the presence of ORF1p. We examine several Ty3 NC mutants to identify the roles of functional regions of the protein in its chaperone activity. This research was supported in part by funding from INSERM and ANRS (France).

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The Codes of "Non-Coding" ncRNA in Epigenetics: Episcription and Hermeneutics of the Genome in the Entangled Cancer-Angiogenesis-Tolerance Epigenome

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OBJECTIVE: The genome as 3D-nucleic acid-[glyco-]protein nanomachine orchestrates translation of genetic [Mendelian] and epigenetic [non-Mendelian] information into phenotypical structural patterns. It is not restricted to transcription *and* translation of DNA scripts into proteins which matter only 2% of transcriptional output. By episcription, same genotypes [e.g. twins] deliver plethoras of not identical epigenetic phenotype variations. Functions of *endogenous RNA* ***isolated*** from total transcriptional output upon cell activation were investigated in vascular remodeling and tolerance phenomena. Structural codes *[3D-episcripts]* for epigenetic phenotyping were found escaping deciphering, so far. ***METHODS:*** Wissler et al, Protides Biol.Fluids **34**:517-536,1986; Materialwiss.Werkstofftech.**32**:984-1008,2001; Ann.N.Y.Acad.Sci.**961**:292-297,2002; **1022**:163-184,2004; **1137**:316-342,2008. ***RESULTS: Functional small ncRNA*** [<200n] were found upon cell activation by extrinsic environmental factors, including mitogenic, cell-mediated immune memory, metabolic [hypoxia] and [physical] shear stress reactions. They comprise conventional models for epigenetic remodeling variations directed rather to proteinaceous gene expression and regulation than genomic DNA sequence changes. Some edited, modified, *redox- and metalloregulated small hairpin* nc-shRNA bioaptamers are ***not complimentary*** to protein-coding transcripts, but feature 3D-episcripts fitting or misfitting to distinct protein conformers. Some address homologous helix-nucleating structural [proteomic] domains, termed **K**/**R**xxx**H** [**K**/**R**3**H**], i.e.-t/s/x**K**/**R**/q/n/hxxx**H**/y/n/q/e/d/r/kx$_{7\text{-}9}$h/xx$_{7\text{-}9}$h/xx$_{5\text{-}20}$**K**/**R**/q/n/e/h- with accessory canonical basic [R/K]$_n$, R/K-zipper, SR/K/RS, EF-hand and/or HxxxH/y/n/q segments, shared in several epigenetic regulator proteins entangled in growth, metabolic syndrome, vascularization, cancer epi- and genetic information indexing of the epigenome. ***CONCLUSIONS:*** Results suggest epigenetic [non-Mendelian] codes consisting of two different associated imprints: [1] Nucleic acid 3D-episcripts which some are not directly retranslatable to protein-coding transcripts. [2] Defined domains in epigenetic regulator protein and nucleic acid matrices as interaction address with [1] comprising all mutational, variant, polymorphism, infectious [viral] and Mendelian disease implications. This suggests tolerated mess-chaotic tumor vascularization associated with *bioaptamer disorders* in ncRNA-switched proteinaceous genetic and epigenetic processes.

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Linking Yeast Transcription Factor Structural Class and Detailed Binding Preferences with in vivo Regulatory Functions

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Cellular responses to internal and external stimuli through changes in gene expression are in part controlled by the binding of regulatory transcription factors (TFs) to specific sequences of DNA. These TFs belong to a wide variety of DNA binding domain (DBD) structural classes. Sequence specificity is diverse between structural classes, but TFs within each class often have apparently redundant sequence preferences. To understand how cells use these regulators to coordinate networks of responses, it is essential to determine how regulatory function is shared and partitioned between factors of similar and different structures and DNA binding specificities. Are certain structural classes better suited for certain functions? Are detailed differences in binding preferences among apparently similar TFs relevant to *in vivo* function? Using genome-wide datasets, we have described trends in biological function and regulatory mechanisms within TF structural classes in the yeast *Saccharomyces cerevisiae*. These trends suggest general ways in which TF function may be distributed across structural classes according to the biophysical constraints dictated by each DBD structure. Such analyses do not show, however, how specific details of an individual TF's binding specificity might affect its biological function. New data from protein binding microarrays (PBMs) provide such detailed TF binding preference information at all possible 8 base-pair DNA sequences. By combining these PBM data with *in vivo* binding locations measured by chromatin immunoprecipitation (ChIP-chip) experiments, we can infer the functional importance of specific types of TF binding sites (ie low and high affinity sites). This and other analyses made possible by the high resolution PBM data, when combined with observed functional trends in structural classes, will demonstrate how the cell utilizes both general and specific biophysical TF properties to accomplish cellular functions.

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Dissecting the High Rate Constant for the Binding of a Ribotoxin to the Ribosome

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Restrictocin belongs to a family of site-specific ribonucleases that kill cells by inactivating the ribosome. The restrictocin-ribosome binding rate constant was observed to exceed 10^{10} $M^{-1}s^{-1}$ [1]. We have developed a transient-complex theory to model the binding rates of protein-protein and protein-RNA complexes [2, 3]. The theory predicts the rate constant as $k_a = k_{a0} \exp(-U_{el}^*/k_BT)$, where k_{a0} is the basal rate constant for reaching the transient complex, located at the outer boundary of the bound state, by random diffusion, and U_{el}^* is the average electrostatic interaction energy within the transient complex. Here we applied the transient-complex theory to dissect the high restrictocin-ribosome binding rate constant. We found that the binding rate of restrictocin to the isolated sarcin/ricin loop is electrostatically enhanced by ~300-fold, similar to results found in protein-protein and protein-RNA complexes [2, 3]. Ribosome provides an additional 5000-fold rate enhancement. Two mechanisms work together to provide the dramatic additional enhancement. First, with the ribosome the transient complex is formed with relative separations and orientations where local electrostatic interactions with sarcin/ricin loop are particularly favorable. Second, distant parts of the ribosome provides additional electrostatic attraction with the ribotoxin. Our results quantitatively rationalize the experimental results for salt dependences and mutational effects of the binding rates of restrictocin with the isolated sarcin/ricin loop and the ribosome.

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Insight into the Roles of the 140-149 Catalytic Loop and the Zinc-Binding Domain for HIV-1 Integrase Activity

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Compounds targeting HIV-1 integrase multimerization process represent an interesting approach with likely no cross-resistance with other compounds such as strand-transfer inhibitors (INSTI). To get deeper insight into the role of the zinc-binding domain into the multimerization process, we have studied the effect of a zinc ejector, DIBA-1(2,2′-dithiobisbenzamide-1), on integrase activity. In presence of Mg^{2+}, DIBA significantly decreases the Hill number characterizing integrase-DNA interaction, as measured by fluorescence anisotropy, with only modest effects on the overall affinity, suggesting that zinc ejection primarily perturbs protein-protein interactions and then the nature of the active oligomer. In presence of Mn^{2+}, we found that the DNA-binding of integrase was less cooperative and therefore the Mn^{2+}-dependent 3′-processing is not expected to be influenced by zinc ejection. Nevertheless, the Mn^{2+}-dependent disintegration activity performed by truncated integrase lacking the zinc-binding domain remains sensitive to DIBA-1. One residue, Cys56, was identified as playing an important role for DIBA efficiency in the Mn^{2+} context. In contrast, no effect of the C56 mutation was observed in presence of Mg^{2+}, suggesting that, in this context, DIBA primarily inhibits integrase by a zinc ejection mechanism. A catalytic mutant Q148H was also studied due to its key role in the resistance to Raltegravir, an INSTI currently used in clinic against viruses resistant to other antiretroviral compounds. An additional mutation, G140S, was found to be associated with Q148H in Raltegravir-resistant patients. We found that resistance was mainly due to Q148H as compared to G140S. However, Q148H alone ''freezes'' integrase into a catalytically inactive state. In contrast, the conformational transition from the inactive to the active form is rescued with the double mutation G140S/Q148H. Consequently, when combined together, G140S/Q148H lead to a highly resistant integrase with improved catalytic efficiency.

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Single Molecule Measurements Of The Role Of Tetramer Opening In LacI-mediated DNA Looping

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Lac repressor (LacI) controls transcription of the genes involved in lactose metabolism. A key role in LacI function is played by its ability to bind simultaneously to two operators, forming a loop in the intervening DNA. Recently, several lines of evidence (both theoretical and experimental) have suggested the possibility for the LacI tetramer to adopt different structural conformations by flexing about its C-terminal tetramerization domain. At present it remains unclear to what extent different looping geometries are due to DNA binding topologies rather than distinct protein conformations. We address these questions by employing single molecule tethered particle motion on LacI mutants with intratetramer crosslinking at different positions along the cleft between the two dimers. Measurements on wild-type LacI reveal the existence of three distinct levels of effective tether length, most likely due to the presence of two different DNA looped structures. Restricting conformational flexibility with protein by cross-linking induces clear changes in the tether length distributions, indicating profound effects of tetramer opening (and its limitation due to cross-linking) on the looping conformations available to the system. Our data suggest an important role for large-scale conformational changes of LacI in the looping structures and dynamics.

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The Enfolding Arms of EcoRI Endonuclease as Probed by ESR Experiments

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Our research focuses on adducing general principles applicable to site-specific protein-DNA interactions by linking function to structural, thermodynamic and dynamic properties. We use as a model the interaction of EcoRI endonuclease with specific, miscognate (EcoRI*), and nonspecific DNA sequences. The crystal structure of the specific complex shows that the EcoRI "arms", invisible (disordered) in the structure of the apoenzyme, enfold cognate DNA upon binding. We are using four pulse Double Electron-Electron Resonance (DEER) FT-ESR experiments to map distances and distance distributions between nitroxide spin labels placed on cysteine-substituted residues in the two "arms" of the EcoRI homodimer, between Cu^{2+} ions bound near the active sites, and between nitroxide to Cu^{2+} positions. Our data show that the mean point-to-point distances between the "outer arms", between the "inner arms" and from the "outer arm" to the main domain are the same in specific, EcoRI*, and nonspecific complexes. This implies that the EcoRI arms must enfold the DNA in all three classes of complexes. However, an increase in the breadth of distance distributions is observed for noncognate complexes relative to that observed for the tightly complementary specific complex. These results are consistent with inferences from our thermodynamic analyses that the equilibrium ensemble of conformational microstates is larger for noncognate than specific complexes. Our continuous wave (CW) ESR experiments probing the dynamics of the arms support this hypothesis. Nonspecific complexes have been shown to have an important function in accelerating the location of correct recognition sites. It is striking that the EcoRI arms also embrace the DNA in the sliding nonspecific complex.

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Nuclear RISC Originates from Cytoplasmic Loaded RISC in Human Cells

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Studies of RNA interference (RNAi) provide evidence that in addition to the well characterized cytoplasmic mechanisms, nuclear mechanisms also exist. The mechanism by which the nuclear RNA-induced silencing complex (RISC) is formed in mammalian cells, as well as the relationship between the RNA silencing pathways in nuclear and cytoplasmic compartments is still unknown. Here we show by applying fluorescence correlation and cross-correlation spectroscopy (FCS/FCCS) in vivo that two distinct RISC exist: a large ~3 MDa complex in the cytoplasm and a 20-fold smaller complex of ~158 kDa in the nucleus. We further show that nuclear RISC, consisting only of Ago2 and a short RNA, is loaded in the cytoplasm and imported into the nucleus. The loaded RISC accumulates in the nucleus depending on the presence of a target, based on an miRNA-like interaction with impaired cleavage of the cognate RNA. Together, these results suggest a new RISC shuttling mechanism between nucleus and cytoplasm ensuring concomitant gene regulation by small RNAs in both compartments.

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A Dna Mimic Caught In The Act: 3D Electron Microscopy Shows Ecoki Methyltransferase In Complex With The T7 Antirestriction Protein Ocr.

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Type I DNA restriction-modification (R/M) systems are important agents in limiting the transmission of mobile genetic elements responsible for spreading bacterial resistance to antibiotics. EcoKI, a Type I R/M enzyme from Escherichia coli, acts by methylation- and sequence-specific recognition, leading to either methylation of the DNA target or translocation, followed by cutting at a random site, often hundreds of base pairs away. Consisting of one specificity subunit, two modification subunits, and two DNA translocase/endonuclease subunits, EcoKI is inhibited by the T7 phage antirestriction protein Ocr. Ocr mimics DNA with a pseudo-helical arrangement of charges, and is bent at a similar angle to that predicted for target DNA. We present a 3D density map generated by negative stain electron microscopy of the central core of the restriction complex, M.EcoKI M2S1 methyltransferase, bound to dimeric Ocr. Single particle analysis was carried out in IMAGIC and EMAN and resulted in a 3D reconstruction at ~18 Å resolution. An atomic model of all 5 subunits was generated by automated docking and homology modelling. This was computationally fitted into the EM density, giving excellent agreement. Ocr binds through the center of the M.EcoKI complex, spanning the two DNA recognition sites and matching the path predicted for its substrate DNA. We also present a complete atomic model of M.EcoKI in complex with its cognate DNA giving a clear picture of the overall clamp-like operation of the enzyme. The model is consistent with a large body of published experimental data on EcoKI spanning 40 years.

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Investigation of Dnmt1-DNA Interaction using Fluorescence Fluctuation Spectroscopy

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DNA methyltransferase 1 (dnmt1) is an important factor in the epigenetic process of DNA methylation. It is responsible for the regulation of tissue-specific patterns of methylated cytosine residues. Pathological changes in these methylation patterns are connected with various diseases, for example certain types of cancer.

We investigated the functional nature of the interaction between dnmt1 and DNA. A construct was formed, consisting of a synthetic DNA strand, labeled with a synthetic fluorescent dye, and dnmt1, labeled with Green Fluorescent Protein (GFP).

To determine whether the functional form of dnmt1 is monomeric, dimeric or consists of even larger complexes, we measured the ratio of GFP to synthetic dye molecules using Fluorescence Fluctuation methods such as Fluorescence Cross Correlation spectroscopy (FCCS), stoichiometry determination from a Burst Analysis experiment as well as Photon Counting Histograms (PCH) analysis.

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Thermodynamic Characteristics of pre-mRNA Splice Site Recognition

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Here, we reveal that an unusually large enthalpy-entropy compensation underlies recognition of polypyrimidine (Py) splice site signals. Competitive binding to Py tract splice site signals represents a prevalent means for alternative pre-mRNA splicing. The thermodynamic forces driving association of splicing factors with single-stranded (ss) pre-mRNAs represents a gap in the current understanding of splice site selection. We compared Py tract interactions among three splicing factors: (1) $U2AF^{65}$, an essential pre-mRNA splicing factor that recognizes constitutive 3′ splice site signals; (2) Sex-Lethal, a prototypical alternative splicing factor that antagonizes $U2AF^{65}$; and (3) TIA-1, an alternative splicing factor that promotes use of specific 5′ splice sites. All three proteins bound polyuridine (U_{20}) sequences with comparable or higher affinity than natural splice site sequences in fluorescence anisotropy assays. Consistent with the ability of Sex-Lethal to outcompete $U2AF^{65}$ during splice site selection, $U2AF^{65}$ displayed the lowest and Sex-Lethal the highest affinities for the RNA sites.

The enthalpic and entropic contributions were investigated in detail using ITC, initially using the homogeneous U_{20} site to avoid sequence-dependent complications of the binding isotherms. All three splicing factors exhibited an unusually large enthalpy-entropy compensation underlying U_{20} binding, with magnitudes ~10-fold greater than those of typical protein-protein or protein-ligand complexes. Given that full thermodynamic characterizations of protein association with single-stranded RNAs are rare, this raised the question of the source of this unusual thermodynamic signature: Is a large enthalpy-entropy