Translation matters: protein synthesis defects in inherited disease

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Abstract | The list of genetic diseases caused by mutations that affect mRNA translation is rapidly growing. Although protein synthesis is a fundamental process in all cells, the disease phenotypes show a surprising degree of heterogeneity. Studies of some of these diseases have provided intriguing new insights into the functions of proteins involved in the process of translation; for example, evidence suggests that several have other functions in addition to their roles in translation. Given the numerous proteins involved in mRNA translation, it is likely that further inherited diseases will turn out to be caused by mutations in genes that are involved in this complex process.

A-site
One of three binding sites for tRNAs on the ribosome, the acceptor site (A-site) is the one into which amino-acyl-tRNAs are recruited to decode the next codon of the mRNA.

5′ cap structure
A modified nucleotide attached to the 5′ end of precursor mRNA within the nucleus. It has key roles in the function of the mRNA, for example, in its translation.

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An extensive and growing catalogue of human diseases are caused by alterations in a diverse range of components that function in the translation of mRNA. Some of these diseases arise from mutations in specific mRNAs that affect the sophisticated mechanisms that govern their translation, leading to changes in expression of the proteins that they encode. Others involve mutations that affect essential components of the translation machinery, such as protein components of the ribosome, tRNAs and amino-acyl-tRNA synthetases, which attach amino acids to their cognate tRNAs. Further disorders arise from alterations in the translation factors that mediate the process of mRNA translation, or in the cellular components that control them. All of the diseases of this type that have been identified so far are 'rare' — defined as affecting <200,000 people in the US population (Office of Rare Disease) or affecting <1 person in 2,000 (Orphanet) — and, for some of them, only a few or even single cases have been reported.

Because of their fundamental role in the process of translation, mutations that affect key components of the translation machinery or translation factors might be expected to have similar phenotypic effects that involve a broad range of tissues. However, it has become clear that mutations in such genes lead to a wide variety of diseases, and that the range of tissues and organs that are affected in each disease varies. Studies of the cellular and molecular bases of these diseases are leading to new insights into the functions of the affected proteins, and into the translation process in general. Here we provide a synthesis of these studies and the insights that they have generated. We begin by providing a brief overview of the process of translation. We then focus on diseases that result from defects in protein synthesis in the cytosol. Finally, we discuss diseases that involve defects in mitochondrial translation.

Translation and its regulation

Protein synthesis is mediated by ribosomes, which act as RNA-based enzymes (ribozymes) to facilitate peptide-bond formation. Ribosomes comprise two subunits (in the cytosol, these are the 'large' subunit (60S in mammals) and the 'small' subunit (40S in mammals)). Amino acids are brought to the ribosome as conjugates with the appropriate tRNA, which possesses an anticodon that is complementary to a codon for that amino acid. These amino-acyl-tRNAs are first recruited into the acceptor site, or A-site, on the ribosome. Other tRNA-binding sites on the ribosome are the P-site, which binds peptidyl-tRNA (tRNA that is bound to the nascent peptide), and the E-site, which binds a free tRNA before it exits the ribosome. The process of translation itself requires several other proteins that are extrinsic to the ribosome, which are known as translation factors. mRNA translation is conventionally divided into three stages (FIG. 1). During initiation, the ribosome and the tRNA for the first amino-acid residue, methionine, are positioned at the first (or 'start') codon (AUG) of the mRNA. During elongation, the polypeptide chain is assembled step-by-step as dictated by the ORF of the mRNA. When the ribosome encounters a 'stop' codon, termination occurs, resulting in release of the completed polypeptide and the ribosomal subunits.

Accuracy is essential at all of these stages, perhaps particularly in locating the correct start codon. For most mRNAs, this involves a process whereby the 40S ribosomal subunit is recruited to the mRNA's 5′ cap structure.

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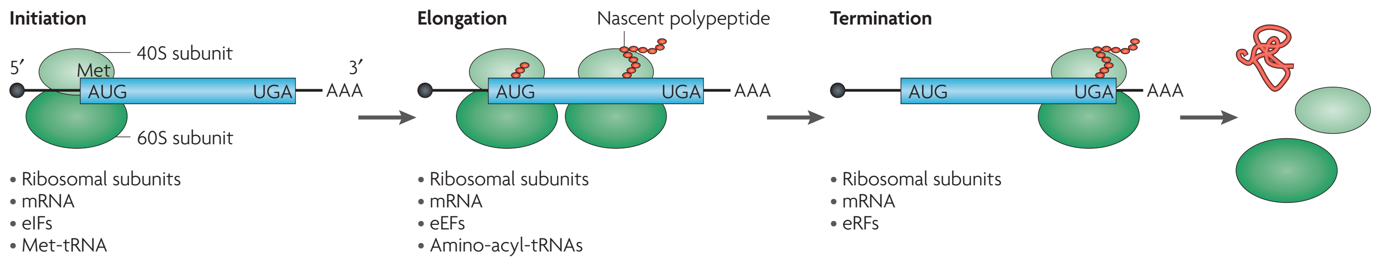

Figure 1 | **Protein synthesis.** The three stages of mRNA translation in eukaryotes. The ORF is indicated by a blue rectangle with the AUG start codon and a stop codon (UGA), untranslated regions are shown as black lines and the 5′ cap structure as a black circle. Note that mitochondrial mRNAs are uncapped. AAA indicates the poly(A)-tail. The ribosomal subunits (40S and 60S) are indicated in green and the growing (or released) polypeptide in red. Essential components of the translational machinery are indicated for each step. How recycling of ribosomes occurs in eukaryotes remains obscure[101], and no known diseases have been linked to this step of the translation process. eEFs, eukaryotic elongation factors; eIFs, eukaryotic initiation factors; eRFs, eukaryotic release factors; Met, methionine.

Together with the methionyl-tRNA and certain translation initiation factors, the 40S subunit then scans along the 5′ untranslated region (5′ UTR or 'leader') of the mRNA to find the start codon (FIG. 2). Some mRNAs contain upstream ORFs (uORFs), which usually impair the translation of the main ORF, because ribosomes that assemble at an upstream AUG generally dissociate from the mRNA after translating the uORF, and therefore fail to reach the genuine start codon. In some cases, features within the 5′ UTR allow the 40S subunit to enter downstream of the 5′ cap, close to or at the start codon, largely obviating the need for scanning. Such 'internal ribosome entry sites' (IRESs)[1] were first found in certain viral RNAs, but also occur in a subset of human cellular messages. Both 5′ and 3′ UTRs can contain other elements that modulate the efficiency of mRNA translation. Furthermore, sequences in the 3′ UTR can affect translation through interaction of the poly-A-binding protein with eukaryotic initiation factor (eIF) 4F (REF. 2), leading to circularization of the mRNA or binding of proteins that inhibit this interaction[3].

Diseases that affect cytosolic translation

Diseases that result from altered translation of specific mRNAs. Most known disease-causing mutations in mRNAs occur within the ORF and thus affect the sequence or reading frame of the encoded protein. However, several diseases are caused by mutations in 5′ UTRs, which lead to alterations in the translation of those mRNAs and, therefore, the expression of the encoded protein (FIG. 3) (for reviews, see REFS 4,5,6). In general, the pathophysiology of the related diseases can be explained by the function of the encoded protein, as described in the examples below.

Excessive production of the iron-storage protein ferritin, leading to hereditary hyperferritinaemia or cataract syndrome, is caused by mutations in a stem–loop in the 5′ UTR of ferritin mRNA. These mutations abrogate a mechanism that normally suppresses translation of the mRNA when iron levels are low. Controlling the expression of proteins at the level of translation, rather than transcription, has the important advantage of allowing a much faster response. In the case of ferritin, it is essential that the cell sequesters free iron to avoid oxidative damage, so it is important to adjust ferritin expression rapidly depending on the availability of iron. It is likely that translational control mechanisms have evolved to allow the rapid control of the expression of other proteins involved in responses to stress or other environmental changes. Translational regulation is likely to be exerted through regions outside the ORF, and such sequences might be missed in searches for disease-causing mutations, which generally focus on coding regions.

Increased translation also occurs in hereditary thrombocythaemia, a disease in which loss of an upstream start codon in the 5′ UTR of the thrombopoietin mRNA causes excessive production of thrombopoietin and, thus, platelets[4]. In another example of increased translation that involves a different mechanism, a single C>T mutation in the IRES of the *MYC* mRNA occurs in a high proportion of multiple myeloma patients[7]. This mutation increases the ability of the mRNA to bind a number of proteins; in particular, heterogeneous nuclear ribonucleoprotein K (HNRPK). This results in enhanced translational efficiency[8–10] (FIG. 3).

The loss of IRES-mediated translation seems to be involved in the aetiology of X-linked Charcot–Marie–Tooth disease (CMT), a peripheral neuropathy, through mutations in a nervous-system-specific variant of the connexin 32 (also known as GJB1) mRNA[11]. A similar mechanism has also been suggested to have a role in X-linked dyskeratosis congenita[12], a rare and often fatal condition caused by mutations in *DKC1*. This gene encodes dyskerin, which combines with other proteins to form a pseudouridine synthetase (which, in this case, modifies ribosomal RNA (rRNA)) or a telomerase complex[13]. The predominant consequence of *DKC1* mutations in human cells seems to be telomere erosion during proliferation rather than reduced rRNA modification or ribosome synthesis[14]. However, deficiency of dyskerin in mice has been shown to cause defective translation of IRES-dependent mRNAs, including those encoding cell-cycle regulators (such as $p27^{Kip1}$) and negative regulators of apoptosis (Bcl-XL and X-linked inhibitor of apoptosis (XIAP)). Importantly, cells from patients with X-linked dyskeratosis congenita also show defective translation of the XIAP and $p27^{Kip1}$ mRNAs[12]. This might explain

Methionyl-tRNA
A tRNA to which a methionine residue is attached: a specific 'initiator' methionyl-tRNA recognizes the AUG start codon and provides the first amino acid of the new polypeptide.

Eukaryotic initiation factors
Proteins involved in the first stage of mRNA translation.

Telomerase
An enzyme that modifies the ends of eukaryotic chromosomes.

Telomere erosion
A decrease in the number of repeat sequences at the ends of chromosomes.

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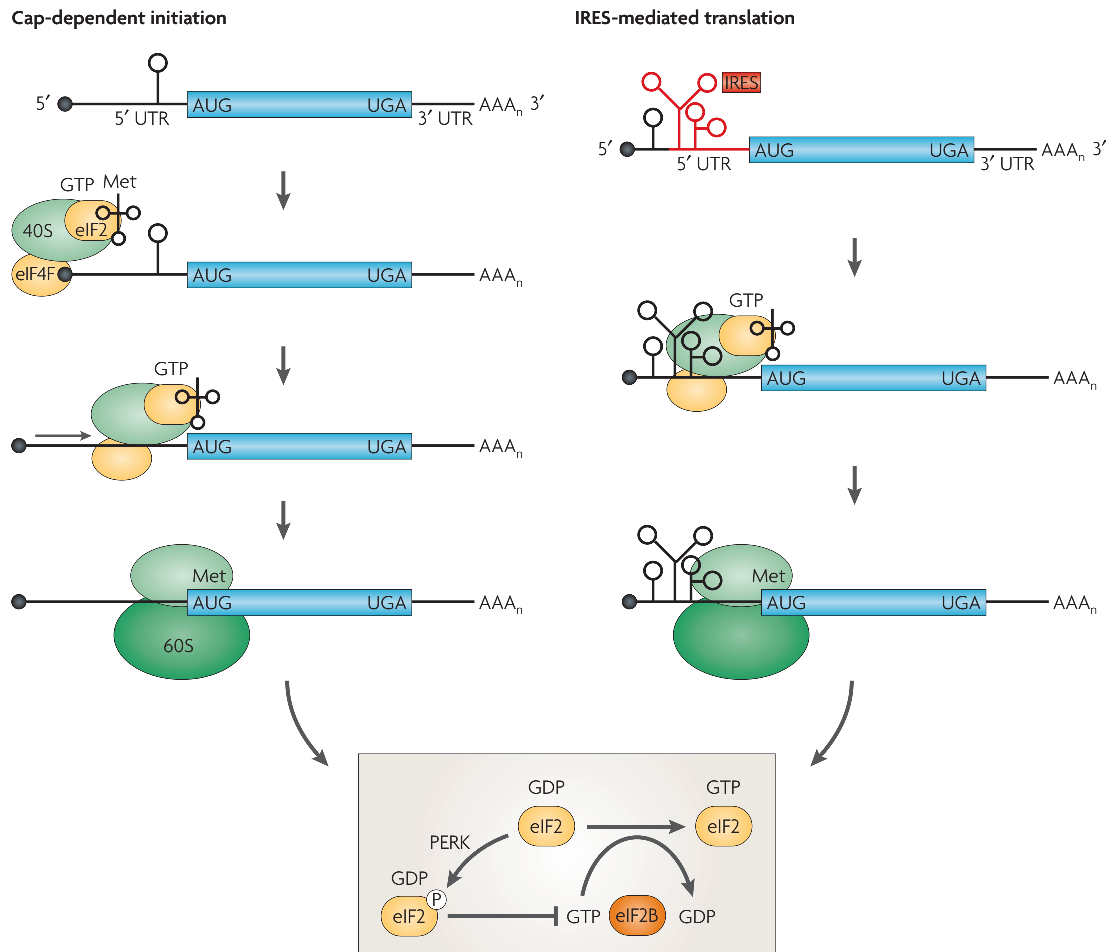

Figure 2 | **Translation initiation.** In cap-dependent translation, the small ribosomal subunit (40S) is brought to the 5′-terminal cap structure (represented as a black circle) through the interaction of eukaryotic initiation factor (eIF) 3 (not shown) on the ribosome with the cap-binding complex eIF4F. The ribosome is thought to migrate ('scan') along the 5′ untranslated region (5′ UTR, shown as a black line), removing any inhibitory stem–loops, until it encounters the first start codon (AUG) in a favourable 'Kozak' context. The codon–anticodon interaction and the initiation factors eIF2, eIF1 and eIF1A all have a role in start codon selection. eIF1/1A and several other initiation factors are omitted from the figure for clarity. During internal ribosome entry site (IRES)-mediated initiation, the ribosomal complex is not assembled at the 5′ end of the mRNA, but at an internal site (indicated in red) formed by secondary structures in the 5′ UTR. After recognition of the start codon of the ORF (represented as a blue rectangle), a functional ribosome is formed through binding of the large ribosomal subunit (60S). During this process, eIF2-bound GTP is hydrolysed to GDP and initiation factors are released. 'UGA' indicates one of the three possible stop codons of the ORF, UTRs are indicated by black line, and the poly(A)-tail at the 3′ end of the mRNA by 'AAAn'. One of the rate-limiting steps in the initiation process is the formation of the ternary complex Met-tRNAi–eIF2–GTP. To enable eIF2 to bind initiator Met-tRNA and participate in the next round of translation, the GDP has to be replaced by GTP. As Met-tRNAi is normally present in abundance, the eIF2B-catalysed GDP–GTP exchange is thought to be rate limiting (bottom). Phosphorylation of eIF2 on its α-subunit by eIF2-specific kinases (for example, pancreatic endoplasmic reticulum-resident kinase (PERK)) inhibits eIF2B.

both the predisposition to cancer and the bone marrow failure that are associated with this disease, the latter being a result of increased death of haematopoietic progenitor and stem cells[15]. Translation defects can contribute to the more severe symptoms of X-linked dyskeratosis congenita in comparison with those of dominant inherited forms, in which only the telomerase complex is affected[16]. It is unclear how IRESs recruit ribosomes or why altered rRNA pseudouridylation affects IRES-driven translation.

Reduced translational efficiency can also occur when inhibitory upstream AUG codons are created by nucleotide changes in the 5′ UTR of the mRNA. In the case of cyclin-dependent kinase inhibitor 2A (CDKN2A or p16)[17], mutations have been identified that impair 40S subunit scanning to the authentic AUG codon and, therefore, the synthesis of the corresponding protein. The resulting decrease in expression of p16, a tumour suppressor, causes dysregulation of the cell cycle and leads to melanoma[18].

Kozak consensus sequence
gccgcc(A/G)ccAUGG: the most favourable sequence to serve as translational start site (AUG, underlined). The purine at position −3 from the AUG start codon and the 'G' at +1 are the most important determinants.

Pseudouridylation
A post-transcriptional nucleotide modification found in RNAs.

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Trans-acting factors
RNA-binding proteins that regulate translation of specific mRNAs.

Mutations that affect translation initiation factors and their regulators. Translation factors assist in specific steps of the translation process, and around a dozen such factors are required for translation initiation in mammalian cells. One of them, eIF2, brings the initiator methionyl-tRNA (Met-tRNA$_i^{Met}$) to the ribosome to locate the start codon[19] (FIG. 2). Only GTP-bound eIF2 interacts with Met-tRNA$_i^{Met}$ and, during each cycle of initiation, this GTP is hydrolysed to GDP. Regeneration of active eIF2–GTP is catalysed by eIF2B, a guanine nucleotide exchange factor (GEF) comprising five different subunits, α–ε (FIG. 2). eIF2 activity is controlled by phosphorylation of the α-subunit of eIF2 — eIF2α (REF. 20); this modification inhibits eIF2B, resulting in a general inhibition of translation. The kinase pancreatic endoplasmic reticulum-resident kinase (PERK; also known as EIF2AK3) phosphorylates eIF2 as part of the 'unfolded protein response' (UPR), which is activated when unfolded or misfolded proteins accumulate in the endoplasmic reticulum (ER) (BOX 1). The role of PERK and eIF2B in the UPR is a further example of the utility of controlling translation to achieve rapid responses to changing conditions.

Mutations in any of the genes for the eIF2B subunits, *EIF2B1–5*, lead to a severe autosomal recessive neurodegenerative disorder termed 'leukoencephalopathy with vanishing white matter' (VWM) or 'childhood ataxia with central hypomyelination' (CACH)[21]. VWM usually begins in childhood and leads to both chronic progressive and episodic neurological deterioration. The phenotypic variation for this disease is extremely wide, ranging from antenatal onset involving multiple organs and early death[22,23] to adult onset with slow disease progression[24,25,26].

Although eIF2B activity is decreased by up to 20–30% in lysates from VWM-patient-derived lymphoblasts compared with controls[27], this does not seem to affect protein synthesis rates[28,29]. This suggests that eIF2B activity is not limiting in all cells — a surprising conclusion given the presumed role of eIF2B in regulating translation under stress conditions, a function that has been taken to imply that eIF2B activity limits the rate of translation[20]. In post-mortem brain tissue obtained from VWM patients, UPR activation is evident[30,31], and VWM-patient-derived fibroblasts show a heightened stress response under conditions of perturbed ER function[29]. UPR activation in VWM is probably related to enhanced expression of stress-activated transcription factors, such as activating transcription factor 4 (ATF4) and C/EBP-homologous protein (CHOP; also known as DDIT3)[30], resulting from decreased eIF2B activity. CHOP sensitizes cells to ER stress[32] and, in VWM cells, the UPR might therefore be more readily triggered by minor stresses than it is in healthy cells. Several explanations have been offered for the selective involvement of glia in VWM: the importance of ER function in oligodendrocytes, which produce vast amounts of myelin components; the sensitivity of astrocytes to apoptosis under increased CHOP levels; and the translational control of glia-specific mRNAs that contain uORFs or IRESs[33,34].

Regulation of translation through eIF2 activity also has a role in Wolcott–Rallison syndrome (WRS)[35,36,20], an extremely rare autosomal recessive disorder caused by mutations in the *EIF2AK3* gene, which encodes PERK. The highest expression of PERK occurs in secretory cells, including insulin-secreting β-cells, as might be expected from its location on the ER, and WRS patients suffer from infantile onset diabetes mellitus. All of the known WRS mutations either impair or abolish PERK activity, which might allow overproduction of insulin beyond the capacity of the ER of the β-cells to process it, leading to UPR activation and β-cell apoptosis[37]. Surprisingly, it was recently shown using a conditional knockout mouse model that deletion of *PERK* after the early neonatal stage did not lead to diabetes,

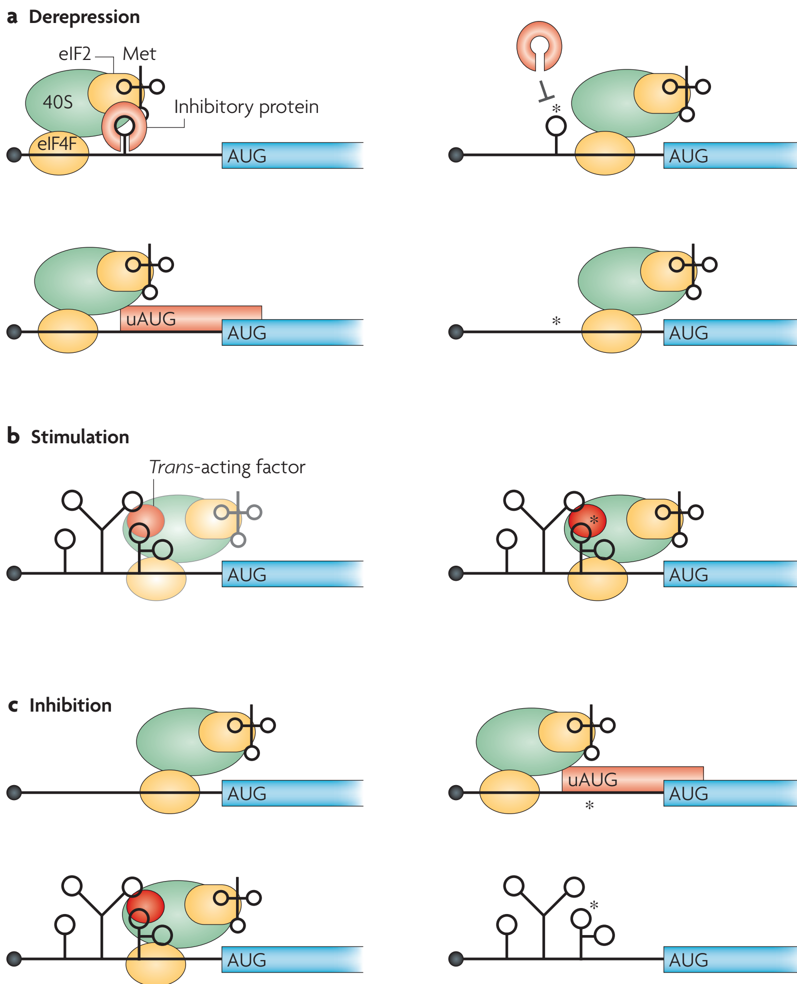

Figure 3 | **Mutations in 5′ untranslated (5′ UTR) regions. a** | Mutations that remove inhibitory sequences. Nucleotide changes (indicated by the asterisk in the right-hand part of the figure) in the 5′ UTR (black line) can destroy the binding site for an inhibitory protein (top) or remove inhibitory upstream AUG codons (bottom), leading to derepression of translation initiation. **b** | Increased internal ribosome entry site (IRES)-mediated translation. Mutations in the 5′ UTR can affect the binding of *trans*-acting factors. Such *trans*-acting factors are especially important for IRES-mediated initiation. **c** | Mutations leading to decreased protein synthesis. The creation of a spurious upstream AUG codon (top) or mutations that affect IRES sequences, either by impairing the proper folding of the 5′ UTR or by reducing the binding of *trans*-acting factors (bottom) can severely inhibit initiation at the start codon of the main ORF.

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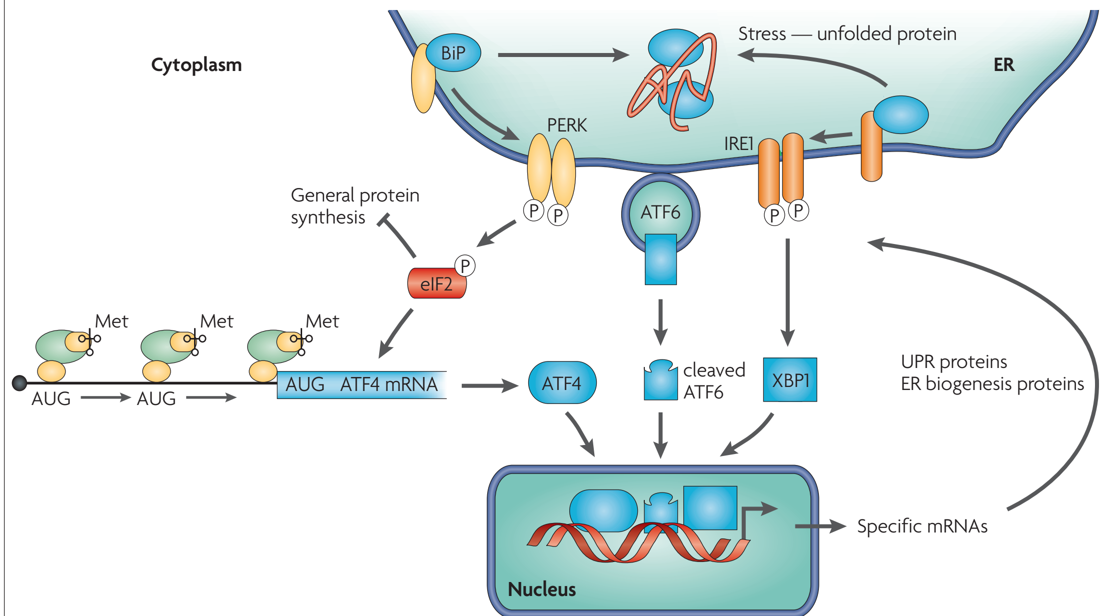
Box 1 | The unfolded protein response



The endoplasmic reticulum (ER) processes polypeptides that are made in the cytosol and are destined for secretion, such as insulin, and contains a range of proteins that help with folding and post-translational modification of the polypeptides. If the load of client proteins exceeds the capacity of the ER to process them, unfolded proteins accumulate — an undesirable outcome. The response of cells to counteract the accumulation of toxic amounts of denatured or unfolded proteins is called the unfolded protein response (UPR). Under these conditions, three proteins are activated: PERK (pancreatic endoplasmic reticulum-resident kinase), ATF6 (activating transcription factor 6) and IRE1 (endoplasmic reticulum-to-nucleus signalling 1). PERK (also called PEK (PKR-related ER-resident kinase)), which spans the ER membrane, is one of the four mammalian eukaryotic initiation factor 2 (eIF2)-α kinases. One domain (related to the protein IRE1) resides in the ER lumen, whereas the second, which contains the kinase domain, is cytosolic. The chaperone protein BiP (also known as HSPA5) prevents activation of PERK and IRE1 by binding to its luminal domain[97]. PERK is activated when unfolded or misfolded proteins accumulate in the lumen of the ER[20,98], leading to dissociation of BiP and subsequently to dimerization of PERK through the luminal domain. Dimerization leads to kinase activation, phosphorylation of eIF2, and inhibition of cytosolic translation. This resulting decrease in new protein production allows the ER to 'catch up'.

Translation of upstream ORFs does not always lead to dissociation of the ribosome. Under conditions in which the activity of eIF2B is decreased and, consequently, the amount of eIF2 carrying Met-tRNA is low, some of the subsequent upstream AUGs, which are inhibitory under normal conditions, become poorly recognized, and the ribosome is allowed to reach the start codon of the major ORF. So, paradoxically, inhibition of eIF2B by phosphorylated eIF2 serves to enhance the translation of certain mRNAs, such as that of the transcriptional regulator ATF4 (activating transcription factor 4) in mammalian cells[99]. Increased expression of ATF4 is achieved through such a regulatory mechanism involving upstream AUGs in its mRNA. ATF4 is a key regulator of the transcription of many genes for proteins that help the cell cope with unfolded proteins, including other transcription factors (ATF6, X-box-binding protein 1 (XBP1)), which in turn activate genes involved in restoring ER homeostasis.

and that PERK is required for proliferation of insulin-secreting β-cells only during the fetal and early neonatal periods[38].

Mutations that affect elongation factors. No known inherited human diseases are associated with mutations in cytosolic elongation factors (eEFs); however, such mutations have been shown to cause specific phenotypic defects in mice. eEF1A brings charged amino-acyl-tRNAs to the ribosomal A-site. Mice possess two isoforms of eEF1A: eEF1A-1 and eEF1A-2. eEF1A-2 activity is abolished by the *wasted* mutation, which causes profound neuromuscular problems shortly after weaning[39]. The specific involvement of the CNS in this phenotype can be explained by a switch in isoform expression. During early postnatal development, the eEF1A-1 (or EF1α) isoform is expressed ubiquitously; subsequently, in the brain, heart and skeletal muscle, a switch occurs such that the eEF1A-2 (or S1) isoform becomes the major form[40]. In *wasted* mice, the complete absence of eEF1A-2 activity in neurons, heart and skeletal muscle 20 days after birth leads to lethal abnormalities in these tissues. eEF1A-1 has additional non-canonical roles in actin binding and bundling, microtubule bundling and severing, and cellular transformation processes, whereas eEF1A-2 has a role in resistance to oxidative-stress-induced death in terminally differentiated cells[41].

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Mutations that affect release factors. Translation termination is mediated by release factors (eRFs) upon recognition of the stop codon, and significantly reduced eRF function might lead to ribosome stalling and reduced protein synthesis rates. A GGC expansion in exon 1 of eRF3 is associated with gastric cancer[42]. The most common allele for this protein encodes 10 glycines, whereas a 12-glycine allele has been detected exclusively in cancer patients (allelic frequency, 5%) and is associated with a 20-fold increased risk of gastric cancer. The molecular pathogenesis of the cancer phenotype is unknown. Surprisingly, eRF3 has been shown to function in the control of chromosome segregation and in regulating apoptosis, independently of its role in translation[43]. These functions could explain the involvement of eRF3 mutations in cancer, although disruption of its role in translation has not been ruled out as a possible cause.

Paradoxically, in some diseases, patients might actually benefit from decreased translational accuracy at stop codons. Suppression of ribosomal proof reading by aminoglycoside antibiotics allows incorporation of an amino acid and permits translation to 'read through' premature stop codons caused by 'nonsense' mutations, thus facilitating the synthesis of full-length proteins. Although this treatment is still under development, it might be applicable, for example, in the 5–10% of cystic fibrosis patients who have premature termination codons in the *CFTR* gene[44,45].

Ribosomal proof reading
Accurate recognition of the codons that enter the ribosomal A-site to prevent misincorporation errors during polypeptide synthesis.

Mutations that affect ribosomal components. Ribosomes are composed of three to four rRNA molecules and several dozen proteins. In mammalian cells, many additional genes encode proteins or RNAs that are involved in the complicated process of ribosome assembly. Few human diseases are related to changes in the many genes that encode these components, probably because they are so important for normal cell function that mutations are lethal early in development.

Approximately 25% of cases of Diamond–Blackfan anaemia (DBA) are due to mutations in ribosomal protein S19 (RPS19)[46], a component of the 40S ribosomal subunit. DBA is a congenital erythroid aplasia that usually presents in infancy, and is genetically heterogeneous: about 2% of *RPS19* mutation-negative probands have mutations in another ribosomal protein, RPS24 (REF. 47). RPS19 is required for the maturation of 40S ribosomal subunits[48,49], leading to the suggestion that DBA might result from a general decrease in ribosome levels. In support of this theory, in DBA patients who lack *RPS19* mutations (and for which the genetic defect is unknown), 28 ribosomal protein mRNAs (including *RPS19*) are expressed at lower levels than in controls[50]. However, earlier studies suggested that RPS19 and RPS24 might be involved in binding eIF2 (REF. 51), and the possibility that DBA involves the impairment of mRNA translation initiation has not been ruled out.

Three other bone marrow failure syndromes — X-linked dyskeratosis congenita (mentioned above), cartilage-hair hypoplasia and Shwachman–Diamond disease — are also linked to genes that are involved in ribosome biogenesis (TABLE 1). Recent work on the yeast homologue of *SBDS*, the affected gene in Shwachman–Diamond disease, showed that it has a key role in the maturation and translational function of ribosomes[52]. *RMRP* gene mutations, which are involved in cartilage-hair hypoplasia, impair ribosomal assembly and alter cyclin-dependent cell-cycle regulation. The cartilage-hair hypoplasia mutation affects both pathways intermediately whereas, in anauxetic dysplasia, B-cyclin mRNA levels are not affected but ribosomal assembly is severely incapacitated[53]. The proteins that are affected in these diseases might function in other processes in addition to ribosome biogenesis, making it difficult to determine whether a translational defect is the primary cause of these bone marrow failures, affects their severity or underlies the other symptoms that are seen in these conditions. In other forms of dyskeratosis congenita, the mutations that have been identified point clearly to telomerase defects as an underlying cause of the disease[54,55], suggesting that effects on translation do not underlie bone marrow failure. However, the fact that both genes that are mutated in DBA encode ribosomal proteins strengthens the case that the disease phenotype is caused by a defect in ribosome biogenesis (for detailed reviews, see REFS 13,56).

tRNAs and their charging enzymes. To ensure fidelity of translation elongation, it is clearly important to attach the right amino acid to the tRNA, and to ensure that the tRNA recognizes, through its anticodon, the correct codon in the ribosomal A-site. Incorporation of an incorrect amino acid into the nascent polypeptide could cause misfolding and production of defective or dominant interfering proteins. Amino acids are attached to tRNAs by amino-acyl-tRNA synthetases, each of which is specific for a single amino acid. However, as there can be several codons and several different tRNAs for a single amino acid, an amino-acyl-tRNA synthetase can 'charge' several different tRNAs. Accuracy is a key aspect of amino-acyl-tRNA synthetase function.

Several mutations in amino-acyl-tRNA synthetases cause human neurological disorders[57–59], including the peripheral neuropathy CMT (mentioned in a previous section). CMT is divided into groups and subtypes on the basis of electrophysiological and neuropathological criteria. CMT1 comprises the demyelinating polyneuropathies, whereas CMT2 comprises the axonal polyneuropathies; intermediate forms are also recognized. Mutations in *GARS*, which encodes glycyl-tRNA synthetase, are linked to CMT disease type 2D (CMT2D) and distal spinal muscular dystrophy V[60]. *GARS* mutations affect the localization of glycyl-tRNA synthetase in granules within the cell bodies and neurite projections of neuronal cells[60], although the significance of this mislocalization and the effects on enzymatic activity await characterization. In a mouse model, a missense mutation in *GARS* resulted in a CMT2D phenotype without affecting amino-acylation activity[61].

Mutations that affect the activity and axonal distribution of tyrosyl-tRNA synthetase are linked to dominant intermediate CMT type C[58]. The localization of tyrosyl-tRNA

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Table 1 | **Heritable defects caused by mutations in nuclear genes involved in translation or its regulation**

Gene	Protein	Disease or phenotype	References
Cytosolic proteins			
EIF2B1–5	eIF2B subunits α–ε	Vanishing white matter; childhood ataxia with central nervous system hypomyelination (chronic progressive, an episodic encephalopathy)	21,102
EIF2AK3	eIF2α kinase PERK	Wolcott–Rallison syndrome (neonatal or early childhood diabetes mellitus, epiphyseal dysplasia, kidney and liver dysfunction, mental retardation, central hypothyroidism and dysfunction of the exocrine pancreas)	103
GARS	Glycyl-tRNA synthetase	Charcot–Marie–Tooth type 2D (slowly progressive axonal polyneuropathy)	60
YARS	Tyrosyl-tRNA synthetase	Dominant intermediate Charcot–Marie–Tooth type C (slowly progressive polyneuropathy with a mixed demyelinating-axonal phenotype)	58
RPS19, *RPS24*	Ribosomal protein S19, Ribosomal protein S24	Diamond–Blackfan anaemia (abnormalities of the thumb, short stature, ventricular septal defects, kidney hypoplasia and congenital glaucoma)	46,47
GSPT1	Eukaryotic release factor 3	Gastric cancer	42
PUS1	Pseudouridine synthase 1	Mitochondrial myopathy, sideroblastic anaemia, mental retardation, microcephaly and dysmorphic features	81,104
DKC1	Dyskerin	X-linked dyskeratosis congenita (ectodermal abnormalities, bone marrow failure and increased susceptibility to cancer)	105
SBDS	Shwachman–Bodian–Diamond syndrome	Shwachman–Diamond Syndrome (exocrine pancreatic insufficiency, bone marrow dysfunction, skeletal abnormalities and short stature)	106
RMRP	RNA component of ribonuclease mitochondrial RNA processing (RNase MRP)	Cartilage-hair hypoplasia	107
Mitochondrial proteins			
MRPS19	MRPS19	Agenesis of corpus callosum and dysmorphism and fatal neonatal lactic acidosis	85
TSFM	Elongation factor Ts	Encephalomyopathy; hypertrophic cardiomyopathy	90
GFM1	Elongation factor G1	Liver dysfunction, hypoplasia of corpus callosum and delayed growth	91
TUFM	Elongation factor Tu	Lactic acidosis, diffuse cystic leukoencephalopathy, polymicrogyria, liver involvement and early death	89
SPG7	Paraplegin	Hereditary spastic paraplegia	108
DARS2	Mitochondrial aspartyl-tRNA synthetase	Leukoencephalopathy with brain stem and spinal cord involvement and elevated lactate	83
LARS2	Mitochondrial leucyl-tRNA synthetase	Susceptibility to diabetes mellitus	57
PUS1	Pseudouridine synthase 1	Mitochondrial myopathy, sideroblastic anaemia, mental retardation, microcephaly and dysmorphic features	81,104
Mouse models			
AARS$^{sti/sti}$	Alanyl-tRNA synthetase	'*Sticky*' phenotype (sticky appearance of fur), cerebellar Purkinje cell loss and ataxia	62
EIF2AK3$^{-/-}$	eIF2α kinase PERK	Diabetes mellitus and exocrine pancreatic dysfunction	109
GCN2$^{-/-}$	eIF2α kinase GCN2	Liver steatosis	110
HRI$^{-/-}$	Haem-regulated inhibitor	Iron-deficiency-induced anaemia with erythroid hyperplasia	111
4E-BP1$^{-/-}$, *4E-BP2*$^{-/-}$	eIF4E-binding protein 1/2	Sensitivity to diet-induced obesity	112
EIF2$^{+/Ser51Ala}$	eIF2α	Diet-induced obesity, type 2 diabetes mellitus	113
EEF1A2$^{-/-}$	eukaryotic elongation factor 1A2	'*Wasted*' phenotype: mice are characterized by wasting and neurological and immunological abnormalities	39
SBDS$^{-/-}$	Shwachman–Bodian–Diamond syndrome	Embryonic lethality	114
RPS19$^{-/-}$	Ribosomal protein 19	Lethal prior to implantation	115
DKC1$^{-/-}$	Dyskerin	Embryonic lethality	116

eIF, eukaryotic initiation factor; MRP, mitochondrial ribosomal protein; PERK, pancreatic endoplasmic reticulum-resident kinase.

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synthetase in granular structures in the growing processes of differentiating neuronal cells appears to be similar to the localization of glycyl-tRNA synthetase, but is not typical of other tRNA synthetases[58], suggesting these two enzymes have additional axon-terminal-specific functions that, when disrupted, cause the specific phenotypes that are seen in these diseases.

Amino-acyl-tRNA synthetases often accept amino acids that are smaller than the correct cognate amino acid, but reject bulkier ones[62]. Subsequently, an editing function that is carried out by the same enzymes removes smaller amino acids that have been inappropriately attached to the tRNA. If this function were defective, certain codons would become ambiguous, resulting in the synthesis of misfolded proteins, which could aggregate to form inclusions and induce further protein misfolding. Misfolding and protein aggregation are thought to underlie several neurodegenerative disorders[63].

The mouse *sticky* mutation affects the editing domain of alanyl-tRNA synthetase, leading to charging of $tRNA^{Ala}$ with serine and resulting in ataxia due to apoptotic loss of Purkinje cells[62]. The cerebellum of the *sticky* mouse contains electron-dense structures, which are probably protein inclusions, and shows increased expression of chaperones, indicating accumulation of misfolded proteins. The specific loss of Purkinje cells is unexplained, but is in line with the fact that diseases characterized by unstable DNA repeats and protein aggregates often selectively affect the cerebellar cortex[64].

Amino-acyl-tRNA synthetases not only charge tRNAs but also participate in processes such as transcription, splicing, inflammation, angiogenesis and apoptosis[65]. They interact with multifunctional proteins to form complexes with novel functions that are unrelated to their role in protein synthesis, acting as cytokines that control angiogenesis, immune response and wound repair, and as crucial regulators of cell proliferation and DNA repair (reviewed in REF. 65). Detailed studies of the effects of mutations in tRNA synthetases on their translational and non-canonical functions are needed to understand the pathophysiology of the diseases that are linked to such proteins.

Defects in mitochondrial translation

Human cells possess two different genomes: nuclear DNA, present in two copies in each cell, and the much smaller mitochondrial genome (mtDNA), present in 1,000–10,000 copies per cell. Because the mitochondrial genome is relatively small, allowing comprehensive sequencing in individuals, diseases that are caused by mutations in mtDNA have been studied widely[66]. The proteins involved in mitochondrial protein synthesis are all encoded by nuclear genes, whereas the two mitochondrial rRNAs and 22 tRNAs are encoded by mtDNA. The components of the mitochondrial protein synthesis machinery — ribosomes and translation factors — are quite distinct from their cytosolic counterparts, generally being more similar to those of bacteria. However, many of the mammalian mitochondrial ribosomal proteins do not have bacterial homologues, and seem to have evolved separately[67]. The mitochondrial translational apparatus makes only 13 proteins, all of which are components of the respiratory chain. Other proteins of the respiratory chain complexes and all other mitochondrial proteins are encoded by nuclear genes, synthesized in the cytosol and directed to the mitochondrion by targeting sequences.

Defects in mitochondrial metabolism lead to a wide range of human diseases[68,69]. Several recent reviews have addressed the relationship between mitochondrial dysfunction and the vulnerability of different tissues or organs[70,71], as well as the role of mutations in the mitochondrial versus the nuclear genome in mitochondrial disease[66,72]. mtDNA defects most commonly affect muscle and nervous system tissues; it is often suggested that this reflects their substantial requirement for ATP, but in fact almost all tissue types can be affected (TABLES 1,2).

An important issue in the case of mutations in the mitochondrial genome is heteroplasmy, the presence of a mixture of more than one mtDNA genotype in a cell or individual (BOX 2). Because each eukaryotic cell contains many mitochondria and hundreds of copies of mtDNA, mutations frequently affect only a proportion of the copies, and the number of affected copies can vary between different cells in one individual. The 'threshold' — that is, the number of affected mitochondria and cells needed to cause organ dysfunction — varies from tissue to tissue.

Defects in mitochondrial tRNAs. Mitochondrial tRNA mutations are associated with various diseases. Disease phenotypes have been linked with all mitochondrial tRNAs, except $tRNA^{Met}$ and $tRNA^{Cys}$ (TABLE 1). The best-studied mutation in a mitochondrial tRNA is related to MELAS, a disease characterized by myopathy, encephalopathy, lactic acidosis, stroke-like episodes and other neurological and non-neurological symptoms. Eighty percent of patients with MELAS carry a maternally inherited A>G mutation in nucleotide 3243 of mtDNA, affecting the $tRNA^{Leu(UUR)}$ gene, and a further 10% have a 3271T>C transition in the same gene[73]. The 3243A>G mutation has also been observed in patients with ophthalmoplegia and other neurological signs, but without stroke-like episodes, in patients with diabetes mellitus and deafness without neurological problems, and in asymptomatic family members, reflecting threshold and heteroplasmy effects[74].

A key concept in translation is that of 'wobble', whereby a single tRNA can decode two codons, provided that they differ only in the third position, so that a pyrimidine is replaced by another pyrimidine or a purine with another purine. For example, the tRNA with the anticodon UAA can decode the codons UUA and UUG (for leucine). Mitochondrial tRNAs undergo a strikingly high number of post-transcriptional modifications, and the first base of the anticodon (which pairs in an antiparallel fashion with the wobble base) of mitochondrial $tRNA^{Leu(UUR)}$ and $tRNA^{Lys}$ is post-transcriptionally modified with a taurine group[75]. The 3243A>G MELAS mutation affects the stability of $tRNA^{Leu(UUR)}$ (REF. 76) and impairs the efficiency with which the tRNA is charged with leucine[74,76]. In addition, both this mutation and 3271T>C prevent taurine

Taurine
An amino-sulphonic acid that can modify the wobble position of mitochondrial tRNAs.

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Table 2 | **Disease phenotypes caused by mutations in mitochondrial tRNA and rRNA genes**

Gene	RNA	Disease or phenotype
MTTA	$tRNA^{Ala}$	CPEO, dysphagia and proximal myopathy; myotonic dystrophy-like myopathy; mitochondrial myopathy
MTTN	$tRNA^{Asn}$	Ophthalmoplegia; renal failure, mental retardation, ataxia, muscle weakness, seizures; myopathy
MTTR	$tRNA^{Arg}$	Mitochondrial encephalomyopathy [117]; hearing loss (in mice)
MTTD	$tRNA^{Asp}$	Myopathy
MTTC	$tRNA^{Cys}$	Deafness; encephalomyopathy
MTTQ	$tRNA^{Gln}$	Myopathy; sensorineural deafness and migraine; MELAS syndrome
MTTE	$tRNA^{Glu}$	Myopathy with diabetes mellitus
MTTG	$tRNA^{Gly}$	Hypertrophic cardiomyopathy; exercise intolerance, with progressive muscle fatigue and myalgia; sudden death
MTTH	$tRNA^{His}$	Idiopathic dilated cardiomyopathy; pigmentary retinopathy and sensorineural deafness; MERRF/MELAS overlap syndrome
MTTI	$tRNA^{Ile}$	Cardiomyopathy; truncal ataxia, dysarthria, severe hearing loss, mental regression, ptosis, ophthalmoparesis, and diabetes mellitus; familial progressive necrotizing encephalopathy; hypertension, hypercholesterolaemia, and hypomagnesaemia
MTTK	$tRNA^{Lys}$	MERRF syndrome; CPEO with myoclonus; diabetes mellitus–deafness syndrome; MNGIE syndrome; cardiomyopathy and deafness
MTTL1	$tRNA^{Leu(UUR)}$	MELAS syndrome; MERRF syndrome; cardiomyopathy; encephalomyopathy; chronic progressive external ophthalmoplegia, proximal myopathy; skeletal myopathy; sudden (infant) death syndrome; neuropsychiatric disorder and early-onset cataract; Kearns–Sayre syndrome (external ophthalmoplegia, pigmentary retina degeneration, progressive encephalopathy and cardiac conduction block); myelodysplastic syndrome
MTTL2	$tRNA^{Leu(CUN)}$	Encephalomyopathy; cardiomyopathy; myopathy
MTTM	$tRNA^{Met}$	Myopathy
MTTF	$tRNA^{Phe}$	MELAS syndrome; MERRF syndrome
MTTP	$tRNA^{Pro}$	Myopathy; susceptibility to Parkinson disease
MTTS1	$tRNA^{Ser(UCN)}$	MERRF/MELAS overlap syndrome; palmoplantar keratoderma with deafness; sensorineural deafness
MTTS2	$tRNA^{Ser(AGY)}$	Cerebellar ataxia, cataract and diabetes mellitus
MTTT	$tRNA^{Thr}$	Susceptibility to Parkinson disease
MTTW	$tRNA^{Trp}$	Leigh syndrome (encephalopathy with prominent signs of brain stem and basal ganglia dysfunction) ; myopathy ; neurogastrointestinal syndrome
MTTY	$tRNA^{Tyr}$	Exercise intolerance; chronic progressive external ophthalmoplegia with myopathy; focal segmental glomerulosclerosis and dilated cardiomyopathy
MTTV	$tRNA^{Val}$	Ataxia, progressive seizures, mental deterioration and hearing loss; neonatal death — cardiomyopathy
MTRNR1	12S rRNA	Aminoglycoside-induced deafness, non-syndromic deafness
MTRNR2	16S rRNA	MELAS; deafness

Sources: OMIM (Online Mendelian Inheritance in Man) and Mitomap. Note: some of the disease phenotypes in mitomap are provisional and await further studies for confirmation. CPEO, chronic progressive external ophthalmoplegia; MELAS, myopathy, encephalopathy, lactic acidosis, stroke-like episodes; MERRF, myoclonic epilepsy with ragged-red fibres; MNGIE, myoneural gastrointestinal encephalopathy.

modification in the anticodon. To distinguish the effect of the primary mutation from that of the absence of the taurine modification, Kirino *et al.*[73] used an elegant approach termed 'molecular surgery' to remove the normal taurine-modified wobble base and replace it with the unmodified nucleotide in the framework of the normal, unmutated tRNA. The data clearly show that the unmodified tRNA displays a major defect in decoding UUG codons, but not UUA codons, whereas the MELAS mutations themselves caused a modest decrease in the usage of both codons. This study gave new insights into the importance of tRNA modifications for the basic mechanism of translation: the taurine modification at the wobble uridine in mitochondrial $tRNA^{Leu(UUR)}$ has a crucial role in stabilizing the wobble base pairing.

An 8344A>G mutation in mitochondrial $tRNA^{Lys}$ causes myoclonic epilepsy with ragged-red fibres (MERRF)[77]. This mutation affects amino acylation[78] and taurine modification of the wobble nucleotide of the anticodon[73]. Loss of the taurine modification abrogates codon–anticodon pairing on the ribosome, explaining why MERRF patients show a marked defect in overall mitochondrial translation[73].

It has been suggested that some of the differences in the symptoms of MELAS and MERRF can be explained by codon usage in the *ND6* mRNA. The *ND6* gene encodes a subunit of respiratory complex 1 (NADH-coenzyme Q reductase), and the mRNA contains eight UUG codons (42.1% of its leucine codons). Thus, it is tempting to speculate that defective translation of ND6, and therefore reduced levels of complex 1, might

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Box 2 | **Heteroplasmy**

The entire mitochondrial genome of each human individual, both males and females, is inherited from the mother. This is due to the fact that, during egg fertilization, the sperm cell contributes almost no cytoplasm to the zygote. Mitochondrial disorders with mitochondrial DNA (mtDNA) mutations thus follow maternal inheritance. A mother carrying a mtDNA mutation will transmit it to all her children, males and females, but only her daughters will pass it on to their progeny.

Cells can contain many mitochondria, with the actual number differing in relation to the energy requirements of the cell type. Each mitochondrion contains, on average, five copies of the mitochondrial genome. Consequently, there are many copies of mtDNA in each cell. In normal individuals, all copies of mtDNA are identical (homoplasmy) but, in the case of mutations, there can be two populations of mtDNA (heteroplasmy), one being wild type and the other being mutant. During cell division, mitochondria (and mtDNA) are distributed randomly between daughter cells. As a consequence, the proportion of mutant mitochondrial genomes can shift in daughter cells. Therefore, the proportion of mutant DNA versus normal DNA can vary markedly, both among siblings and in different tissues of the same person, and can alter over the course of time. Whether or not the mtDNA mutation is actually expressed is largely determined by the relative proportion of normal versus mutant genomes in a given tissue. A minimum critical number of mutant DNAs is necessary to impair energy metabolism severely enough to cause dysfunction of that particular organ or tissue. This phenomenon is known as the 'threshold effect'. The number of affected mitochondria and cells that are needed to cause organ dysfunction varies from tissue to tissue depending on the vulnerability of any given tissue to impairments of oxidative phosphorylation. The relative reliance of tissues on oxidative phosphorylation energy decreases in the following order: nerve tissue, skeletal muscle, heart, kidney and liver. As a result of heteroplasmy, unequal mitotic segregation and the threshold effect, different individuals in the maternal lineage can differ in symptomatology and organ involvement, or can even be asymptomatic. A recent report studied the association between mutation load in mitochondria in blood and muscle cells and the phenotype of patients with the 3243A>G mutation in the tRNA$^{Leu(UUR)}$ gene[100]. All subjects with a muscle mutation load above 50% showed reduced maximal oxygen uptake during exercise and abnormal morphology of muscle fibres, whereas a mutation load of 65% correlated with diabetes mellitus and hearing impairment. The phenotype was not correlated with mutation load in blood, highlighting the importance of tissue-specific distribution of mutation-carrying mitochondria on disease outcome[100].

underlie the MELAS phenotype. Mutations in the *ND6* gene itself give rise to MELAS-like features[79], supporting this idea.

Another post-transcriptional modification of tRNAs is pseudouridylation, which is carried out by a pseudouridylate synthetase encoded by the nuclear *PUS1* gene. Pseudouridylation in the stem or the anticodon of the tRNA strengthens base pairing[80]. Mutation of *PUS1* is associated with mitochondrial myopathy, lactic acidosis and sideroblastic anaemia (MLASA)[81]. Protein synthesis in the mitochondria of fibroblasts derived from MLASA patients is markedly reduced, and several clinical symptoms of MLASA patients can be attributed to mitochondrial dysfunction.

Mitochondrial amino-acyl-tRNA synthetases. The autosomal recessive disease leukoencephalopathy with brain stem and spinal cord involvement and elevated lactate (LBSL)[82] has recently been found to be caused by mutations in *DARS2*, which encodes mitochondrial aspartyl-tRNA synthetase (mtAspRS). *In vitro* amino-acylation experiments with bacterially expressed proteins show that these mutations affect the enzyme's activity[83]. Mutations in the mitochondrial gene that encodes aspartyl-tRNA might be expected to have a similar phenotype. Surprisingly, however, a mutation in the tRNA^{Asp} gene has been associated with an isolated case of mitochondrial myopathy[84], whereas defects in mtAspRS affect only the CNS. An important difference is that tRNA synthetases are encoded by nuclear genes, whereas mitochondrial tRNAs are encoded in mtDNA. A mutation load of almost 100% in the tRNA^{Asp} gene was found in the muscle of the myopathy patient mentioned above, whereas the mutation load in fibroblasts and lymphocytes was less than 3% (REF. 84). This again illustrates the differences between mitochondrial and nuclear mutations and the role of heteroplasmy in the resulting phenotype.

Mitochondrial ribosomes. Little is known about the phenotypic consequences of mutations that affect mitochondrial ribosomes. However, mutations in the gene encoding MRPS16 (REF. 85), a protein of the small subunit of mitochondrial ribosomes, lead to dysmorphism, agenesis of the corpus callosum, severe neonatal encephalopathy and fatal lactic acidosis. This mutation markedly decreases 12S rRNA levels, probably resulting from reduced formation of the small subunit and degradation of this rRNA, and a defect in mitochondrial translation has been clearly demonstrated for patients with *MRPS16* mutations. Several other MRP genes are putative candidates for other syndromes[86].

Insights into how mitochondrial ribosome biogenesis defects might cause disease are more advanced. Loss of function of the protease paraplegin is linked to hereditary spastic paraplegia. The m-AAA-protease, of which paraplegin is a subunit, is involved in maturation of mitochondrial ribosomal protein MRPL32 by removing the first 71 amino acids from the cytosolically synthesized precursor polypeptide[87]. These amino acids target the precursor to the mitochondrion. Paraplegin is required for maturation of the nuclear-DNA-encoded MRPL32 protein and thus for correct assembly of mitochondrial ribosomes. The selective involvement of specific neurons in this hereditary spastic paraplegia might be explained by the variable cell- and tissue-specific subunit composition that is seen in mitochondrial m-AAA protease complexes, so that reduced protease activity would have differential effects in different cells and tissues[88].

Mitochondrial translation elongation factors. A few patients have been described with mutations in genes encoding components of the mitochondrial translation elongation machinery, including elongation factor EFTu (*TUFM*)[89], EFTs (*TFSM*)[90] and EFG1 (*GFM1*)[89,91,92]. These patients have severe disease, leading to early fatality, and show combined mitochondrial complex deficiencies. Decreased mitochondrial translation was reported in all cases; however, this function could be restored by overexpressing the corresponding wild-type protein. These mutations occur in nuclear-encoded genes, so heteroplasmy does not have a role. Nevertheless, clinical symptoms vary greatly between patients, even between those with mutations in the

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same gene. The single patient with mutations in *TUFM* showed lactic acidosis, a diffuse cystic leukoencephalopathy, polymicrogyria and mild liver involvement. The clinical course of one child with mutations in the *GFM1* gene was dominated by neonatal lactic acidosis and rapidly evolving neurological failure, without any symptoms of hepatic involvement[89]. Another patient with *GFM1* mutations suffered postnatal liver failure and encephalopathy[91]; one study showed that EFG1 was undetectable in that patient's liver[92]. The phenotypic differences might result from mutation-specific effects on the stability of the protein in different tissues, and differences in the abundance of other elongation factors have been suggested to have a role in this variability[92]. Indeed, two studies have shown that the relative abundance of elongation factors is important in determining mitochondrial translation efficiency[90,92].

Conclusions

Studies of diseases that result from defects in mRNA translation have begun to provide new insights into the fundamental process of translation. For example, it has become clear that proteins that regulate the UPR are particularly important in certain tissues, such as PERK during differentiation and proliferation of β-cells and eIF2B in white matter cells. In addition, an increased understanding of the roles of individual ribosomal proteins in ribosome biogenesis has come from studies on DBA and SBDS. Although the roles of many proteins involved in the translation process have been characterized, further studies on translation factors that are linked to disease are likely to provide further insights.

Perhaps one of the most puzzling issues surrounding diseases that are caused by defects in the translation machinery or translation factors is the cell specificity of many of them. In most cases, it remains to be addressed whether this is due to specialized tissue-specific functions of these factors, altered translational regulation of tissue-specific mRNAs, or differential expression of the affected proteins. This is a key area for further study. It is already clear that many components involved in translation are not just 'mere house keepers', because additional functions besides their canonical roles in protein synthesis can underlie disease phenotypes. In many cases, the actual effect of the causal mutations on protein synthesis has not yet been defined.

It is evident that mutations in genes that encode components of the translational machinery can give rise to a wide spectrum of diseases; that is, there is no single 'translational' phenotype. However, some organs seem to be affected more frequently than others. Brain and muscle are commonly affected in mitochondrial diseases, an observation that has been attributed to the high energy requirements of muscle cells and neurons, although a diverse range of tissues and organs are affected in these diseases as a whole. In many of the diseases and animal models in which cytosolic translation factors are involved, diabetes mellitus is one of the main symptoms. This could be the consequence of disturbed ER function induced by faulty translational control, which would especially affect insulin-secreting β-cells. By contrast, there is still little insight into why specific cells within the nervous system are preferentially affected in, for example, VWM or CMT. It should be noted that the nervous system comprises a range of different cell types; care should be taken not to group all encephalopathies and all peripheral neuropathies together as representing only two phenotypes. Furthermore, although many studies have been carried out with cell types that can be readily obtained from patients — that is, lymphoblasts and fibroblasts — these are often not the affected cell types. Translational regulation differs by cell type, depending for instance on differences in sensitivity to stress and the cell-specific expression of components of the translational machinery or mRNAs. To understand the pathophysiology of the many diseases that we have described here, generation of new animal models will be of great importance and allow defects to be studied in the relevant cells and tissues.

It is likely that more disorders that result from inherited defects in mRNA translation remain to be identified. Because of the ease of sequencing the mitochondrial genome, most of the known mitochondrial disorders are caused by mutations in mtDNA[93]. However, oxidative phosphorylation disorders are most often inherited in an autosomal manner, suggesting that nuclear gene mutations have an important role in mitochondrial diseases[94]. Nuclear-DNA-encoded factors that are involved in mitochondrial translation have been predicted to be likely candidates for causing mitochondrial diseases[72]. For example, LBSL is the first known disease to be directly linked to mutations in a nuclear-DNA-encoded mitochondrial amino-acyl-tRNA synthetase. It is likely that mutations in other mitochondrial amino-acyl-tRNA synthetases and ribosomal proteins will be found to be associated with human disease.

Diseases that result from other kinds of defects in translation are also likely to be more prevalent than is currently realized. For example, reports of mutations that affect the control of translation of specific mRNAs are rare. This might reflect the fact that mutational analysis of disease-linked genes focuses on changes in mRNA coding regions, so that mutations in untranslated regions, which could affect the processing, translation and/or stability of the mRNA, are likely to be overlooked. Furthermore, the diseases mentioned here are predominantly monogenic diseases. Translation factors could also have a role in more common complex diseases, as has been suggested for Parkinson disease[95]: mtDNA mutations that impair mitochondrial translation or respiratory chain activity might modify the age of onset. mtDNA mutations might also contribute to neurodegeneration in Alzheimer disease[96]. Similarly, the above-mentioned mutations in UTRs might cause qualitative effects on protein levels, thereby contributing to complex disease. SNPs in these regions should therefore be considered in gene association studies.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
connexin 32 | CFTR | DARS2 | DKC1 | EIF2AK3 | GARS | GFM1 | MYC | ND6 | PUS1 | RMRP | RPS19 | SBDS | TUFM
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Alzheimer disease | anauxetic dysplasia | cartilage-hair hypoplasia | Diamond–Blackfan anaemia | LBSL | leukoencephalopathy with vanishing white matter | MELAS | MERRF | MLASA | Parkinson disease | Shwachman–Diamond disease | Wolcott–Rallison syndrome | X-linked Charcot–Marie–Tooth disease | X-linked dyskeratosis congenita
UniProtKB: http://ca.expasy.org/sprot
ATF4 | CDKN2A | CHOP | HNRPK | MRPL32 | MRPS16 | paraplegin | PERK | $p27^{Kip1}$ | RPS19 | RPS24 | thrombopoietin

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