Disorders of mitochondrial protein synthesis

Howard T. Jacobs*

Institute of Medical Technology and Tampere University Hospital, University of Tampere, Finland and Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK

Received August 7, 2003; Revised and Accepted August 13, 2003

Mitochondrial tRNA gene mutations, including heteroplasmic deletions that eliminate one or more tRNAs, as well as point mutations that may be either hetero- or homoplasmic, are associated with a wide spectrum of human diseases. These range from rare syndromic disorders to cases of commoner conditions such as sensorineural deafness or cardiomyopathy. The disease spectrum of mutations in a given gene, or even a single mutation, may vary, but some patterns are evident, for example the prominence of cardiomyopathy resulting from tRNA^{Ile} defects, or of MERFF-like disease from tRNA^{Lys} defects. Molecular studies of many laboratories have reached a consensus on molecular mechanisms associated with these mutations. Although precise details vary, loss of translational function of the affected tRNA(s) seems to be the final outcome, whether by impaired pre-tRNA processing, half-life, base-modification or aminoacylation. However, a mechanistic understanding of the consequences of this for the assembly and function of the mitochondrial OXPHOS complexes and for the physiological functions of the affected tissues is still a distant prospect. This review presents some views of possible downstream consequences of specific tRNA deficiencies.

A SHORT HISTORY OF MITOCHONDRIAL tRNA AND rRNA GENE DISEASE

Heteroplasmic deletions of the mitochondrial genome were the first class of pathological mtDNA mutations to be discovered (1), yet remain the least understood. The enormous variety in disease severity, even between patients carrying an identical rearrangement, most obviously the well-known 4977 bp common deletion, is an enduring puzzle. The precise pathological features of disease do not bear any obvious relationship to the break-points of mitochondrial genome rearrangements, nor to the set of tRNA (or protein-coding) genes deleted. The only genotype–phenotype relationship that seems to hold up for deletions is that the more severe cases appear to harbour easily detectable amounts of partially duplicated as well as deleted mtDNA molecules carrying the same primary rearrangement (2), these and other molecular forms being interconvertible by homologous recombination. This puzzle should caution against trying to draw firm mechanistic conclusions relating mitochondrial genotype to phenotype, in the case of point mutations affecting mitochondrial tRNA genes.

The field of mitochondrial tRNA gene disease began with the discovery and characterization of the eponymous MERRF and MELAS mutations, A8344G and A3243G, respectively, in the late 1980s and early 1990s (3–5). Since that time, many other tRNA mutations have been reported in association with disease, although linkage with pathology has not been rigorously documented in every case. Some reports of such mutations have turned out to be harmless polymorphisms, or at least to have questionable associations with disease, but the majority can be regarded with a reasonable degree of confidence as pathological, based on criteria such as phylogenetic conservation, reports of the same mutation in multiple families, or functional studies (see www.mitomap.org for a current listing). Taken together, mitochondrial disorders associated with mtDNA deletions or point mutations affecting translation represent a common class of neurological disease in different populations (6,7). A recent estimate of the prevalence of just one point mutation, A1555G, affecting the 12S rRNA, found it at a frequency of approximately 2% amongst patients with prelingual deafness (8). In some populations it may occur at an even higher frequency (9,10) (I. del Castillo, personal communication). In a recent study of British and Italian patients with prelingual deafness, causative point mutations of mtDNA have been found amongst more than 5% of all subjects, making mtDNA the second most prevalent cause of hearing impairments after connexin-26 (H.T Jacobs, J. Walker, T. Käppi, T. Hutchin, P. Gasparini, S. Melchionda, M. Zeviani, unpublished data).

Some curious patterns emerge. Firstly, the disease phenotype bears a striking correlation to the gene affected (Fig. 1). The two clearest examples of this are tRNA^{Ile}59, different mutations in which usually lead to MERRF or a MERRF-like disease, e.g.

*To whom correspondence should be addressed at: Institute of Medical Technology, FIN-33014 University of Tampere, Finland. Tel: +358 32157731; Fax: +358 32157710; E-mail: howy.jacobs@uta.fi

Human Molecular Genetics, Vol. 12, Review Issue 2 © Oxford University Press 2003; all rights reserved
T8356C, G8342A and G8363A, as well as A8344G (11–13), and tRNAIle, which in virtually every report (e.g. A4300G, A4295G and A4269G) seems to be implicated in an isolated cardiomyopathy (14–17), or occasionally a syndromic disorder in which cardiomyopathy is a cardinal feature (e.g. C4320T). Mutations affecting tRNASer(UCN), such as A7445G, 7472insC, T7510C and T7511C also show a striking association with sensorineural deafness (18–21), although some of the more severely affected patients may also exhibit other neurological features, tending towards an overlap with MERFF. Mutations in tRNALeu(UUR), widely regarded as mutation hotspot (22), show a corresponding diversity of phenotypes, although muscle involvement or even pure skeletal myopathy seems to be a common finding. Some A3243G patients have the classic MELAS syndrome (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes), whereas others can show a much milder presentation affecting only one tissue, although this can vary quite widely, including ocular or more generalized skeletal myopathy (23), isolated deafness and/or diabetes (24).

What underlies these associative patterns? This review will consider some possible explanations, based on knowledge of how pathological mutations of mtDNA affect cellular function.

**BIOCHEMICAL CONSEQUENCES OF MITOCHONDRIAL PROTEIN SYNTHESIS DEFECTS**

The primary, biochemical effects of a deficiency of mitochondrial protein synthesis are easy to predict, given that the 13 mitochondrial translation products are all well characterized...
subunits of four of the OXPHOS complexes of the inner mitochondrial membrane (complexes I, III and IV of the respiratory chain, plus ATP synthase, complex V). Cells harbouring pathological tRNA mutations of mtDNA show decreased activities of the OXPHOS complexes, decreased consumption of oxygen, reduced growth rates on substrates such as galactose, which need to be metabolized via mitochondrial oxidation, and enhanced production of lactate (due to greater reliance on glycolysis). In many cases, these effects have been conclusively shown to be conferred by the mutant mtDNA alone, since they are retained or even revealed by cybrid transfer of mutant mitochondria to the control nuclear background of a standard rho-zero tester cell-line (i.e. one that does not contain its own mtDNA).

Just like the overall clinical phenotype, the details of the biochemical defect provoked by a given mutation seem to vary according to which precise mitochondrial tRNA is affected by the mutation. For example, tRNA\textsuperscript{Leu(UUR)} mutations appear to affect primarily complex I (25), whereas tRNA\textsuperscript{Lys} mutations most dramatically affect complex IV (26–28).

ASCERTAINMENT BIAS?

The most prosaic explanation for associations between a given clinical phenotype and a mutation affecting a specific tRNA gene would be that they are not real at all, in other words, are wholly attributable to ascertainment bias. For example, following initial reports of A7445G, investigators studying patients with isolated sensorineural deafness showing apparent maternal inheritance have focused their attention on tRNA\textsuperscript{Ser(UCN)}, and thus found a set of novel, causative mutations in that gene, as well as further cases of the original mutation. However, they may not have looked systematically in other tRNA genes, hence did not discover examples of A3243G or A8344G patients with isolated deafness, nor did they report novel mutations in the 19 other tRNA genes that may equally well be associated with the disorder.

As time goes by, this explanation begins to look a little threadbare. Nowadays, most patients studied for mtDNA involvement in disease are analysed either by complete mtDNA sequencing or by whole-genome DHPLC in combination with follow-up sequencing. Enough patients have been studied that it is now highly unlikely that A8344G has simply been missed in those with isolated deafness, or that A7445G has been missed in patients with MERRF and so on. The one case where ascertainment bias may still have influenced conclusions concerns tRNA\textsuperscript{Leu(UUR)} mutations, and in particular A3243G. The diversity of symptoms reported may partly reflect the relatively high frequency of A3243G patients, combined with the fact that screening for the mutation has been undertaken by clinicians focused on different aspects of an undoubtedly complex phenotype. Therefore, some studies have found the mutation to be predominantly associated with maternally inherited diabetes and/or deafness plus short stature, whereas others have found it predominantly in patients with muscle disease, running to full-blown MELAS. One major, unresolved issue concerns the degree to which clinical A3243G phenotypes run ‘true’ in maternal pedigrees. If they do so, this must indicate the importance of other polymorphisms in the mtDNA as genetic modifiers. However, the question remains open. The modifier issue will be revisited below.

Given the prevalence and clinical importance of this mutation, an unbiased, population-based analysis is surely needed, in order to determine the true spectrum of phenotypes associated with A3243G. This would also reveal the degree to which they are influenced by heteroplasmia level and other genetic factors, including mtDNA haplotype.
HETEROPLASMY AND SEGREGATION

Some of the phenotypic variability, especially that seen between patients with the same mtDNA mutation, is undoubtedly attributable to differences in the level and ‘evolution’ of heteroplasmic within an individual, although this obviously cannot be invoked for homoplasmic mutations. Cybrid studies have revealed that nuclear background can influence the direction and rate of mitotic segregation (29–31). If applicable in vivo, a critical threshold for expression of a molecular phenotype might or might not be reached in a given tissue during life. It is unclear whether this phenomenon is primarily attributable to genetic differences or to developmental programming. Recent studies in a mouse model of heteroplasmia certainly indicate that segregation of different mtDNA genotypes can differ systematically between tissues (32), and that this effect is governed by nuclear genes (33), although specific genes responsible have not yet been identified.

MOLECULAR MECHANISMS OF PATHOGENESIS

Much progress has been made in recent years in understanding the molecular effects of specific, pathological mutations in mitochondrial tRNAs, again based mainly on the results of rho-zero cybrid studies. These point towards a common theme, i.e. that they are all loss-of-function mutations, although the exact mechanistic details vary. Although this deepens the mystery of why they can result in such varied clinical phenotypes, it does at least downstage more complicated hypotheses based on gain-of-function, or long-distance effects on to developmental programming.

Table 1. Usage of specific codons in human mitochondrial genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number of amino acids</th>
<th>Number of UUR codons</th>
<th>Number of UUG codons</th>
<th>UUR codon density (%)</th>
<th>Number of UCN codons</th>
<th>Number of UCN codon pairs</th>
<th>UCN codon density (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND1</td>
<td>318</td>
<td>6</td>
<td>1</td>
<td>1.9</td>
<td>18</td>
<td>0</td>
<td>5.7</td>
</tr>
<tr>
<td>ND2</td>
<td>347</td>
<td>9</td>
<td>1</td>
<td>2.6</td>
<td>23</td>
<td>2</td>
<td>6.6</td>
</tr>
<tr>
<td>COI</td>
<td>513</td>
<td>7</td>
<td>0</td>
<td>1.4</td>
<td>28</td>
<td>2</td>
<td>5.5</td>
</tr>
<tr>
<td>COII</td>
<td>227</td>
<td>5</td>
<td>1</td>
<td>2.2</td>
<td>9</td>
<td>0</td>
<td>4.0</td>
</tr>
<tr>
<td>A8</td>
<td>68</td>
<td>2</td>
<td>1</td>
<td>2.9</td>
<td>4</td>
<td>0</td>
<td>5.9</td>
</tr>
<tr>
<td>A6</td>
<td>226</td>
<td>5</td>
<td>1</td>
<td>2.2</td>
<td>10</td>
<td>0</td>
<td>4.4</td>
</tr>
<tr>
<td>COIII</td>
<td>360</td>
<td>9</td>
<td>2</td>
<td>1.2</td>
<td>16</td>
<td>0</td>
<td>6.2</td>
</tr>
<tr>
<td>ND3</td>
<td>115</td>
<td>10</td>
<td>0</td>
<td>8.8</td>
<td>5</td>
<td>1</td>
<td>4.4</td>
</tr>
<tr>
<td>ND4L</td>
<td>98</td>
<td>1</td>
<td>0</td>
<td>1.0</td>
<td>8</td>
<td>0</td>
<td>8.2</td>
</tr>
<tr>
<td>ND4</td>
<td>459</td>
<td>9</td>
<td>1</td>
<td>2.0</td>
<td>33</td>
<td>3</td>
<td>7.2</td>
</tr>
<tr>
<td>ND5</td>
<td>603</td>
<td>9</td>
<td>2</td>
<td>1.5</td>
<td>36</td>
<td>0</td>
<td>5.9</td>
</tr>
<tr>
<td>ND6</td>
<td>174</td>
<td>14</td>
<td>6</td>
<td>8.1</td>
<td>5</td>
<td>0</td>
<td>2.9</td>
</tr>
<tr>
<td>Cyt b</td>
<td>378</td>
<td>9</td>
<td>2</td>
<td>2.4</td>
<td>25</td>
<td>1</td>
<td>6.6</td>
</tr>
</tbody>
</table>

hypermodification of the wobble-base U of the anticodon of the mutant tRNA_{Leu(UUR)} is lost, or at least greatly diminished (37) (T. Yasukawa and K. Watanabe, personal communication). Other base-modifications can also be affected (40). The mutant tRNA binds poorly to mitoribosomes (35), and is thus believed to be dysfunctional for translation. The extent of the protein synthesis defect appears to vary between cybrid clones (41), suggesting that compensation mechanisms (34) might operate to mitigate some of these effects.

The dramatic effects on both mitochondrial translation and on respiratory function of very high levels of A3243G mutant mtDNA in A549 lung carcinoma cybrids can be compensated by modest heteroplasm for a suppressor mutation, G12300A, in tRNA_{Leu(UUR)}\(^{\text{Ser(UCN)}}\). This creates a novel tRNA with the appropriate anticodon to substitute for the one affected by the A3243G mutation (39). The suppressor tRNA is aminoacylated (34), and at least a proportion of it carries the same taurine hypermodification (42) at the wobble-U as wild-type tRNA_{Leu(UUR)}\(^{\text{Leu(UUR)}}\) (T. Suzuki, personal communication), rendering it functional for translation. These findings indicate that the major effects of the A3243G mutation on bioenergy metabolism, at least in this cell background, are due to a loss of UUR decoding capacity. The mRNA most affected by both the A3243G and T3271C mutations has been construed to be ND6 (43,44), with a possible effect also on ND3. This may reflect an unusual codon usage, in particular, a high content of UUR and especially UUG codons (Table 1). Indeed, a point mutation in the structural gene for ND6 has recently been reported in a patient with a MELAS-like phenotype (45). Complex I appears to be the primary biochemical target of the A3243G mutation (25,43).

Similarly, the A8344G MERRF mutation has been reported to impair the aminoacylation of tRNA\(^{\text{Lys}}\) (26) although this may not apply to the same degree in vivo, at least in muscle (36). Effects on steady-state level of the tRNA are modest, but hypermodification of the wobble-base U is again lost (46), along with translational activity in vitro (47). The effects on translation of different mitochondrial mRNAs correlates well with their content of lysine codons (26), consistent with the idea that the major molecular consequence of the mutation is a functional deficiency of decoding capacity for the specific (AAR) codon group.
Mutations affecting both tRNA\textsuperscript{Ile} and tRNA\textsuperscript{Ser(UCN)} seem to exert their primary effects on tRNA biosynthesis, leading to a drop in the steady-state levels of the affected tRNAs (48,49). A7445G is probably the clearest case, since the mutation lies outside of the tRNA itself and does not affect its primary structure. Aminoacylation should be normal, unless it is functionally linked to tRNA maturation in some way. In vitro pre-tRNA processing assays indicate that the mutation completely blocks the pathway of endonucleolytic 3′ processing (50). Since the tRNA is still present in vivo at 30–35% of its wild-type level, it has been suggested that one or more alternate pathways of 3′ end maturation must operate for tRNA\textsuperscript{Ser(UCN)}, and perhaps for all mitochondrial tRNAs, based upon a trimming exonuclease such as in eubacteria (50). The mutation has also been suggested to impair the light-strand RNA processing pathway(s) required for the production of ND6 mRNA (49), although the details of the latter have not been elucidated.

The 7472insC mutation also reduces the steady-state level of tRNA\textsuperscript{Ser(UCN)} by ~70% at the level of tRNA biosynthesis (51,52). Analysis of tRNA termini in vivo and pre-tRNA processing in vitro indicate that the mutation reduces the efficiency of both 5′ and 3′ processing (M. Toompuu and H. T. Jacobs, unpublished data). In this case, the aminoacylation of tRNA\textsuperscript{Ser(UCN)} by leucine is also impaired, albeit to a modest degree (~25%), contributing to the functional deficiency (52). Similarly, four different mutations in tRNA\textsuperscript{Ile} affect its biosynthesis by impairing the efficiency of its 3′ end maturation (53). However, at least one of them, A4269G, also renders tRNA\textsuperscript{Ile} unstable both in vivo and in vitro (54), which is partly attributable to a reduced binding affinity for EF-Tu (T. Suzuki, personal communication).

Studies of protein synthesis defects in cells carrying tRNA\textsuperscript{Ser(UCN)} mutations do not show a systematic, differential effect on the various translation products (48,49,51). This may simply reflect the fact that UCN codons are common in all of the mitochondrial protein-coding genes (Table 1): even successive UCN codon pairs are found widely. Based on UCN codon number, ND5, ND4, COI and cytochrome b would be predicted to be the most affected by tRNA\textsuperscript{Ser(UCN)} ‘starvation’, although ND4L might also be sharply affected, having the highest density of UCN codons.

Regarding deletions, cybrid studies confirm that they also lead to a loss of mitochondrial protein synthesis above a certain heteroplasmic threshold, typically around 50–60% mutant mtDNA (55). It is presumed that this is the result of an ‘imbalance’ of the different mtDNA-encoded tRNAs, i.e. a combined deficiency of those tRNAs within the deleted region, possibly compounded by a relative excess of others. This would be equally true of partial duplications of mtDNA as of deletions. Frameshifting in response to tRNA imbalances is a recognized phenomenon in bacteria (56). Although the above findings on tRNA\textsuperscript{Ser(UCN)} do seem to support the idea that rather modest changes in tRNA abundance can impair mitochondrial function, the loss of protein synthesis consequent upon a 50% drop in the level of a rather small (and variable) subset of tRNAs is still somewhat surprising, and suggests that other explanations should still be considered to account for the pathogenicity of deletions. One long-standing idea, that has not been explored in detail, is that fusion peptides or RNAs encoded across rearrangement junctions might have toxic effects on mitochondrial protein synthesis and/or OXPHOS function, by analogy with CMS in plants (57).

**PHYSIOLOGICAL CONSEQUENCES OF tRNA DEFICIENCIES**

If all, or at least the vast majority of pathological tRNA mutations impair mitochondrial protein synthesis by provoking a functional deficit of a specific tRNA, the question still remains as to why they result in such varied clinical phenotypes. This fundamental question remains unanswered. In this last section four radically different hypotheses to account for this phenotypic variability are summarized and evaluated, and suggestions put forward as to how they might be tested.

Firstly, as already indicated, the lack of specific tRNAs impairs the synthesis of the mitochondrial translation products to different extents, according to their content of specific codons. This is most clearly established for tRNA\textsuperscript{Lys} mutations, but is probably general, although it may not be straightforward, e.g. some codons of a group might be more affected than others, or specific runs of codons might be problematic in cases where isolated codons are not. The spectrum of translation products affected may thus be subtly different in each case, leading to variable patterns of reduced activity of the four OXPHOS complexes which are dependent upon mitochondrial translation products. Since it is already well established that the contribution of the different OXPHOS complexes to respiratory control in different tissues can vary (58), the lack of specific subunits may have a dramatic effect in one tissue, but none or almost none in others. One obvious variable is the degree to which different tissues, or even the same tissue under different physiological conditions, may depend upon substrates metabolized via the reduction of NAD\textsuperscript{+}, i.e. supplying electrons to the respiratory chain via complex I, versus succinate (or other substrates that are metabolized to succinate), and thus supply electrons to the respiratory chain via complex II (which lacks mtDNA-encoded subunits). Although there are only four enzyme complexes supplied by mitochondrial translation products, combinatorial effects could generate many more than four possible patterns of tissue involvement. This hypothesis can, at least in principle, be addressed by determining the tissue patterns of respiratory control, and testing whether they reflect measurable effects of different tRNA deficiencies in a control cell background. It may also be helpful to extend the range of cellular backgrounds used for rho-zero cybrid studies, including those most relevant to the clinical phenotypes observed.

A second hypothesis is based on the concept of differential tissue-toxicity of the specific, premature translation products that are synthesized, when translation is arrested at specific codons. Assuming that mitoribosomes behave like those of other systems, failure to find the correct tRNA should lead to programmed frameshifting and release of the ribosome and truncated/frameshifted polypeptide, once a stop codon is encountered. Many of the peptides thus generated might be expected to exert toxic effects by interfering with assembly pathways, since they will contain domains from *bona fide*
subunits of the OXPHOS complexes. However, they may also have diverse toxic effects based upon interactions with other mitochondrial or even non-mitochondrial components. One attractive feature of this hypothesis is that it can account for the fact that clinical phenotypes can manifest, and mitochondrial OXPHOS functions are often impaired, under conditions where it is not possible to detect any quantitative abnormality of mitochondrial protein synthesis, e.g. in cybrid cells containing 80% A3243G mtDNA (which nevertheless exhibit a complex I defect), or those harbouring the 7472insC mutation (43,51).

However, it is not easy to design a strategy to test this hypothesis experimentally, since by definition these toxic peptides (or equally well, aberrant RNAs) might be present only at very low abundance. However, where their sequences can be predicted, e.g. in the case of the well-documented tRNA\textsubscript{Lys} deficiency caused by A8344G, it may be possible to design (inducible) transgenes to express them and target them to mitochondria, so see if their effects on cellular function phenocopy precisely those of tRNA mutations.

A third possible explanation for the tissue-specificity of mitochondrial tRNA mutations would lie in differential tissue-expression of components of the apparatus of mitochondrial gene expression, such as aminoacyl-tRNA synthetases, RNA endonucleases or base-modifying enzymes. Thus, a specific defect in any of these processes that is associated with a given tRNA mutation may only be expressed phenotypically in a tissue where the relevant activity is already limited. For example, the fact that a mutant pre-tRNA may be a poor substrate for an RNA processing enzyme might not matter in a tissue where the relevant enzyme is highly expressed, but could be critical if the enzyme is present only at a very low abundance. A variant of this idea is that there are, in some critical cases, tissue-specific isozymes (e.g. created by alternative splicing) whose ability to interact with mutant tRNA substrates may vary. Although such ideas are attractive, they are supported by virtually no solid evidence at this time.

Even though there are obvious candidate genes for such involvement, many of them remain uncharacterized in mammalian cells. Therefore, the best approach to test this idea is probably a transcriptomic or proteomic one. However, since the full mitochondrial proteome has not yet been determined in any cell-type, this approach remains in its infancy (59). Moreover, transcriptomic approaches alone cannot distinguish mRNAs for mitochondrially destined polypeptides. Although functional genomics may eventually reveal if this idea can explain the tissue-specificity of mitochondrial disease, it will require a vast and painstaking data-gathering exercise.

Finally, differential physiology downstream of OXPHOS could also play some role, though this need not be exclusive of any of the above hypotheses. Some cells are susceptible to undergo apoptosis in response to OXPHOS dysfunction, whereas others are relatively resistant to apoptotic stimuli emanating from mitochondria or can even be protected from apoptosis by virtue of the absence of a functioning respiratory chain (60). Some cells also produce large amounts of specific, secondary metabolites in response to OXPHOS deficiency, the most obvious example being lactate excreted by skeletal muscle. Such metabolites may have effects on tissues or organs at distant sites, thus making it difficult to trace the chain of cause and effect that leads from a mitochondrial tRNA mutation to a specific pathological symptom. The contribution of such effects to disease cannot easily be ascertained without a whole organism model. In the case of mitochondrial tRNA mutations, such disease models remain only a distant prospect.

MODIFIER LOCI

A yeast model of the 12S rRNA A1555G mutation, which affects the ribosomal accuracy centre and causes aminoglycoside ototoxicity in humans, has revealed modifier loci (61,62) which can influence the phenotypic outcome in ways highly suggestive of a relevance to the human mutation (which can also result in hearing loss in individuals not known to have been treated with aminoglycosides). These modifier genes (in yeast) encode tRNA base-modifying enzymes that are believed to influence decoding specificities. Their human homologues are strong candidates to influence the expression of the A1555G mutation (63). In cultured cell models, the expression of the associated biochemical phenotype does seem to be under nuclear genetic control (64), and a putative modifier has recently been mapped to human chromosome 8 (65).

Such modifiers may also be highly relevant to tRNA point mutation disorders in humans. Based on the foregoing, differential activity or regulation of any of a large number of proteins could influence the clinical phenotypes resulting from pathological tRNA mutations, and underlie the variability seen between individuals within the same family, even carrying the same mutation and at the same level of heteroplasmy. The list of candidates includes RNA processing endonucleases, base-modifying enzymes, aminoacyl-tRNA synthetases, translational elongation and release factors, ribosomal and other proteins involved in mitochondrion assembly including MRPS12 (66,67), mRNA-specific specific translation factors that might be the analogues of those characterized in yeast (68), and proteases involved in the turnover of abnormal peptides in mitochondria (69).

The possible role of other polymorphisms in mtDNA as modifiers has already been touched upon. The elucidation of the molecular mechanisms of action of the main disease mutations leads to some interesting possibilities. Silent polymorphisms that might create (or remove) ‘difficult’ codons or runs of such codons could have a significant impact on overall translational efficiency. Even rather distant polymorphisms that affect the folding of the primary mtDNA transcript, hence its availability for processing via different pathways, might interact with pathological mutations such as A7445G (49), 7472insC or A4269G, that act mainly at the level of RNA processing. One intriguing observation concerns the polymorphism T16189C in the non-coding region (D-loop), which has been found at a significantly elevated level in several groups of patients with common heterogeneous disorders, such as type II diabetes or cardiomyopathy (70,71). The mutation creates an oligo C tract of sufficient length to generate length heteroplasmy within individuals carrying it, presumably because of slippage-mispairing during DNA replication. Extended homopolymeric tracts may affect the efficiency of mtDNA replication, transcription or RNA processing, favouring a pathological outcome.
Functional genomics approaches will undoubtedly be of great value in identifying nuclear genetic modifiers, but some pitfalls need to be addressed. Probably the most serious of these is the fact that almost all of the commonly used cybrid cell backgrounds used as rho-zero testers are karyotypically unstable. This has been clearly demonstrated in regard to heteroplasmic segregation, where trisomy for chromosome 9 was associated with one episode of transient instability of heteroplasmic segregation, where trisomy for chromosome 9

rho_0 backgrounds used as

preference for the financial support of my research.

European Union (GENDEAF, MitAGE and MitEURO projects) for the financial support of my research.

ACKNOWLEDGEMENTS

I wish to thank the members of my laboratory and many other colleagues whose work and ideas I quote here for much valuable discussion. I am indebted to the Academy of Finland, Tampere University Hospital Medical Research Fund, and the European Union (GENDEAF, MitAGE and MitEURO projects) for the financial support of my research.

REFERENCES


