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Competing interests statement
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SUPPLEMENTARY INFORMATION
See online article: S1 **(movie)**

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Inventory control: cytochrome *c* oxidase assembly regulates mitochondrial translation

David U. Mick, Thomas D. Fox and Peter Rehling

Abstract | Mitochondria maintain genome and translation machinery to synthesize a small subset of subunits of the oxidative phosphorylation system. To build up functional enzymes, these organellar gene products must assemble with imported subunits that are encoded in the nucleus. New findings on the early steps of cytochrome *c* oxidase assembly reveal how the mitochondrial translation of its core component, cytochrome *c* oxidase subunit 1 (Cox1), is directly coupled to the assembly of this respiratory complex.

The four respiratory chain complexes in the mitochondrial inner membrane use electrons from NADH or $FADH_2$ as energy to generate a proton gradient across the membrane that drives ATP production by the F_1F_o-ATP synthase (sometimes referred to as complex V). Electrons are passed through the redox systems of these complexes and are finally transferred to molecular oxygen by cytochrome *c* oxidase (complex IV). Thus, the energy of the oxyhydrogen reaction is used in an indirect way by the mitochondrial respiratory chain to produce ATP.

Three of the mitochondrial respiratory complexes (I, III and IV) and the F_1F_o-ATPase are made up of subunits of dual genetic origin. The majority of respiratory chain subunits are encoded by nuclear genes, the protein products of which are translated on cytosolic ribosomes and imported into mitochondria by the translocase of the outer membrane (TOM) and translocase of the inner membrane (TIM) import machineries[1,2] (FIG. 1). By contrast, the mitochondrial genome encodes only a small number of core complex subunits, most of which are exceedingly hydrophobic. In *Saccharomyces cerevisiae*, mitochondrial DNA (mtDNA) encodes seven subunits of those that make up complexes III, IV and V, whereas in humans, mtDNA encodes 13 subunits of those that make up complexes I, III, IV and V.

To express their small organellar genomes, mitochondria have maintained a transcription–translation apparatus during evolution, the components of which are mainly encoded by nuclear DNA and represent almost one-third of the mitochondrial proteome[3]. Transcription and translation apparently occur at the matrix side of the mitochondrial inner membrane. The protein oxidase assembly 1 (Oxa1), a member of a widely conserved family of translocases, is a main constituent of the mitochondrial protein export machinery, which facilitates the insertion of hydrophobic domains into the bilayer and the export of hydrophilic protein domains to the mitochondrial intermembrane space[4] (FIG. 1). The carboxy-terminal domain of Oxa1 binds to mitochondrial ribosomes[5,6] near their exit-tunnel[7,8], which is consistent with a co-translational mechanism for the insertion of mitochondrially synthesized proteins into the inner membrane. Oxa1 also assists the post-translational insertion of some cytoplasmically synthesized proteins into the inner mitochondrial membrane after their import into the matrix[9,10]. This is essential for membrane protein domains that do not sort laterally through the TIM23 complex.

It is unclear whether the mitochondrial import of cytoplasmically synthesized respiratory chain subunits is spatially coordinated with the export and insertion into the membrane of those synthesized inside mitochondria. It is clear, however, that respiratory complex subunits do not simply self-assemble in the inner membrane. The assembly of cytochrome *c* oxidase is the most well-studied system, in which many factors that assist at different steps of the assembly process have been genetically identified in yeast[11], and distinct assembly intermediates have been detected in yeast and human organelles (BOX 1).

The goal of this Progress article is to discuss new insights into the molecular mechanism of cytochrome *c* oxidase biogenesis and to highlight recent findings that link this assembly process to the regulation of the translation of its central subunit, cytochrome *c* oxidase subunit 1 (Cox1).

The cytochrome *c* oxidase complex

Cytochrome *c* oxidase shuttles electrons from cytochrome *c* to molecular oxygen to capture energy in the membrane potential by asymmetric proton uptake and proton pumping. The complex is assembled from 13 subunits in humans but 11 in *S. cerevisiae*. These subunits have a non-unified nomenclature (Supplementary information S1 (figure)). The three core subunits, Cox1, Cox2 and Cox3, which are encoded inside mitochondria, are highly conserved among all respiring organisms. The remaining subunits are encoded in the nucleus and imported into mitochondria from the cytosol. The overall dimeric structure of the bovine enzyme reveals that the redox cofactors haem and copper are inserted into the core proteins Cox1 and Cox2 (REF. 12) (Supplementary information S1 (figure)). Cox1 carries two haem molecules and a copper ion representing the CuB site, whereas the CuA site is formed by two copper ions in Cox2. The electrons from cytochrome *c* enter the enzyme from the CuA site in Cox2 and end at the CuB site that, together with the haem a_3 molecule, forms the active centre that is deeply embedded in the membrane. Little is known about the enzymatic function of Cox3. As the cytochrome *c* oxidase subunits that are encoded by the nucleus are less conserved than those that are encoded by mitochondria, the nomenclature differs depending on the organism (Supplementary information S1 (figure)). For simplicity, we will use the yeast nomenclature for cytochrome *c* oxidase proteins in this article.

Given the complexity of this multi-protein, multi-cofactor enzyme, and the fact that its subunits enter the assembly process from two sides of the mitochondrial membrane, it is not surprising that the assembly of cytochrome oxidase requires a large number of assembly factors. Although the functions of many assembly factors remain largely enigmatic, some factors have been assigned functions in distinct processes such as translational regulation, haem *a* synthesis and copper or haem insertion (Supplementary information S2 (table)).

One of the best-characterized and highly conserved assembly factors is the SURF1 homologue of yeast (Shy1). In *S. cerevisiae*, loss of Shy1 leads to a severe reduction of cytochrome oxidase and a growth defect on non-fermentable carbon sources[13]. Recent analyses support the idea that Shy1 (or its homologues in other organisms, for example, surfeit locus 1 (SURF1)) plays a part in the insertion step for haem a_3. However, it remains unclear whether Shy1 acts as a protein chaperone or has a more direct role in haem insertion, as is suggested by the haem-binding activity of a bacterial homologue[14,15].

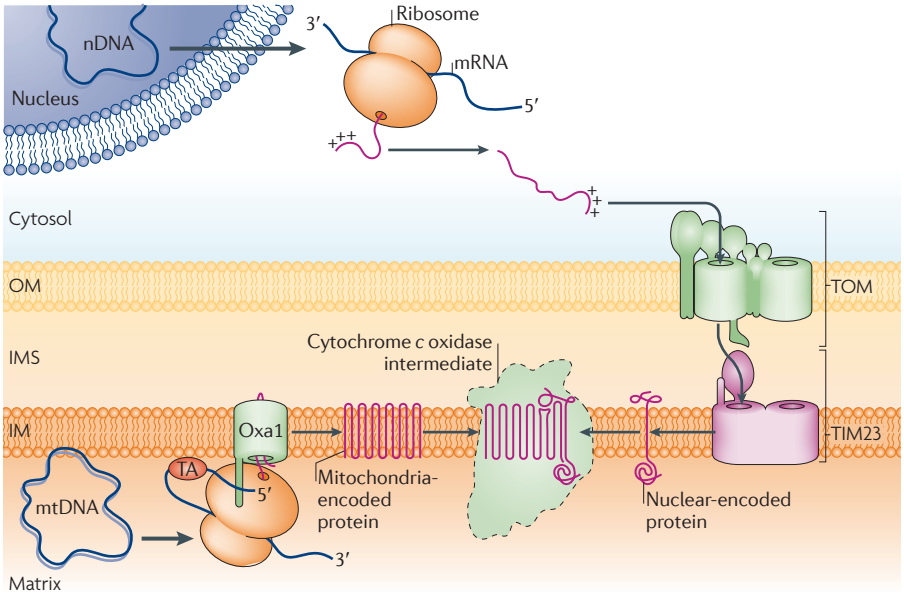


Figure 1 | **Dual origin of cytochrome *c* oxidase subunits.** Respiratory chain subunits are encoded by both nuclear DNA (nDNA), such as cytochrome *c* oxidase subunit 5 (Cox5), and mitochondrial DNA (mtDNA), such as Cox1. Nuclear-encoded proteins are translated on cytosolic ribosomes as precursor proteins that contain information that targets them for mitochondria (+++). Precursors are recognized and transported across the mitochondrial membranes by the translocase of the outer membrane (TOM) and the presequence translocase of the inner membrane (TIM23) complexes, which cooperate in this process. mtDNA is associated with the inner mitochondrial membrane (IM), and transcription of this DNA, as well as translation of the resulting mRNA, occurs in close proximity to the IM. Mitochondrial ribosomes translate mRNAs that are bound to the membrane by specific translational activators (TA), such as Pet309 in the case of mitochondria-encoded Cox1. The translocase oxidase assembly 1 (Oxa1) inserts nascent polypeptides in a co-translational manner into the membrane and helps these proteins to attain their correct topology. After the insertion of both mitochondria- and nuclear-encoded proteins into the membrane, these subunits can assemble into a functional complex. IMS, intermembrane space; OM, outer mitochondrial membrane.

Genetic and biochemical studies in yeast, taking Shy1 as a starting point, have led to the identification of several new factors involved in cytochrome *c* oxidase assembly (COA)[16–20], including Coa1–Coa4. Although Coa2 and Coa4 appear to cooperate with Shy1 for haem insertion[16,19,21], Coa1 and Coa3 link Shy1 functionally to early assembly intermediates of Cox1. In the following sections we focus on the factors and processes that link the translation of the central subunit Cox1 to these early assembly intermediates.

From mitochondrial mRNA to protein

Translation of mitochondria-encoded mRNAs occurs on mitochondrial ribosomes that are bound to the inner membrane, where they interact with several membrane proteins[5,6,22–25]. The intimate link between mitochondrial translation and the inner membrane is underlined by the protein synthesis defects observed in mitochondria deficient in anionic phospholipids[26].

In *S. cerevisiae*, translation of mitochondria-encoded mRNAs specifying subunits of respiratory complexes requires dedicated translational activator proteins, which recognize the 5′ untranslated regions (UTRs) of their cognate mRNAs[27] (BOX 1). In the case of cytochrome *c* oxidase subunits, Pet309 and the mitochondrial splicing suppressor protein 51 (Mss51) act as translational activators for *COX1* mRNA (FIG. 2); Pet111 is required for *COX2* mRNA translation; and Pet54, Pet122 and Pet494 together promote the translation of *COX3* mRNA. Although these functions can be separated genetically in *S. cerevisiae* mutants, in the wild type the activators are actually associated with each other and are believed to colocalize the translation of these cytochrome *c* oxidase mRNAs[28,29]. Indeed, the 5′ UTRs of the *COX2* and *COX3* mRNAs contain topogenic information that is presumably recognized by their activator proteins[30], which direct the mRNAs to the membrane where subsequent translation occurs.

Little is known about the biochemical mechanism of mitochondrial translational

Box 1 | **The cytochrome *c* oxidase assembly line**

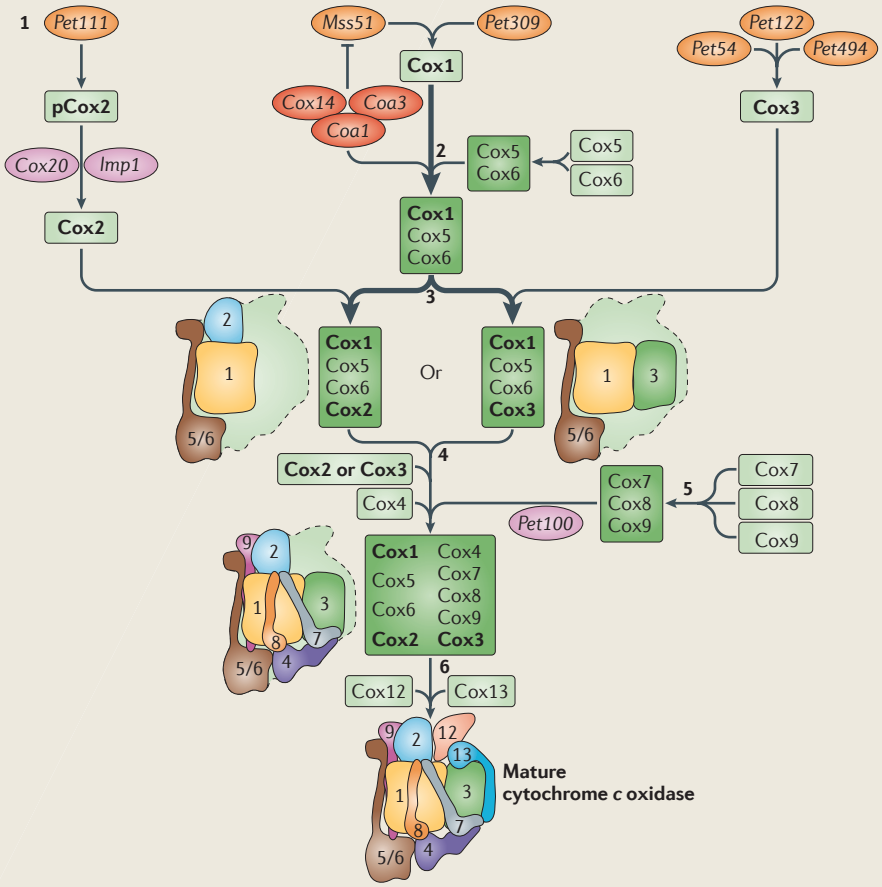
The mRNAs coding for the mitochondria-encoded respiratory chain subunits cytochrome *c* oxidase subunit 1 (Cox1), Cox2 and Cox3 (bold font) have specific translational regulators (orange ovals) that have been defined in yeast and assembly factors (pink and red ovals), such as Cox14, cytochrome *c* oxidase assembly 1 (Coa1) and Coa3, that positively or negatively regulate translation (see the figure, part **1**). The remaining cytochrome *c* oxidase subunits are encoded by the nucleus.

Mitochondrial splicing suppressor protein 51 (Mss51) inactivation by the Cox14–Coa1–Coa3 complex (red ovals) shuts off Cox1 translation, preventing the assembly of cytochrome *c* oxidase (see main text). In yeast, Cox2 is translated as a precursor (pCox2) with an amino-terminal 15 amino acid extension. Both termini are transported across the membrane[4] , pCox2 is processed by the mitochondrial inner membrane peptidase subunit 1 (Imp1) and Cox2 is kept in an assembly-competent state by Cox20 (REF. 63).

Next, Cox1 assembles with the first nuclear-encoded subunits, Cox6 and Cox5 (REFS 47–49) (part **2**). Whether this association occurs before or after the insertion of cofactors into Cox1 is unclear. Mutant analyses revealed that the Cox1–Cox5–Cox6 complex can subsequently form different assembly intermediates in the absence of either Cox2 or Cox3 (part **3**). A proposed Cox1–Cox5–Cox6–Cox2 intermediate was identified in human patients lacking Cox3 (REF. 64), whereas a Cox1–Cox5–Cox6–Cox3 intermediate has been characterized in yeast *cox2* mutants[48]. Copper insertion into Cox2 is a prerequisite for its association with the Cox1–Cox5–Cox6 complex[65], whereas incomplete cytochrome *c* oxidase lacking Cox2 was found to associate with complex III of the respiratory chain[18].

Little is known about the sequence of events that follow the association of Cox2 and Cox3 with the Cox1–Cox5–Cox6 complex, as no stable assembly intermediates have been found (part **4**). However, Cox7, Cox8 and Cox9 form a complex before their incorporation, which is mediated by Pet100 (REF. 66) (part **5**), although the arrangement of this subcomplex is difficult to reconcile with the structure of mature cytochrome *c* oxidase.

The incorporation of Cox12 and Cox13 concludes the formation of the complex (part **6**). However, neither is essential for the enzymatic activity of the complex, which remains stable in their absence[67,68]. Light green boxes denote structural subunits and dark green boxes denote reported assembly intermediate complexes. Schematic drawings represent assembly intermediates in a structural context. The subunits are numbered after their respective protein (for example, 1 stands for Cox1) and are colour-coded as in Supplementary information S1 (figure); for simplification, the Cox5–Cox6 subcomplex (5/6) is shown in brown.



activation but the translational activators of Cox1 have been found to be involved in a regulatory feedback cycle. The *COX1* mRNA activator Pet309 is a member of the pentatricopeptide repeat (PPR) protein family, which is often associated with RNA metabolism[31]. Complete loss of Pet309 prevents the translation and stabilization of *COX1* mRNA, whereas mutations in the PPR motifs selectively affect translation[31,32]. The second *COX1*-specific activator, Mss51, is a multifunctional pioneer protein, the sequence of which does not contain any known functional domains or motifs. One target of action, which it shares with Pet309, is the 5′ UTR of the *COX1* mRNA[33,34]. However, Mss51 also has a second target that maps genetically to the *COX1* open reading frame (ORF) and allows it to promote Cox1 synthesis and/or assembly[35]. Moreover, Mss51 interacts with newly synthesized but unassembled Cox1. Thus, Mss51 appears to interact both with *COX1* mRNA and with Cox1 protein that has not been assembled into the cytochrome *c* oxidase complex. These dual activities allow Mss51 to couple the synthesis of Cox1 to the assembly of cytochrome *c* oxidase.

Regulation of *COX1* translation

The synthesis of Cox1 by budding yeast mitochondrial ribosomes is reduced by mutations in most other genes encoding proteins that are involved in cytochrome *c* oxidase assembly (for example, Cox2 maturation). This suggests the existence of a regulatory feedback mechanism coupling *COX1* translation to cytochrome *c* oxidase assembly[36]. Such a mechanism would ensure that Cox1, the core platform for cytochrome *c* oxidase assembly, is synthesized at an appropriate rate. This is crucial, given that certain unassembled Cox1 species are deleterious to cells because they act as pro-oxidants[37]. The dual activities of Mss51 appear to place it at the nexus of an assembly feedback regulatory system in which the levels of Mss51 available to activate Cox1 synthesis are limited by the amount of Mss51 sequestered by Cox1 protein in cytochrome *c* oxidase assembly intermediate complexes[17,18,33,35,36].

Sequestration of Mss51 into Cox1 complexes. It is unclear whether Mss51 molecules that interact with the *COX1* mRNA 5′ UTR are transferred directly (in *cis*) to nascent Cox1 chains emerging from the associated ribosomes. However, when the *COX2* mRNA 5′ UTR is placed upstream of the *COX1* ORF, Mss51 can still interact with Cox1 in *trans*[33]. This suggests that Mss51 is

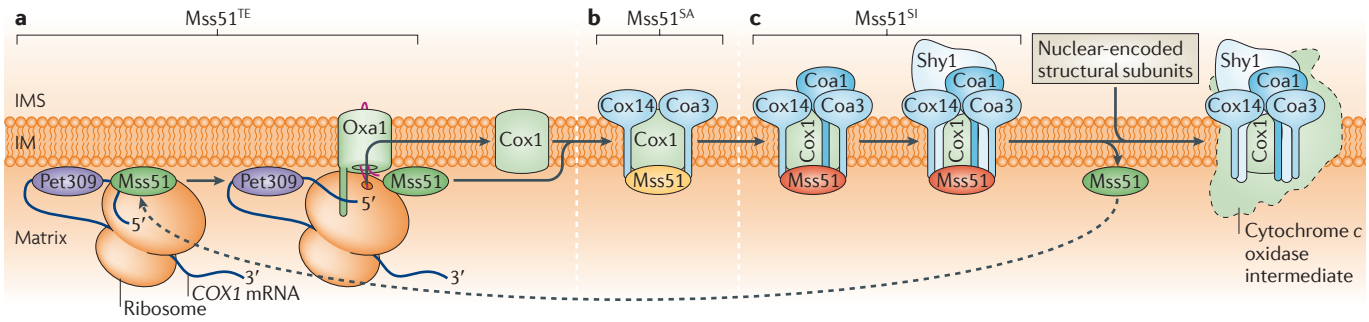


Figure 2 | **Mechanistic model for the translational regulation of Cox1.** Mitochondrial splicing suppressor protein 51 (Mss51) regulates the translation of cytochrome *c* oxidase subunit 1 (Cox1) by interacting with *COX1* mRNA and Cox1 protein that has not yet been assembled into the mature cytochrome *c* oxidase complex. **a** | Cox1 is synthesized by mitochondrial ribosomes upon activation by the translational activators Pet309 and Mss51. Here, Mss51 is in a translation-effective state ($Mss51^{TE}$; shown in green). Cox1 is inserted into the inner mitochondrial membrane (IM) co-translationally by the oxidase assembly 1 (Oxa1) translocase. **b** | Mss51 is also required for Cox1 translation independently of the 5′ untranslated region (UTR). It interacts with newly synthesized Cox1 and the transmembrane proteins Cox14 and cytochrome *c* oxidase assembly 3 (Coa3), which promote the Cox1–Mss51 interaction. In this sequestered active state ($Mss51^{SA}$; shown in yellow), which has been observed in cells lacking the assembly factor Coa1, Mss51 may be able to initiate further rounds of Cox1 synthesis on mitochondrial ribosomes (not shown) or it might be loosely associated with this complex so that some Mss51 can be released to activate translation. **c** | The association of Coa1 with the Cox1–Mss51–Cox14–Coa3 complex in wild-type cells converts Mss51 to a sequestered inactive state ($Mss51^{SI}$; shown in red), which prevents it from activating translation. Moreover, Coa1 association promotes the binding of the SURF1 homologue of yeast (Shy1) to Cox1, which might positively regulate the insertion of the haem cofactor into Cox1. It is currently debated whether Shy1 and Mss51 exist in the same complex with Cox1, as different experimental approaches lead to opposite results. Subsequent addition of further, nuclear-encoded subunits of cytochrome *c* oxidase, such as Cox6, leads to the release of Mss51 from the assembly intermediates, which allows further rounds of Cox1 synthesis. The mitochondrial heat shock protein 70 (Hsp70) chaperone Ssc1, which has been suggested to associate with Mss51 in the 'TE' and in the 'SI' states, is not shown. IMS, intermembrane space.

involved in Cox1 biogenesis independently of its ability to bind to the 5′ UTR of *COX1* mRNA. In any event, newly synthesized Cox1 also rapidly associates with Cox14 and Coa3, two small inner membrane proteins with domains that are exposed to the mitochondrial matrix and the intermembrane space[20,36,38,39]. It should be noted that Fontanesi *et al.* referred to Coa3 as Cox25 in a later study[39]. Newly synthesized Cox1 promotes the dynamic association of Mss51, Cox14 and Coa3; these proteins then form a complex with Cox1 that is thought to keep Mss51 in a sequestered active state ($Mss51^{SA}$) (FIG. 2). Based on this, it is tempting to speculate that Mss51 in this complex can associate with ribosomes. In the absence of Cox1 synthesis, interactions between Mss51 and Cox14 or Coa3 are not detected and the interaction between Cox14 and Coa3 is severely reduced, indicating that these factors only associate upon insertion of Cox1 into the membrane[20,33]. Furthermore, Cox14 and Coa3 are both required to ensure a stable interaction between Mss51 and newly synthesized Cox1 (REFS 20,33). This association is apparently required to prevent Mss51 from activating Cox1 synthesis, as *cox14Δ* and *coa3Δ* mutants both exhibit hyperactive Cox1 synthesis that is uncoupled from cytochrome *c* oxidase assembly[20,36]. However, Cox1 synthesized in the absence of Cox14 or Coa3 is rapidly degraded before it reaches a state in which it can act as a pro-oxidant, indicating that these assembly factors stabilize Cox1. The degradation process is likely to involve AAA proteases of the inner membrane[40,41] or the metalloendopeptidase Oma1 (REFS 16,21), as it has been shown that the pro-oxidant Cox1 is formed in Coa2-deficient cells, and that the degradation of Cox1 in these cells is dependent on Oma1 and AAA proteases. The necessity for the cell to maintain a pool of unassembled Cox1 for complex assembly on the one hand, and to degrade a pro-oxidant form of Cox1 on the other, leads to the idea that different activities for Cox1 degradation may exist that are regulated by its assembly state.

Interestingly, the mitochondrial heat shock protein 70 (Hsp70) chaperone Ssc1 is also associated with Mss51 in both a binary complex comprising Mss51 and Ssc1 as well as in larger complexes that also contain Cox1, Coa3 and Cox14 (REFS 39,42). Synthesis of all three mitochondria-encoded cytochrome *c* oxidase subunits was reduced in *ssc1* mutants[43]. However, Ssc1 does not associate with newly synthesized Cox1 but instead does so with the mitochondria-encoded proteins ATP synthase subunit 9 (Atp9) and Var1 (REF. 43). Although it is difficult to imagine Ssc1 having a role in the folding of the 12 Cox1 transmembrane domains, it could affect the structure of the C-terminal domain of Cox1, which is exposed to the mitochondrial matrix.

A role for Cox1 in its own regulation was directly shown by generating mutations that truncated the Cox1 C terminus by 11 or 15 residues[44]. These mutations did not prevent assembly of active cytochrome *c* oxidase. However, they did eliminate assembly feedback regulation of Cox1 synthesis and weaken the interaction between Mss51 and Cox14 (the interaction of Mss51 with Coa3 was not tested). Thus, the C-terminal domain of Cox1 appears to participate in Mss51 sequestration upon its emergence from the ribosome.

Mss51 inactivation by assembly intermediates. The current models for the regulation of Cox1 synthesis propose that physical sequestration of Mss51 into assembly intermediate complexes containing newly synthesized Cox1, Cox14, Coa1 and Coa3 prevents it from activating *COX1* mRNA translation. Steps in this important pathway have been inferred by the examination of assembly intermediate complexes in solubilized extracts of wild-type and mutant mitochondria from *S. cerevisiae* using pull-down experiments, blue native gel electrophoresis and sedimentation[14,17,18,20,42,44]. These data have generated a hypothetical framework for early events, but not a consensus on the pathway (FIG. 2). Furthermore, it is conceivable that, in addition to sequestration, Mss51 translational activity may be altered through structural changes.

In the absence of Coa3 or Cox14, Mss51 does not associate with Cox1 or any other factors except Ssc1, and may be in a fully translation-effective form ($Mss51^{TE}$). Coa1, an additional player in the regulatory cycle, associates with Cox1, Coa3, Cox14 and Mss51 (REFS 17,18). $Mss51^{SA}$ accumulates in the absence of Coa1. In *coa1* mutants, most of the detectable Mss51 is in this complex[14,20], but Cox1 synthesis is neither reduced nor subject to normal assembly feedback inhibition[17,18]. This suggests that in a complex lacking Coa1, Mss51 may not be fully inactivated and that binding of Coa1 is required to generate sequestered, translationally inactive Mss51 ($Mss51^{SI}$). Interestingly, Coa1 is not absolutely required for cytochrome *c* oxidase assembly, as the *coa1Δ* phenotype can be suppressed through an unknown mechanism by adding copper to the growth medium[17], and minute amounts of mature cytochrome *c* oxidase can be formed in the mutant[18].

In the absence of a kinetic analysis of Cox1 biogenesis, the formation of $Mss51^{SA}$ and $Mss51^{SI}$ as distinct pathway intermediates in wild-type *S. cerevisiae* remains hypothetical. However, the currently available data are consistent with such a stage model: at steady state, Mss51 complexes that could correspond to $Mss51^{TE}$ and $Mss51^{SI}$ are detectable[14,16,20,42]. Thus, under normal conditions, Mss51 is likely to exist in different states, the equilibrium of which can be shifted according to the requirements for newly synthesized Cox1.

Connecting Mss51 release to complex assembly. The mechanism that promotes Mss51 release from assembly intermediate complexes remains enigmatic, although data suggest that Shy1 might contribute to this process. The presence of Coa1 in the $Mss51^{SI}$ complex promotes the recruitment of Shy1 to Cox1 (REFS 14,17,18). Moreover, a lack of Shy1 traps Mss51 together with Coa1 in the sequestered-inactive state[17]. Shy1, and its homologues in other species, are likely to play a part in the maturation of functional Cox1 through incorporation of haem a_3 (REFS 14,15,45), either directly[15] or by a chaperone-like activity that maintains Cox1 in an assembly competent state[14,18]. Mutants lacking Shy1 display reduced Cox1 synthesis. This phenotype is partially rescued by the missense mutations *mss51-T167R* and *mss51-F199I*, or by Mss51 overexpression[36,46]. Moreover, recent studies have shown that overexpression of certain nuclear-encoded cytochrome *c* oxidase subunits also restores growth of *shy1Δ* mutants on nonfermentable medium[47]. Thus, the absence of Shy1 appears to produce a bottleneck in complex assembly that sequesters Mss51, but Shy1 itself may not be necessary for Mss51 release. Interestingly, deletion of the nuclear *COX6* gene caused the greatest reduction in Cox1 synthesis among a set of cytochrome *c* oxidase mutants, as judged by pulse-labelling and by the expression of a fused reporter gene[44]. This suggests that the addition of Cox6, which along with Cox5 is thought to be the first subunit to assemble with newly synthesized yeast and human Cox1 (REFS 48,49), may be a key step in the ejection of Mss51 from assembling complexes.

Cox1 in humans and disease

The malfunction of cytochrome *c* oxidase has severe implications for cellular energy metabolism and causes increased production of reactive oxygen species with a variety of deleterious consequences in humans[50]. A number of severe disorders, collectively referred to as mitochondrial encephalomyopathies, are caused by cytochrome *c* oxidase deficiency resulting from mutations in mitochondrial genes encoding structural subunits or impaired assembly of the complex due to nuclear mutations affecting assembly factors. The high demand of certain tissues, such as neurons and muscles, for oxidative phosphorylation apparently explains why mitochondrial encephalomyopathies are associated with muscle and nervous system dysfunction.

Several assembly factors functioning in yeast have human orthologues[11] (Supplementary information S2 (table)). For example, defects in the human copper chaperones SCO1 and SCO2, which were first identified in yeast, cause a severe cytochrome *c* oxidase deficiency and death shortly after birth[51,52]. Other cytochrome *c* oxidase deficiencies cause Leigh syndrome, a neurological disorder with bilaterally symmetrical lesions in subcortical brain regions. The most frequent causes of this disorder are mutations affecting the SURF1 protein[53,54], which is associated with the early steps of Cox1 biogenesis. However, factors clearly implicated in the translational regulation of Cox1 were only identified in mammals very recently.

As mammalian mitochondrial mRNAs lack significant 5′ UTRs, mechanisms regulating human Cox1 synthesis must differ from those used in budding yeast. However, an activity similar to that of yeast Mss51, the binding of which to newly synthesized Cox1 allows continued synthesis and/or assembly[35], could be present in mammals and couple cytochrome *c* oxidase assembly to Cox1 synthesis. Although no clear Mss51 orthologue is encoded in mammalian genomes, the mammalian protein zinc finger MYND domain-containing 17 (ZMYND17), of unknown function, is significantly similar.

In any event, post-transcriptional regulation appears to be a feature of mammalian mitochondrial gene expression, based on studies of human patients with Leigh syndrome and cytochrome *c* oxidase deficiency. The French-Canadian variant of this disease is caused by mutations affecting the nuclear gene for the Leu-rich PPR motif-containing protein (*LRPPRC*; also known as *LRP130*), which encodes a mitochondrial protein that is distantly related to yeast Pet309 (REFS 55,56). LRPPRC is an RNA-binding protein[57] that is associated in a mitochondrial ribonucleoprotein with the SRA stem-loop-interacting RNA-binding protein (SLIRP) and, at least, the mitochondria-encoded *COX1* and *COX2* mRNAs[58]. Reduced LRPPRC levels cause lower steady-state levels of most mitochondrially encoded mRNAs and a selective reduction in cytochrome *c* oxidase levels[58]. More recently, analyses of a Leigh syndrome patient revealed a specific reduction in Cox1 synthesis owing to a mutation in a gene named translational activator of cytochrome *c* oxidase 1 (*TACO1*), the protein product of which appears to be a *COX1*-specific translational activator in humans[59]. The identification of TACO1 highlights the importance of human genetics in identifying missing assembly factors and shows that similar mechanisms of translation control may regulate the synthesis of respiratory complex subunits in phylogenetically diverse eukaryotes.

Perspectives

Coupling of organellar gene expression at the level of translation to the assembly of organellar gene products into energy-transducing complexes may be a widespread phenomenon. In yeast mitochondria, translation of the bicistronic mRNA encoding the hydrophobic Atp6 and Atp8 subunits of the F_0 sector of ATP synthase was recently shown to depend on the presence of soluble F_1 sector subunits α and β, or a partially assembled complex containing them[60]. Although the mechanism of this regulation is unclear, it may prevent the formation of membrane subassemblies containing Atp6 and Atp8 that could dissipate the membrane potential if they were not assembled into a complex with F_1. In chloroplasts of

Chlamydomonas reinhardtii, translation of organellar-encoded mRNAs specifying certain key subunits of photosystem I, photosystem II and the cytochrome b_6f complex is also coupled to the assembly of those complexes[61]. In parallel to the mechanism proposed for Mss51 function in yeast, assembly feedback regulation of cytochrome *f* synthesis depends on C-terminal residues of cytochrome *f* itself, which could interact with a protein that also regulates translation[62].

More detailed understanding of assembly feedback control in the mitochondria of model organisms and humans may emerge from further study of the sequences in which assembly intermediates form, their precise compositions and structure and the interaction dynamics of their constituents. Clearly, a more biochemical understanding will require the development of a true mRNA-dependent *in vitro* translation system derived from mitochondria, as opposed to the current methods of studying translation in isolated intact organelles.

An important health-related goal is to elucidate control mechanisms that operate in human mitochondria. The identification of as-yet-undiscovered nuclear-encoded components that regulate respiratory chain assembly and dynamics may emerge from large-scale gene inactivation studies in cultured cells. The study of their mitochondria-encoded regulatory targets would be advanced by the development of methods to genetically transform and alter animal mitochondrial genomes in a directed fashion.

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Competing interests statement
The authors declare no competing financial interests.

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