Symposium 16: Genome Organization and Dynamics

3155-Symp
Stoichiometry of Active DNA Replication Machinery Within Living Escherichia Coli Cells
David Sherratt, Rodrigo Reyes-Lamothe, Mark Leake.
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One of our missions is to explore the biology of the bacterial chromosome at single molecule resolution in live cells in close to real-time. Our focus is to analyze the assembly, action and disassembly of the molecular machine that replicates DNA [the replisome] in living *E. coli* cells and then use targeted protein ablation, using specific degron tags, to remove individual replisome components. The composition and architecture of the replisome in actively replicating cells is unknown, despite extensive characterization of the major replication components and their use to reconstitute bacterial and bacteriophage replication *in vitro*. By using a novel fluorescence microscopy technique ['slimfield'-microscopy], with single molecule sensitivity, simultaneous multi-color capture and 3 ms temporal resolution, on living cells expressing fluorescent derivatives of eight replisome components from their endogenous promoters, we show that active *Escherichia coli* replisomes *in vivo* contain one clamp loader complex associated with three molecules each of polymerase and DnaX. Comparison of the spatial distribution of the three sliding clamps and polymerases, shows that ~3/4 of replisomes have only two elongating polymerases associated with a sliding clamp at any one time. HolC and HolD, which heterodimerize to link the clamp loader to Ssb, are most frequently present as 2-5 molecules per replisome. Turnover of Ssb, which coats the single-stranded lagging DNA template, is ~2-fold faster in replicating cells than in replication-inhibited cells, with most active replisomes being associated with 5-11 Ssb tetramers. Replication-inhibited replisomes have a ~2-fold increase in Ssb associated with each fork.

3156-Symp
The Genomic Code for Nucleosome Positioning
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Eukaryotic genomes are packaged into nucleosome particles that occlude the DNA from interacting with most DNA binding proteins. We have discovered that genomes care where their nucleosomes are located on average, and that genomes manifest this care by encoding an additional layer of genetic information, superimposed on top of other kinds of regulatory and coding information that were previously recognized. The physical basis of this novel genetic information lies in the nucleosomes'intrinsic DNA sequence preferences, through sequence-dependent mechanics of DNA itself. We have developed a partial ability to read this nucleosome positioning information and predict the in vivo locations of nucleosomes. Most recently, we showed that the distribution of nucleosomes reconstituted on yeast genomic DNA in a purified in vitro system closely resembles that in vivo, implying that much of the in vivo nucleosome organization is explicitly encoded in the genomic DNA sequence itself, through the nucleosomes' DNA sequence preferences. A statistical model based only on the in vitro nucleosome DNA sequence data is significantly predictive of the detailed distribution of nucleosome locations in yeast, C. elegans, and human, suggesting that there may exist a universal genomic code for nucleosome positioning. Our results suggest that genomes utilize the nucleosome positioning code to facilitate specific chromosome functions, including to delineate functional versus nonfunctional binding sites for key gene regulatory proteins, and to define the next higher level of chromosome structure.

3157-Symp
DNA Accessibility in Nucleosomes
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The cell nucleus is a highly dynamic reaction center for many DNA-based cellular processes, as well as an effective compaction center for DNA storage. The basic packing unit of chromatin is the nucleosome. Even this lowest level of genomic compaction presents a strong barrier to many DNA-binding cellular factors. In a coordinated and regulated fashion, DNA associated with nucleosomes must be made transiently accessible to permit many essential processes. Nucleosome stability and structure thus are intimately involved in the regulation of genetic information storage and retrieval. Single molecule mechanical studies are ideally suited to probe these processes and have become powerful complementary tools to traditional biochemical and molecular biological approaches. I will discuss our progress in mechanical studies of nucleosome stability and structure, as well as how DNA in nucleosomes may be accessed by motor proteins.

3158-Symp
Chromatin Higher Order Structure and Regulation of its Compaction
Sara Sandin, Andrew Routh, **Daniela Rhodes**.
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During the past decade it has become evident that histone post-translational modifications are key regulators of nuclear processes whose substrate is DNA. Whilst the effects of, for instance, histone post-translational modification on transcription are well-documented, there is no mechanistic understanding of how such modification regulate chromatin condensation directly, or indirectly. Such an understanding is dependent on knowledge of the three-dimensional structure of chromatin. Although the structure of the first level of DNA folding, the nucleosome core, is known at atomic resolution, the structure of the second level of folding, whereby a string of nucleosomes folds into a fibre with an approximate diameter of 30 nm remains undetermined. I will describe our studies on the higher orders structure of chromatin with two primary aims:
1) Determination of ''30nm'' chromatin fibre structure to provide an understanding of fibre topology.
2) Biophysical characterization of the effects of the linker histone and histone modifications on the compaction of chromatin higher order structure.
EM measurements define the dimensions of the ''30nm'' chromatin fiber: Evidence for a compact, interdigitated structure
Robinson, J. J. P., Fairall, L., Huynh, V. A. T. and Rhodes, D.
(2006) Proc. Natl. Acad. Sci. USA 103, 6506-11
30 nm chromatin fibre decompaction requires both H4-K16 acetylation and linker histone eviction
Robinson PJ, An W, Routh A, Martino F, Chapman L, Roeder RG and Rhodes D.
(2008) J. Mol. Biol. 381: 816-25
Nucleosome repeat length and linker histone stoichiometry determine chromatin fiber structure
Routh A, Sandin S and Rhodes D.
(2008) Proc. Natl. Acad. Sci. U S A. 105: 8872-7
Single-molecule force spectroscopy reveals a highly compliant helical folding for the 30-nm chromatin fiber
Kruithof M, Chien FT, Routh A, Logie C, Rhodes D, van Noort J.
(2009) Nat. Struct. Mol. Biol.16:534-40

Symposium 17: Membranes as Barriers in Combating Infection

3159-Symp
Biological Roles and Therapeutical Potential of Cationic Host Defence Peptides
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Cationic antimicrobial peptides (AMPs; synonyms: host defence peptides, HDPs; defensins) are most frequently occurring antibiotics and are produced by virtually every life form as a first line of host defence. They do have both direct antibiotic activities which lead to rapid killing of microbes as well a immunomodulatory actvities which lead to upregulation of defence pathways and recruitment of immune cells (1). Both actvities are highly interesting for the development of innovative antiinfective strategies. However, in spite of considerable development activities, AMP-based antibiotics did not reach the market so far, and it may be necessary to better understand their molecular activities for AMP based rational drug design.
Unlike conventional antibiotics which act via defined target molecules, antimicrobial host defence peptides are assumed to act unspecifically by permeabilising cell membranes. We used various naturally occurring HDPs and synthetic AMPs to study their antimicrobial activity in more detail. We found that the antibiotic activity of cationic amphiphilic peptides is based on multiple inhibitory activities and that the perturbation of the membrane lipid bilayer may be just one of the multiple activities. In general terms, AMPs appear to disturb the coordinated function of highly dynamic, membrane bound multi-enzyme machineries such as the cell wall biosynthesis machinery and respiratory chains. Defensins *sensu strictu*, as defined by the presence of a disulfide-bridged γ-core motif such as the human ß-defensins 2 and 3 (hBD2, hBD3) may, in addition, gain specificity for defined molecular targets. In particular, the fungal defensin plectasin, which binds with high affinity to the bacterial cell wall precursor Lipid II and thus blocks cell wall biosynthesis is taken forward through preclinical antibiotic development.
1) Hancock, R.E.W., Sahl H.G.: Antimicrobial and host defence peptides as novel anti-infective therapeutic strategies. Nature Biotechnology, **24**, 1551-1557 (2006)