Mitochondrial Biogenesis: Cell-Cycle-Dependent Investment in Making Mitochondria

Mitochondria cannot be made *de novo*, so pre-existing mitochondria must be inherited at each cell division. A new study demonstrates cell-cycle-dependent regulation of the activity of the TOM translocase complex to induce mitochondrial biogenesis during the M phase of the cell cycle.

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In all eukaryotes, pre-existing mitochondria are used as templates to build more mitochondrial mass, ahead of cell division or in response to increased metabolic demand [1–8]. Mitochondrial biogenesis requires the import of up to 1,000 different proteins into the organelle, and all proteins imported across the outer mitochondrial membrane enter through a protein translocase called the TOM complex [2]. This complex is a remarkable nanomachine composed of a core formed by the β-barrel channel Tom40 and additional subunits, each of which has a single α-helical transmembrane segment.

Four of the subunits surrounding the Tom40 β-barrel — Tom5, Tom6, Tom7 and Tom22 — are tail-anchored proteins [2]. For Tom7 and Tom22 it is certain that they were present in the ancestral TOM complex, early in the evolution of mitochondria [9]. The other two tail-anchored proteins, Tom5 and Tom6, are attached onto newly integrated Tom40 molecules at an early step in the assembly of the TOM complex into the outer membrane [10], a process catalyzed by the sorting and assembly machinery (the SAM complex) [1,2,5,6,10]. A new study by Harbauer *et al.* [11] now demonstrates that regulation of the activity of the TOM complex, and thus mitochondrial protein import, is coordinated with the cell cycle.

The yeast *Saccharomyces cerevisiae* has served as a key experimental model for diverse aspects of cell biology, including the discoveries on the cell cycle that led to a Nobel Prize [12]. *S. cerevisiae* has two mating types — **a** and α — and yeast strains of mating type **a** can be arrested in the G1 phase of the cell cycle by treatment with the mating pheromone α-factor. A culture of yeast that has been synchronized in this way will progress together through the phases of the cell cycle and can be sampled at regular intervals to determine cell-cycle-dependent phenotypes. A genome-wide analysis of the dynamic transcriptome in the cell cycle using this strategy [13] revealed that, of the various components of the TOM complex, only the mRNA for Tom6 increases during M phase. This change in transcript levels could be a result of a cell-cycle-dependent increase in transcription of the *TOM6* gene, or a result of a post-transcriptional mechanism that reduces the turnover of the *TOM6* mRNA in a cell-cycle-dependent manner. Harbauer *et al.* [11] found that there was a very clear increase in the steady-state level of Tom6 protein in the M phase of the cell cycle.

How is this increase in Tom6 protein level enacted? The simplest explanation is that the increased *TOM6* mRNA levels give rise to increased translation of Tom6 protein (Figure 1). While this is indeed true, the steady-state level of membrane proteins also depends on the efficiency with which the very hydrophobic protein can be targeted to the correct membrane (in the case of Tom6, this means reaching the mitochondrial surface), as well as the efficiency with which it is assembled into the membrane (in this case, the mitochondrial outer membrane). There is a growing awareness of the mechanisms involved in controlling the efficiency of targeting and assembly of mitochondrial membranes, including the action of the mRNA-localizing

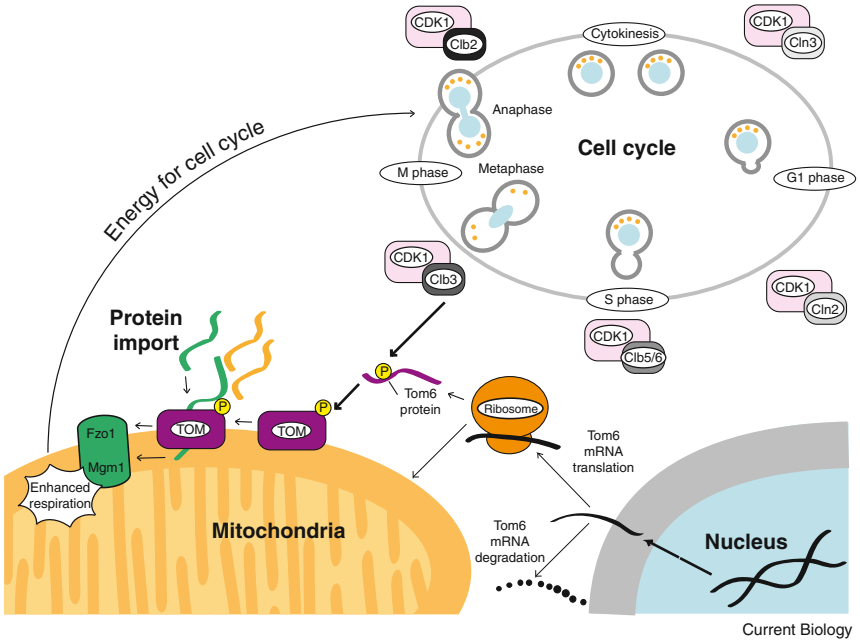

Figure 1. Cell-cycle-dependent regulation of Tom6 and energy production.

In M phase, changes in transcription and/or mRNA degradation lead to an increased level of mRNA encoding Tom6. There is an increase in the level of Tom6, which might be due to increased engagement of ribosomes and/or improved access to the mitochondrial surface, and also an increased phosphorylation of the Tom6 protein. The phosphorylation reaction is induced in M phase through activation of CDK1 by the cyclin Clb3. The phosphorylated form of Tom6 enhances the steady-state level and activity of the TOM complex in the mitochondrial outer membrane, promoting protein import. This includes an M-phase-specific increase in the relative abundance of the regulatory GTPases Fzo1 and Mgm1 in mitochondria, enhancing respiration-driven energy production by mitochondria.

protein Puf3 (the mRNA encoding Tom6 has a Puf3-binding site) [14] and localized translation of membrane proteins at the mitochondrial surface [15]. In the new study, Harbauer *et al.* [11] now show that, in the case of Tom6, a key regulatory mechanism is exerted through the cyclin-dependent kinase CDK1, a master regulator of the cell cycle in all eukaryotes, with CDK1 mediating cell-cycle-dependent phosphorylation of Tom6.

Previously, Schmidt *et al.* [16] discovered and catalogued phosphorylation sites in the various components of the TOM complex, including the serine residue at position 16 in Tom6. Some of these phosphorylation events are mediated by casein kinase 2 and the cAMP-dependent protein kinase in response to various environmental stimuli. Harbauer *et al.* [11] developed biochemical assays to directly demonstrate that the phosphorylation of Tom6 is mediated by CDK1, and that phosphorylated Tom6 is assembled into the mitochondrial outer membrane with increased efficiency (Figure 1). What's more, the increased levels of Tom6 serve to promote an increased assembly of Tom40 into the outer membrane: in this regard, the steady-state level of Tom6 serves as a rheostat to 'dial-up' or 'dial-down' the amount of TOM complex carried by mitochondria. This rheostat is in turn under the control of the cyclin Clb3, which is the specific trigger to activate CDK1 to phosphorylate Ser16 of Tom6. Clb3 expression is tightly controlled to be active only in M phase [17,18].

The compelling findings from this paper rely on several lines of investigation. In addition to the classic use of cell synchronization to produce extracts to monitor protein levels and to purify active mitochondria for *in vitro* analysis, the authors have made use of phosphomimetic forms of Tom6 in both biochemical assays and *in vivo* phenotypic analysis. For *in vivo* studies, there is great utility in the use of a glutamate substitution in place of a serine to mimic the phosphorylated form of the protein of interest. Likewise, an alanine or valine substitution for serine can be useful as a proxy for a non-phosphorylated protein form. In the new study [11], expression of the phosphomimetic form of Tom6, Tom6(S16E), revealed that the protein was imported with increased efficiency, accumulated to higher steady-state levels and promoted the increased level and activity of the TOM complex, mimicking the effect of phosphorylated Tom6. In a yeast strain expressing a non-phosphorylatable form of Tom6, Tom6(S16A), this protein was imported with decreased efficiency, was present at lower steady-state levels and failed to promote the activity of the TOM complex, mimicking the effect of constitutively de-phosphorylated Tom6.

Since the TOM complex is the gateway for protein import into mitochondria, higher levels of the TOM complex can open the gates for the import of more protein, to build more mitochondria. The paper by Harbauer *et al.* [11] makes a further contribution to our understanding of cell-cycle-dependent regulation of mitochondrial biogenesis. By specifically looking at proteins increased 'per unit mitochondria', a remarkable increase was seen in two regulatory GTPases, Fzo1 and Mgm1, which play a role in mitochondrial membrane fusion events that can increase cellular ATP production even further [19].

Now, the stage is set to approach a detailed, systems-level understanding of 'why' and 'how' mitochondrial biogenesis is kicked into top gear. This matters in cell biology. The primary function of mitochondria in most organisms is to maximize the returns in energy currency from investments made in securing carbon from the environment. Via the action of mitochondria, carbon derived from sugars and fats can be used to produce a maximal amount of ATP, but, at a systems level, there is a great paradox in the use of this organelle. To translate the thousand or so proteins needed to build mitochondrial mass and to transport, fold and assemble these proteins into a mitochondrion costs an enormous amount of ATP. It is also costly, in term of ATP consumption, to replace the electron-transport complexes burnt out by oxidative damage in the course of ATP production [19], and to replicate the mitochondrial DNA and transcribe the mitochondrial RNAs that encode several of the electron-transport proteins [20]. It is little wonder then that the amount of mitochondrial mass accumulated in each round of the cell cycle would be judiciously controlled so as to be just sufficient to ensure maximum dividends to the cell's energy investments.

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http://dx.doi.org/10.1016/j.cub.2014.12.006