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Although glycolytic enzymes can be isolated as cytosolic components, some of them are known to bind to proteins from the cytoplasm and other cellular compartments, such as the mitochondria, the nucleus, or the plasma membrane. In some cases these protein-protein interactions are considered significant for the regulation of energy metabolism, as well as for the modulation of other cell functions. Among glycolytic enzymes, phosphofructokinase (PFK) is thought to play a fundamental role in the control of this pathway, because of the number of metabolic signals that can regulate its complex allosteric behavior. No X-ray structure of a characteristic eukaryotic PFK is available yet. However, sequence data indicated that the eukaryotic enzyme originated by duplication, fusion and divergence of an ancestral prokaryotic gene, such that the duplicated fructose 6-phosphate catalytic site in the C-terminal half became an allosteric site for the activator fructose 2,6-bisphosphate. It has been suggested that both sites are shared in the interface between subunits aligned in an antiparallel orientation. To test the contribution of each terminal domain to these two binding sites, chimeric mammalian PFKs involving exchange of their terminal domains have been found to exhibit affinity properties for fructose 6-phosphate and fructose 2,6-bisphosphate that resembled those of the native isozyme that donated the N-terminal half and the C-terminal half, respectively. Further mutational analyses of muscle PFK led us to investigate the composition of the fructose phosphate binding sites and to gain insight into the structural organization of eukaryotic PFK. Data will also be presented evidencing the relationship between mitochondrial function and the operation of glycolysis, as observed after impairment of mitochondrial DNA transcription.

1052-Wkshp
How Mitochondrial Structure Can Affect Energy Metabolism: Insights From Electron Microscopic Tomography
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The idea that the internal structure of mitochondria might influence energy metabolism has its origins in electron microscopic studies in the 1960s and 1970s of morphologic changes during respiratory state transitions and ligand binding to adenine nucleotide translocator. In the ensuing years, the variety of known morphologic transitions displayed by mitochondria, in test tubes and in cells, has increased, as has information about the corresponding three-dimensional membrane topologies, provided by electron tomography. Experiments and simulations indicate that mitochondrial inner-membrane topology influences mitochondrial metabolic rates and other functions by altering internal diffusion pathways for ions, metabolites and soluble proteins. At the same time, the roles of certain proteins (e.g. ATP synthase) and lipids (e.g. cardiolipin) in determining inner-membrane topology are being uncovered. The emerging hypothesis is that mitochondrial inner-membrane topology is regulated by the cell to optimize mitochondrial performance in response to different stimuli (such as metabolite concentrations, apoptotic signals, and reactive oxygen). As techniques for cryo-specimen preparation (e.g. using focused ion beams) and imaging improve, it will become possible to apply electron tomography to whole cells and tissues in near-native, frozen-hydrated state. These enhanced imaging capabilities should provide new information about not only in-situ membrane topology, but also the nature of the physical contacts between mitochondrial outer and inner membranes; the nature of the tethers that regulate the spacing between mitochondria and endoplasmic reticulum (which in turn regulates mitochondrial calcium uptake, which in turn activates matrix dehydrogenases); the organization of the mitochondrial matrix; and possibly even the structure of respiratory supercomplexes in the mitochondrial inner membrane. (The Resource for Visualization of Biological Complexity is supported as an NCRR Biomedical Technology Research Resource by NIH grant P41-RR01219.)

1053-Wkshp
Molecular Architecture of Pyruvate Dehydrogenase Complexes
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Pyruvate dehydrogenase multienzyme complexes are among the largest multifunctional protein assemblies within cells, catalyzing the four-step conversion of pyruvate to acetyl CoA using an assembly comprised of multiple copies of E1, E2 and E3 enzymes, Using cryo-electron microscopy, we have shown that the outer shell enzymes (E1 decarboxylase and E3 pyruvate dehydrogenase) are separated from the inner icosahedral core (E2 acetyl transferase) by an annular gap that is about ~ 100 Å wide. The presence of the gap is a key structural element in the spatial organization of the three enzymes in the complex, and allows the ''swinging arm'' lipoyl domain to shuttle between the active site of the E2 at the inner core and the active sites of E1 and E3 enzymes at the outer shell to carry out synthesis of acetyl CoA. Using a combination of circular dichroism, analytical ultracentrifugation and solution NMR studies we have also demonstrated that the peptide corresponding to the linker region has an extended conformation with a persistence length of ~75-89 Angstroms, consistent with the observed size of the gap. Cryo-electron tomography of individual complexes with varying occupancies of enzymes in the outer shell confirmed unequivocally that the annular gap between the core and the outer shell was maintained even at very low E1 or E3 occupancies. These studies demonstrate unambiguously that it is the linker, rather than interactions between the outer shell enzymes, that are responsible for holding the subunits above the core. The prospect of using cryo-electron tomography to map the locations of individual enzymes within single multi-enzyme complexes could be a powerful approach to obtain structural information, without molecular averaging, on large and structurally heterogeneous biological assemblies that are not amenable to analysis by NMR or X-ray crystallographic techniques.

1054-Wkshp
Electroneutral And Electrogenic Catalysis By Diheme-Containing Succinate:Quinone Oxidoreductases
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Membrane proteins can support both the generation and utilisation of a transmembrane electrochemical proton potential (''proton-motive force''), either by transmembrane electron transfer coupled to protolytic reactions on opposite sides of the membrane or by transmembrane proton transfer. In the case of the diheme-containing quinol:fumarate reductase (QFR) of *Wolinella succinogenes* [1], both theoretical and experimental results, reviewed in [2], supported, but did not prove, a previous hypothesis [3] that both of these mechanisms are combined in a single membrane protein complex, so as to facilitate transmembrane electron transfer by transmembrane proton transfer. Results of measurements on proteoliposomes will be presented, providing evidence for the presence of this unprecedented transmembrane proton transfer pathway (''E-pathway'') in the wild-type enzyme and its non-functionality in a variant QFR where a key glutamate residue has been replaced [4]. The ''E-pathway'', discussed on the basis of the 1.78-Å-resolution crystal structure of QFR, is essential for life under the conditions of fumarate respiration. Results will be compared to those obtained with the diheme-containing succinate:menaquinone reductase from the Gram-positive bacterium *Bacillus licheniformis* [5,6].
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