Symposium 13: Epigenetic Control of Gene Expression

2170-Symp
Molecular Mechanisms of Lysine Methylation
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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
Tatiana Kutateladze.
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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.
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No Abstract.

2173-Symp
Epigenetic Link Between DNA Methylation and Histone Modifications
Xiaodong Cheng.
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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.
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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