structure is caused by the differences among the bond distances between CaO and MnO, as well as those between different Mn and O atoms. Among these, the bond distances between O5 and its surrounding metal ions are especially longer than those between other oxo-atoms and metal ions, suggesting that the bonding of O5 to other metal ions is weak, which in turn suggests that O5 has a higher reactivity and may be easily released during the water-splitting reaction. Importantly, four water molecules were found to be associated with the Mn_4CaO_5-cluster, two of them are associated with the Ca ion and the other two are associated with Mn4. No other water molecules were found to associate with other Mn ions, suggesting that the water oxidation reaction takes place within these four water molecules, which are located in an area surrounded by Ca, Mn4, O5, two OEC-ligands D1-Glu189, and D1-Asp170, and three additional residues D1-Tyr161, D1-Phe182 and D1-Val185. There were several additional water molecules found in this area, suggesting that this region is highly hydrophilic, and the additional water molecules may serve as sources for the substrates in the next reaction cycle.

The high resolution structure of PSII revealed the presence of around 2800 water molecules in a dimer, some of them formed extensive hydrogen-bond networks linking the Mn_4CaO_5-cluster to the protein surface. These hydrogen-bond networks may serve as proton exit paths from, or substrate water inlet channels to, the Mn_4CaO_5-cluster.

This work was carried out in collaboration with Drs. Y. Umena, K. Kawakami and N. Kamiya.

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P6

Structure and mechanism of respiratory complex I

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NADH-ubiquinone oxidoreductase (complex I) is the first and the largest enzyme in the respiratory chain of mitochondria and most bacteria. Mutations in complex I lead to the most common human genetic disorders. It is an L-shaped assembly, with the hydrophobic arm embedded in the membrane and the hydrophilic arm protruding into the bacterial cytoplasm. We have determined all currently known atomic structures of complex I. Initially, we have solved the crystal structure of the hydrophilic domain of complex I from *Thermus thermophilus*, revealing the arrangement of NADH, flavin and nine Fe–S clusters in an electron transfer chain. Structure of the hydrophilic domain reduced by NADH revealed significant conformational changes at the interface with the membrane domain.

The mechanism of coupling between the electron transfer and proton translocation in complex I is currently not established. Recently, we have determined the crystal structure of the membrane domain of complex I from *Escherichia coli*, showing unusual novel fold of antiporter-like subunits. We have also described the low-resolution structure of the entire complex I from *T. thermophilus*. The overall architecture of complex I, thus revealed, provides strong clues about the coupling mechanism, involving long-range conformational changes.

Currently, from the core complement of 14 conserved complex I subunits, only the structure of subunit Nqo8/NuoH/ND1 remains unknown. This subunit is involved in quinone binding and is key to the coupling mechanism. The main challenge now is to solve the atomic structure of the entire, intact complex, including the quinone-binding site. Progress towards this goal will be described and mechanistic implications will be discussed.

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P7

Damage control — How the PINK1/Parkin pathway can regulate removal of impaired mitochondria by autophagy

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The products of two genes mutated in autosomal recessive forms of Parkinson's disease, PINK1 and Parkin, have been identified in Drosophila to work in the same pathway to maintain healthy flight muscles and dopaminergic neurons. PINK1 is a kinase located on mitochondria whereas Parkin is an E3 ubiquitin ligase normally located in the cytosol. Upon mitochondrial damage PINK1 recruits cytosolic Parkin to mitochondria to mediate mitophagy suggesting that in mammalian cells PINK1 can work in the same pathway as Parkin to mediate mitochondrial quality control. PINK1 appears to be constitutively imported to the inner mitochondrial membrane of healthy mitochondria and degraded by PARL and downstream proteases. When import is impaired by mitochondrial damage PINK1 accumulates on the outer mitochondrial membrane where it can recruit Parkin from the cytosol. This sensing mechanism allows the detection and selective removal of damaged mitochondria within a cell. We have found that PINK1 on the outer mitochondrial membrane is stably bound to the TOM complex. However, the TOM complex does not appear crucial for PINK1 to recruit Parkin as inducible targeting of PINK1 to peroxisomes that lack the TOM complex mediates Parkin recruitment to peroxisomes and induces pexophagy. If mitochondria recover membrane potential PINK1 is rapidly reimported into mitochondria and degraded suggesting that PINK1 binding to TOM allows rapid downregulation of PINK1 and salvage of mitochondria from the mitophagy pathway.

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P8

Electron tunneling in biological energetics

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Component parts of photosynthetic and respiratory energy conversion machinery are linked by electron-transfer, a quantum

mechanical tunneling process. Well-tested rules of electron tunneling in proteins have yielded reliable tools for comprehensive analysis of tunneling networks in multi-cofactor containing proteins engaged in energy conversion and associated oxidation–reduction catalysis. Here we apply these tools to assess how electron tunneling is engineered in oxygenic and non-oxygenic photosystems of plants and allied microorganisms. Comprehensive mathematical analysis of natural photochemical reaction center proteins and a range of informative theoretical models reveal that natural photochemical efficiencies, defined as the yield of a photon-generated charge-separated state multiplied by the fraction of the absorbed photon energy preserved as a difference in redox potentials between associated electron donors and acceptors, ultimately substrates and products, are substantially suboptimal. In anoxygenic photosynthetic bacterial membranes, as much as 250 meV of the absorbed photon energy can be coupled to a transmembrane electric potential ($\Delta\varphi$, typically ~90% of the prevailing electrochemical proton gradient, $\Delta\tilde{\mu}_H+$), thereby increasing the overall photochemical efficiency substantially. In oxygenic PSII and PSI chloroplast membranes, $\Delta\varphi$ is low ($\Delta\tilde{\mu}_H+$ predominantly ΔpH), which leaves a significant fraction of the photon energy utilization unaccounted for. Following recent proposals [1], we evaluate whether reaction centers of oxygenic PSII and adjacent PSI make use of this free energy to select redox potentials of cofactors that dodge and minimize the destructive effects of singlet oxygen or partly reduced oxygen (reactive oxygen species; ROS). Both nonoxygenic/oxygenic photosynthetic reaction centers cooperate with cytochrome bc_1/b_6f complexes to generate $\Delta\tilde{\mu}_H+$. Our analyses highlight and contrast the molecular processes adopted for $\Delta\tilde{\mu}_H+$ generation and for ROS suppression/regulation by these two very different classes of energy conversion proteins that nevertheless share many aspects of redox chemistry and the same electron tunneling engineering.

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P9

Electron transfer and catalytic reactions of cytochrome bc_1

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Cytochrome bc_1 (mitochondrial complex III), an important component of biological energy conversion, provides an electronic connection between the quinone and cytochrome *c* redox pools of electron transport chain. It reversibly oxidizes hydroquinone and reduces cytochrome *c* coupling these reactions to proton translocation across the membrane. Homodimeric structure of this enzyme builds in four catalytic quinone binding sites and four chains of cofactors that, connected by a centrally located two-heme b_L bridge, form a symmetric H-shaped electron transfer system. The molecular mechanism of operation of this complex system is not fully understood and several issues, including the electron transfer through the bridge, remain a subject of intense debate. This talk will discuss mechanistic and physiological perspectives on the role of symmetry in cytochrome bc_1 operation. This perspective emerges from recent experiments designed to expose all the electron transfer paths within a dimer for kinetic testing. The evidence supporting the existence of catalytically-competent electron transfer between the two heme b_L will be presented. This will be complemented by spectroscopic data that shed new light on the mechanism of catalytic quinol oxidation.

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P10

The alternative complex III: Properties and possible mechanisms for electron transfer and energy conservation

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Alternative complexes III (ACIII) are recently identified membrane-bound enzymes that replace functionally the cytochrome bc_1/b_6f complexes. In general, ACIII are composed of four transmembrane proteins and three peripheral subunits that contain iron–sulfur centers and C-type hemes. ACIII are built by a combination of modules present in different enzyme families, namely the complex iron–sulfur molybdenum containing enzymes. A historical perspective on the investigation of ACIII will be presented, followed by an overview of the present knowledge on these enzymes. Electron transfer pathways within the protein will be discussed taking into account possible different locations (cytoplasmic or periplasmic) of the iron–sulfur containing protein and their contribution to energy conservation. In this way several hypotheses for energy conservation modes will be presented including linear and bifurcating electron transfer pathways.

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P11

Mitochondria and the control of insulin secretion

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Oscillations in plasma membrane potential play a central role in glucose-induced insulin secretion from pancreatic β-cells and related insulinoma cell lines. We have employed a novel fluorescent plasma membrane potential ($\Delta\psi_p$) indicator in combination with indicators of cytoplasmic free Ca^{2+} ($[Ca^{2+}]_c$), mitochondrial membrane potential ($\Delta\psi_m$), matrix ATP concentration and NAD(P)H fluorescence to investigate the role of mitochondria in the generation of plasma membrane potential oscillations in clonal INS-1 832/13 β-cells. Elevated glucose caused oscillations in plasma membrane potential and cytoplasmic free Ca^{2+} concentration over the same concentration range required for insulin release, although considerable cell-to-cell heterogeneity was observed. Exogenous pyruvate was as effective as glucose in inducing oscillations, both in the presence and absence