are viable and do not develop tumors (*5*).

By using degenerate polymerase chain reaction approaches, two groups have now identified two new proteins—p40 and p51—both encoded by an additional *p53* family member (*6, 7*). The new family members are the products of alternatively spliced mRNAs encoded by a single gene located on chromosome 3q. For simplicity, I refer to the gene as *p51*. Preliminary data suggest that p51, like p53 and p73, can activate transcription and induce apoptosis (*6*). The primary sequence of p51 and p73 resemble each other more closely than either resembles p53. Unlike p53, but in keeping with p73, p51 is rarely mutated in human cancers. p51 mutations were seen in only 3 of 101 evaluable primary tumors and tumor cell lines (*6*). All three of these mutations were in places that would be predicted to abrogate DNA binding. As it turns out, the rat *Ket* gene, initially thought to be the rat homolog of *p73*, is more closely related to *p51* (*8*).

Why is p53 recurrently mutated in human cancers, whereas the other members of this protein family—with similar functional abilities—are not? A similar conundrum exists with respect to two other proteins associated with human tumors. pRB and p16 (see the figure). Both p107 and p130 can, like their well-known family member pRB, interact with members of the E2F transcription factor family and induce a cell-cycle block. Yet pRB, but not p107 and p130, is recurrently mutated in human cancers. Likewise, all of the p16 family members can inhibit cdk4 kinase activity and block progress of the cell cycle; yet only p16 among these proteins is recurrently altered in human cancer. A simple explanation is that certain critical afferent or efferent functions are in fact not shared by these various family members. In this regard, p53 is induced by DNA damage, whereas p73 is not, and so it is possible that p53 is uniquely recruited to execute a response to DNA damage.

A more insidious explanation would be that the similarities within these families are more apparent than real. First, when studying proteins that belong to families, it is necessary to establish that reagents such as antibodies and nucleic acid hybridization probes react with specific family members. For example, it is unknown whether some antibodies to p53 will cross-react with p51 or p73.

Second, firm establishment of family relationships also requires critical evaluation of the procedures used to study protein function. For example, many of the functional studies of p73, p51, and even of p53 have relied on protein overproduction, in which high concentrations of the proteins may have obscured true physiological differences. High protein levels can also induce an apparent loss-of-function if the stoichiometry of a particular multiprotein complex is disturbed, for example, when overproduced protein causes transcriptional squelching by sequestration of transcriptional activators.

Finally, other family members can also complicate the selective inhibition of a particular protein's function. Certain dominant-negative p53 mutants used in past experiments might also interfere with the function of p51, or p73, either by occupying p53 DNA-binding sites or by inducing heteroligomer formation.

Even gene-knockout studies provide an imperfect solution to addressing function because they only reveal functions that are not shared among family members and because they can induce a potentially compensatory change in the expression of another family member. [For example, p107 is up-regulated in fibroblasts without *RB-1* (*9*).] Strain differences in the phenotype of genetically altered mice can complicate interpretation; knockout of *p130* causes no phenotype in one strain of mouse and yet is lethal in another (*10, 11*). In addition, some functions, such as that of pRB, cannot be extrapolated from mice to humans insofar as *RB-1* +/- mice do not develop retinoblastoma.

In conclusion, the normal functions of p73 and p51 remain to be elucidated, as do their potential roles in cancer. Paradoxically, their potential as therapeutic targets might be augmented if they prove to be largely vestigial copies of p53 that are rarely mutated or altered in human carcinogenesis. Because these p53 homologs can cause p53-defective tumor cells to undergo apoptosis, therapies that triggered the transcription of these genes might restore one measure of p53 function in tumor cells.

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PERSPECTIVES: STRUCTURAL BIOLOGY

Secret Life of Cytochrome bc_1

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The inner membrane of the mitochondrion houses the power plant of the cell. Here, the fundamental chemistry of respiration takes place to generate energy for aerobic life. Together three large protein complexes in this membrane—NADH dehydrogenase, cytochrome bc_1, and cytochrome oxidase—transfer electrons to molecular oxygen from NADH produced by oxidation of food molecules. Cytochrome bc_1, the central electron-transfer complex, oxidizes the membrane-soluble electron carrier ubiquinol and reduces the water-soluble carrier cytochrome c.

This deceptively simple function belies the complexity of events that occur within the cytochrome bc_1 molecule, including a bifurcated electron transfer pathway and translocation of protons across the mitochondrial inner membrane. Although intensively studied, the respiratory complexes have been very difficult to isolate in pure form because of their size and complexity; for instance, cytochrome bc_1 is a dimer of 11-subunit monomers. But finally, a wealth of data from the crystal structures of cytochrome bc_1 and cytochrome oxidase is illuminating the extensive biochemical and spectroscopic data, repaying decades of crystallization effort.

Exactly 2 years from the day S. Yoshikawa and H. Michel announced structures of cytochrome oxidase to much fanfare at the Bioenergetics Gordon Conference on 4 July 1995, Xia *et al.* presented the first structure of cytochrome bc_1 (*1*). In the ensuing year, a more complete, detailed, and fascinating picture of cytochrome bc_1 has emerged. The latest installment, from Iwata *et al.* (*2*), appears on page 64 of this issue.

The initial report by Deisenhofer and co-workers of an 80% complete, 2.9-Å crystal structure of bovine cytochrome bc_1 established the organization of the complex and positions for the metal centers (*1*). Berry and co-workers have just published the structure of the chicken bc_1 complex, nominally at 3 Å, including 9 of 11 subunits and complete chain tracing for the functional extrinsic domains of the cy-

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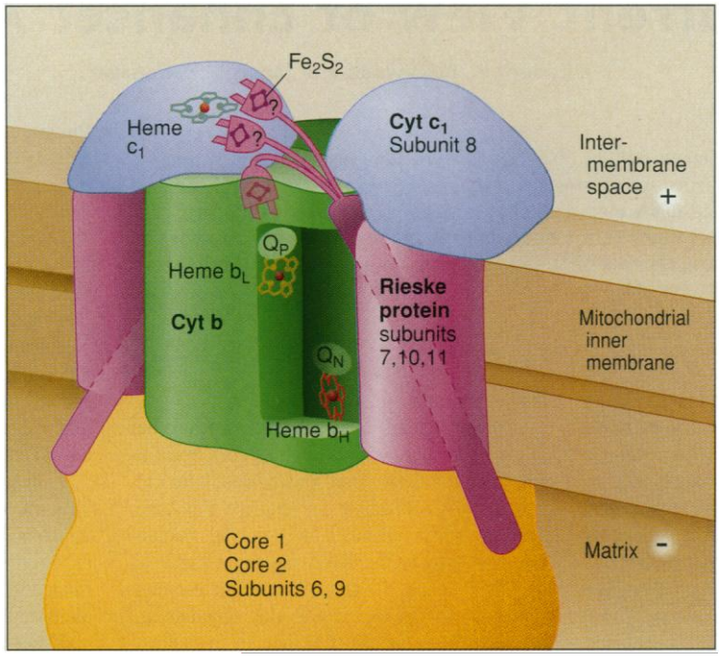
tochrome c_1 and Rieske subunits (*3*). Now, Jap and co-workers report refined, 3-Å structures for two new crystal forms of the bovine bc_1 complex (*2*). Both new structures include all 11 subunits.

These four different crystal structures of cytochrome bc_1 are snapshots along the reaction pathway of this dynamic molecule and also allow lattice-independent inference of biological function: The positions of hemes b_L and b_H and heme c_1 are invariant with respect to one another and the membrane bilayer. The position of the Rieske Fe_2S_2 center is still controversial, although motion is now recognized as a feature of its function (*2–4*).

A major conclusion from the crystal structures is that the dimer is essential to the chemistry of cytochrome bc_1. The "essential dimer" notion is manifest in the location of the Rieske intrinsic and extrinsic domains in opposite monomers of the dimer but has special relevance to the enigmatic, protonmotive Q cycle. The Q cycle hypothesis explains how transfer of two electrons through cytochrome bc_1 is linked to translocation of four protons across the membrane (*5*). The players in the Q cycle, all bound to the transmembrane domain of cytochrome b, are hemes b_L and b_H and their adjacent ubiquinol/ubiquinone sites, Q_P and Q_N. At Q_P, the electron transfer pathway is thought to bifurcate, with one electron of ubiquinol transferred to the Rieske extrinsic domain and the other cycled through the b hemes back to ubiquinone at Q_N.

Is the structure consistent with diffusion of ubiquinone species in and out of cytochrome bc_1 as well as efficient transfer between Q_P and Q_N? Yes, in a clever and unexpected way. The transmembrane helices of the bc_1 dimer are arranged to form two symmetric, bilayer-accessible "caves," with walls made from transmembrane helices and floor and ceiling from extrinsic domains (see the figure). Tucked into the walls of each cave are one Q_P and one Q_N. Thus, in a very simple way, the cave facilitates diffusion of ubiquinone species between Q_P and Q_N, while still providing outside access through its 25-Å-wide mouth. The dimer is essential, because Q_P of one monomer shares a cave with Q_N of the other. The cave and its relevance to the Q cycle were discussed in the original structure report (*1*), but until positions for all subunits of the bc_1 complex were identified, bioenergeticists were uncharacteristically reluctant to interpret it. With a complete bc_1 structure in hand (*2*), it's now open season on interpretation of the cave and the Q cycle. Stay tuned for a flurry of new ideas on the Q cycle mechanism.



A cave for chemistry. Cytochrome bc_1 contains a hollow between its two monomers (the essential dimer) that allows easy shuttling of ubiquinol/ubiquinone to and from the complex and between Q_P and Q_N. The Rieske extrinsic domain (plug) also shuttles between a site near Q_P (socket) and cytochrome c_1. For every electron transferred from Q_P through Rieske and cytochrome c_1 to cytochrome c, two protons are deposited on the electropositive side of the membrane.

The Rieske extrinsic domain is unexpectedly mobile, a major unanticipated feature of cytochrome bc_1 function. The Rieske extrinsic domain shuttles 20 Å between the sites for ubiquinol oxidation and cytochrome c_1 reduction. The Fe_2S_2 cluster is at one end of the domain, with the two histidine ligands protruding like the prongs of an electrical plug (see the figure). In one conformation, the "plug" is inserted into a cytochrome b "socket" adjacent to Q_P. This is presumably the position of Rieske when it oxidizes ubiquinol. Release of the plug from the socket is controlled by the ligand state of Q_P (*3, 4*). There is general agreement that Rieske unplugs from the socket after ubiquinol oxidation. What happens next is controversial. Rieske has been seen in at least three positions near cytochrome c_1 and in one intermediate position. The Deisenhofer group argues for no fixed, unplugged position of Rieske, citing various Rieske positions near cytochrome c_1 and stabilization of all well-ordered, unplugged Rieske positions by crystal lattice contacts (*4*). The Jap and Berry groups propose a unique Rieske–cytochrome c_1 association, on the basis of a hydrogen bond from an Fe_2S_2 histidine ligand to heme c_1 (*2, 3*). The Rieske extrinsic domain in the intermediate position is proposed as yet a third functionally relevant position by the Jap group (*2*).

Perhaps even more important is the conformational hinge within the Rieske extrinsic domain, which was discovered from analysis of the new structures (*2*). In the intermediate position, the domain is more open than when proximal to cytochrome c_1 or when isolated from the bc_1 complex (*6*). This flexibility raises a question for future investigations. Could hinging in the Rieske extrinsic domain be part of its function?

Finally, the new structures explain recognition of the consensus sequence motif for mitochondrial targeting by mitochondrial processing proteases. The Core 1 and Core 2 subunits of bc_1 are homologs of mitochondrial processing proteases β and α subunits. Subunit 9, which was formerly the mitochondrial targeting presequence of the Rieske protein, binds to the Core 2 subunit in a manner that explains conserved elements of the motif.

Is the structural story of cytochrome bc_1 finished? Most definitely not. The nature of the Rieske–cytochrome c_1 interaction, the pathways of proton transfer, the difference in reduction potential of ubiquinone in Q_P and Q_N, and a structure-based mechanism for the Q cycle remain to be determined. The clues reside in details of structure at substantially higher resolution than reported to date. The fun has just begun.

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