

Movin' on. A model for the evolutionary fate of male-biased genes in *Drosophila* based on new experimental data (7) and previous theoretical predictions (5). (A) Male-biased genes (vertical arrows) emerge at a higher rate on the X chromosome (red). (B) However, they move preferentially to the autosomes (blue) from the X chromosome. Male-biased X-linked genes may also experience a faster rate of inactivation than other X-linked genes. Under equilibrium conditions, the net effect is underrepresentation of male-biased genes on the X chromosome, which has been termed demasculinization of the X chromosome. (The thickness of the horizontal arrow represents the speed of gene movement; the thickness of the chromosome indicates the number of male-biased genes it contains.)

deleterious mutations on the X chromosome are purged more efficiently, making it unlikely that X-linked genes are inactivated at a higher rate. Furthermore, only male-biased genes are found in greater numbers on the autosomes than on the X chromosome. Hence, simple models that assume equal functional importance for all genes cannot explain a preferential loss of male-biased genes from the X chromosome. If X-linked male-biased genes are on average less important than autosomal genes, then this could explain X-chromosome demasculinization.

Irrespective of the reason for the lower

number of male-biased genes on the X chromosome, it is intriguing that the Parisi *et al.* findings seem to contradict earlier theoretical work (5). According to the notion that the presence of a single X chromosome in males renders selection more effective for X-linked genes, Rice (5) predicted that new (partially) recessive genes that are beneficial for males are less likely

to be lost if they are located on the X chromosome. The prediction is that, over the long term, this would result in a greater number of male-biased genes on the X chromosome (see the figure). Although genes that are beneficial for males are “fixed” in the X chromosome at a faster rate than in the autosomes, they are also lost more rapidly from the X chromosome. Depending on the relative rates of fixation and loss of male-biased genes, the number of such genes could easily differ between the X chromosome and autosomes.

How general is the phenomenon of demasculinization of the X chromosome? The worm exhibits a similar male-biased gene expression pattern to that of the fruit fly, with almost no sperm-specific or germline-specific genes found on the X chromosome (6). On the other hand, mice harbor an excess of male-biased genes on the X chromosome (7). Future studies comparing sex-biased gene expression on a genome-wide scale among a range of different species will reveal the mechanisms that underlie the nonrandom distribution of male-biased genes.

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movement of male-biased genes from the X chromosome to the autosomes (see the figure), and (iii) loss of function of male-biased genes occurring at a faster rate on the X chromosome than on autosomes.

The first evolutionary scenario is supported by a recent study of retroposed genes in *Drosophila* (3). Retroposed genes are derived by insertion of a processed RNA into the genome. They lack introns and have traces of the polyadenylated tail characteristic of messenger RNAs. It seems that retroposed genes often acquire a male-biased expression pattern and are overrepresented on autosomes relative to the X chromosome. Nevertheless, the number of retroposed genes is probably too small to result in a pattern that could be described as “demasculinization of the X chromosome.”

The second hypothesis—the preferential movement of male-biased genes from the X chromosome to autosomes—can only be tested when the genome of a third insect species becomes available enabling the observed differences to be polarized. In this respect, it is remarkable that those male-biased genes that are X-linked in the fruit fly but autosome-linked in *A. gambiae* are better conserved than male-biased genes that are X-linked in both species (1).

Finally, the expression of male-biased genes could be preferentially lost on the X chromosome, although such a situation is hard to reconcile with our current understanding of molecular evolution. Recessive X-linked genes conferring a male advantage are selected for more efficiently because males have only one X chromosome and therefore only one set of X-linked genes (4). Thus, recessive

STRUCTURAL BIOLOGY

Complex II Is Complex Too

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In aerobic eukaryotic cells, the generation of energy in the form of adenosine triphosphate (ATP) is mainly driven by the activity of the respiratory chain enzymes of the mitochondrial inner membrane. The respiratory chain—composed of enzyme complexes I to IV, ubiquinone, cytochrome c, and ATP synthase (complex V)—transfers electrons from NADH and succinate at one end to molecular oxygen at the other (1). On page 700 of this issue, Yankovskaya *et al.* (2) present the x-ray crystal structure at 2.6 Å resolution of the bacterial version of eukaryotic complex II. This enzyme, called succinate:quinone

oxidoreductase (SQR) in *Escherichia coli*, couples the two-electron oxidation of succinate forming fumarate, to the reduction of ubiquinone (3). SQR, which is also the succinate dehydrogenase of the Krebs cycle, is crucial for intermediary metabolism and energy production in eukaryotic cells and bacteria under aerobic conditions.

Eukaryotic complex II and *E. coli* SQR are composed of four protein subunits and five prosthetic groups. The two largest subunits bind to one flavin adenine nucleotide (FAD) and three iron-sulfur cluster prosthetic groups. These two hydrophilic protein subunits are linked to a pair of hydrophobic protein subunits, which form a membrane anchor that binds to one heme group and provides ubiquinone with a binding site. The SQR structure reveals that electrons are transferred in a path more than 40 Å in length from the succinate oxidation site at the FAD, by way of the three

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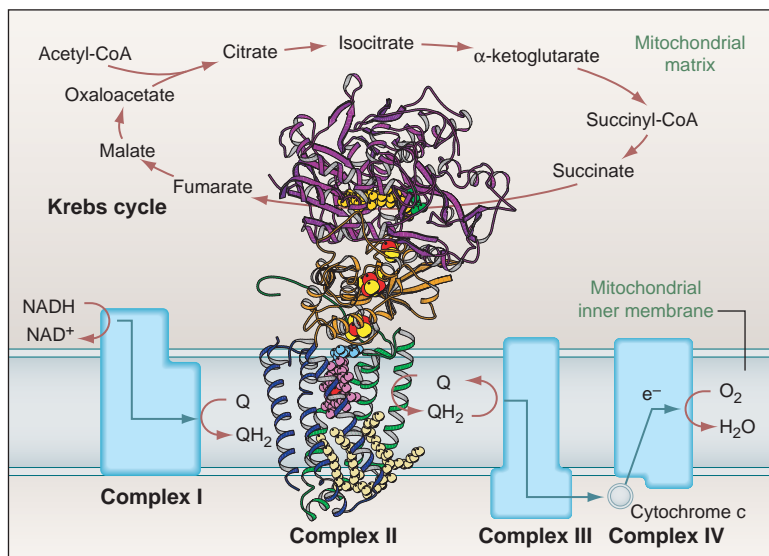
PERSPECTIVES

iron-sulfur clusters, to the ubiquinone binding site (see the figure). One iron-sulfur cluster, the heme group, and bound ubiquinone are positioned at the corners of a triangle. The edge-to-edge distance between individual pairs of these redox components is 11 Å or less, a distance appropriate for rapid electron transfer. The heme in the *E. coli* SQR can be reduced by succinate, but based on the structure, this does not seem to be obligatory for the transfer of electrons from succinate to ubiquinone.

Yankovskaya and colleagues present the crystal structure of *E. coli* SQR in a complex with either ubiquinone or an inhibitor that blocks binding of ubiquinone. The two structures elegantly pinpoint where on SQR the reduction of ubiquinone takes place. Other revelations include the presence of two tightly bound, well-ordered phospholipids; the architecture of the ubiquinone-binding pocket; and the spatial arrangement of heme and ubiquinone. It is reassuring to find that the crystal structure of *E. coli* SQR is consistent with most of the predictions based on other types of experimental data (4).

Dehydrogenases that oxidize an organic substrate and reduce ubiquinone often consist of only one polypeptide and a flavin moiety as the prosthetic group. Examples of such simple membrane-associated enzymes in *E. coli* include NADH dehydrogenase type II, D-lactate dehydrogenase, and aerobic glycerol-3-phosphate dehydrogenase (5). The enzymatic tasks of these dehydrogenases and of succinate dehydrogenase (SQR) seem similar, so it is all the more surprising that SQR has five different prosthetic groups. Perhaps SQR carries out additional, as yet unrecognized, tasks such as sensing the amount of molecular oxygen (6). Saraste emphasized the intricate nature of SQR, compared with respiratory complexes I, III and IV, in his phrase “complex II is complex too.” Despite its intricacies, SQR is not directly involved in energy transduction (the coupling of electron transfer to the formation of a transmembrane proton gradient that drives ATP synthesis).

The composition of *E. coli* SQR is also puzzling when compared with that of its close relative fumarate reductase (QFR).



The intricacies of complex II. Eukaryotic complex II (equivalent to SQR in *E. coli*) directly connects the respiratory chain of the inner mitochondrial membrane with the Krebs cycle enzymes in the mitochondrial matrix. Complex I (NADH:quinone oxidoreductase) and complex II reduce ubiquinone (Q) to ubiquinol (QH₂). Complex III (quinone:cytochrome c reductase) uses ubiquinol to reduce cytochrome c, which is reoxidized by reduction of molecular oxygen to water catalyzed by complex IV (cytochrome c oxidase). Complexes I, III, and IV couple electron transfer to the formation of a transmembrane proton gradient that drives ATP synthesis by complex V (not shown). The x-ray crystal structure of *E. coli* SQR (2) with its four protein subunits (purple, orange, green, and blue ribbons) is shown in the position of complex II. Visible in the crystal structure is FAD (gold), oxaloacetate (green), three iron-sulfur clusters (red, iron; yellow, sulfur), ubiquinone (light blue), heme b (magenta), and cardiolipin (pale yellow). (The respiratory chain complexes are not drawn to scale.)

Detailed comparison is now possible as crystal structures are available for the QFRs of *E. coli* and another bacterium, *Wolinella succinogenes* (7, 8). The extracellular domains of SQR and the two QFRs are very similar in composition and overall structure, but the membrane-anchor domains differ in their structure and particularly in the number of heme groups that they bind. *E. coli* QFR contains no heme but has binding sites for two menaquinone molecules, Q_P and Q_D; the *W. succinogenes* QFR contains two heme groups, b_P and b_D. The position of the single heme group in the *E. coli* SQR roughly corresponds to that of Q_P and heme b_P in the QFRs. Intriguingly, a cavity in SQR filled with two acyl chains of a cardiolipin phospholipid molecule corresponds to the location of Q_D and heme b_D in the QFRs. Thus, the three available structures reveal considerable diversity in redox components within a protein scaffold of common evolutionary origin. Although the SQR and QFR of *E. coli* can functionally replace each other, SQR operates under aerobic conditions and QFR under anaerobic conditions. The SQR crystal structure of Yankovskaya *et al.* reveals a possible explanation for why SQR is the favored enzyme when oxygen is present.

Under aerobic conditions, reduced *E. coli* QFR is autooxidized at the FAD, producing large amounts of reactive oxygen species, in-

cluding superoxide anion radical ($\cdot\text{O}_2^-$) and hydrogen peroxide (H_2O_2) (9). The production is first order with respect to oxygen concentration, and the rate-limiting step is the formation of $\cdot\text{O}_2^-$. The extent of reduction of the enzyme influences whether the released product is mainly $\cdot\text{O}_2^-$ or H_2O_2 . In sharp contrast, *E. coli* SQR reacts poorly with molecular oxygen, producing modest amounts of $\cdot\text{O}_2^-$ and no H_2O_2 . Differences in accessibility of the flavin moiety to molecular oxygen, the redox properties of FAD, and the properties of neighboring redox groups and cooperative electromagnetic interactions between these groups affect the reactivity of the flavin moiety. On the basis of their crystal structure and other data, Yankovskaya *et al.* argue that the heme group in SQR is pivotal in preventing the formation of reactive oxygen species at FAD.

They also point out that mutations identified in human complex II—for example, those causing tumors in patients with hereditary paraganglioma—could lead to increased production of reactive oxygen species by SQR, resulting either directly or indirectly in tissue damage and disease.

Crystal structures are now available for seven of the eight enzymes of the Krebs cycle and for the entire succinate oxidase system, that is, complexes II, III, and IV, and cytochrome c. But obtaining the structures of complex I and parts of complex V remain formidable challenges. Although complex II has yet to reveal all of its secrets, the Yankovskaya *et al.* crystal structure of *E. coli* SQR brings us a giant step closer to a full appreciation of the intricacies of this enzyme.

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10. This article is dedicated to Matti Saraste (1949–2001).