The peroxisome: a production in four acts

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A cell regulates the number, size, and kind of each organelle it possesses in response to its particular role in an environment or tissue. Yet we still know little about how the molecular signaling networks within each cell perform such regulation. In this issue, Saleem et al. (Saleem, R.A., B. Knoblach, F.D. Mast, J.J. Smith, J. Boyle, C.M. Dobson, R. Long-O'Donnell, R.A. Rachubinski, and J.D. Aitchison. 2008. *J. Cell Biol.* 181:281–292) show for the first time how groups of kinases and phosphatases are organized to control when and how a cell assembles one kind of organelle, the peroxisome.

Many of the processes in cells involve macromolecules and assemblies zooming around and interacting on a millisecond timescale. There are also processes, such as the building of new cellular megastructures, that require seconds to hours and involve major rearrangements at all levels of the cell's architecture. Consider an amoeba's pseudopod, a growing neuronal dendrite, or the generation of a new organelle. These processes involve the coordinated interactions of hundreds of different components and require controls with incredible levels of finesse, yet very little is known about such controls.

Take, for example, the assembly of the peroxisome. Found in most eukaryotic cells, peroxisomes are vesicle-like packets bounded by a single membrane that surrounds a compartment termed the matrix. However, unlike most organelles, peroxisomes can vary dramatically in their abundance and size depending on the cell's type and its environment. Within the matrix are sets of specialized enzymes so concentrated that they sometimes crystallize. Peroxisomes are so named because of the function of some of their matrix enzymes in ridding the cell of peroxides and other toxins, including the ethanol that remains in our blood after a night on the town. They also play a role in numerous other metabolic reactions, including the β-oxidation of very-long-chain fatty acids, which allows the utilization of fats as fuel. Defects in peroxisome biogenesis are associated with numerous serious diseases, such as the fatal congenital abnormality Zellweger syndrome (Weller et al., 2003; Steinberg et al., 2006; Platta and Erdmann, 2007). It seems curious that, despite the key cellular roles the peroxisome plays, most of the principles underlying the coordination of its assembly and maintenance, established long ago for other organelles, have yet to be determined (Platta and Erdmann, 2007).

In the model organism *Saccharomyces cerevisiae* (baker's yeast), peroxisomes are low in number during growth in which sugars are the source of carbon. However, yeast respond by rapidly inducing peroxisome biogenesis when switched to a medium in which fatty acids supply the carbon (Fig. 1), enabling researchers to identify many genes required for peroxisome assembly. Importantly, mutants in these genes often mimic human peroxisome biogenesis disorders including Zellweger syndrome (Subramani, 1997).

Until recently, studies of peroxisomes have, of necessity, proceeded one gene, one protein at a time, trying to fathom the diverse effects of the disruption of a single component in the machineries that regulate peroxisome assembly and function. Notwithstanding the tremendous impact such single-item studies have had, they necessarily miss the emergent properties of the whole peroxisome biogenesis pathway, just as detailed examinations of a steering wheel alone cannot trace the assembly or elucidate the mechanism and purpose of the entire car to which it is attached. However, recent advances in proteomics and genomics have built resources of complete genome sequences and suites of high-throughput techniques to analyze the thousands of dynamic interactions between proteins, DNA, and RNA that regulate cellular responses. The study of these molecular information networks and the emergent properties arising from them is termed systems biology—the "holistic" version of molecular biology many of us have long wished we could practice but could not until recently. At the heart of systems biology are the efforts to define the complex and shifting information networks within living cells as they develop and react to their environment (Saleem et al., 2006).

Armed with such new approaches, Saleem et al. (see p. 281) have set out with the goal to understand, quantitatively and at a systems level, how a switch to a fatty acid medium induces yeast to begin assembling peroxisomes. The environmental cues involved are complex. The cell must read the melee of metabolic stresses resulting from the depletion of glucose and the addition of fatty acids into the sole correct response: the induction of peroxisomes. Because phosphorylation is the most important and predominant mechanism for signal transduction, Saleem et al. (2008) decided to determine the role of kinases and phosphatases in the control of peroxisome biogenesis. They began their study by assembling a comprehensive collection of some 250 strains, encompassing the majority of such proteins, from each of which was removed

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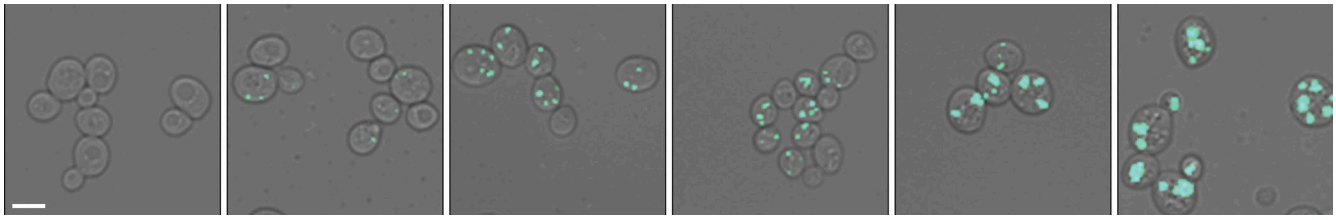

Figure 1. **Induction of yeast peroxisomes over a 20-h period in oleic acid, visualized by the Pot1p-GFP reporter, showing how yeast can fill themselves with many peroxisomes in a remarkably short time.** This image appears courtesy of R. Saleem and J. Aitchison. Bar, 5 µm.

a specific regulatory phosphatase or kinase. Each strain in this collection was then genomically tagged so that it expressed a fluorescent chimera of a peroxisomal matrix protein, Pot1p-GFP, from the *POT1* locus, leaving the coding sequence and upstream transcriptional control sequences intact. The activity of the *POT1* promoter is controlled by the available carbon source. Thus the level of expression of the fluorescent Pot1p-GFP chimera reports upon the activity of peroxisomal protein gene promoters. Moreover, the localization of Pot1p-GFP to the peroxisome reports on the number and morphology of assembling peroxisomes. Each strain was grown in three carbon sources: glucose (a sugar), glycerol (a sugar alcohol metabolized differently from either sugars or fatty acids), and oleic acid (a fatty acid). The *POT1* gene is fully repressed during growth on glucose, being expressed at <1% of its maximal level on oleic acid. *POT1* expression rises a little on glycerol to ~10% of its maximal level as the effects of glucose repression are removed. Using FACS to measure the amount of the Pot1p-GFP reporter in cells, Saleem et al. (2008) were able to assay the effects of glucose inhibition, glycerol derepression, and oleic acid induction on the conditional mutants. At the same time, the degree of peroxisome assembly in the mutants could be measured by microscopically determining the volume and number of the peroxisomes as well as the reporter signal intensity within. The time course of reporter induction and localization was quantified for each of the mutants, and the resulting data were statistically analyzed and superimposed on the existing yeast genetic and protein–protein interaction databases to determine the functional kinase and phosphatase modules responsible for controlling each step.

Importantly, most of the kinases and phosphatases tested had no significant effect on the expression or localization of the reporter, indicating that peroxisome biogenesis is under the tight control of a specific subset of signaling pathways. Indeed, many of the identified regulatory proteins were those that might be expected to have a role in peroxisome biogenesis (such as Snf1p; Navarro and Igual, 1994), although many others revealed previously unexpected links that underscore the tremendous scope of the architectural rearrangements a cell must undertake to accommodate the formation of new organelles. One possibility would be for the cell to read a single set of environmental cues into an uninterrupted linear flow of instructions that culminate in organelle biogenesis, rather like one initial push toppling a row of dominoes. However, Saleem et al. (2008) have instead shown that at least three discrete environmentally regulated modules, each a distinct control network, function at different stages in the induction pathway. Each module acts at the transcriptional level via kinase and phosphatase cascades, turning transcription factors on or off, and together the modules lead to the commitment by the cell to begin forming peroxisomes.

The first module maintains the repression of a set of genes in the presence of glucose and was revealed by a group of mutants that failed to keep the reporter fully repressed during growth in glucose. Most of these mutants are unaffected by growth in oleic acid, indicating that oleic acid induction and glucose repression are largely controlled by separate modules. Interestingly though, three mutants are defective in both glucose repression and oleic acid induction. Two, Ctk1p and Ssn3p, are regulatory components of the RNA pol II complex and so might be expected to be generally deficient in transcriptional responsiveness. But the third is Pho85p, which was also found to play key roles in gene derepression and oleic acid induction and so is perhaps a "master" controller of the whole peroxisome biogenesis process.

The second module ensures that peroxisomal protein genes are properly derepressed upon removal of glucose, presumably signaling for the clearing away of the glucose repression machinery at these genes' promoters. Mutants lacking the genes making up this control module could not induce the reporter in either oleic acid or glycerol. Several components of this network are also involved in controlling sphingolipid and phosphatidylinositol production, two lipid species whose roles as signal messenger molecules regulating metabolism are beginning to be revealed (Fernandis and Wenk, 2007; Strahl and Thorner, 2007).

Next, several of the mutants were defective in reporter induction on oleic acid but not in increased reporter expression on glycerol, revealing a third module controlling gene activation upon detection of fatty acids. These mutants led to both hypo- and hyperinduction of peroxisomal genes, perhaps pointing to some analogue control mechanism allowing the cell to exquisitely regulate the number and size of peroxisomes. Moreover, many of these regulatory proteins link the transcriptional response to processes such as the general stress response and the cell cycle, which may reflect the cell's preparation to slow down growth in reaction to a poorer energy source.

These three modules act stepwise, first turning off glucose repression after glucose removal, then derepressing these genes, and finally turning the appropriate genes on in response to the addition of oleic acid. It also appears that a fourth control module acts later, during the actual phase of peroxisome assembly, and is controlled by the presence of lipids in the environment.

Mutants in this network can begin to assemble peroxisomes but fail to correctly regulate their size and number, for example having too many small ones or too few large ones. Again, kinases and phosphatases regulating phosphatidylinositol metabolism are components of this module, as are regulators of the actin cytoskeleton, which is known to participate in the assembly of peroxisomes (Platta and Erdmann, 2007).

This work represents a first of its kind, presenting a genome-wide analysis of how phosphorylation regulates the production of an organelle. It also forms an essential first step toward a systems-wide understanding of the signaling pathways controlling peroxisome biogenesis. Many more of these steps need to be elucidated, including those whose control is not mediated through phosphorylation. Nevertheless, by isolating and identifying some of the key signaling control modules involved in peroxisome biogenesis, Saleem et al. (2008) have set the stage for a systematic dissection of the molecular mechanisms underlying each module. Such studies will shed light on how the flow of information from the environment to the genome is transduced into instructions for a cell to assemble an organelle or coordinate other large-scale changes. Moreover, by understanding the plasticity of peroxisomes, their different functions, and the details of their biogenesis in response to peroxisome proliferators, we will also gain a valuable understanding of the contributions made by the organelle to many peroxisome-related human conditions and so should help point the way to treatments for these diseases.

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