a proof of concept we focused on the prototypical human β2-adrenergic receptor, β2AR, reconstituted in lipid vesicles. Our approach allowed us to extract the dimerization equilibrium constant of the β2AR to be −4 ± 0.2 KbT.

[1] Bouvier, M. Oligomerization of G-protein-coupled transmitter receptors. Nat Rev Neurosci 2, 274-286 (2001).
[2] Smith, N.J. & Milligan, G. Allostery at G protein-coupled receptor homo- and heteromers: uncharted pharmacological landscapes. Pharmacol Rev 62, 701-725 (2010).
[3] Fung, J.J. et al. Ligand-regulated oligomerization of beta(2)-adrenoceptors in a model lipid bilayer. EMBO J 28, 3315-3328 (2009).
[4] Stamou, D. et al. Amphipathic motifs in BAR domains are essential for membrane curvature sensing. Embo Journal 28, 3303-3314 (2009).
[5] Stamou, D. et al. How curved membranes recruit amphipathic helices and protein anchoring motifs. Nature Chemical Biology 5, 835-841 (2009).

Symposium: Chromosome Architecture and Function

1174-Symp
Bacterial Chromosome Structure and Function
Sankar Adhya.
National Institutes of Health, Bethesda, MD, USA.
We previously showed that the transcription profile of the E. coli chromosome is dictated by its structure. We proposed that the chromosome has defined structures which change the transcriptability of the constituent promoters. We are exploring to understand the structure of the E. coli chromosome by a variety of approaches.
HU, an abundant highly conserved chromosome-associated protein in E. coli, has been implicated to play an architectural role in the chromosome, and to constrain supercoils in DNA. We will discuss the contribution of HU in chromosomal structure and, as a result, in global gene expression. We will also describe RNA molecules that bind to HU, and a potential role of the RNA/HU complexes in chromosome compaction.
In addition, we performed chromosome conformation analysis, and discovered that GalR, a regulon specific repressor, connects distal segments of the chromosome by GalR-GalR interactions. We believe this network formation organizes the chromosome in space, thereby contributing to chromosome compaction. This is the first evidence that a transcription factor participates in folding the chromosome into a 3-D structure.

1175-Symp
Mechanisms of Gene Regulation Over a Distance on DNA and in Chromatin
Vasily M. Studitsky.
UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA.
The majority of eukaryotic genes and some prokaryotic genes are positively regulated by activator-binding DNA sequences (enhancers) that can efficiently communicate and directly interact with their targets (promoters) over extended regions of spacer DNA organized into chromatin structure (see (1) for a review). The mechanisms for mediating enhancer-promoter interaction in chromatin include tracking and looping models.
Previously we have shown that DNA supercoiling greatly facilitates EPC over a large distance (2-4). Our studies have also suggested that chromatin structure per se can support highly efficient communication over a distance by a looping mechanism and functionally mimic the supercoiled state characteristic for prokaryotic DNA (5). However, the mechanisms allowing efficient communication in chromatin remain unknown.
More recently, we have established defined polynucleosomal chromatin templates allowing quantitative analysis of the rate of distant EPC and have identified components of the system and protein factors that strongly affect the rate of communication in chromatin. These studies will be presented at the meeting.
References
1. Bondarenko, V. A., Liu, Y. V., Jiang, Y. I., and Studitsky, V. M. (2003) Biochem. Cell. Biol. 81, 241-251.
2. Liu, Y., Bondarenko, V., Ninfa, A., and Studitsky, V. M. (2001) Proc. Natl. Acad. Sci. USA 98, 14883-14888.
3. Bondarenko, V. A., Jiang, Y. I., and Studitsky, V. M. (2003) EMBO J. 22, 4728-4737.
4. Polikanov, Y. S., Bondarenko, V. A., Tchernaenko, V., Jiang, Y. I., Lutter, L. C., Vologodskii, A., and Studitsky, V. M. (2007) Biophys. J. 93, 2726-2731.
5. Rubtsov, M. A., Polikanov, Y. S., Bondarenko, V. A., Wang, Y. H., and Studitsky, V. M. (2006) Proc. Natl. Acad. Sci. USA 103, 17690-17695.

1176-Symp
Single-Molecule Sorting of DNA Repair Machines
Maria Spies, Ph.D.
Biochemsitry, University of Illinois, Urbana-Champaign, Urbana, IL, USA.
Often, molecular machines orchestrating distinct types of DNA repair share motor components known as DNA helicases, ubiquitous vectorial enzymes that use chemical energy of ATP to translocate directionally on the DNA, transiently unwind duplex DNA or remodel protein-nucleic acid complexes. Participation in different pathways may require a helicase to switch between different activities (for example, between being a bona fide helicase versus a DNA translocase that dismantles protein-nucleic acid complexes), to down- or up-regulate its processivity or to recognize a different substrate. Activity switching in DNA repair enzymes is often controlled by reversible post-translational modifications. Addition or removal of these modifications may rapidly activate or inactivate one or several activities of a target helicase or may alter its molecular interactions.
To address the role of post-translational modification and specific interactions in directing a helicase to a particular genome maintenance event, we developed an experimental system we refer to as single-molecule sorting. This single-molecule total internal reflection microscopy (TIRFM) approach discriminates between subpopulations of differentially modified enzymes and correlates the presence of each modification to the biochemical function and therefore to the biological role of a particular helicase. Application of single-molecule sorting to deciphering the molecular basis of pro- and anti-recombinogenic activity of human DNA repair helicase Fbh1 and its regulation will be discussed.

1177-Symp
Mechanisms of Nuclear, Spindle and Mitotic Chromosome Scaling
Rebecca Heald[1], Dan Levy[2], Jeremy Wilbur[2], Rose Loughlin[2], Esther Kieserman[2].
[1]Department Molecular/Cell Biology, University of California, Berkeley, Berkeley, CA, USA, [2]University of California, Berkeley, Berkeley, CA, USA.
Cell size varies widely among different organisms as well as within the same organism in different tissue types and during development, placing variable functional demands on internal structures. A fundamental question is how essential subcellular components such as the nucleus, mitotic spindle and chromosomes are regulated to accommodate cell size differences. Xenopus frogs offer two physiological contexts in which we can investigate this question. First, we can compare Xenopus laevis to the smaller, related species Xenopus tropicalis, which lays smaller eggs and has proportionally smaller cells throughout development. Second, we can compare different stages of Xenopus laevis embryogenesis, as the ~1 millimeter diameter egg rapidly cleaves to form smaller blastomeres, which by the 15th division are reduced to 40 microns across. A unique aspect of our approach is to prepare cytoplasmic extracts from eggs and embryos that recapitulate organelle scaling in vitro, which we can use to identify molecular differences that underlie size changes. We identified two factors, importin α and Ntf2, whose levels alter nuclear import and are largely responsible for the difference in nuclear size between the two frog species. With respect to spindle size regulation, based on predictions from a 2-D meiotic spindle simulation, we identified katanin-dependent MT severing as an activity reduced in X. laevis compared X. tropicalis. Interestingly, X. tropicalis lacks an inhibitory Aurora B phosphorylation site in the p60 catalytic subunit of katanin found in X. laevis at Ser131, which is largely responsible for the difference in spindle length. Mitotic chromosomes must also decrease in size to permit their proper segregation in smaller cells. We have established an in vitro system that recapitulates chromosome scaling during development, and are now poised to elucidate the underlying molecular mechanisms.

Symposium: Stretching and Bending Lipid Membranes

1178-Symp
Using Tension to Map Free-Energy Landscapes that Govern Pore Nucleation in Membranes
Evan Evans[1,2].
[1]Physics, Boston University, Boston, MA, USA, [2]University of British Columbia, Vancouver, BC, Canada.
Because of the large energy, thermal activation is needed to open nanopores in membranes yet activation energy can change significantly with tension. Although models exist describing the impact of tension, experiments have failed to cover the ranges of tension and pore nucleation rate required to examine theories. Hence, a micromechanical method was established to assay rates at which unstable nanopores form in giant single-bilayer vesicles in relation to