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Regulation of Organelle Biogenesis

Review

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One hallmark of eukaryotic cells is that they contain many functionally distinct membrane-bounded compartments, or organelles. Each organelle carries out highly specialized functions because it possesses its own unique combination of proteins, lipids, and cofactors. The endoplasmic reticulum (ER), mitochondria, and peroxisomes each have a unique set of receptors that allow them to select faithfully their characteristic set of proteins. Although there are only a few initial entry points from the cytosol into subcellular organelles, many proteins are sorted and distributed further before reaching their final destinations. The ER, for example, serves as entry point into all compartments on the secretory and endocytic pathways (Palade, 1975), and proteins entering the mitochondria are subsequently distributed into mitochondrial subcompartments. There is, however, no protein traffic among the ER (or compartments of the secretory pathway), the mitochondria, and the peroxisomes, possibly because each of the three organelles is of distinct evolutionary origin (Blobel, 1980). Nevertheless, all three of these organelles coexist in the cell, and their functions must be coordinated and adapted to cellular needs. Interorganellar communication is established via metabolites that can move between organelles. Such metabolic communication renders organelles dependent upon one another for their function and requires that their biogenesis and turnover be coordinated so that homeostasis is achieved.

De novo organelle synthesis is never required; organelles always grow by proliferation of preexisting organelles. Thus, the templates that allow their proliferation are maternally inherited by endowing each daughter cell with a complete set of organelles during cell division (Palade, 1983; see accompanying review by Warren and Wickner). The amount and composition of a given organelle, however, is not fixed but has to be responsive to the particular needs of the cell. An increased demand for energy upon repeated muscle contraction, for example, results in proliferation of mitochondria (Hood et al., 1994). In many known examples, this regulation is achieved by transcriptional networks that respond to a change in demand for the function of a particular organelle with an appropriate modulation of expression of genes encoding organellar proteins.

In some cases, the signals that influence organellar biogenesis originate from inside the cell or even from within the organelle itself; in other cases, the signals originate from extracellular locations and may result from developmental cues or changes in nutrient availability. The net result of regulation of organelle biogenesis can be seen clearly when different cell types within an organism are compared. In mammals, for example, the ER is vastly abundant in exocrine pancreatic secretory cells, where the demand for the secretion is enormous, whereas the ER is virtually absent in reticulocytes, where there is no appreciable demand for the secretion of any protein (Bolender, 1974). Using representative examples primarily from the yeast Saccharomyces cerevisiae, we highlight in this review some recent insights into the biogenesis of organellar proteins and lipids and discuss some of the known mechanisms that are involved in regulation of organelle biogenesis.

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Organelle Protein Targeting Occurs by Redundant Pathways

Proteins are targeted to organelles by signal sequences (Blobel and Sabatini, 1971). These sequences are recognized by specific targeting factors that mediate their delivery to the appropriate organelle. In most cases, soluble cytosolic targeting factors work together with receptors bound to the surface of the organelles. These components pass the proteins to a translocation apparatus in the organellar membrane, which in turn catalyzes the movement of the protein across the membrane into the organelle or, as it is the case for integral membrane proteins, catalyzes their proper membrane integration.

Recent developments have shown that targeting of proteins to organelles is more complicated than was originally appreciated. It is now clear that, in the case of the ER, mitochondria, and peroxisomes, more than one targeting pathway exists for each organelle. There are at least two pathways that deliver proteins to the ER: one pathway utilizes the signal recognition particle (SRP) and its receptor to deliver nascent proteins as they emerge from ribosomes and thus operates by a cotranslational mechanism (Hann and Walter, 1991; Ogg et al., 1992; Walter and Johnson, 1994); the other pathway utilizes the cytosolic chaperones hsp70 and eukaryotic DnaJ homologs to deliver completely synthesized proteins after they are released from ribosomes and thus operates by a posttranslational mechanism (Hansen et al., 1986; Rothblatt and Meyer, 1986; Waters and Blobel, 1986). The role of SRP and SRP receptor is to target ribosomes synthesizing the appropriate proteins to the ER, whereas the presumed role of the chaperones is to maintain the proteins partially unfolded to facilitate their subsequent translocation. There are also at least two targeting pathways to mitochondria (Ryan and Jensen, 1995; Lithgow et al., 1995). For this organelle, both pathways are posttranslational: one is dependent upon the recently identified cytosolic factor MSF (for mitochondrial import–stimulating factor) and the outer membrane complex Mas70p–Mas37p (Gratzer et al., 1995; Hachiya et al., 1995; Söllner et al., 1990), and the other is dependent on the outer membrane receptor complex Mas20p–Mas22p (Kiebler et al., 1993; Lithgow et al., 1994). Similarly, multiple targeting routes to peroxisomes are known, each using a different receptor protein (Rachubinski and Subramani, 1995; Purdue and Lazarow, 1994; Subramani, 1993).

For all three organelles, the available pathways seem to operate in parallel (but see, for example, Lithgow et al., 1995). Although most proteins are routed preferentially into one of the targeting pathways, they are at least partially redundant, because most proteins can be targeted, albeit often inefficiently, by an alternative route. Yeast cells, for example, can live in the complete absence of SRP and SRP receptor, although the cells grow more slowly (Hann and Walter, 1991; Ogg et al., 1992). Therefore, functional redundancy can provide a fail-safe mechanism, in case one pathway fails or one pathway becomes saturated under certain physiological conditions. In these situations, cross-talk between the different pathways may regulate their activity in response to an increase in demand. This type of regulation occurs for ER targeting, where cells adapt to a loss of SRP function, in part, by up-regulating the expression of cytosolic chaperones (Arnold and Wittrup, 1994).

There are several other possible reasons why more than one delivery route for proteins to organelles may have evolved. Certain proteins, for example, may have special requirements for their targeting and translocation that are best fulfilled by a particular pathway. For example, some proteins may fold rapidly or display a strong tendency for aggregation and, thus, might require specialized factors to maintain their translocation competence. It is unlikely that multiple targeting pathways are required to deliver proteins to specialized translocation apparatuses, because most targeting routes seem to converge at common translocation components in the respective membranes (e.g., the Sec61p complex in the ER, and Mas22p, Isp42p, Mim44p, and other associated proteins in the mitochondrial inner and outer membranes) (Pfanner et al., 1994; Rapoport, 1992).

An exciting possibility is that multiple targeting pathways could play direct regulatory roles in organelle biogenesis. The regulation of a particular pathway could modify the targeting and translocation efficiency of a specific subset of proteins and thus might result in the functional differentiation of the organelle. For example, in the case of protein targeting to the ER, regulating the activity of SRP could selectively control the synthesis of proteins whose signal sequences are recognized by it. Indeed, in vitro, SRP, by directly interacting with ribosomes, can modulate the translation elongation rate of proteins (Walter and Johnson, 1994; Wolin and Walter, 1989).

Lipids Travel between Organelles by Different Mechanisms

Compared with the delivery of proteins to organelles, the corresponding traffic of lipid molecules is less well understood. Whereas organelles import a specific complement of proteins that confer to them their structural and functional identity, identical lipid molecules are found in most membranes. Every organelle, however, has a unique composition of lipids, and because lipids are synthesized at different sites within the cell, they must travel between the membranes of different organelles (Daum and Paltauf, 1990; Trotter and Voelker, 1994; van Meer, 1993). For example, glycerolipids are synthesized in the ER and in mitochondria, whereas sphingolipid synthesis takes place in the Golgi apparatus. This traffic is not only important for distributing lipids from their sites of synthesis, but also for adjusting the surface area of any particular membrane according to need.

Lipids travel between organelles by different mechanisms. The lipid flux between the organelles that comprise the endocytic and secretory pathways involves the vesicle-mediated transport mechanism by which these organelles communicate, as lipids are an integral constituent of the membranes of the vesicles. For the movement of lipids between organelles that are not connected by vesicular transport (i.e., the ER, mitochondria, and peroxisomes), specialized lipid transport pathways are required. Soluble lipid carrier proteins that mediate the shuttling of lipids from one organelle to another are present in the cytosol (Wirtz, 1990).

Recent evidence points to a possible third mechanism by which organelles exchange lipids. An example is the biosynthetic pathway by which phosphatidylcholine (PC) is produced. This pathway requires the close cooperation of the ER and mitochondria. Phosphatidylserine (PS) is synthesized on the cytosolic face of the ER (Jelsema and Morre, 1978; Vance and Vance, 1988). It is then transported in an ATP-dependent process from the ER to the mitochondrial inner membrane, where the enzyme PS-decarboxylase converts it to phosphatidylethanolamine (PE) (Ardail et al., 1993; Simbeni et al., 1991; Voelker, 1985). PE is then transported back to the ER, where it can be converted to PC by PE-methyltransferase. Surprisingly, in permeabilized mammalian cells, the transport of PS from the ER to the mitochondria exhibits no sensitivity to dilution (Voelker, 1990). This and similar experiments suggest that the transport mechanism does not involve soluble lipid transfer proteins or small diffusible vesicles (Voelker, 1993). ER subfractions can be isolated that are enriched over bulk ER in lipid biosynthetic enzymes, suggesting that specific regions of the ER are specialized for lipid synthesis (Rusinol et al., 1994; Vance, 1990; Zinser et al., 1991). Moreover, mitochondria are often found in close juxtaposition to the ER (Ardail et al., 1993). Thus, when these observations are taken together, a model emerges where the transport of PS from the ER to mitochondria occurs in a specialized space where the two organelles come into close contact (Brown, 1992; Trotter and Voelker, 1994).

Organelles Can Self-Regulate Their Abundance and Composition through Intracellular Signaling Pathways

Individual organelles can monitor their functional status and adjust accordingly their own abundance and the relative amounts of their constituent components (Figure 1A). This allows organelles to compensate for an increase in demand for a particular function that they fulfill for the cell. An example of such a regulatory pathway is the unfolded protein response that leads to a proliferation of the protein-folding apparatus in the lumen of the ER. When unfolded proteins accumulate in the ER, the transcription of genes encoding ER-resident proteins is induced (Shamu et al., 1994). These proteins function to catalyze the folding, assembly, and modification of proteins that enter the ER membrane and include the hsp70 homolog BiP (for binding protein), or Kar2p

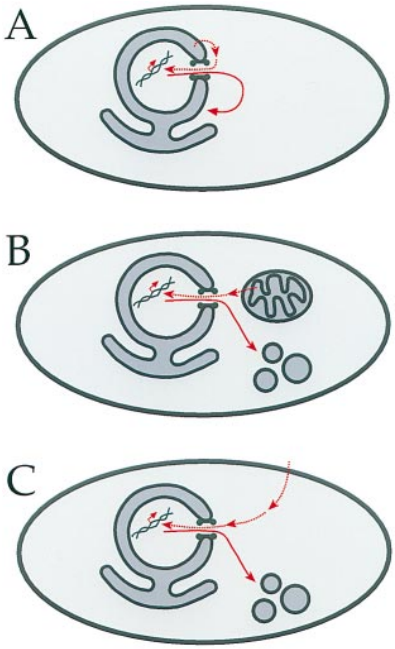

Figure 1. Different Pathways for the Regulation of Organelle Biogenesis

The different regulatory pathways are depicted by red arrows.

(A) Organelles regulate their own biogenesis through intracellular signaling pathways. The unfolded protein response pathway is schematically depicted where unfolded proteins present in the ER lumen cause a signal to be transduced across the ER membrane to activate the transcription in the nucleus of genes encoding resident ER proteins.

(B) Organelles regulate the biogenesis of one another by transcriptional activation. The retrograde pathway is schematically depicted where the loss of mitochondrial respiratory function causes an increase in the transcription of genes encoding peroxisomal proteins.

(C) Extracellular signals regulate organelle biogenesis by modulating the transcription of genes encoding organellar proteins. The control of peroxisomal biogenesis by extracellular nutrients is schematically depicted.

(in yeast), and protein disulfide isomerase. Thus, cells sense the accumulation of unfolded proteins in the ER, and the greater need for the protein-folding machinery in the ER results in an increase in the production of such enzymes.

The unfolded protein response was first discovered in mammalian cells, where ER lumenal proteins were termed glucose-regulated proteins (GRPs) because of their transcriptional up-regulation upon glucose starvation (Shiu et al., 1971). It is now clear that glucose starvation leads to glycosylation defects, which in turn result in the accumulation of misfolded proteins in the ER lumen and induction of the unfolded protein response (Kozutsumi et al., 1988; Resendez et al., 1988). A corresponding pathway exists in yeast, where the specific promoter element (the unfolded protein response element [UPRE], which mediates transcriptional induction) has been identified (Mori et al., 1992; Normington et al., 1989; Rose et al., 1989). The UPRE functions as an autonomous upstream activating sequence (UAS) that is both required and sufficient to render transcription of a promoter responsive to the status of the ER lumen. The unfolded protein signal is transmitted from the ER lumen to the nucleus by a transmembrane kinase, Ire1p, with its kinase domain in the cytoplasm or nucleus (Cox et al., 1993; Mori et al., 1993). Ire1p is structurally related to growth factor receptor kinases found in the plasma membrane of mammalian cells, and the mechanism of signal transduction probably resembles that of these kinases. Thus, it is likely that unfolded proteins—by binding directly as ligands to Ire1p or by an indirect route through other proteins such as BiP—trigger the oligomerization of Ire1p, which in turn becomes activated and phophorylates a downstream component(s). The target of Ire1p is still unknown; it could be a factor that upon phosphorylation enters the nucleus to activate transcription directly, or the pathway could be more complicated and, like many other signaling pathways, involve a cascade of multiple components and possibly other kinases (Shamu et al., 1994).

Interestingly, Ire1p not only regulates the transcription of ER-resident proteins, but may control membrane biogenesis as well. This possibility is suggested because *ire1* mutant cells are inositol auxotrophs (Nikawa and Yamashita, 1992). In yeast, inositol-containing lipids are the major phospholipid component of membranes, and the level of free inositol in the cell regulates the synthesis of phospholipids (Plautauf et al., 1992). Thus, activation of Ire1p in response to unfolded proteins may cause the coordinate biogenesis of both the protein and lipid components of the ER; as more ER lumenal content proteins are made, more membrane is made to house them.

Protein and membrane surface area is also coregulated when HMG-CoA reductase, an integral membrane protein of the ER, is overproduced (Wright et al., 1990). Under these conditions, membrane is vastly expanded and, in yeast, forms karmellae, which are extended sheets of ER membrane that wrap around the nucleus. This provides an experimental handle that should help decipher the mechanism of how membrane expansion can be triggered and controlled.

Different Organelles Can Affect One Another's Biogenesis through Signaling Pathways

Changes in the status of organelles can produce signals that modulate the expression of genes required for the biogenesis of other organelles (Figure 1B). Metabolites are often shared among a number of organelles. Thus, an altered function in one organelle that results in a decrease in a particular metabolite may be compensated for by an alteration of another organelle that maintains the metabolite at a physiologically required concentration. An example of such a regulatory network is the so-called retrograde pathway, which responds to a decrease in mitochondrial output and leads to an increase in peroxisome biogenesis (Shyjan and Butow, 1993).

In yeast, the mitochondrial tricarboxylic acid (TCA) cycle is metabolically linked to the peroxisomal glyoxylate cycle through shared metabolites, such as citrate and malate. Two isoforms of the enzyme citrate synthase are encoded by the *CIT1* and *CIT2* genes (Lewin et al., 1990; Suissa et al., 1984). Cit1p is localized to

mitochondria, where it functions in the TCA cycle, whereas Cit2p is localized to peroxisomes, where it functions in the glyoxylate cycle. When the mitochondrial TCA cycle is disrupted by deleting *CIT1*, *CIT2* is transcriptionally induced (Liao et al., 1991). This results in an increase in peroxisomal Cit2p activity that can partially compensate for the loss of Cit1p activity by increasing the flux of carbon sources, such as citrate, to the mitochondria (Liao et al., 1991). Interestingly, the transcriptional activation of *CIT2* also occurs in cells that are lacking other enzymes of the TCA cycle and in cells that are generally defective in respiration (i.e., rho^o cells) (Liao et al., 1991). Therefore, the loss of mitochondrial respiratory function must produce a signal that is responsible for mediating the increase of *CIT2* transcription.

Activation of *CIT2* in response to decreases in mitochondrial function requires a specific promoter element (UAS_r, for upstream activating sequence, regulatory) and two *trans*-acting factors, encoded by *RTG1*, whose product is a transcription factor, and *RTG2*, whose product is a protein as yet unknown in function (Liao and Butow, 1993). Interestingly, *RTG1* and *RTG2* are also required for the transcriptional activation of genes encoding other peroxisomal proteins, indicating that they play a general role in peroxisomal biogenesis and in maintaining the functional balance between the mitochondria and the peroxisome (Chelstowska and Butow, 1995; Kos et al., 1995). The nature of the signal leading to *CIT2* activation is still unknown. It is possible that signaling occurs through a pathway that is similar to the unfolded protein response pathway; a transmembrane kinase localized to the mitochondria might sense a decrease in mitochondrial function and transduce a signal across the mitochondrial envelope to the nucleus. Alternatively, the signal could be mediated via mitochondrially derived metabolites that either accumulate or are missing when mitochondrial function decreases (Shyjan and Butow, 1993). A paradigm for this latter scenario is the transcriptional activation of genes encoding mitochondrial proteins involved in electron transport, such as *CYC1*. Here, heme synthesized in the mitochondria directly regulates *CYC1* transcription by binding and activating the transcription factor Hap1p (Forsburg and Guarente, 1989).

Changes in the Extracellular Environment Can Regulate the Abundance and Composition of Organelles

Changes in the extracellular environment of a cell often alter the demand for particular functions that are provided by organelles (Figure 1C). In response to environmental changes, cells can adjust the biogenesis of organelles. One relatively simple example of this regulation is found for peroxisomes. The metabolic demand for peroxisomal biosynthetic pathways is dictated by the type of extracellular nutrients that is available, and these, in turn, regulate the abundance of peroxisomes in the cell (Subramani, 1993; Van den Bosch et al., 1992). In yeast, for example, peroxisomes both increase in volume and proliferate in number when cells are grown on oleate as the sole carbon source, because the enzymes of the β-oxidation pathway that are required to metabolize oleate reside in the peroxisome (Veenhuis et al., 1987). This response is regulated through the transcriptional control of genes encoding peroxisomal proteins (Einerhand et al., 1992). Activation of transcription by oleate requires at a minimum a specific promoter element, termed the oleic acid–responsive element (ORE) (Einerhand et al., 1993; Filipits et al., 1993; Wang et al., 1994), and a dedicated transcription factor or factors likely to interact with the ORE. How the oleate signal is sensed by the cell is still unknown; it could involve either a bona fide signal transduction pathway that initiates from the cell surface (or from the cytosol after uptake of the nutrient), or, in the simplest scenario, oleate might bind directly to the transcription factor to cause its activation. In mammalian hepatocytes, peroxisomal biogenesis is regulated by a mechanism that resembles this latter possibility. A variety of extracellular ligands bind to a set of transcription factors, termed the peroxisome proliferator–activated receptors (PPARs), that are part of the steroid receptor family (Green and Wahli, 1994). Binding of these ligands activates the PPARs and leads to transcriptional induction.

The regulation of peroxisomal biogenesis is also controlled by other interdependent pathways. Changes in extracellular nutrients, such as glucose, drastically affect peroxisomal biogenesis in yeast; this response is mediated in part through relatively well-characterized glucose repression/derepression pathways (Einerhand et al., 1992). These regulatory networks globally affect the cell by modulating the transcription of genes that also encode, for example, cytosolic and mitochondrial proteins (Johnston and Carlson, 1992). In this way, peroxisomal biogenesis becomes coordinated with other cellular metabolic pathways. Activation of the ORE can be modulated by the SNF1/SNF4-dependent transcriptional regulatory pathway, which is required for derepression of glucose-repressible genes (Johnston and Carlson, 1992; Simon et al., 1992). Another regulatory pathway involves Adr1p, a positive regulatory factor for peroxisomal biogenesis that is inactivated in high glucose (Johnston and Carlson, 1992). Adr1p was initially found to be required for the derepression of a cytosolic enzyme, Adh2p (Denis and Young, 1983), but was subsequently shown to play an essential role in peroxisomal biogenesis as well (Simon et al., 1991, 1992).

Thus, multiple pathways converge at the transcriptional level where, depending upon the elements contained in any given promoter (e.g., OREs, *ADR1*-responsive sites, *SNF1*/*SNF4*-responsive sites, and retrograde UAS_r-responsive sites), different transcription factors interact in a combinatorial manner to regulate both the biogenesis and the functional composition of the organelle (Einerhand et al., 1992; Hill and Treisman, 1995; Kos et al., 1995).

Perspectives

We have emphasized in this review some recent advances in our understanding of interorganellar communication. Cells regulate the abundance, composition, and position of each organelle. At the most fundamental level, this regulation coordinates the balance of organelle functions in the context of the cell. This balance

is not static, however, but is responsive to conditions imposed from the environment, thus allowing the cell to adapt to changing conditions. Most of these regulatory pathways are likely to be basic housekeeping mechanisms that are common to every cell. Some aspects of the pathways described in this review for yeast cells are therefore likely to be universally conserved. However, multicellular organisms pose another level of complexity. They are made of many different cell types, each having a unique intracellular identity that is established during differentiation in response to developmental signals. The future challenge will be to decipher how this regulation is achieved and whether it is due to modulation of general house-keeping pathways or is unique to each particular cell type.

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