during inflammation and nerve injury. TRPV2 is constituted of four subunits, each with six transmembrane (TM) spanning helices with a pore loop between the fifth and sixth TM helices. To date, little is understood about the complex biophysical properties or the 3D architecture of the TRPV2 channel. We used single particle electron cryo-microscopy (cryo-EM) to solve the 3D structure of the tetrameric TRPV2 channel. To obtain sufficient quantities of purified channel proteins for structural and functional analysis, we used heterologous expression in the budding yeast, *S. cerevisiae* and immunoaffinity chromatography to purify TRPV2. The purified channel was vitrified in the presence of amphipathic polymer, amphipol 8-35, which was used to substitute for solubilizing detergent in TRPV2 preparations. This procedure allowed us to achieve optimal ice thickness and uniform particle distribution in the cryospecimen. Vitrified TRPV2 samples were imaged using an FEI Polara electron cryo-microscope operated at 300 keV at liquid-nitrogen temperature. Images of the channel were acquired on a 4k x 4k CCD camera (TVIPS) at 59,000 nominal magnification of microscope using low-dose mode and a defocus range of 2-5 µm. The 3D reconstruction of TRPV2 was generated with 20,608 particle images extracted from 431 CCD frames, and image processing was performed using EMAN2. The resulting structure was validated using tilt-pair analysis, and is interpretable to ~15 Å based on "gold standard" resolution criterion. Supported by grants from NIH (R21AR063255, R01GM072804, P41GM103832, T32KD007696, R01EY07981) and by AHA (12GRNT10510002).

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A CRYO-EM Structure of a Minimal Translation Initiation System: Deletion in Self-Sufficient IRES Prevents Translocation

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Translation occurs on the ribosome, a ribonucleoprotein machine that exists in many copies per cell and is conserved among all forms of life. In canonical eukaryotic translation initiation, a suite of protein factors is required for many essential processes: for messenger RNA (mRNA) to bind the ribosome and to align itself with the translation initiation site, for the first aminoacylated tRNA to bind the start codon, for the ribosome to shift the mRNA downstream by one codon, and more. These processes occur on and near the mRNA's start codon immediately downstream of a 5' modified-nucleotide cap.

In a departure from canonical catalytic and location requirements, some eukaryotes and many viruses encode regions of highly structured RNA that can initiate translation without a 5' cap and at least some initiation factors. These RNAs are called internal ribosomal entry sites, or IRESes. We study a type of IRES found in the Dicistroviridae virus class. This type of IRES initiates translation of the second of two gene clusters in the continuous viral RNA genome, and, uniquely, it requires no initiation factors to hijack eukaryotic host ribosomes.

Here, we present a three-dimensional reconstruction of one such IRES–the Cricket Paralysis Virus intergenic IRES–bound to the eukaryotic yeast ribosome. The structure was obtained by cryo-electron microscopy and single-particle reconstruction techniques. This particular IRES sample is a mutant lacking three consecutive nucleotides in a loop region; it cannot undergo the initial translocation event, when mRNA shifts to allow a new aminoacylated tRNA into the A-site. We examine how the deletion prevents translocation, and thus how the intact IRES, a unique minimal translation system, might enable translocation, an essential part of initiating the universal translation process that is not wholly understood.

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Serial Block-Face Scanning Electron Microscopy for Nanoscale Characterization of Tissue Ultrastructure

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Serial block-face scanning electron microscopy (SBF-SEM) can be used to image large (100-micrometer size) volumes of biological tissue, and thus complements standard transmission electron tomography, as well as scanning transmission electron tomography. SBF-SEM is attractive not only for its ability to generate high-resolution three-dimensional ultrastructure, but also for its relative ease of implementation. Whereas electron tomography traditionally requires the tissue block to be manually sectioned, SBF-SEM makes use of an automated ultramicrotome built in to the SEM's specimen chamber; and tissues optimally stained with heavy metals and imaged with backscattered electrons, show equivalent contrast to TEM images of conventionally stained sections (Denk and Horstmann, 2004). Using SBF-SEM it is possible to image the organization and geometrical parameters of complex tissues, extending over 100-micrometer dimensions, in three dimensions at 10-nm spatial resolution in the plane of the block face and 25-nanometer resolution in the perpendicular direction. For example, we have reconstructed entire mouse pancreatic islets of Langerhans, allowing us to determine the distribution of cell types, average numbers of insulin granules in beta cells, numbers of glucagon granules in alpha cells, and the organization of capillaries. Moreover, since acquisition of such data sets can be performed relatively quickly, it is possible to obtain results from many samples and to compare wild-type and knock-out islets.

W. Denk and H. Horstmann, PLoS Biology 2(11): 1900-1909 (2004).

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Examining Drp1 Conformational Changes and Domain Interactions in the Mitochondrial Fission Complex using Cryo-Em

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Mitochondria are essential organelles involved in the generation of chemical energy, maintenance of calcium levels and induction of apoptosis. Mitochondria are also highly dynamic, undergoing continuous cycles of fission and fusion. This dynamic cycle is integral to mitochondrial proliferation, distribution, organelle morphology, bioenergetics and cell death. Excessive fusion leads to interconnected, collapsed and dysfunctional mitochondria. Conversely, excessive fission promotes mitochondrial fragmentation and the release of apoptotic proteins that promote cell death. Many neurodegenerative diseases, including Alzheimer's, Parkinson's and Huntington's display increased levels of mitochondrial fission. The protein responsible for mitochondrial fission in mammalian cells is dynamin-related protein 1 (Drp1). Drp1 is a 80kDa GTPase that is thought to constrict the outer mitochondrial membrane to mediate fission. However, the mechanism of Drp1 assembly at mitochondrial constriction sites is unclear. Recent studies have shown that lipid and nucleotide interactions facilitate Drp1 self-assembly into ordered oligomers that mimic interactions found at the surface of the outer mitochondrial membrane. Cryo-EM is being utilized to solve the 3D structure of this fission complex. Specific Drp1 mutants will be used to identify key interactions within the oligomerization interfaces. In this way, the roles of distinct domains will also be elucidated. A clear understanding of the role of Drp1 in mitochondrial fission is essential for the development of future therapies that prevent mitochondrial dysfunction.

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Structure Determination of Small Macromolecular Complexes by Cryo-Electron Microscopy

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Application of emerging methods in single particle cryo-electron microscopy (cryo-EM) has led to the determination of the structures of a variety of macromolecular complexes at sub-nanometer resolutions. The overwhelming majority of these are large complexes, mostly with high symmetry. Extending cryo-EM methods to routinely determine the structures of small macromolecular complexes with sizes of $<$ 500 kDa to sub-nanometer resolution still remains an exciting frontier in modern structural biology. Working with small protein complexes has certain inherent challenges such as conformational flexibility and beam induced movement during exposure to electrons which can significantly reduce resolution. Further, during image processing, errors can be introduced from model-bias, over-refinement, and inaccuracy in orientation determination. Although these errors are problematic for all reconstructions, they are particularly detrimental for structural analysis of complexes that are small and have either low or no internal symmetry. We show a step-wise systematic workflow for structure determination by cryo-EM at ~ 6 Å resolution of □ß-galactosidase, a 464 kDa protein, whose structure has previously been determined at atomic resolution by X-ray crystallography. Our approach employs a number of recent developments in cryo-EM technology that include the use of direct electron detectors, the ability to fractionate the dose to use only those portions of the exposure that are recorded before there is significant radiation damage, and the use of image processing procedures that can track and correct for the movement of single molecules during the course of the exposure.