# Compartmentation of cellular activities

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In the yeast *Saccharomyces cerevisiae*, almost one third of cellular function is concerned with maintaining the compartmentation of cellular activities. From classic studies in yeast genetics we have come to understand a great deal of the processes driving the delivery of proteins into these compartments and the metabolic advantages that this provides. With the publication of the yeast genome sequence, '-omics' level studies began to provide further detail on the compartmentation of yeast cells. Very recent technological advances, including new applications in mass spectrometry, NMR, cryo-electron microscopy and the use of live-cell imaging have also been applied to yeast, because of the comparative analyses that can be done on yeast mutants. The mitochondrion is a complex compartment, carrying more than a thousand proteins that must be transported into and then distributed between, four sub-mitochondrial compartments. Essential molecular machinery in the outer and inner membranes, the intermembrane space and the matrix of mitochondria, drive protein transport, sorting and assembly. A glimpse of how *S. cerevisiae* and other microbes have provided understanding of cellular compartments is the aim of this review.

At a cellular level the feature that distinguishes eukaryotes is a diversity of subcellular compartments. These compartments collectively represent a complex set of intracellular membranes; the membrane of each compartment houses a set of protein activities (Figure 1). Maintaining this specific protein distribution in these compartments is a major commitment for eukaryotic cells: in the yeast *S. cerevisiae* ~16% of all genes encode proteins that function in organelle organisation and biogenesis, and a further 14% encode transporters that are needed to drive small molecules into these compartments [1]. Thus, almost one third of the known cellular functions in this eukaryote are concerned with maintaining the compartmentation of cellular activities.

## The mitochondrion as a model organelle

Mitochondrial compartments contain around a thousand proteins in yeast (and in humans), and only ~1% of these are synthesised within the compartment; the majority are imported into the organelle by virtue of a series of protein translocases in the outer and inner mitochondrial membranes (Figure 2) [2,3]. The translocase in the outer membrane, the TOM complex, was first identified in biochemical studies undertaken with the filamentous fungus *Neurospora crassa*, using assay systems largely developed with *S. cerevisiae*. Being readily and economically cultured to large scale meant that large quantities of mitochondrial outer membranes could be produced from *Neurospora* and used to raise antibodies to each of the outer membrane proteins. The antibodies were used to pre-treat isolated mitochondria, which were then assayed for protein import. Antibodies against the outer membrane proteins Tom20 and Tom70 inhibited precursor protein import [4,5]. Genetic analysis using *S. cerevisiae* revealed the interplay between Tom20, Tom70 and the third 'receptor' of the TOM complex, a protein called Tom22 [6-15]. We and others used yeast mutants to determine that Tom22 has three domains, each participating in functionally distinct aspects of protein transport: an N-terminal receptor domain to bind protein substrates [10,11,15];

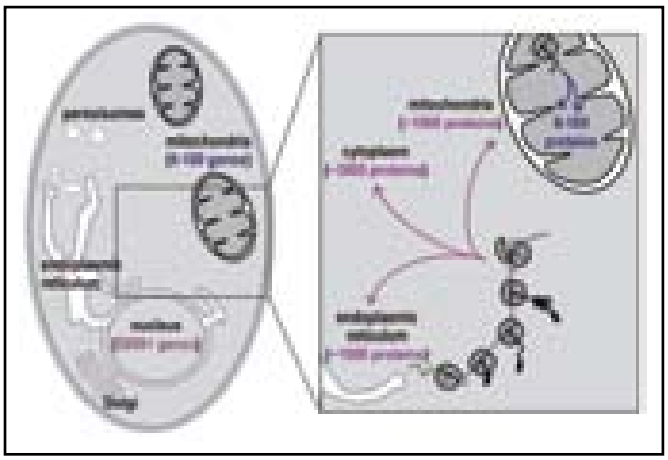

**Figure 1. Cellular compartments in a 'typical' eukaryotic microbe. A representation of a eukaryote with ~5000 protein-coding genes in its nuclear genome is shown. Based on work from the yeast *S. cerevisiae,* genes within the nucleus code for ~1000 proteins that are targeted to the endoplasmic reticulum to be distributed through the endomembrane system, ~3000 proteins that fold in the cytoplasm (though might then be redistributed to the peroxisomes or nuclear compartment) and ~1000 proteins that are transported into the mitochondrial compartments [1]. A further set of proteins are encoded in the mitochondrial genome. In many yeast, the mitochondrial genome codes for ~10 proteins. In the unicellular flagellate *Reclinomonas americana* there are ~100 genes, while microsporidians have no mitochondrial genome.**

a central transmembrane segment that organises other subunits within the TOM complex [15] and a C-terminal domain that might function as an entropic spring, to drive protein transport through the outer membrane [16].

Detailed comparative analyses have demonstrated how well yeast serves as a model for studying protein transport. For example, we used bioinformatic and biochemical assays to show the receptor Tom20 in *Saccharomyces,* and *Neurospora* is similarly structured to that found in humans and other mammals [17]. Work done in Toshi Endo's lab described the three dimensional structure of the mammalian protein, and explained how Tom20 recognises and binds the targeting signals that dictate protein import into mitochondria [18, 19].

## Does only *S. cerevisiae* provide the goods?

*S. cerevisiae* was the yeast of choice for early studies on protein targeting, but other microbes have proven their worth. In the area of peroxisomal protein transport, our current knowledge on disorders such as Zellweger's syndrome, that result from defects in peroxisomal protein import, is built in large part on studies done by Denis Crane and others with the yeast *Pichia pastoris* [20]. Microsporidia are fungi or close relatives of fungi, and recent work on the microsporidian *Encephalitizoon cuniculi* revealed a tiny genome, as befits an intracellular parasite [21]. Comparative analyses show that this curious pathogenic fungus has a pared down protein import machinery, with only the essential, core components driving protein transport into the mitochondrion [22].

But it is not just fungi that provide insight into protein targetting. Other, bizarre microbes like *Giardia* and *Trichomonas*, were previously thought to have so anciently diverged from the eukaryote lineage that they missed the acquisition of mitochondria. Not so. *Giardia* and *Trichomonas*, like all known eukaryotes, have mitochondria. These microbes carry mitochondria that have become metabolically specialised and are operationally named 'mitosomes' where they are the size of transport vesicles and without invaginations in their inner membrane (e.g. in *Giardia*), or 'hydrogenosomes' if they are responsible for liberating hydrides as the end product of their energy-generating metabolic activities (e.g. in *Trichomonas*). Recently we showed that these mitosomes and hydrogenosomes are specialised mitochondria, finding common mitochondrial protein transport machines in the membranes of all these organelles [23,24]. These findings, as well as the discovery of Golgi bodies and nuclear envelopes in *Giardia* and *Trichomonas*, demonstrate that compartmentation of cellular activities was already sophisticated in the last common ancestor to all eukaryotes.

## Microbial models: is anything left to be done?

In addition to studies focused on protein compartmentation in mitochondria, fungal systems have served broadly as models for understanding the transport of proteins into the nucleus, endoplasmic reticulum (and throughout the endomembrane system) and peroxisomes. Very recent technological advances

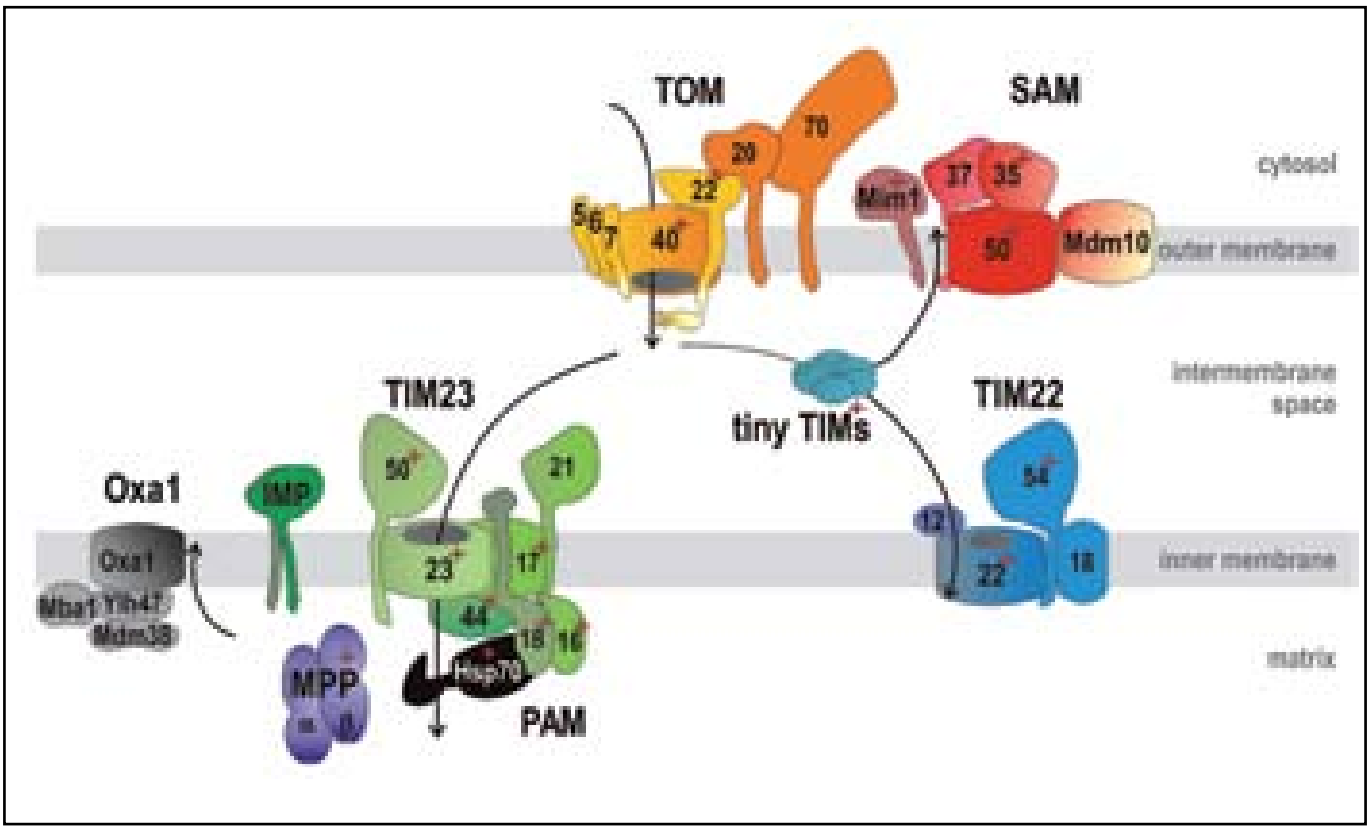


Figure 2. The mitochondrial protein transport machinery of *Saccharomyces cerevisiae.* Arrows indicate the direction of protein transport to each of the sub-mitochondrial compartments. See reviews by Kaye Truscott [3] and Carla Koehler [2] for more details, or the author, Trevor Lithgow, to define symbols.

in mass spectrometry, cryo-electron microscopy and the use of live-cell imaging have also focused on yeast, again because of the comparative analyses that can be done on yeast mutants [25,28]. Many of these topics will be under discussion at the 23rd International Conference on Yeast Genetics and Molecular Biology (Melbourne, 01 – 06 July 2007).

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