NADH:ubiquinone oxidoreductase (complex I) of the electron transport chain is a multimeric mitochondrial enzyme of approximately 1000 kDa consisting of 46 different proteins encoded by both the mitochondrial and nuclear genomes. Little is known about the cellular mechanisms and protein chaperones that guide its assembly. In this issue of the JCI, Ogilvie et al. use genomic sequence data to compare the proteins produced by yeasts with and without complex I in order to generate a list of proteins whose human orthologs might serve as complex I assembly proteins (see the related article beginning on page 2784). The gene encoding one of these candidate proteins, B17.2L, was found to harbor a nonsense mutation in one of 28 patients with a deficiency of complex I. B17.2L associated with subcomplexes that are seen when complex I assembly is incomplete. The research described here combines clever model organism genomics and bioinformatics with sophisticated human molecular and biochemical genetics to identify the first mammalian protein required for the normal assembly of complex I.

Complex I of the mitochondrial respiratory chain

Of all the components of the electron transport chain, NADH:ubiquinone oxidoreductase (complex I) is by far the biggest and contains the largest number of structural proteins (1). Complex I contains the mitochondrial NADH-dehydrogenase (EC 1.6.99.3) activity responsible for accepting electrons from NADH and passing them to ubiquinone (2). The gene encoding one of these candidate proteins, B17.2L, was found to harbor a nonsense mutation in one of 28 patients with a deficiency of complex I. B17.2L associated with subcomplexes that are seen when complex I assembly is incomplete. The research described here combines clever model organism genomics and bioinformatics with sophisticated human molecular and biochemical genetics to identify the first mammalian protein required for the normal assembly of complex I.

Researchers have not yet been able to bring the full power of genetic analysis to elucidating the structure and function of complex I because the usual eukaryotic workhorse for such approaches, the yeast Saccharomyces cerevisiae, lacks complex I. Although there has been a lot progress in using prokaryotes or eukaryotic fungi such as N. crassa as alternative model systems (7), the pace of progress seems to now be accelerating through the use of Yarrowia lipolytica, an obligate aerobic yeast and a powerful new model organism for studies of complex I structure and function (1). Complex I from Y. lipolytica appears to be very similar to mammalian complex I in structure and composition, and its identification has facilitated the usual opportunities for genetic manipulation in yeast, such as mutagenesis screens for functional mutants, facile site-directed mutagenesis, and expression of tagged proteins suitable for affinity chromatography and proteomic analysis (8).

Disorders of oxidative phosphorylation in humans

Human diseases caused by defects in oxidative phosphorylation are rare (approximately 1 per 10,000 live births) but often take the form of devastating neurological conditions (9). Symptoms can vary from fatal lactic acidosis in the neonate to mental and physical retardation with cardiomyopathy, skeletal myopathy, and hepatic failure in childhood, to acute painless loss of vision (Leber hereditary optic neuropathy) in young adults, to a form of Parkinson disease later in life. One-third of the defects in the electron transport chain that cause genetic oxidative phosphorylation diseases occur in complex I (9, 10). Only a minority of the molecular abnormalities that cause complex I deficiency are known. Laboratories that do extensive molecular diagnostic analysis for complex I deficiency report that only approximately 20–25% of such patients have homoplasmic or heteroplasmic mutations in 1 of 4 mitochondrial-encoded complex I subunit genes; another 20–25% are the result of mutations in 1 of 9 nuclear-encoded complex I subunit genes (10).

Nonstandard abbreviations used: BN-PAGE, blue native PAGE; complex I, NADH:ubiquinone oxidoreductase.

Conflict of interest: The author has declared that no conflict of interest exists.

Citation for this article: J Clin Invest. 115:2689–2691 (2005). doi:10.1172/JCI26625.
complex I deficiency and found 1 patient, the child described above, who appeared to be homozygous for a nonsense mutation (C182T) in exon 2 of B17.2L that caused premature termination of translation. Her mother was heterozygous for this mutation, but the mutation was not found in her father, suggesting that he was likely to be heterozygous for a deletion allele that the proband inherited from him as her paternal allele. The functional complex I deficiency and defective assembly in this patient, as determined by enzyme assay and 2D BN-PAGE, was corrected by transduction with a vector expressing B17.2L cDNA. Finally, Ogilvie et al. demonstrated that the B17.2L protein associates with a particular 830-kDa subcomplex of complex I that accumulates in a variety of patients with mutations in genes encoding structural components of complex I, but not with the normal intact complex I itself. Based on these data, the authors concluded that B17.2L is a component of the cellular machinery that is involved in the assembly of complex I without being a part of the mature complex I itself and that loss of function of this protein leads to complex I deficiency (Figure 1).

Humans stand at the opposite end of the spectrum from yeast in terms of serving as an easily manipulated genetic system. However, the study of human genetics has much to offer, not only because of the direct involvement with human disease, but also because of the depth of phenotypic richness and the locus and allelic heterogeneity that human genetic disease provides. Indeed, one of the more striking themes of modern molecular genetics has been how progress in understanding fundamental biological processes has come time and again from the marriage of model organism research with careful human genetic studies. The research reported here by Ogilvie and colleagues (17) is an excellent example of just such a successful marriage.

Acknowledgments
The author is supported by the Intramural Research Program of the National Human Genome Research Institute, NIH.

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Figure 1
Schematic diagram of complexes I through IV of the electron transport chain and ATP synthase. The red line traces the path of electrons as they enter and move along the electron transport chain. Complex I is shown at the far left as an L-shaped structure with one portion extending down into the mitochondrial matrix and the other portion embedded in the inner mitochondrial membrane. Ogilvie and colleagues used bioinformatics to perform a virtual whole genome subfraction of yeasts with or without complex I to find candidate complex I assembly factors, identified the human orthologs of these proteins, and showed that one of these orthologs, B17.2L, is a component of the cellular machinery that is involved in the assembly of complex I without being a part of the mature complex I itself and that loss of function of this protein leads to complex I deficiency (Figure 1).

In this issue of the JCI, Ogilvie et al. report the first human protein required for assembly of human complex I (17).

Ogilvie et al. (17) report a female child, born to a normal, nonconsanguineous couple, who developed progressive neurologic disease affecting many portions of her central nervous system beginning around 1 year of age and suffered relentless neurologic deterioration until her death at 13.5 years of age. Her disease was associated with elevation of cerebral spinal fluid lactate because of the depth of phenotypic richness and the locus and allelic heterogeneity that human genetic disease provides. Indeed, one of the more striking themes of modern molecular genetics has been how progress in understanding fundamental biological processes has come time and again from the marriage of model organism research with careful human genetic studies. The research reported here by Ogilvie and colleagues (17) is an excellent example of just such a successful marriage.

structural subunit genes (9, 11–13). The molecular defects in the remaining 50–60% of patients with deficiencies in complex I, but without obvious mutations in genes encoding complex I structural subunits, still remain largely undetermined. It seems a very reasonable supposition that some of these complex I defects without structural subunit mutations are caused by defects in auxiliary proteins required for multimer assembly, as has already been demonstrated in some patients with severe encephalopathy and failure of other organ systems due to mutations in genes such as SURF1 and SCO2 that affect complex IV assembly (14) or mutations in BCS1L affecting complex III assembly (15).

Of the 2 assembly proteins that have been shown to be required for N. crassa complex I assembly, 1 has a human ortholog; however, no defects in that gene have been found in patients with complex I deficiency (16). In this issue of the JCI, Ogilvie et al. report the first human protein required for assembly of human complex I (17).

Ogilvie et al. (17) report a female child, born to a normal, nonconsanguineous couple, who developed progressive neurologic disease affecting many portions of her central nervous system beginning around 1 year of age and suffered relentless neurologic deterioration until her death at 13.5 years of age. Her disease was associated with elevation of cerebral spinal fluid lactate and a deficiency of complex I enzyme activity in muscle mitochondria (approximately 38% of control complex I activity) and cultured fibroblasts (less than 20% of control complex I activity). Taking a clever bioinformatics approach in the appropriate model organisms, these researchers carried out a subtraction in silico of genes found in Y. lipolytica and another aerobic yeast with a complex I, Debaryomyces Hansenii, but not in other yeasts that lack a complex I, and used the resulting protein sequences to search for human orthologs containing mitochondrial targeting sequences. Their analysis ultimately yielded 14 genes, 1 of which was B17.2L, a paralog of B17.2, which encodes a known structural subunit in the matrix arm of human complex I (18). They sequenced B17.2L in 28 patients with complex I deficiency and found 1 patient, the child described above, who appeared to be homozygous for a nonsense mutation (C182T) in exon 2 of B17.2L that caused premature termination of translation. Her mother was heterozygous for this mutation, but the mutation was not found in her father, suggesting that he was likely to be heterozygous for a deletion allele that the proband inherited from him as her paternal allele. The functional complex I deficiency and defective assembly in this patient, as determined by enzyme assay and 2D BN-PAGE, was corrected by transduction with a vector expressing B17.2L cDNA. Finally, Ogilvie et al. demonstrated that the B17.2L protein associates with a particular 830-kDa subcomplex of complex I that accumulates in a variety of patients with mutations in genes encoding structural components of complex I, but not with the normal intact complex I itself. Based on these data, the authors concluded that B17.2L is a component of the cellular machinery that is involved in the assembly of complex I without being a part of the mature complex I itself and that loss of function of this protein leads to complex I deficiency (Figure 1).

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PDGF signaling in pulmonary arterial hypertension

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The pathobiology of pulmonary arterial hypertension (PAH) includes endothelial cell dysfunction and proliferation and migration of VSMCs. As PDGF has been implicated in these processes, Schermuly et al. hypothesized that altered PDGF signaling may be involved in the vascular remodeling observed in PAH. To explore this notion further, the authors evaluated the effects of the PDGF receptor inhibitor ST1571 in 2 different animal models of pulmonary hypertension (see the related article beginning on page 2811). In both models, after development of pulmonary vascular disease, administration of ST1571 reversed pulmonary vascular changes. These studies provide preclinical proof of concept for the clinical development of a PDGF inhibitor as a targeted therapy for PAH patients.

Pulmonary arterial hypertension (PAH), a disorder limited to the pulmonary circulation, is characterized by pulmonary vascular obstruction and variable pulmonary vasoconstriction leading to increased pulmonary vascular resistance and death. PAH can be idiopathic, or unexplained (formerly termed primary pulmonary hypertension); PAH can also occur in association with connective tissue diseases, HIV infection, congenital heart disease, portal hypertension, and appetite suppressant exposure. Idiopathic PAH occurs more often in women than in men, with a median survival of 2.8 years if untreated. The mean age at diagnosis is 35 years, i.e., it occurs most often in young adults with no other comorbid conditions. Although current treatment options have markedly improved overall quality of life and survival in PAH, 5-year survival remains at 50% for this devastating disease. As discussed below, over the past 2 decades, we have learned a great deal about the pathobiology of PAH; however, we still do not know what initiates this disease with its subsequent progressive pulmonary vascular obstruction.

PAH has a multifactorial pathobiology. Vasoconstriction, remodeling of the pulmonary vessel wall, and thrombosis contribute to increased pulmonary vascular resistance in PAH. The process of pulmonary vascular remodeling involves all layers of the vessel wall and is complicated by cellular heterogeneity within each compartment of the pulmonary arterial wall (Figure 1). Indeed, each cell type (endothelial, smooth muscle, and fibroblast), as well as inflammatory cells and platelets, may play a significant role in PAH. Endothelial dys-function is considered a key element in the pathobiology of PAH, with increased levels of endothelin occurring concomitantly with decreased NO and prostacyclin levels (1). Although it remains unclear whether excessive vasoconstriction is associated with the endothelial dysfunction, many of the perturbations associated with the endothelial dysfunction promote vascular remodeling in addition to increasing pulmonary vascular tone. Prostacyclin, NO, endothelin, angiotensin I, serotonin, cytokines, chemokines, and members of the TGF-β superfamily have all been implicated in the pathobiology of PAH. As a result, 3 of the currently approved therapeutic modalities for the treatment of PAH — prostacyclin, phosphodiesterase inhibitors, and endothelin receptor antagonists (2–4) — target prostacyclin and NO deficiencies and increased endothelin levels, respectively, in PAH patients.

Distal extension of smooth muscle cells into small, peripheral, normally nonmuscular pulmonary arteries is a hallmark of PAH. However, the processes causing this migration as well as the formation of a layer of microfibril matrix and extracellular matrix (termed the neointima) between the endothelial cells and the internal elastic...