Complements of the house

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Commentary

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Finding mutations in nuclear genes responsible for disorders in the mitochondrial oxidative phosphorylation system has been a tedious matter. A "Venn diagram" approach — not unlike a classic complementation experiment — reported in this issue will now make the search easier (see the related article beginning on page 837).

Forty-six in the first, 4 in the second, 11 in the third, and 13 in the fourth, for a total of 74. Sounds like the score, by quarters, for an erratic college basketball team. Of course, it's not — it's the number of subunits in each of the 4 complexes of the human mitochondrial respiratory chain (Figure 1). And that number does not even include the 16 subunits of the fifth complex, ATP synthase, which uses the protons produced by the respiratory chain to fuel oxidative ATP synthesis.

And therein lies a problem. In the last 15 years, we have begun to recognize that defects in the mitochondrial respiratory chain/oxidative phosphorylation system (OxPhos) are responsible for a panoply of human disorders, ranging from sporadic myopathies to fatal encephalomyopathies. Those disorders can be maternally inherited, as a result of mutations in any one of the 13 polypeptides encoded by mitochondrial DNA (mtDNA), or they can be Mendelian, as a result of mutations in the 77 polypeptides encoded by nuclear DNA (nDNA). To make matters worse, there are at least 30 other proteins — all nDNA encoded — required for the proper assembly and functioning of these 5 complexes.

With more than 120 potential culprit genes responsible for OxPhos disorders, what's a geneticist to do? In the old days — say, 5 years ago — the problem was simple. We didn't have the human genome available online, so we spent lots of time looking for mutations in mtDNA. After all, how hard is it to find mutations in a genome that's only 16.6 kb in size? In relatively short order, mutations were found in all 13 polypeptides (as well as in both mtDNA-encoded ribosomal RNAs and in 21 of the 22 transfer RNAs) (1). On the other hand, if anyone wanted to find the cause of a Mendelian OxPhos disorder — and there are plenty of them, almost all lethal in early infancy or childhood and almost all recessively inherited (for example, Leigh syndrome) — the task was daunting, for at least two reasons: first, there were, at a minimum, 120 candidate genes; where do you start? Second, there are few large pedigrees, so linkage analysis is usually not an option.

In spite of these obstacles, progress came, albeit slowly. Today, we know of mutations in at least 22 nDNA-encoded polypeptides, of which 8 are assembly proteins (Figure 1). Of the 22, nine are structural subunits of complex I, the largest by far of the 5 OxPhos complexes, which consists of 46 subunits (7 encoded by mtDNA and 39 by nDNA). There is no reason to believe that there could not be mutations in any of the 46 subunits or in complex I assembly protein. In fact, it appears that more patients with respiratory chain disorders have deficits in complex I than in all the other complexes combined (2). What would be the most expeditious way of finding these culprit genes, other than by brute force DNA sequencing?

One path to the solution of this problem was revealed by Eric Shoubridge in Montreal and then by Massimo Zeviani in Milan, both of whom identified mutations in SURF1, a complex IV assembly protein that was responsible for fatal infantile cytochrome *c* oxidase (COX) deficiency. Both groups had the unusual advantage of access to large pedigrees, which enabled them to map the culprit locus to chromosome 9. They narrowed down the locus using monochromosomal hybrids and microcell-mediated chromosome transfer, in which human chromosomes are "inserted" into patient COX-deficient fibroblasts in order to find the one chromosome (or piece of a chromosome) that could complement the defect. Once the region had been narrowed down to a manageable size (actually a few megabases!), some brute force, plus intelligent choices of candidate genes for sequencing, yielded the responsible gene, namely, *SURF1* (3, 4).

A different approach was taken by Eric Lander in Boston and Brian Robinson in Toronto, who collaborated to look for a gene responsible for another recessive COX deficiency prevalent in individuals from the Saguenay Lac St. Jean region of Quebec. Using a combination of genomics, proteomics, and bioinformatics, they were able to identify mutations in another COX assembly gene called *LRPPRC* (5).

In this issue of the *JCI*, David Thorburn, Denise Kirby, and colleagues have combined elements of both of these approaches to determine, first, the prevalence of genes mutated in a series of patients with complex I deficiency of unknown origin, and second, the identity of one such new gene, using what might be called a "Venn diagram" approach (Figure 2) (6). They fused complex I–deficient cells from individual patients in pairwise fashion and asked whether fused pairs could rescue the respiratory-deficient phenotype; in other words, they performed a classical complementation experiment. In a second round of fusions, Kirby et al. asked whether patient cells could rescue function in ρ⁰ cells, which are cells containing mitochondria that are devoid of mtDNA (and hence devoid of respiratory function). Fusions that were rescued did so because the ρ⁰ nucleus complemented the mutation in the patient cell's nucleus, which implied that the patient's defect resided in a nuclear gene. On the other hand, fusions that failed to rescue the complex I deficiency did so because the mtDNA in the patient's mitochondria could not rescue the respiratory deficiency arising from the ρ⁰ cell's lack of mtDNA, which suggests that the patient's genetic error resided in the mtDNA, not the nDNA.

Using this Venn diagram approach, Kirby et al. (6) were able to pigeonhole
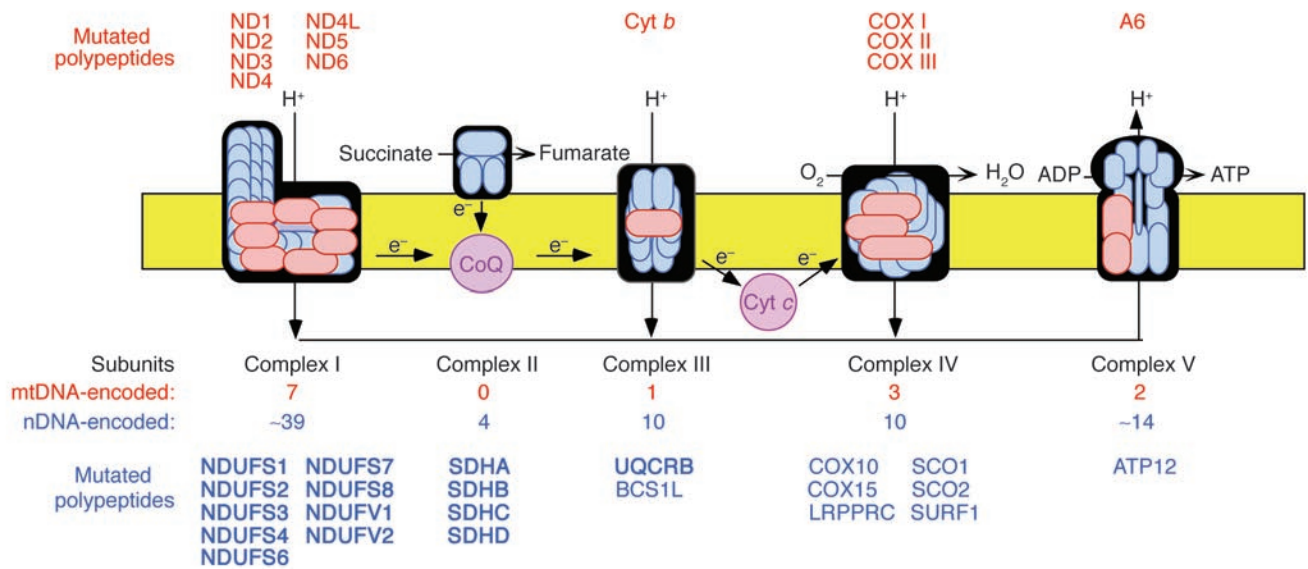
Figure 1
The OxPhos system, consisting of NADH dehydrogenase-CoQ oxidoreductase (Complex I), succinate dehydrogenase-CoQ oxidoreductase (Complex II), CoQ-cytochrome *c* oxidoreductase (Complex III), cytochrome *c* oxidase (Complex IV), and ATP synthase (Complex V). Nuclear DNA–encoded subunits are shown in blue; mtDNA-encoded subunits are shown in red; mutated OxPhos complex subunit polypeptides that cause mitochondrial disease are shown in bold; mutated assembly polypeptides are shown in plain text; Cyt *c*, cytochrome *c*.

cells from 10 patients into 8 complementation groups, including 8 patients with mutations at 7 chromosomal loci and 2 patients with mutations in mtDNA-encoded genes (which were identified rapidly, by sequencing the mtDNA). The power of the approach was demonstrated in the 2 patients who shared the same complementation group. Following analysis by homozygosity mapping, microcell-mediated chromosome transfer, and tran-

scriptome analysis, both patients were found to have mutations in *NDUFS6*, a complex I subunit gene residing on chromosome 5 to which no pathogenic mutation had been assigned previously.

As demonstrated by Kirby et al. (6), the integrated approach of 3-way complementation, biochemistry, genetics, microarrays, and bioinformatics, although cumbersome, offers a relatively straightforward path to deducing the molecular

basis of recessive respiratory chain disorders (or any other recessive disorder, for that matter). As for dominant disorders, or those due to multifactorial causes (uncommon, but not unknown in the field of mitochondrial diseases), however, this approach will fail. Tennis, anyone?

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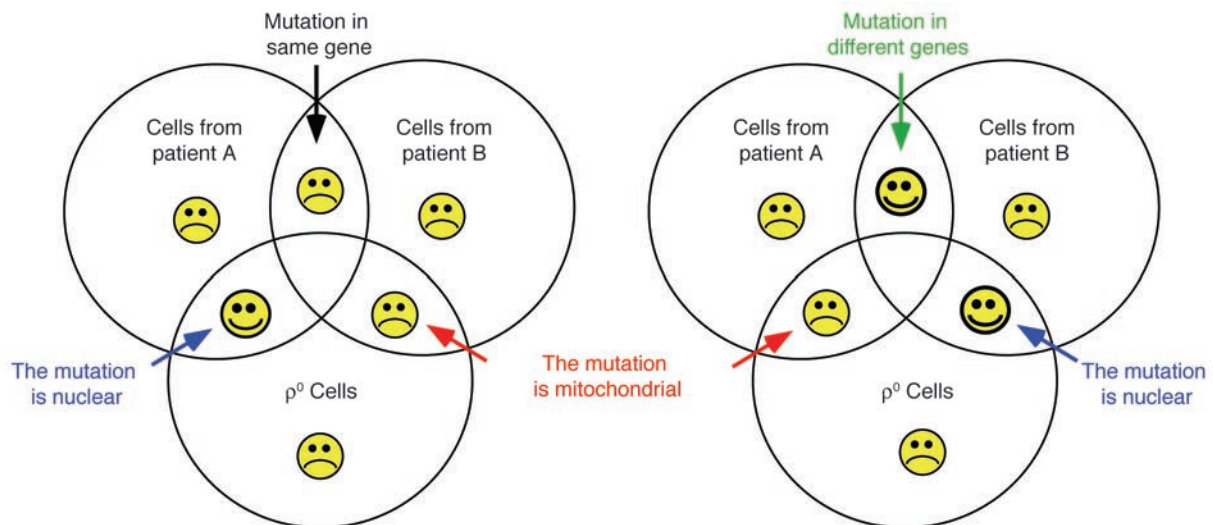
Figure 2
Venn diagram approach used by Kirby et al. (6). Smiling and frowning faces indicate elevated complex I activity (indicating rescue of the phenotype) and reduced complex I activity, respectively. The diagrams show only some of the possible combinations of outcomes.

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The link between IFN-γ and allograft arteriopathy: is the answer NO?

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Vascularized organ transplants often fail because of smooth muscle cell migration and proliferation in the intima of graft arteries, leading to progressive lumenal narrowing and resultant ischemic damage. Graft arterial disease is caused by IFN-γ secreted by alloreactive T cells. New evidence indicates that IFN-γ dysregulates expression of the enzymes eNOS and iNOS in graft-infiltrating leukocytes (see the related article beginning on page 846). Dysregulated NO synthase expression occurs prior to and is causally linked to intimal smooth muscle cell accumulation.

Graft arteriosclerosis is the major cause of late allograft failure. Pathologic features include arterial intimal hyperplasia due to recruitment and proliferation of smooth muscle cells (SMCs), which eventually causes lumenal obstruction and allograft ischemia (Figure 1, A and B). Although the mechanisms underlying graft arteriosclerosis are not well understood, the condition most likely results from a form of ongoing immune rejection, and most evidence suggests critical dependence on host T cell alloantigen recognition (1). Nevertheless, graft arteriosclerosis is resistant to standard immunosuppressive therapies. Of a plethora of candidate effector molecules, IFN-γ — a proinflammatory cytokine produced by effector T cells — has long been suspected of being a major player in graft arteriosclerosis.

IFN-γ causes graft arterial disease
To dissect the molecular mechanisms underlying this process, in vivo models that recapitulate the changes observed following human organ transplants have

been developed; indeed, murine cardiac allografts develop arterial changes pathologically identical to the human disease. Using this model, arteriopathy does not develop in allografts transplanted into IFN-γ–deficient hosts or after treatment with anti–IFN-γ; this finding provided the first direct evidence that IFN-γ is *necessary* to induce graft arteriosclerosis (2–4). However, the relevance of findings in a mouse model to human arteriopathy must be weighed in light of known interspecies differences in vascular cell phenotype and function, including those in endothelial cells (ECs) and SMCs (5).

Jordan Pober and colleagues previously addressed the issue of species differences using a model of graft arterial disease involving human arterial segments interposed in the infrarenal aorta of lymphocyte and NK cell–deficient (SCID/beige) mice (6–8). These arterial xenografts remain histologically normal in the immunodeficient host mice. However, if human IFN-γ is administered, intimal thickening occurs as a result of SMC accumulation. Because the host mice are immunodeficient and murine cytokines do not have biological activity on human cells, the results indicate that IFN-γ is also *sufficient* to induce graft arterial disease. Moreover, human arterial grafts in SCID/beige hosts injected with allogeneic

PBMCs also develop arteriopathy, associated with human IFN-γ–expressing T cell infiltrates. Administering anti–IFN-γ reduces the intimal thickening caused by PBMC injection (6–8).

NO and arterial dysfunction
Although the data from murine allograft and xenograft models clearly establishes a central role for IFN-γ in graft arterial disease, the pathologic mechanisms downstream of IFN-γ are not clear. One possibility is that IFN-γ dysregulates vascular wall production of and response to NO (9, 10). ECs synthesize NO from L-arginine via constitutively expressed eNOS; leukocytes can synthesize NO through iNOS. Induction of vasodilation (through vascular SMC relaxation) is one of the principal roles of NO synthesized by ECs via eNOS. However, NO does have other activities related to vascular wall function including inhibiting SMC proliferation and reducing platelet and leukocyte adhesion (11).

Dysregulated eNOS production — reflecting a more global EC dysfunction — can be assessed by measuring the vasomotor responsiveness of arteries to eNOS-dependent vasodilators. EC dysfunction is implicated in atherosclerosis and graft arteriosclerosis. In addition, failure of SMCs to relax in response to NO (SMC dysfunction) is also implicated in graft arteriosclerosis. Since both IFN-γ and dysregulation of NO responses are associated with graft arterial disease, a logical question to ask is whether IFN-γ is the cause of EC and vascular SMC dysfunction. In a report by Pober, Koh, and colleagues in this issue of the *JCI*, elegant experiments that establish this causal link are described (12).