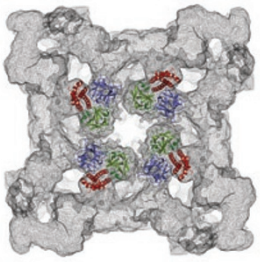
Muscle talk



Ryanodine receptors (RyR) are large, tetrameric channels that regulate the release of calcium from the endoplasmic reticulum (ER). RyR1 is found in skeletal muscle and is required for excitation-contraction coupling. Mutations of this receptor can result in severe diseases and disorders, which can now be explained by the crystal structure of the three N-terminal domains of RyR1 obtained by Van Petegem and colleagues. Domain A has a β-trefoil motif, in line with previous reports, as does domain B, and domain C forms a bundle of five α-helices. The three N-terminal domains interact with each other through hydrophilic interfaces, and docking of the RyR1 crystal structure into electron microscopy maps show that the domains are located in the cytoplasmic part of the channel. Together, they form a vestibule that surrounds the fourfold symmetry axis. Thirty-three disease-associated mutations could be mapped to the RyR1 structure, resulting in three groups of effects: the first group destabilizes the interface between each of the three N-terminal domains, the second affects the folding of each domain, and the third alters the interface between the domains and the rest of the receptor. From this information, a model is proposed whereby the opening of RyR1 changes the position of the three N-terminal domains, and the mutations act to destabilize the closed state and promote the open state, producing either 'leaky' channels or ones that are more easily activated. (*Nature* doi:10.1038/nature09471, published online 3 Nov 2010) *MH*

Islets in the stream

Comprehensive genome-wide mapping of epigenomic modifications has the potential to increase understanding of a multitude of diseases, including type 2 diabetes (T2D). However, to date few tissues have been thoroughly examined with regard to epigenomic status. Collins and colleagues have now undertaken extensive profiling of human pancreatic islets to gain insight into gene regulation in the endocrine pancreas. Using DNase-seq and ChIP-seq, the authors characterized open chromatin, several histone methylation marks and CTCF binding sites across the genome. Over 18,000 potential transcription start sites were identified (of which over 30% were not previously annotated), along with at least 34,000 distal regulatory elements. CTCF binding sites, which often function as insulators, comprised 22% of these distal elements. Many putative distal regulatory elements were unique to the islet and were clustered together in the genome, indicating that they may function together to regulate islet gene expression. Surprisingly, several highly expressed islet hormone genes lacked the usual chromatin conformation and histone methylation patterns found at active genes. Four predicted regulatory elements unique to the islet functioned as transcriptional enhancers. Two of those elements, found within the genes *TCF7L2* and *WFS1 (Wolfram syndrome 1)*, contained known T2D-associated single-nucleotide polymorphisms (SNPs) that conferred allele-specific differences in enhancer activity, indicating they may have altered function in T2D. The abundance of regulatory elements unique to the islet raises intriguing questions about how gene expression is regulated in the pancreas and what role it may play in the etiology of diabetes. This extensive characterization will be foundational for studies probing gene regulation in normal and diseased human pancreatic islet tissue. (*Cell Metab.* **12**, 443–455, 2010) *SM*

Written by Inês Chen, Maria Hodges & Steve Mason

Subtle shifts

For protein synthesis, the ribosome requires additional protein factors, including several GTPases. One such protein is the elongation factor EF-Tu, which takes aminoacyl-tRNA to the ribosome where it forms a complex with GTP, elongation factor G and initiation factor 2. Hydrolysis of GTP is known to be accompanied by a change in conformation of the switch I and II regions of GTPase. The question that has remained unanswered for nearly 40 years is, how does the ribosome activate GTP hydrolysis? The crystal structure of EF-Tu bound to the 70S ribosome with Trp-tRNA^{Trp} in the presence of the antibiotic paromycin and a GTP analog, solved by Ramakrishnan and colleagues, reveals the active form of EF-Tu. In this state, the switch I region of the GTPase remains ordered, and no large-scale conformational rearrangements are seen. Instead, the catalytic histidine shifts subtly within the active site to a position where it can act as a general base coordinating the nucleophilic water, ready to attack the γ-phosphate of GTP. Residue A2662 of the sarcin-ricin loop of the 23S ribosomal RNA is important for the positioning of the active site histidine. This is likely to be a conserved mechanism because all translational GTPases interact with this loop and the catalytic histidine. (*Science* **330**, 835–838, 2010) *MH*

Going retro

When a polypeptide is terminally misfolded in the lumen of the ER, the cell translocates it back to the cytoplasmic compartment for proteasomal degradation, in a process called ERAD (for ER-associated protein degradation). Despite extensive research in this area, how the polypeptide crosses the ER membrane remains unclear. Retrotranslocation of the ERAD substrate is coupled to its polyubiquitination, but both the RING domain of the E3 ligase of Hrd1p and the Cdc48p ATPase, thought to drive polypeptide translocation, reside in the cytosolic side of the ER membrane. This raises the question of how the substrate gets access to this location in the first place. ERAD components in the ER membrane include Hrd1p and associated proteins, but their specific functions remain unclear. Now, Carvalho, Stanley and Rapoport reveal that Hrd1p is the core component of the retrotranslocation complex in ER membrane. They show that Hrd1p overexpression can support ERAD in the absence of other ER membrane proteins (Hrd3p, Usa1p and Der1p). By performing *in vivo* cross-linking with a site-specific labeled model substrate for ERAD, the authors find that the polypeptide makes specific contacts with Hrd1p regions within the membrane, and these require Hrd1p E3's ligase activity and Cdc48p. Substrate contacts with Hrp3p and Der1p are also observed, but not with Usa1p or Sec61p. The latter observation dispels previous proposals that Sec61, the translocon channel component that transports polypeptides into the ER, might also be involved in shuttling of misfolded polypeptides for ERAD. Along with previous data, this work allows the proposal of a model for ERAD retrotranslocation, whereby the RING domain of Hrd1p and Cdc48p ATPase regulate conformational changes in Hrd1p. These changes allow the interaction of Hrd1p with the substrate on the luminal side of the ER membrane and the initial translocation of the substrate. (*Cell* **143**, 579–591, 2010) *IC*