Track: Protein and Ligand - A New Marriage Between an Old Couple

ABS312 | Human sperm TMEM95 facilitates membrane fusion with eggs

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TMEM95 encodes a sperm acrosomal membrane protein, whose knockout has a male-specific sterile phenotype in mice. How TMEM95 plays a role in gamete membrane fusion has remained elusive. Here, we show that human TMEM95 binds hamster eggs, providing evidence for a TMEM95 receptor. We determine a 1.5 Å X-ray crystal structure of TMEM95 and reveal an evolutionarily conserved, positively charged area as a putative receptor-binding surface. Amino-acid substitutions within this region of TMEM95 ablate egg-binding activity. We identify monoclonal antibodies against TMEM95 that inhibit fusion of human sperm to hamster eggs. Strikingly, these antibodies do not block binding of sperm to eggs. Taken together, these results provide strong evidence for a specific, receptor-mediated interaction of sperm TMEM95 with eggs and suggest that this interaction may have a role in facilitating membrane fusion.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS313 | Structural and Kinetic Mechanism of Substrate Specificity Change due to Enzyme Filamentation

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We use a model system, the sequence dependent endonuclease SgrAI, to investigate important mechanistic and biological questions regarding enzyme filamentation and enzyme regulation. SgrAI forms structured assemblies of heterogeneous stoichiometries under conditions where its DNA cleavage activity is 200-1000 fold accelerated, and surprisingly, its DNA sequence specificity is altered (Biochem. 49, 8818); SgrAI cleaves a secondary set of DNA sequences but only in the presence of its primary site, which induce the observed assemblies of SgrAI. We have shown that these assemblies are helical filaments composed of SgrAI bound to DNA (Biochem. 52, 4373, Structure 21, 1848), and that filamentation stabilizes an activated conformation of SgrAI resulting in the binding of a second divalent cation in the active site leading to rapid DNA cleavage (Structure 27, 1, JBC, 298, 101760). We have also carried out a complete kinetic investigation to create a full computational model of the entire DNA cleavage pathway including filamentation and all forward and reverse rate constants for each step (JBC 293, 14585 & 14599). This model has allowed us to predict the behavior of SgrAI within a cell, at biologically relevant concentrations, to show that the filamentation mechanism, and in particular the slow filament assembly step, allows SgrAI to target invading DNA while minimizing damage to its host genome (J. Virol. 93, e01647). We now show, with new data kinetic and global modeling data, that the highly unusual alteration of DNA sequence specificity exhibited by SgrAI when in the filamentous form is due to a 5-17-fold stabilization of the low activity (and non-filamenting) state when SgrAI is bound to its secondary site DNA sequence. Structural studies show the likely origin of this preferential stabilization in the form of sequence-dependent DNA structure, complementarity of the protein-DNA interface, and a disorder-to-order protein structural transition.

Track: Structure and Dynamics Perspectives on Enzyme Function

ABS314 | Filamentation as a New Level in Enzyme Regulation

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It is becoming clear that many enzymes, from diverse biological pathways and all branches of life, form linear, helical, or tubular polymers (or filaments) with altered function and/or activities. We present the collection of known filament-forming enzymes including their structure, function, as well as any known effects on enzyme activity resulting from filamentation. In many cases, effects on enzyme activity are not known, while in others, enzymes are activated, inhibited, show altered substrate specificity, altered cooperativity, altered response to allosteric effectors, or in some cases show completely new enzymatic or biological activity. Many filament-forming enzymes are medically important, controlling important steps in metabolic pathways and where dysfunction, including changes in filamentation, can be related to disease states. The relationship of enzyme polymeric filaments to the larger scale "filaments" (membraneless self-assemblies) seen in cells via fluorescence microscopy is also discussed.