

Animal models for respiratory chain disease

Nils-Göran Larsson and Pierre Rustin



The elucidation of pathogenesis in respiratory chain diseases is of great importance for developing specific treatments. The limitations inherent to the use of patient material make studies of human tissues often difficult and the mouse has therefore emerged as a suitable model organism for studies of respiratory chain diseases. In this review we present an overview of the field and discuss in depth a few examples of animal models reproducing pathology of human disease with primary and secondary respiratory chain involvement.

Rapid advances have been reported in our understanding of molecular defects causing human mitochondrial disease during the past decade. Numerous point mutations and rearrangements of mitochondrial DNA (mtDNA) as well as nuclear mutations have been identified as the primary cause of respiratory chain disease in affected patients¹⁻³. Despite considerable progress in elucidating the underlying gene defects, the molecular pathogenesis events linking the mutated gene to the observed clinical phenotype are poorly understood.

In most cells, the respiratory chain produces the majority of the cellular energy in the form of ATP, through the process of oxidative phosphorylation. The respiratory chain comprises five complexes consisting of a total of ~80 different proteins¹. Regulation of respiratory chain biogenesis is unique in its bipartite dependence on both the nuclear and mitochondrial genomes. The mtDNA encodes 13 subunits of the respiratory chain complexes I, III, IV and V, 2 rRNAs, and 22 tRNAs, whereas nuclear genes encode the remaining respiratory chain subunits as well as all proteins that control replication and transcription of mtDNA.

Why do we need animal models?

Therapeutic intervention to ameliorate or cure respiratory chain diseases is hampered by our poor understanding of key pathogenesis events. It is often assumed that ATP deficiency is the main cause of pathology, but there is as yet no conclusive experimental evidence for this hypothesis. Deficient respiratory chain function could induce other pathogenesis events besides ATP deficiency [e.g. alterations of cellular reduction–oxidation (redox) status, induction of the mitochondrial pathway for apoptosis or increased production of reactive oxygen species (ROS)], which all could be important determinants of pathogenesis. The limited availability of human tissues makes it necessary to perform in-depth studies of molecular pathogenesis events in model organisms. Lower model organisms

such as budding yeast, fruit flies and worms have shed light on basic biochemical defects induced by a variety of nuclear and mtDNA mutations, but the profound differences in physiological processes between humans and these model organisms preclude any conclusions regarding human pathogenesis. The mouse offers several advantages because mice and humans display quite similar gene content, comparable types of internal organs and physiological processes. Furthermore, genetic manipulation of the mouse utilizes rather straightforward, albeit labor-intensive, and well-established protocols. There are several ongoing projects using gene traps in embryonic stem (ES) cells and ethylnitrosourea (ENU) mutagenesis that will increase dramatically the number of available mouse mutants with respiratory chain dysfunction in the future. Despite important limitations inherent to the mouse, such as some differences in metabolic pathways, short life span and less evolved brain functions, the mouse is the most suitable model organism available for studies of pathogenesis in respiratory chain disease. A variety of transgenic mice with impaired mitochondrial function have been generated⁴⁻¹⁵ and can be classified, according to Smeitink *et al.*^{1,2} as summarized in Table 1. In this review, we discuss mouse models reproducing pathology found in humans with single large mtDNA deletion (Δ mtDNA) syndromes directly affecting respiratory chain function and in Friedreich's ataxia (FRDA) patients with secondary respiratory chain involvement.

Mice reproducing pathophysiology of human Δ mtDNA syndromes

Δ mtDNA syndromes are usually spontaneously occurring and display pleiotropic clinical manifestations^{1,16}. There is always heteroplasmy (i.e. a mixture of wild-type and Δ mtDNA); the levels of Δ mtDNA vary dramatically between different tissues of the individual and even between different cells of single organ¹. A certain minimal threshold level of Δ mtDNA is required for the induction of a respiratory chain deficiency¹⁷. Heavy cellular load of Δ mtDNA affects tRNA genes by abolishing mitochondrial protein synthesis, which results in lack of all mtDNA-encoded respiratory chain subunits and severe respiratory chain deficiency. Thus, the distribution of Δ mtDNA participates to determine the organ-specific patterns of respiratory chain

Nils-Göran Larsson*
Dept of Medical Nutrition,
Karolinska Institutet,
NOVUM, Huddinge
Hospital, S-141 86
Huddinge, Sweden.
*e-mail: Nils-Goran.
Larsson@mednut.ki.se

Pierre Rustin
Unité de Recherches sur
les Handicaps Génétiques
de l'Enfant, INSERM
U393, Hôpital des Enfants-
Malades, Paris, France.

Table 1. Animal models for mitochondrial disorders

Knockout mice	Biochemical defect	Phenotype	Refs
Adenosine nucleotide translocator, <i>Ant1</i>	Impaired mitochondrial transport of ATP/ADP, defect in coupled respiration	Cardiac hypertrophy, mitochondrial myopathy	6
Mitochondrial superoxide dismutase, MnSOD or <i>Sod2</i>	Loss of mitochondrial superoxide activity	Dilated cardiomyopathy, lipid accumulation in liver and skeletal muscle, metabolic acidosis	4
Mitochondrial transcription factor A, <i>Tfam</i>			
Germ line <i>Tfam</i> knockout	mtDNA depletion respiratory chain deficiency	Embryonic lethality at embryonic day (E)9.5, mutant phenotype with absence of heart and optic disc, delayed neural development, caspase-dependent apoptosis	7
Heart-specific <i>Tfam</i> knockout	mtDNA depletion, respiratory chain deficiency	Dilated cardiomyopathy, atrioventricular heart conduction blocks, caspase-dependent apoptosis, induction of ROS defenses	8, 9
β -cell-specific <i>Tfam</i> knockout	mtDNA depletion, respiratory chain deficiency	Diabetes, insulin deficiency, impaired stimulus-secretion coupling in β -cells in young knockouts, loss of β -cells in older knockouts	10
Frataxin, <i>Frda</i>			
Germ line <i>Frda</i> knockout	Loss of frataxin	Embryonic lethality at E7–8	11
Tissue-specific <i>Frda</i> knockouts	Loss of frataxin, decreased activity of complex I, II, III and aconitase	Ataxia, loss of proprioception, hypertrophic cardiomyopathy, weight loss	14
Transmitochondrial mice			
Heteroplasmic mice	None	Maternal transmission of a neutral mtDNA polymorphism	5
Chloramphenicol resistance (CAP-R)	Not demonstrated	Ocular abnormalities, myopathy, cardiomyopathy, growth retardation, perinatal lethality	12
Δ mtDNA mice	Respiratory chain deficiency	Growth retardation, kidney failure, mitochondrial myopathy	13
Spontaneous mutants			
Defective nuclear-mitochondrial communication	Not demonstrated	Deafness	15

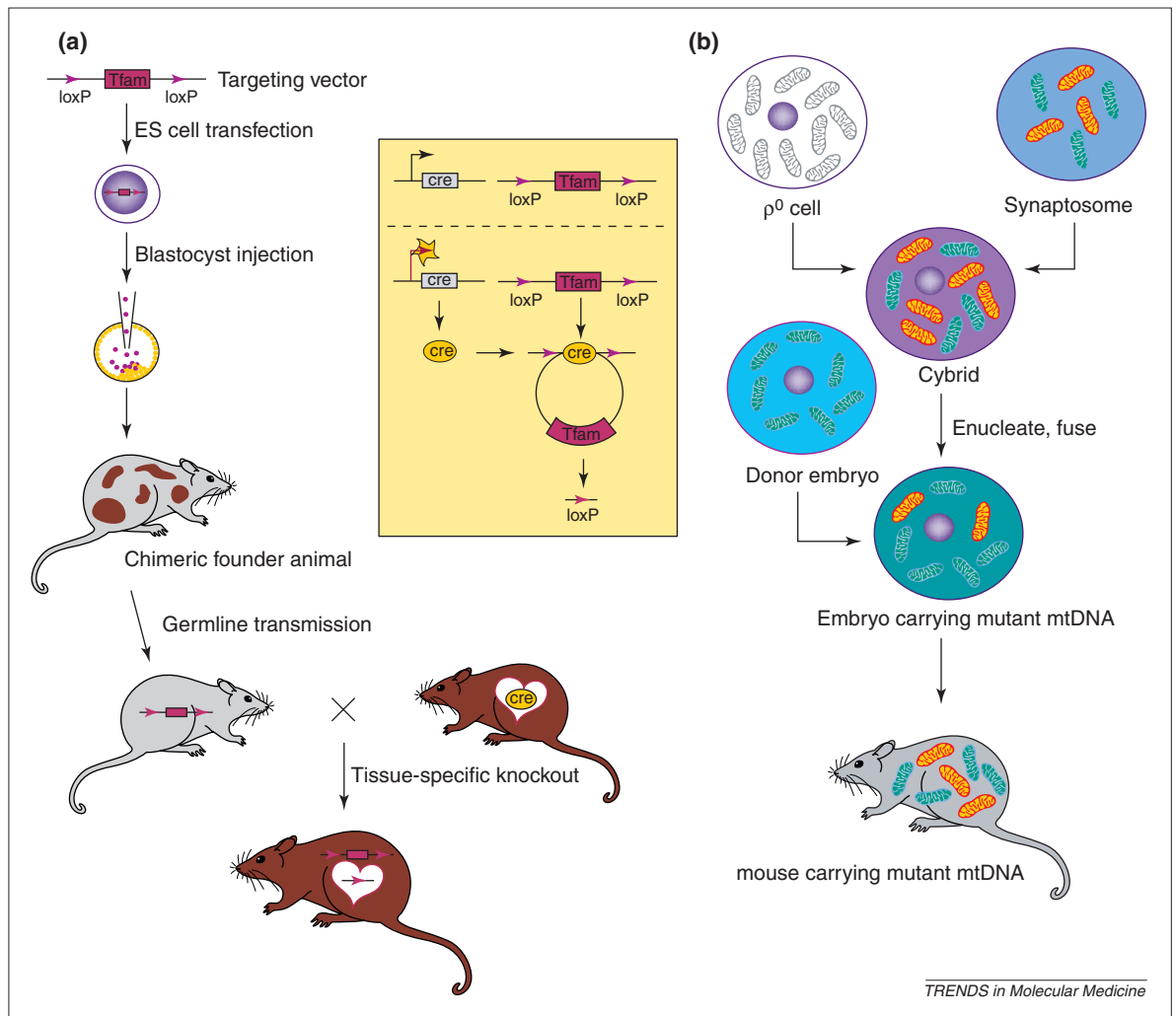
deficiency. It should be noted that there is no evidence that Δ mtDNA has a dominant function in causing disease, but rather, it is the absolute levels of wild-type mtDNA that determines the occurrence of respiratory chain deficiency¹⁷.

In a technical *tour de force*, Hayashi and co-workers introduced exogenous mouse mitochondria with Δ mtDNA into mouse zygotes and demonstrated that the Δ mtDNA could be transmitted through the germ line¹³. Naturally occurring Δ mtDNA was isolated from enucleated somatic cells or synaptic nerve cell endings (synaptosomes), fused with mtDNA-less (p^0) mouse cells and propagated. Transmitochondrial mice were subsequently generated by electrofusion of cytoplasts containing Δ mtDNA to pronuclear-stage mouse embryos containing wild-type mtDNA (Fig. 1). Surprisingly, a partially duplicated mtDNA molecule was identified in the transmitochondrial mice despite being undetectable in the cybrid cells used to create the mice. It remains unclear whether the Δ mtDNA molecules were transmitted intact through the

germ-line and subsequently recombined with the wild-type mtDNA to generate the partially duplicated molecules, or whether partially duplicated mtDNA molecules were transmitted and later resolved via intramolecular rearrangements to yield Δ mtDNA.

The Δ mtDNA mice had heteroplasmic distribution with high levels of rearranged mtDNA in most tissues and respiratory chain deficiency in heart, skeletal muscle and kidney¹³. The Δ mtDNA mice reproduce several important features of early-onset multi-system mitochondrial disease with widespread tissue distribution of high levels of Δ mtDNA in most tissues. Interestingly, several human families have been described with childhood multisystem disorders owing to maternal transmission of rearranged mtDNA. However, it should be noted that adult-onset Δ mtDNA syndromes usually occur spontaneously and are associated with high levels of Δ mtDNA, mainly in postmitotic tissues such as heart, brain and skeletal muscle, and low or undetectable levels of Δ mtDNA in blood. The Δ mtDNA mouse is clearly an important breakthrough and will provide a valuable tool to

Fig. 1. Schematic drawing of the construction of (a) conditional *Tfam* knockout mice and (b) transmitochondrial mice. (a) The conditional *Tfam* knockout mice were generated by initially using a targeting vector to transfect embryonic stem (ES) cells. Clones of ES cells with appropriate recombination at the *Tfam* locus were identified and injected into blastocysts. Chimeric founder animals were mated to obtain germ line transmission of the loxP-flanked *Tfam* allele. Animals were bred to homozygosity for the loxP-flanked *Tfam* allele and mated with *cre*-transgenic animals (two successive crosses) to obtain tissue-specific knockout animals that are homozygous for the loxP-flanked *Tfam* allele and heterozygous for the *cre*-transgene. The inserted box shows how *cre*-recombinase specifically recognizes the loxP-sequences flanking two crucial exons of the *Tfam* gene to generate the knockout allele. (b) The transmitochondrial mice harboring deleted mtDNA (Δ mtDNA) were generated by first isolating synaptosomes containing Δ mtDNA from old mouse brain. The synaptosomes were fused to cells lacking mtDNA (ρ^0 cells) to generate cybrids containing a mixture of Δ mtDNA and normal mtDNA. The cybrids were enucleated and fused to normal mouse embryos to generate transmitochondrial cells carrying Δ mtDNA. The Δ mtDNA was subsequently transmitted through the maternal germ line.



investigate pathogenesis factors such as principles for germ-line transmission of rearranged mtDNA, regulation of tissue distribution of Δ mtDNA and occurrence of *in vivo* complementation between Δ mtDNA and wild-type mtDNA¹⁸. The limitation of this model is that it does not reproduce well the pathology found in adult patients with Δ mtDNA. It might also be less suited to study pathogenesis or evaluate treatments, because levels of Δ mtDNA are likely to vary from animal to animal. The widespread tissue-distribution of Δ mtDNA in all cells of a given tissue will also make it very difficult to study cell-type-specific pathology.

It is possible to generate the same type of respiratory chain deficiency as the one caused by Δ mtDNA by disrupting the nuclear gene for mitochondrial transcription factor A (*Tfam*) (Ref. 7). Loss of *Tfam* causes depletion of mtDNA, loss of mitochondrial transcripts, loss of mtDNA-encoded polypeptides and severe respiratory chain deficiency⁷. It should be noted that the levels of wild-type mtDNA genomes probably determine whether a respiratory chain deficiency will occur in patients with Δ mtDNA^{1,2}. A conditional knockout strategy utilizing the *cre-loxP* recombination system has been developed to disrupt *Tfam* (Fig. 1). This system allows

spatial and temporal control of the knockout and makes it possible to create respiratory chain deficiency in selected cell types of the mouse⁸⁻¹⁰. In Δ mtDNA syndromes, uneven distribution of Δ mtDNA create a mosaic respiratory chain deficiency in affected tissues¹⁹. Similarly, conditional knockout of *Tfam* will create a mosaic respiratory chain deficiency because *cre-loxP*-mediated recombination rarely, if ever, is 100% efficient⁸⁻¹⁰.

Disruption of *Tfam* in cardiomyocytes results in severe heart-specific mosaic respiratory chain deficiency. Interestingly, this mimics very closely a phenotype found in human Δ mtDNA patients (i.e. dilated cardiomyopathy with atrioventricular heart conduction blocks)^{8,9}. Furthermore, respiratory chain-deficient cardiomyocytes display increased caspase-dependent apoptosis and induction of defenses against ROS, suggesting that this could be important in the pathogenesis²⁰. Disruption of *Tfam* in pancreatic β -cells causes impaired insulin release in response to glucose stimulation (impaired stimulus-secretion coupling) in young knockouts. This is followed later by β -cell death in older knockouts¹⁰, which is very similar to the phenotype observed in mitochondrial diabetes patients. These experiments have clarified that the distribution of respiratory

chain deficient cells is a main determinant of the phenotype in Δ mtDNA syndromes. Future use of the conditional *Tfam* knockout mice will include dissection of molecular pathogenesis downstream of the respiratory chain deficiency by genetic and pharmacological approaches. In contrast to the Δ mtDNA mouse the conditional *Tfam* knockout mouse cannot provide any information on the transmission of mtDNA and complementation between different mtDNA molecules. However, the conditional *Tfam* knockout mouse reproduces human pathology better than the Δ mtDNA mouse, and it makes it possible to study effects of disrupted respiratory chain function in individual cell types. This latter point is a clear advantage for studies of pathogenesis in the brain with its mixture of different cell types located in different regions²¹.

Mouse models for Friedreich's ataxia

Friedreich's ataxia is an autosomal recessive neurodegenerative disease characterized by progressive limb and gait ataxia, hypertrophic cardiomyopathy and increased incidence of diabetes mellitus caused by intronic expansions of a triplet nucleotide repeat (GAA) in the nuclear frataxin (*Frda*) gene²². FRDA patients primarily exhibit pathology in the nervous system and the heart. The frataxin protein is localized to mitochondria and reduced activities of respiratory chain complexes I, II and III and of aconitase are found in endomyocardial biopsy specimens of FRDA patients²³. Mostly based on observations in yeast strains deleted for the frataxin homologue gene, it was initially thought that a decrease (or lack) of frataxin primarily resulted in

mitochondrial iron overload. ROS formation triggered by iron overload would in turn specifically target iron-sulfur (FeS) cluster protein, exquisitely sensitive to superoxides. According to this hypothesis, ROS formation would impair the activity of RC complexes I, II, and III and aconitase. However, the homozygous disruption of the mouse frataxin gene causes embryonic lethality but no iron accumulation¹¹.

Tissue-specific disruption of *Frda* in the brain results in ataxia, loss of proprioception, and premature death¹⁴. Disruption of *Frda* in cardiomyocytes resulted in cardiac hypertrophy and degeneration of myocardium¹⁴. There was a progressive reduction in the activity of the FeS cluster-containing respiratory chain complexes I, II, III and aconitase in the heart, later followed by iron accumulation. The clinical and biochemical phenotypes of the tissue-specific knockout mice are very similar to the manifestations observed in human FRDA patients. The results from this mouse model demonstrated that iron accumulation is a late event in the pathogenesis of FRDA. The tissue-specific *Frda* knockouts thus support an alternative hypothesis, suggesting that frataxin might be involved in iron or reactive oxygen species handling, and that lack of frataxin causes decreased synthesis of FeS cluster proteins.

Conclusion

Besides faithfully reproducing major features of mitochondrial diseases, mouse models have already allowed us to reconsider molecular pathogenesis events in several cases. Ongoing drug therapy trials using several of these models might also open the way to new approaches to fight these diseases in humans.

Acknowledgements

We thank Lene Sørensen for designing the figure. N.G.L. is supported by grants from the Swedish Medical Research Council, Funds of Karolinska Institutet, Torsten and Ragnar Söderbergs stiftelse, Human Frontiers Science Program and the Swedish Foundation for Strategic Research. P.R. is supported by the Association Françaises contre les Myopathies (A.F.M.).

References

- Larsson, N. and Clayton, D. (1995) Molecular genetic aspects of human mitochondrial disorders. *Annu. Rev. Genet.* 29, 151–178
- Smeitink, J. *et al.* (2001) The genetics and pathology of oxidative phosphorylation. *Nat. Rev. Genet.* 2, 342–352
- Munnich, A. and Rustin, P. (2001) Clinical spectrum and diagnosis of mitochondrial disorders. *Am. J. Med. Genet.* 106, 4–17
- Li, Y.B. *et al.* (1995) Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat. Genet.* 11, 376–381
- Jenuth, J.P. *et al.* (1996) Random genetic drift in the female germline explains the rapid segregation of mammalian mitochondrial DNA. *Nat. Genet.* 14, 146–151
- Graham, B.H. *et al.* (1997) A mouse model for mitochondrial myopathy and cardiomyopathy resulting from a deficiency in the heart/muscle isoform of the adenine nucleotide translocator. *Nat. Genet.* 16, 226–324
- Larsson, N.G. *et al.* (1998) Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. *Nat. Genet.* 18, 231–236
- Wang, J. *et al.* (1999) Dilated cardiomyopathy and atrioventricular conduction blocks induced by heart-specific inactivation of mitochondrial DNA gene expression. *Nat. Genet.* 21, 133–137
- Li, H. *et al.* (2000) Genetic modification of survival in tissue-specific knockout mice with mitochondrial cardiomyopathy. *Proc. Natl. Acad. Sci. U. S. A.* 97, 3467–3472
- Silva, J.P. *et al.* (2000) Impaired insulin secretion and β -cell loss in tissue-specific knockout mice with mitochondrial diabetes. *Nat. Genet.* 26, 336–340
- Cossee, M. *et al.* (2000) Inactivation of the friedreich ataxia mouse gene leads to early embryonic lethality without iron accumulation. *Hum. Mol. Genet.* 9, 1219–1226
- Sligh, J.E. *et al.* (2000) Maternal germ-line transmission of mutant mtDNAs from embryonic stem cell-derived chimeric mice. *Proc. Natl. Acad. Sci. U. S. A.* 97, 14461–14466
- Inoue, K. *et al.* (2000) Generation of mice with mitochondrial dysfunction by introducing mouse mtDNA carrying a deletion into zygotes. *Nat. Genet.* 26, 176–181
- Puccio, H. *et al.* (2001) Mouse models for Friedreich's ataxia exhibit cardiomyopathy, sensory nerve defect and Fe-S enzyme deficiency followed by intramitochondrial iron deposits. *Nat. Genet.* 27, 181–186
- Johnson, K.R. *et al.* (2001) A nuclear-mitochondrial DNA interaction affecting hearing impairment in mice. *Nat. Genet.* 27, 191–194
- Munnich, A. *et al.* (1996) Clinical presentations and laboratory investigations in respiratory chain deficiency. *Eur. J. Pediatr.* 155, 262–274
- Hayashi, J-I. *et al.* (1991) Introduction of disease-related mitochondrial DNA deletions into HeLa cells lacking mitochondrial DNA results in mitochondrial dysfunction. *Proc. Natl. Acad. Sci. U. S. A.* 88, 10614–10618
- Nakada, K. *et al.* (2001) Inter-mitochondrial complementation: Mitochondria-specific system preventing mice from expression of disease phenotypes by mutant mtDNA. *Nat. Med.* 7, 934–939
- Larsson, N.G. *et al.* (1995) Pathogenetic aspects of the A8344G mutation of mitochondrial DNA associated with MERRF syndrome and multiple symmetric lipomas. *Muscle Nerve* S102–S106
- Wang, J. *et al.* (2001) Increased *in vivo* apoptosis in cells lacking mitochondrial DNA gene expression. *Proc. Natl. Acad. Sci. U. S. A.* 98, 4038–4043
- Sørensen, L. *et al.* (2001) *J. Neurosci.* Late-onset cortico-hippocampal neurodepletion due to catastrophic failure of oxidative phosphorylation in MILON mice. In press
- Campuzano, V. *et al.* (1996) Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science* 271, 1423–1427
- Rotig, A. *et al.* (1997) Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich's ataxia. *Nat. Genet.* 17, 215–217