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1975-Pos

Differential Sensitivity of Sarcomeric and Ubiquitous Isoenzymes of Mitochondrial Creatine Kinase to Oxidative Inactivation

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Oxidative modifications of creatine kinase (CK) isoenzymes are thought to play a critical role during pathologies involving oxidative stress. Reactive oxygen and nitrogen species (ROS, RNS) not only induce enzymatic inactivation, which occurs with all CK isoenzymes, but also specific damage to the mitochondrial CK isoforms, namely interference with their oligomeric state and membrane binding capacity. Using purified recombinant proteins, cell homogenates and mitochondria isolated from rat heart and brain, we have compared the two isoforms of mitochondrial CK (sarcomeric sMtCK expressed in heart and skeletal muscle, ubiquitous uMtCK expressed in other tissues) in respect to their sensitivity to oxidative inactivation induced by the drug doxorubicin or occurring spontaneously after extraction under non-reducing condition. We confirmed that sarcomeric sMtCK shows significantly higher sensitivity to oxidation and that the loss of total CK activity in heart extracts upon storage under non-reducing condition is mainly due to the inactivation of sMtCK. We could also show that the sMtCK dimer is particularly easily inactivated and that solubilization of sMtCK from membrane (promoting dimerization) makes the protein an especially vulnerable substrate for inactivation. The differential susceptibility of the two MtCK isoenzymes has been related to some differences in their molecular structures (e.g. number and surface exposure of cysteine residues).

Cryo-Electron Microscopy & Reconstruction

1976-Pos

The *Trypanosoma Brucei* Flagellum Reveals Unique and Dynamic Structures of a Nanomachine

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The *Trypanosoma brucei* flagellum is vital for the organism's locomotion, pathogenesis and cell division. It contains a microtubular axoneme, a paraflagellar rod (PFR), and connecting proteins bridging these two structures. Our investigation by cryo-electron tomography revealed a characteristic arrangement of the axoneme internal features: the 9+2 arrangement of microtubule doublets displayed radial spoke spacing not found in other organisms. We have determined that the PFR is a quasi-crystal with a unit cell that repeats every 55 nm along the length of the axoneme. Connecting proteins are attached at 55 nm intervals (the spacing of a PFR repeat) along two of the nine doublets. During flagellar bending, the PFR unit cell axial lengths remain constant while the interaxial angles vary to accommodate the quasi-crystal's expansion and compression. RNAi silencing of one of the major PFR proteins completely abolished the assembly of the PFR, and resulted in defective cell motility. Our tomographic data of this mutated flagellum also showed that the microtubule doublets are not properly arranged within the axoneme. Thus the PFR simultaneously provides structural organization to the axoneme and the flexibility and regulation required for productive locomotion.

Acknowledgements: This research has been supported by NIH grants (P41RR02250, training grant in molecular virology T32AI07471) and Singapore National Research Foundation Fellowship to CYH.

1977-Pos

A Uniique Density at the Portal Vertex of HSV Virions Revealed by Asymmetric Averaging of Subtomograms

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The best characterized herpesvirus is the important human pathogen, herpes simplex virus type 1 (HSV-1). The HSV-1 virion comprises 1) an icosahedral capsid which encloses the genome, 2) a surrounding variable proteinaceous layer called the tegument and 3) an enclosing lipid envelope with glycoprotein spikes. The capsid shell has the form of a T=16 icosahedron and has been studied extensively both as an isolated capsid and as the core of the virion. Several proteins have been described as minor capsid components, prominent among which is pUL6. A dodecameric ring of pUL6 proteins forms the portal, which replaces a penton at one capsid vertex. The portal has a central channel and by analogy with tailed bacteriophage, is believed to form the route for transit of the DNA into and out of the capsid. Here we describe a cryo-ET reconstruction of the intact HSV-1 virion that identifies the portal vertex and reveals a previously unsuspected structure that spans the tegument, linking the capsid to the envelope, which we term the ''tegument cylinder''. The position and nature of this structure suggests possible roles in virus assembly or transport.

1978-Pos

Electron Tomography and Molecular Modeling Study of Chemoreceptor Organization

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The movement of bacteria in response to external stimuli represents a paradigm of broad general interest for the understanding of mechanisms underlying signal transduction across cell membranes. Bacterial chemoreceptors respond to changes in concentration of extracellular ligands by undergoing conformational changes that initiate a series of signaling events, leading ultimately to regulation of flagellar motor rotation. Atomic structures for several domains of chemoreceptors, including the periplasmic ligand-binding domain, the cytoplasmic signaling domain and the HAMP domain are available, but the molecular architectures of an intact receptor dimer, or the functionally relevant trimer-of-dimer configuration have remained elusive. Here, we have used cryo-electron tomography combined with 3D averaging to determine the *in situ* 3D structure of receptor assemblies in bacterial cells that have been engineered to overproduce only the receptor for serine chemotaxis, Tsr, and lacking all other chemotaxis receptors and signaling components. We identified two major conformations of the chemotaxis receptors. Through comparative modeling and map-constrained molecular dynamics simulations, we obtained the assembly structures of tsr organized in a two dimensional array. We show that receptors are organized in trimer-of-dimer conformations with peripasmic domain, HAMP domain, and signal traction domain transiting between conformations. It is suggested that the position of the ligand binding domain and the HAMP domain play a pivotal role in mediating signal transduction across the cell membrane.

1979-Pos

Combined Approach Towards Automatic Identification of Protein Secondary Structure Elements in Volumetric Data Sets

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Single particle cryo-electron microscopy studies, but also data from tomographic experiments, often result in volumetric 3D reconstructions of low- to intermediate resolution. Although a direct atomic interpretation is not feasible at these levels of detail, one may be able to extract structural information that describes the overall conformation of the molecular system. Especially a detection of secondary structure elements, would aid in a further description of large macromolecular complexes.

On the other hand, the limited resolution often prohibits a clear visual identification of those elements.

We therefore propose a novel algorithmic tool, which is able to annotate automatically volumetric reconstructions and determines the secondary structure elements inside the maps. Our technique is based on a multi-stage analysis: In a first step, a spatial digital path filtering technique is applied, which is able to enhance local features that may characterize helices or sheets.

In a second step those features are extracted by combining the voxel information and modeling the likelihood of the presence of a secondary structure element at the specific location. To evaluate the performance of our algorithm, we have tested it using both, synthetic and experimental maps. The results show that our software is able to successfully annotate even intermediate-resolution maps. In addition, we have combined the before-mentioned algorithmic technique with our visualization system Sculptor. Sculptor provides a user-friendly environment, which enables not only an interactive pre-processing of the volume data, but also an intuitive exploration of the results.

This work was supported by NIH grant R01GM62968, by a grant from the Gillson Longenbaugh Foundation, and by startup funds of the University of Texas Health Science Center at Houston.