demonstrates that a wide range of biological phenomena can be explained by interactions between TFs and chromatin, and provides a quantitative description of the following processes:

- cooperative binding and synergistic action of non-interacting TFs;
- access of TFs to chromatinized DNA;
- displacement of nucleosomes from regulatory regions;
- rapid evolutionary changes in arrangement and membership of TF-binding sites in eukaryotic regulatory regions.

Strikingly, we found that cooperative binding of TFs to chromatinized DNA is identical to the Monod-Wyman-Changeux model of allosteric cooperativity in hemoglobin, pointing at a general mechanism of cooperativity employed in a range of biological systems. This parallel allowed us to use classical results in biochemistry to gain deep insights into the mechanisms of gene regulation.

2916-Plat

Nucleosome Depleted Region In Promoter Improves Robustness In Gene Expression

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Nucleosomes assembled onto promoters are critical for eukaryotic transcription regulation. Recent genome wide mapping of nucleosome positioning in *Saccharomyces cerevisiae* revealed that many promoters have nucleosome depleted region (NDR) that contains the transcription factor (TF) binding sites (TFBS), presumably to provide access for the factors. However, the necessity and advantage of this configuration is not well understood. We perturbed the relative position between TFBS and nucleosomes on two G1/S cell-cycle regulated promoters, and used time-lapse fluorescence microscopy to probe their transcriptional activity in individual cells through multiple cell cycles. We find that although localization of TFBSs to NDR is not required for transcription activation, it significantly reduces variability in expression between cell cycles. TFBSs localized to NDR activate transcription in every cell cycle with high reliability. In contrast, when the same TFBSs are located within positioned nucleosomes, activation was bimodal: nearly fully active in some cell cycles, and essentially undetectable in others. Interestingly, this ''on/off'' transcription pattern displays short-term ''memory'', or epigenic inheritance, from the previous mother cell cycle. Furthermore, the probability of ''on/off'' cycles could be affected by varying the TF concentration and nucleosome acetylation level. Our results have significant implications for the function and evolution of NDR, and for the relationship between the chromatin structure on promoters and the reliability of gene expression.

2917-Plat

Identifying and quantifying load-dependence in transcription initiation by T7 RNA Polymerase

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To access the genetic code to be transcribed to RNA, RNA polymerases must first melt the DNA, opening a ''transcription bubble''. In single molecule optical tweezers studies of initiation by T7 bacteriophage RNA polymerase (T7 RNAP), we have found that the lifetime of the initiation complex is strongly dependent on tension applied to the upstream DNA, indicating, in accord with structural studies, that initiation by T7 RNAP likely involves mechanical deformation of the DNA. The tension dependence diminishes considerably in the absence of free ribonucleotides, suggesting that this mechanical deformation of the DNA template does not coincide with binding, but instead with the opening of the transcription bubble. A new maximum-likelihood method will be presented, in which data do not have to be grouped into force bins, to identify the force-dependent step or steps in initiation by T7 RNAP and to estimate the associated rates and distances to the transition state from these experiments. This method is generalizable to systems with more complicated kinetic pathways, as long as there are two observable states (e.g. bound and unbound) and an irreversible final transition.

2918-Plat

Single RNA Counting Reveals Alternative Modes Of Gene Expression In Yeast

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Proper execution of gene expression programs requires the coordination of many steps along the gene expression pathway. Preceding all downstream steps, transcriptional control is the most critical step in gene expression regulation. The transcription machinery has to ensure that the two most defining factors of mRNA expression, timing and amplitude of mRNA production, are properly controlled. How precisely the transcription machinery fulfills this task is not known. To address this question directly, we used an improved in situ hybridization approach that detects single mRNA molecules within fixed yeast cells. This approach provides a quantitative measure of mRNA abundance and transcriptional activity for endogenous genes in single cells. We show that expression levels are higher than reported by the literature and vary significantly among cells. Combining the single transcript measurements with stochastic simulations we demonstrate the existence of alternative modes of transcription that lead to different levels of mRNA variation in the cell. Housekeeping genes are expressed by single initiation events but no transcription bursts, resulting in low mRNA variability. In contrast, PDR5, a SAGA regulated gene, is expressed by transcription bursts; multiple transcripts are initiated within a short period of time followed by periods where no transcription occurs, resulting in significantly larger variation. This shows that yeast contains multiple modes of transcription allowing it to modulate its transcriptome according to specific physiological requirements.
To gain even further insight in the kinetic of gene expression, we used a live cell imaging approach that allows us to detect single mRNAs in yeast cells; at the site of transcription as well as in the cytoplasm. Analysis of the expression kinetics of different steps along the gene expression pathway will be presented. Supported by NIH.GM 57071.

2919-Plat

A Powerful Response to Viral Infection

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The key initial step in the response of the innate immune system to viral infection is the induction of the cytokine Interferon-β(IFNβ.) Recently, the cell-to-cell variability of IFNβ mRNA in human dendritic-cells has been studied. Specifically, the number of mRNAs produced by each of the two alleles in a cell in response to infection to by a non-pathogenic avian-flu virus was measured and surprisingly high levels of variability were observed. The induction is known to occur through the slow recruitment of several transcription factors to form an 'enhanceosome' assembly, over a period of hours. This variability referred to as noise was attributed to the dynamics of enhanceosome formation. Noise can arise because of the stochasticity of the chemical reactions due to the small copy numbers of molecules involved (termed ''intrinsic'') or due to fluctuations the number of transcription factors between cells (termed ''extrinsic). The experiment was able to determine the allelic origin of the mRNAs in a given cell and quantify the 'extrinsic noise' as the correlation between the number of alleles produced by each allele, thus isolating the intrinsic noise contribution. They found that a significant fraction (about half) of the total noise came from intrinsic noise.
We have analyzed the measured mRNA distribution and find that the observed long-tailed distribution can be fit by a power-law over roughly three decades at different times of observation. We find that a pulsing model in which the completed enhanceosome switches between a state in which transcription occurs at a very low or zero level and another in which transcription proceeds at a high rate can reproduce the power-law behavior. We discuss under what conditions this model produces mRNA distributions with the observed long-tailed behavior and how these distributions evolve with time.

2920-Plat

How Xenopus Embryos Complete DNA Replication Reliably: Solution to the Random-completion Problem

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DNA replication in *Xenopus* embryos starts at random positions along the genome and at random times during S phase. This spatiotemporal randomness implies fluctuations in the completion times and can lead to cell death if replication takes longer than the cell cycle time (approximately 25 min). Surprisingly, while the typical completion time (approximately 20 min) is close to the cell cycle time, replication failure occurs only about 1 in 300 times. These observations raise an interesting question, known as the ''random-completion problem'': How can *Xenopus* embryos accurately control the replication timing despite the spatiotemporal randomness?
We use a nucleation-and-growth model and extreme-value statistics to address quantitatively the random-completion problem. We first show that *Xenopus* embryos solve the problem by using a large reservoir of potential replication origins that are increasingly likely to initiate, a situation that leads to robust

control of replication timing. We also show that variations in the spatial distribution of origins have minimal effect on the accurate control of replication times. Finally, we compare the replication program in *Xenopus* to the program that minimizes the use of certain replicative proteins; we find them to be similar.

2921-Plat
Dynamics Of DNA Replication Loops Reveal Temporal Control Of Lagging-strand Synthesis
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In all organisms, the protein machinery responsible for the replication of DNA, the replisome, is faced with a directionality problem. The antiparallel nature of duplex DNA permits the leading-strand polymerase to advance in a continuous fashion, but forces the lagging-strand polymerase to synthesize in the opposite direction. By extending RNA primers, the lagging-strand polymerase restarts at short intervals and produces Okazaki fragments. At least in prokaryotic systems, this directionality problem is solved by the formation of a loop in the lagging strand of the replication fork to reorient the lagging-strand DNA polymerase so that it advances in parallel with the leading-strand polymerase. The replication loop grows and shrinks during each cycle of Okazaki-fragment synthesis. Here, we employ single-molecule techniques to visualize, in real time, the formation and release of replication loops by individual replisomes of bacteriophage T7 supporting coordinated DNA replication. Analysis of the distributions of loop sizes and lag times between loops reveals that initiation of primer synthesis and the completion of an Okazaki fragment each serve as a trigger for loop release. The presence of two triggers may represent a fail-safe mechanism ensuring the timely reset of the replisome after the synthesis of every Okazaki fragment.

Platform BE: Protein Folding & Stability II

2922-Plat
The Rop-Dimer: A Folded Protein Living Between Two Alternate Structures
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A central assumption in protein folding is that a protein's native state is unique and stable. The Rop-dimer (Repressor Of Primer) shows strong changes in its folding kinetics and binding ability to RNA upon mutation of its hydrophobic core. Computer simulations investigated the possibility of two competing conformations to explain these results. Given an equivalent energetic bias, both conformations show different kinetic accessibilities in these simulations. Thus Rop's mutational behavior was explained by a preference of the kinetically less favored Wild-Type conformation for slow (un)folding mutants. Faster (un)folding mutants should prefer the kinetically favored conformation. For specific mutants it was suggested that the protein's native state is constituted by two competing conformations. Inspired by these simulations, single-molecule FRET-measurements verified the suggestion of two competing conformations constituting the native ensemble. Despite the need of a large-scale conformational change to get from the one conformation to the other, it shows that for a specific mutant the same dimer can adopt both conformations over time without disassociation of its monomers or changes in environmental conditions.

2923-Plat
Crowded, Cell-like Environment Induces Shape Changes In Aspherical Protein
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Protein dynamics in cells may be different from that in dilute solutions in vitro since the environment in cells is highly concentrated with other macromolecules. This volume exclusion due to macromolecular crowding is predicted to affect both equilibrium and kinetic processes involving protein conformational changes. To quantify macromolecular crowding effects on protein folding mechanisms, here we have investigated the folding energy landscape of an α/β protein, apoflavodoxin, in the presence of inert macromolecular crowding agents using in silico and in vitro approaches. By coarse-grained molecular simulations and topology-based potential interactions, we probed the effects of increased volume fraction of crowding agents (fc) as well as of crowding agent geometry (sphere or spherocylinder) at high fc. Parallel kinetic folding experiments with purified Desulfovibro desulfuricans apoflavodoxin in vitro were performed in the presence of Ficoll (sphere) and Dextran (spherocylinder) synthetic crowding agents. In conclusion, we have identified in silico crowding conditions that best enhance protein stability and discovered that upon manipulation of the crowding conditions, folding routes experiencing topological frustrations can be either enhanced or relieved. The test-tube experiments confirmed that apoflavodoxin's time-resolved folding path is modulated by crowding agent geometry. We propose that macromolecular crowding effects may be a tool for manipulation of protein folding and function in living cells.

2924-Plat
Assessing Mechanical And Thermodynamic Response Upon Allosteric Perturbation
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Allosteric regulation involves changes of protein activity upon ligand binding or covalent modification at a location distinct from the active site. We investigate the underlying mechanisms of allosteric proteins using a minimal Distance Constraint Model (mDCM) [1]. We recently employed the mDCM to assess how relationships between mechanical and thermodynamic descriptions affect intramolecular communication [2]. Here, both the mechanical and thermodynamic response upon allosteric perturbation is assessed. Constraints are introduced at every residue position to mimic the binding of allosteric ligands, and the resulting changes are analyzed using a wide array of response functions. We apply the methodology to several proteins, including calmodulin, CheY, and ras. Interestingly, application of a small number of constraints in one domain of the extended calmodulin structure is sufficient to cause substantial changes in the other, despite the propagation path being channeled through a long connecting helix. Residues identified as important to the binding and function of these dynamic protein systems show acute changes in thermodynamic response. In addition to quantifying changes in free energy, thermodynamic response is decomposed into component enthalpies and entropies. Allosteric response is also quantified by induced changes within mechanical properties, such as flexibility along the backbone, cooperativity correlation between residue pairs, and global rigidity characteristics. Taken together, the mechanical and thermodynamic responses provide insight into the fundamental mechanisms of allosteric communication. This work is supported by NIH R01 GM073082.
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[2] J.M. Mottonen, et al. *PROTEINS* In press (DOI: 10.1002/prot.22273).

2925-Plat
Configuration Entropy Modulates the Mechanical Stability of Protein GB1
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Configurational entropy plays important roles in defining the thermodynamic stability as well as the folding/unfolding kinetics of proteins. Here we combine single molecule atomic force microscopy and protein engineering techniques to directly examine the role of configurational entropy in the mechanical unfolding kinetics and mechanical stability of proteins. We use a small protein GB1 as a model system and constructed four mutants that elongate loop 2 of GB1 by two, five, twenty four and forty six flexible residues, respectively. These loop elongation mutants fold properly as determined by far-UV circular dichroism spectroscopy, suggesting that loop 2 is well tolerant of loop insertions without affecting GB1's native structure. Our single molecule AFM results reveal that loop elongation decreases the mechanical stability of GB1 and accelerates the mechanical unfolding kinetics. These results can be explained by the loss of configurational entropy upon closing an unstructured flexible loop using classical polymer theory, highlighting the important role of loop regions in the mechanical unfolding of proteins. This study not only demonstrated a general approach to investigate the structural deformation of the loop regions in mechanical unfolding transition state, but also provides the foundation to use configurational entropy as an effective means to modulate the mechanical stability of proteins, which is of critical importance towards engineering artificial elastomeric proteins with tailored nanomechanical properties.

2926-Plat
Air/water Interface Induced Folding And Self-assembly Of Amyloid-beta Peptide
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