maternal inheritance suggest that the first laboratory Rattus norwegicus colony was started with two wild norwegicus females and that all other laboratory strains of this species were derived from this colony, without further introduction of other wild females. Alternatively, only two mtDNA types exist in the wild population, and this possibility is being investigated.
Supported by NIH (GM22333) and American Cancer Soc. (NP87J).

396 RESISTANCE TO ANTIMYCIN A : A NEW EXTRACHROMOSOMAL GENETIC MARKER IN MAMMALIAN CELLS. I. Craig and M. Webb, Genetics Laboratory, Oxford.

A cell line which is resistant to the inhibitor of electron transport, Antimycin A has been isolated following the mutagenesis of D98 (HeLa) with N-methyl-N-nitro-N-nitrosoguanidine in the presence of cycloheximide. The variant cell line has been cloned twice in 15 ug/ml antimycin A, a concentration ten times greater than that which kills all parental cells (Webb, M. and Craig, I., 1978, Gen.Soc. ABS). The growth rate and oxygen consumption of the variant line in the presence of 15 ug/ml antimycin A are similar to those of the parental cell in the absence of the drug. However, the succinate cytochrome c reductase activity of membrane preparations from the variant was similar to that of the parental cell and showed full sensitivity to the inhibitor. No cross resistance to triethyl tin or to D-threo chloramphenicol was observed.

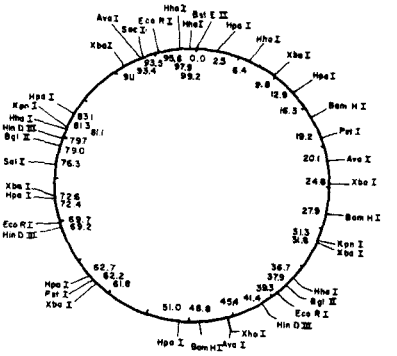
These properties of the variant were found to be stably propagated in the absence of selection for two and a half months.

The mitochondrially synthesized proteins of the variant have been compared with those of D98 and other human cell lines. The variant is characterized by an apparent increased production of one mitochondrial protein of molecular weight (apparent) 29,000 (isoelectric point 4.45). A relative increase in labelling of this protein was observed with both ^{35}-S methionine and ^{14}-C leucine.

The determinant for antimycin resistance has been shown to be cytoplasmically inherited.

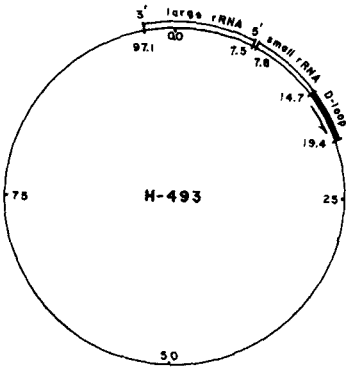
397 PHYSICAL MAP AND NUCLEOTIDE SEQUENCE OF BOVINE MITOCHONDRIAL DNA FROM AN INDIVIDUAL ANIMAL, Philip J. Laipis and William W. Hauswirth, University of Florida, Gainesville, Florida 32610.

Mitochondrial DNA has been isolated from the liver of an individual Holstein cow and a physical map has been derived for the 38 cleavage sites made by restriction endonucleases: Ava I, Bam HI, Bgl II, Bst EII, Eco RI, Hha I, Hin dIII, Hpa I, Kpn I, Pst I, Sac I, Sal I, Xba I, and Xho I. Sufficient mitochondrial DNA (ca 16 mg) could be isolated, allowing this map to serve as the basis for detailed physical, genetic and nucleotide sequence studies in an individual mammal. Initial sequence studies on a region near the 3'-terminus of the large ribosomal gene (clockwise from 95.6 map units) revealed limited sequence heterogeneity between related animals. Additionally restriction endonuclease site comparisons allows an independent estimate of nucleotide sequence divergence of about 1% between these animals.



398 GENETIC MAPPING OF MITOCHONDRIAL DNA FROM AN INDIVIDUAL ANIMAL, William W. Hauswirth and Philip J. Laipis, University of Florida, Gainesville, Florida 32610.

Using a physical map of bovine mitochondiral DNA derived from the liver of a single Holstein cow, we have determined the location of the genes specifying the large and small ribosomal RNAs by hybridization analysis and electron microscopic observations of R-loop forms. The location of the origin of DNA replication (D-loop) has been located by electron microscopy. Additionally, the direction of D-loop expansion and the polarity of the large ribosomal RNA gene were determined. From initial DNA sequence studies, the location of a possible promoter site, a "Pribnow box", has been detected.



399 REPLICATION OF YEAST EXTRACHROMOSOMAL GENETIC ELEMENTS, Virginia A. Zakian, Bonita J. Brewer and Walton L. Fangman, University of Washington, Seattle, WA 98195

The replication of 2 μm plasmid DNA molecules was examined in two ways. A density-transfer experiment with an asynchronous population of cells revealed that all 2 μm duplexes were of hybrid density one generation after transfer from dense to light medium. A majority of the duplexes had single strands which were fully dense or fully light. We interpret these observations to mean that each of the plasmid molecules in a cell replicates once each cell cycle. This pattern of replication is in contrast to the random distribution of replication events found for bacterial plasmid DNAs and mammalian mitochondrial DNAs. Replication of 2 μm DNA was also examined with synchronous populations of cells obtained by arrest with the yeast pheromone α-factor, followed by temperature arrest with a cell cycle (cdc 7) mutant. No 2 μm DNA replication occurred during the arrest periods, but the population of molecules doubled during the synchronous S-phase at the permissive temperature. Moreover, most of the 2 μm DNA replication occurred in early S. Thus, each 2 μm replication origin is activated one time per cell cycle, and this activation is probably limited to the S phase.

The replication of the double-stranded RNAs (dsRNAs) and mitochondrial DNA was also investigated in synchronous cells. The replication of these elements, unlike that of nuclear and 2 μm DNA, continued during the arrest periods. When the cells were allowed to enter the S-phase, replication of mitochondrial DNA continued, but replication of the dsRNAs stopped abruptly. We conclude that controls over the replication of 2 μm DNA are similar to those for nuclear DNA. In contrast, the replication of mitochondrial DNA and dsRNAs can be uncoupled from each other, and both can be uncoupled from the replication of nuclear DNA and 2 μm DNA.

400 A NUCLEAR GENE CONTROLLING ASSEMBLY OF THE YEAST ENERGY TRANSDUCING MEMBRANE, Michael G. Douglas, Phyllis C. McAda and Richard D. Todd. The University of Texas Health Science Center, San Antonio, Texas 78284.

To examine the role of nuclear genes in the expression and assembly of the mitochondrial ATPase complex a detailed analysis of yeast nuclear mutants lacking measurable F_1-ATPase activity has been initiated. The F_1-ATPase is synthesized by the nucleocytoplasmic system and is assembled with partner subunits of mitochondrial origin during biogenesis of the functional complex. Single nuclear mutants which are devoid of mitochondrial F_1-ATPase activity have been characterized to determine the level of ATPase subunits expressed in the mutant. Quantitative immunoprecipitation analysis of one mutant revealed that the mitochondrial membrane of the mutant contained 15% of the level of ATPase antigenic determinants found in the parental strain. Direct comparison of the ATPase immunoprecipitates by electrophoretic analysis on non-reducing SDS slab gels revealed that all the nuclear gene products of the parental enzyme were also present in the mutant in the same stoichiometry. In addition, an in vitro cell free protein synthesizing system was developed to quantitate the relative level of F_1-ATPase messenger RNA in the mutant and parental strain. Quantitation of the F_1-ATPase mRNA from the two strains revealed that the amount of translatable messenger RNA was the same (2.05% and 1.96% of the total poly A RNA for the parental and mutant strains, respectively). Similar analysis of another inner membrane enzyme, cytochrome oxidase, revealed that although the level of enzyme present in the mutant is 5-8% that of the parental strain the level of translatable cytochrome oxidase mRNA was the same. These data indicate that the biogenesis of two major complexes of the membrane are similarly repressed as a result of a single nuclear mutation. Furthermore, conservation of the stoichiometry of the individual cytoplasmically synthesized subunits in the ATPase assembly defective mutant suggests a mechanism of F_1-ATPase subunit synthesis that is coordinated at the translational level. Experiments to assess the mechanism of this control will be provided. Supported by grant GM-25648 from the National Institues of Health.

401 THE SEQUENCE OF THE INVERTED REPEATS OF YEAST PLASMID DNA, James L. Hartley, Brian P. Nichols, and John E. Donelson, University of Iowa, Iowa City, Iowa 52242.

Most yeast strains carry 50-100 copies per cell of an extrachromosomal circular DNA element with a contour length of about 2 μm (about 6000 base pairs). This 2μ DNA may code for several proteins and does not appear to integrate into the nuclear or mitochondrial chromosomes. It has been used as a cloning vehicle in yeast transformations. Each 2μ circle contains 2 copies of a 600 base pair sequence which are located on opposite sides of the circle and are inverted with respect to each other. The inverted repeats may play a role in intra-molecular recombination, in replication, or in other unknown functions of the plasmid.

Using a recombinant plasmid containing 2μ DNA, we have isolated and sequenced an 801 base pair DNA fragment containing one of the inverted repeats. The sequence of about 1/3 of the other repeat was determined by sequencing portions of other restriction enzyme fragments. By comparing the sequence data, the inverted repeat boundaries were located and the repeat length was determined to be 597 base pairs. A 120 base pair region near the center is rich in repeated and inverted sequences, while the boundaries are not distinguished by major symmetries or other unusual features. The portions of the 2 inverted repeats which have been analyzed show 100% homology, indicating a high degree of sequence conservation and suggesting that the repeat sequences have an important functional role.

402 TRANSLATIONAL ANALYSIS OF THE CYTOPLASMIC dsRNA GENOMES OF KILLER YEAST, Donald Tipper, U. Mass. Medical School, Worcester, Ma., Keith Bostian and James Hopper, Brandeis University, Waltham, Ma. and Dave Rogers, McGill University, Montreal, Quebec. Killer (K^+) strains of Saccharomyces cerevisiae produce an extracellular toxin (12,000 daltons) and contain L and M species of dsRNA, (~3 and 1.4 million daltons) separately encapsulated in cytoplasmic virus-like particles. These particles contain a single major capsid protein (88,000 daltons). Neutral strains do not kill (K^-), but like killer strains, are resistant to toxin (R^+). They contain indistinguishable L and M dsRNA's. Suppressive strains contain an S dsRNA derived from M by an internal deletion. In vitro translation of denatured L dsRNA produces the capsid protein as the only identifiable product. Translation of M dsRNA from either killer or neutral strains produces a 32,000 dalton polypeptide that cross reacts with antitoxin antibody and appears to contain the toxin peptide sequence. Translation of S dsRNAs gives an 8,000 dalton peptide that also interacts with antitoxin. These data support the hypothesis that M from killer strains codes for toxin, that M from neutral strains codes for a missense mutant product that fails to mature into active toxin, that the deletion in S covers part of the toxin coding sequence and that L is required for M maintenance because it provides the capsid protein. If the 32,000 dalton in vitro product is produced in vivo, it could be a precursor of both toxin and an immunity factor.

403 EXPRESSION OF YEAST RIBOSOMAL RNA GENES IN E. COLI. Richard A. Kramer, National Cancer Institute, NIH, Bethesda, Maryland 20014

The expression of the yeast ribosomal RNA coding region carried into E. coli on a bacteriophage λ vector has been examined. Transcription of the yeast rDNA was detected by hybridization to cloned rDNA and was shown to be in the same direction as normal transcription in yeast by hybridization to separated DNA strands. The transcription is independent of λ RNA synthesis as shown by several criteria including detection of yeast rRNA sequences in a λ lysogen super-infected with the recombinant phage. Hybridization to restriction fragments of yeast rDNA and mapping by electron microscopy of r-loops formed on the recombinant phage show that, while the yeast-specific transcripts isolated from E. coli are qualitatively different from the mature rRNA species found in yeast, there is some specificity in the synthesis. The yeast sequences made in E. coli are being characterized further to determine whether or not any of the steps in transcription and processing have been carried out correctly.

404 MAPPING OF MITOCHONDRIAL RNA IN XENOPUS LAEVIS. Eva Rastl, Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, Maryland 20014.

Mitochondrial DNA of metazoan animals codes for 2 rRNAs, about 20 tRNAs and a number of poly(A)-containing RNAs that are presumably mRNAs. The location of the sites coding for rRNAs and tRNAs in Xenopus laevis has been determined previously in our group. I shall present a map of the poly(A)-containing RNAs which we obtained using two approaches.

(1) R-loop-mapping of poly(A)-containing RNA on cloned mitochondrial DNA, using mitochondrial rRNAs as orientation markers.

(2) Separating fully denatured (glyoxylated) mitochondrial RNAs on gels, transfering them to RNA binding diazobenzyloxymethyl-paper and hybridizing them to different restriction fragments of mitochondrial DNA.

We have localized at least 9 poly(A)-containing RNAs as well as 2 nonribosomal poly(A)-lacking RNAs and have determined their molecular weights. We have also found rare RNA molecules of higher molecular weight that overlap the mRNA positions and that might be precursors. Most RNAs are transcribed from the heavy strand of the DNA molecule.