Symposium 13: Epigenetic Control of Gene Expression

2170-Symp
Molecular Mechanisms of Lysine Methylation
Raymond C. Trievel[1], Paul Del Rizzo[1], Jean-Francois Couture[2], Lynnette M. Dirk[3], Bethany Strunk[1], Marijo Roiko[1], Joseph S. Brunzelle[4], Robert L. Houtz[3].
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SET domain lysine methyltransferases (KMTs) are S-adenosylmethionine (AdoMet)-dependent enzymes that catalyze the site-specific methylation of lysine residues in histones, transcription factors, chromatin modifying enzymes, and other protein substrates. These modifications mediate protein:protein interactions with signaling factors that possess effector modules which can recognize methyllysines in a sequence-dependent manner. In addition to their site specificity, SET domain KMTs exhibit product specificity, which is defined as their ability to catalyze different degrees of methylation of the lysine epsilon amine group, thus imparting an additional hierarchy in methyllysine signaling. To understand the mechanism underlying the product specificity of SET domain KMTs, we have structurally and functionally characterized two active site mutants of the human monomethylase SET7/9 that alter its specificity to a dimethylase and a trimethylase, respectively. Structures of the SET7/9 mutants in complex with peptides bearing unmodified, mono, di, and trimethylated lysines reveal that water molecules within the active site function as molecular place holders that align the lysine epsilon amine group in a linear geometry with the sulfonium methyl group of AdoMet to promote methyltransfer. As the methylation state of the lysine substrate increases during successive methyltransfer reactions, the water molecules dissociate from the active site, thereby enlarging the lysine binding channel to accommodate the increasing bulk of the multiply methylated epsilon amine group. Taken together, our findings illuminate the catalytic roles of active site water molecules in facilitating lysine multiple methylation by SET domain KMTs.

2171-Symp
Structural Insights into the Histone Specificity of PHD Fingers
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Histone tails are essential components of the chromatin remodeling and gene transcription machinery. They undergo various posttranslational modifications (PTMs), including methylation at Lys and Arg residues, acetylation and ubiquitination at Lys residues and phosphorylation at Ser and Thr residues, and serve as docking sites for protein effectors. The histone marks can be added or removed by histone modifying enzymes, and a few protein domains have been identified to specifically recognize (or read) the tail modifications. The zinc binding PHD (plant homeodomain) finger is a recent addition to the family of epigenetic readers. Here, we characterize binding specificity of the PHD finger family using a set of biochemical, crystallographic and spectroscopic approaches. We compare the crystal structures and the histone binding mechanisms of the PHD fingers which select for histone H3 trimethylated at lysine 4 (H3K4me3) and unmodified H3. Our results provide novel insights into the molecular mechanisms underlying the biological activity of the PHD fingers and further our understanding of how the histone marks are read.
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2172-Symp
Chromatin Marks: Histone-Binding Modules and Catalytic Mechanisms
John Denu.
Univ Wisconsin Madison, Madison, WI, USA.
No Abstract.

2173-Symp
Epigenetic Link Between DNA Methylation and Histone Modifications
Xiaodong Cheng.
Emory Univ, Atlanta, GA, USA.
The methylation of mammalian DNA, primarily at CpG dinucleotides, has long been recognized to play a major role in controlling gene expression and in coordination with the parallel chromatin-marking system that operates at the level of histone modification. I will describe recent studies on, and discusses the resulting biochemical and structural insights into, the DNA nucleotide methyltransferases (Dnmts) and histone lysine methyltransferases (HKMTs) that modulate DNA methylation.

Symposium 14: TRP Channel Multimodal Gating

2174-Symp
Probing Mechanisms of Temperature Sensitivity of Thermo-TRPs
Ardem Patapoutian, Jörg Grandl.
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The sense of touch is unique in perceiving stimuli both physical (temperature, mechanical) and chemical (compounds that cause pain, itch, et cetera) in nature. Recently, we and others have identified and characterized molecules responsible for sensing environmental temperature. These proteins are ion channels activated by distinct changes in thermal energy (in the noxious to innocuous range), thus functioning as the molecular thermometers of our body. To date, the mechanism underlying thermal activation of TRP channels represents a fundamental unknown in the field. As might be expected, thermoTRPs are steeply temperature dependent. While most enzymatic processes have a Q10 of 2-4, thermoTRPs have Q10 values as high as 20. Temperature activation of most thermoTRPs is retained in cell-free membranes, arguing for a mechanism independent of cytoplasmic processes. We have developed a novel high-throughput random mutagenesis screen to identify residues required for the modulation of ion channels or receptors. We are applying this method to isolate thermoTRP temperature-insensitive mutants. Our near-term goal is to catalog all amino acids of thermoTRPs required for thermal activation. Ultimately, we aim to explain the mechanism by which temperature leads to pore-opening.

2175-Symp
The Role of TRP Channels In Mechanosensitive Somatosensory Neurons
Diana Bautista.
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The somatosensory system underlies our ability to detect touch and pain. To understand the molecular and cellular processes governing somatosensation, it is necessary to identify receptors that detect physical stimuli, such as temperature and touch, and determine how they transduce environmental signals into action potentials. TRP channels that mediate sensitivity to heat and cold have been identified from somatosensory neurons. But the role of TRP channels in mammalian mechanosensitive sensory neurons remains controversial. We used a variety of in vitro assays to characterize responses of distinct subsets of mechanosensitive sensory neurons. Using calcium imaging in combination with radial stretch, osmotic stimuli or natural products that target mechanosensitive neurons, we assessed mechanosensitivity of nociceptors and low-threshold mechanoreceptors. The role of various TRP channels in these responses will be discussed.

2176-Symp
Heat and Spice: Setting the Sensitivity of TRPV Channels
Rachelle Gaudet.
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TRPV channels play key roles in pain, thermo- and mechanosensation, and calcium homeostasis. The six mammalian TRPV channels partition into two groups: TRPV1-4, involved in sensory signaling; and TRPV5 and TRPV6, expressed in the intestinal tract and kidneys and important for calcium homeostasis. We determined the crystal structures of the N-terminal ankyrin repeat domain of TRPV1, TRPV2, TRPV4 and TRPV6. Superficially, the structures are similar, as expected from sequence homology, with six ankyrin repeats and unusually long finger loops. The structures do have notable differences in their details, and these structural differences result in drastically different biochemical properties. Our recent work has uncovered that the ankyrin repeats of TRPV1, TRPV2 and TRPV4 bind nucleotides and calmodulin and play a role in modulating channel sensitivity. For instance, the TRPV1 channel is a receptor for both noxious heat ($>42^{\circ}C$) and capsaicin, the compound that gives chili peppers their ''hot'' taste. Intracellular ATP sensitizes TRPV1 to capsaicin and heat, while calmodulin is necessary for desensitization. Furthermore, our latest data indicate that the interaction of calmodulin with the cytoplasmic regions of TRPV1 provides a negative feedback that is essential for setting the sharp temperature threshold of TRPV1.

2177-Symp
Activation of TRPML Channels in the Lysosome
Haoxing Xu.
Univ Michigan, Ann Arbor, MI, USA.
The mucolipin family of Transient Receptor Potential (TRPML) proteins is predicted to encode ion channels of intracellular endosomes and lysosomes. The physiological importance of TRPMLs has been established genetically. Mutations of human *TRPML1* cause type IV mucolipidosis (ML4), a devastating neurodegenerative disease; mutations in the mouse *TRPML3* result in the *varitint-waddler* (*Va*) phenotype with hearing and pigmentation defects. The

broad-spectrum phenotypes of both ML4 and *Va* appear to result from certain aspects of endosomal/lysosomal dysfunction. Lysosomes, traditionally believed to be the terminal ''recycle center'' for biological ''garbage'', are now known to play indispensable roles in membrane traffic and multiple intracellular signaling pathways. The putative lysosomal function(s) of TRPML proteins, however, has been unclear until recently. Studies on animal models and cell lines in which TRPML genes have been disrupted or genetically depleted have discovered roles of TRPMLs in a variety of cellular functions including membrane traffic, signal transduction, and organellar homeostasis. Physiological assays on cells in which TRPMLs are heterologously over-expressed revealed the channel properties of TRPMLs, suggesting that TRPMLs mediate cation (Ca^{2+}/Fe^{2+}) efflux from endosomes and lysosomes in response to unidentified cellular cues. Using our recently developed lysosome patch-clamp technique, we screened a variety of cytosolic and luminal factors that are known to affect endolysosomal functions and have identified an endogenous agonist for TRPML channels. We are currently investigating the activation mechanism in detail.

Platform AI: Micro & Nanotechnology, Nanopores

2178-Plat
Quantized Ionic Conductance in Nanopores
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Ionic transport in nanopores is a fundamentally and technologically important problem in view of its occurrence in biological processes and its impact on novel DNA sequencing applications. Using molecular dynamics simulations we show that ion transport may exhibit strong nonlinearities as a function of the pore radius reminiscent of the conductance quantization steps as a function of the transverse cross section of quantum point contacts. In the present case, however, conductance steps originate from the break up of the hydration layers that form around ions in aqueous solution. We discuss this phenomenon and the conditions under which it should be experimentally observable.
M. Zwolak, J. Lagerqvist, and M. Di Ventra, Phys. Rev. Lett. 103, 128102 (2009)

2179-Plat
Base-By-Base Ratcheting of Single Stranded DNA through a Solid-State Nanopore
Binquan Luan, Glenn Martyna, Gustavo Stolovitzky, Hongbo Peng, Stas Polonsky, Steve Rossnagel.
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The benefits of low-cost, high-throughput human genome sequencing to medical science has inspired recent experimental work focused on DNA translocation through solid-state nanopores. Given that microelectronic fabrication methods permit the integration of nano-electronics devices to sense each DNA base, the genetic code (DNA sequence) could be read out during translocation by measurement of transverse electrical current, voltage signal, ionic current or hydrogen-bond mediated tunneling signal generated by each base in turn. However, DNA translocation inside a solid nanopore remains poorly controlled and DNA moves too rapidly to be detected at the desired single-base resolution. Here we show using realistic atomistic modeling that the recently proposed DNA transistor can achieve single-base control. These simulation results and a simple theoretical model inspired by the numerical studies demonstrate that when pulled by an optical tweezer as in a single molecule experiment or driven by a biasing electric field as in a high-throughput screening mode, the DNA transistor allows single stranded DNA to transits a nanopore in a stick-slip or thermal ratchet-like fashion, i.e. DNA alternatively stops and advances quickly one nucleotide spacing. During a stick state, a DNA base could be positioned before a sensor for an accurate read-out. We expect that the DNA transistor could be utilized in conjunction with a nanopore-based DNA sensing technology to achieve the goal of fast and cheap DNA sequencing.

2180-Plat
Synthetic Mycolic Acid Bilayers with Applications in Nanopore Sequencing
Kyle Langford, Boyan Penkov, Ian Derrington, Jens H. Gundlach.
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To date, work in lipid bilayers has been primarily limited to a handful of small-chain lipid chemistries. We demonstrate and characterize lipid bilayer formation using pure mycolic acid, a long-chain saccharolipid, in circular apertures less than 50 microns in size. The resultant bilayers exhibit high mechanical stability over 12-hour timescales, breakdown voltages exceeding 1 V, as well as electric seals exceeding 400 GOhm, making them particularly useful for nanopore sequencing. We find these bilayers permeable to transmembrane porins and have analysed the insertion charachteristics of the porins MspA, α-Hemolysin and gramicidin, as well as demonstrating ssDNA translocation. Moreover, this result yields further understanding of the outer membrane structure of mycobacteria.

2181-Plat
Novel Nanoscale Tunneling Architectures for DNA Analysis
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Rapid, label-free analysis of individual biopolymers, specifically of individual DNA fragments is of great importance to many areas of biology and medicine. In recent years, translocation experiments within solid-state pores and protein channels combined with ionic current blockade measurements have become the technology of choice when detection is needed at the single molecule level. For linear biopolymers such as DNA and RNA however, detection based on ionic current blockade seem to lack the signal sensitivity necessary to obtain structural information with single base resolution. Transverse (perpendicular to the helix axis) conductance measurements of DNA in nanometer-sized tunneling junctions promise current detection limits within single nucleotide resolution. Yet, the exact alignment of nanoscale electrodes in tunneling regime to a solid-state nanopore has proven to be a significant challenge. We address this shortcoming by developing a novel method for aligning nanopore and tunneling junction in a nanoscale tunneling architecture by electrochemical metal deposition. As a result, tunnelling electrodes can be fabricated with atomic sharpness and precisely aligned to the nanopore. DNA can be driven electrophoretically through the tunneling architecture and it may be possible to detect modulations in the tunneling current specific to each base in the DNA.

2182-Plat
A Novel DNA Sensing Technique using the Nanopore MspA
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Nanopores of both protein and solid-state composition provide an excellent tool for single molecule sensing, particularly for DNA. Nanopores are nanometer-sized holes that provide the only pathway between two ionic baths. DNA is sensed by electrophoretically driving it into the nanopore which temporarily causes a reduction in ionic current as the DNA translocates. The protein nanopore Mycobacteria smegmatis porin A (MspA) has a geometry enabling the discrimination of the four nucleotides using ssDNA. This discrimination is easily observed when ssDNA translocation is briefly interrupted by complimentary oligonucleotides which must dissociate before ssDNA translocation can occur. We show that such duplex-interrupted translocation yields the ability to sense nucleotide composition on various strands of DNA. Such sensing could be useful in next-generation sequencing techniques with nanopores.

2183-Plat
Single Molecule Studies of Polyadenylic Acid Helix-Coil Kinetics using Nanopore
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Polyadenylic acid (poly(A)) forms helical configuration in aqueous solution at neutral and alkaline pH. The transition between its helical and random coil structures has been studied using bulk spectroscopic or calorimetric methods, revealing its thermodynamic properties. Recently, optical tweezers pulling experiments provided further support for the stacked helix structure of poly(A). While the bulk and the single-molecule experiments used 'bare' ssRNA molecules, the biological function of poly(A) entails the interactions with multiple proteins, such as poly(A)-binding proteins (PABP). In this study, we explore the helix-coil dynamics of poly(A) inside a small protein channel (alpha-hemolysin) at the single molecule level. The fluctuations between stacked and unstacked states are directly observed and quantified using statistical averaging over multiple individual events. An extensive temperature-dependent study of the process provides us with activation energies of the helix to coil (and vice versa) transitions, which are found to obey first order kinetics and results agree with bulk measurements. Surprisingly, time scales extracted from the single-molecule measurements are ~3 orders of magnitude longer than temperature-jump kinetics using 'bare' RNA. We provide a model that explains these results based on the protein-nucleic acid interactions inside the β-barrel channel