Review

Dynamic regulation of endosymbiotic organelles by ubiquitination

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Recent work has revealed that mitochondria and chloroplasts are subject to direct control by the ubiquitin-proteasome system (UPS). Ubiquitin E3 ligases are present at the outer membrane of both organelles where they mediate ubiquitination and turnover of other organellar proteins. Both organelles exhibit remarkable structural dynamism and UPS control is particularly concerned with these properties. In mitochondria, the UPS targets factors involved in organellar fission and fusion, with significant impacts upon organellar morphology, mitophagy, and apoptosis. In chloroplasts (and other plastids), the UPS targets components of the protein import machinery, facilitating reorganization of the organellar proteome to determine organellar development and functions. Acquisition of such regulatory control during evolution is perhaps linked to the dynamic characteristics of the two organelles, which are not paralleled in their prokaryotic relatives. Here we discuss our current understanding of the role of the UPS in the regulation of endosymbiotic organelles.

Endosymbiotic organelles and the UPS

Mitochondria and chloroplasts are factories for energy transduction in eukaryotic cells. Chloroplasts in green plants use sunlight energy to fix CO_2 into organic compounds through photosynthesis, the process which supports almost all life on earth. Mitochondria metabolize organic molecules to produce ATP through oxidative phosphorylation, thereby powering cellular activities in animals and other eukaryotes. These organelles additionally have a diversity of other roles; for example, chloroplasts contribute to numerous metabolic pathways [1] and mitochondria are involved in cell differentiation, signaling, and apoptosis [2].

Both organelles originated from prokaryotic ancestors through endosymbiosis (Box 1), and an interesting question is how they evolved to become fully integrated components of eukaryotic cells. Part of the answer may lie in recent findings that the organellar proteomes are dynamically regulated by the principal eukaryotic protein turnover pathway – the UPS (Box 2). The UPS is a conserved proteolytic system in eukaryotes with numerous components, accounting in *Arabidopsis thaliana* for nearly 6% of the proteome [3]. The E1 (ubiquitin activating), E2 (ubiquitin conjugating), and E3 (ubiquitin ligating) enzymes cooperate to mediate attachment of ubiquitin to target proteins, which typically are then recognized and degraded by the 26S proteasome. Notably, proteasomal degradation is not the only destiny of ubiquitinated substrates because ubiquitination plays many other roles, for example in autophagy, dependent upon the ubiquitin linkage type [4].

In this review we discuss recent findings concerning UPS-mediated regulation of dynamic aspects of the endosymbiotic organelles. We particularly focus on the specificity-determining E3 ligases, and summarize similarities and differences between mitochondria and chloroplasts in this area.

The UPS and mitochondrial dynamics

Cycles of fusion and fission dynamically organize mitochondrial shape and networks (Box 1). The main components involved in these processes are: the dynamin-related GTPases mitofusins 1 and 2 (Mfn1, Mfn2) and optic atrophy 1 (Opa1) which control fusion, and dynamin-related protein 1 (Drp1) and mitochondrial fission 1 (Fis1) which mediate fission (Figure 1) [5]. Fusion and fission act antagonistically to control mitochondrial dynamics, and imbalances can lead to dysfunctional mitochondria. For example, loss of mitofusin function causes fragmentation of mitochondria, whereas knockdown of Fis1 results in elongated organelles [6]. This is of physiological importance because such mitochondrial defects have been linked to human neurodegenerative diseases [5]. As detailed below, several mitochondrial E3 ligases have been identified recently (Table 1) [7], and they regulate the balance of fusion and fission by directly targeting the aforementioned proteins. Unless specifically noted otherwise, all proteins discussed in this section are mammalian.

Mitochondrial outer membrane E3 ligases

MARCH5/MITOL is a RING-type E3 ligase with its N-terminal RING domain facing the cytosol, and with four transmembrane domains embedded in the outer mitochondrial membrane (OMM) [8–11]. It has been successively shown to interact with four different dynamics-related proteins, namely Mfn2, Fis1, Drp1, and Mfn1 [8–11]. Thus, although its role in mitochondrial dynamics is clear, the identity of its physiological substrate(s) is controversial. Two earlier studies suggested that MARCH5 activates fusion, with loss of MARCH5 function stimulating mitochondrial

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Keywords: chloroplasts; E3 ligases; mitochondria; plastids; ubiquitin-proteasome system.

0962-8924/$ – see front matter
 http://dx.doi.org/10.1016/j.tcb.2013.04.008

Box 1. Evolution and dynamics of endosymbiotic organelles

Mitochondria and chloroplasts share several characteristics. Both organelles are surrounded by a double-membrane system, of distinct composition, and both are semiautonomous – containing their own genomes and protein synthesis systems. These features reflect their prokaryotic ancestry and their entrance into the eukaryotic lineage via endosymbiosis. Mitochondria appeared first as a result of the engulfment of an α-proteobacterium by an early eukaryote [97]. During evolution, the bacterium lost its independence and became an organelle controlled by the host [98]. Later, chloroplasts evolved in a similar fashion from a cyanobacterial ancestor. Both organelles relinquished many of their genes to the host genome, and thus can produce a very limited number of proteins on their own. The majority of their proteins are now encoded by the nuclear genome and are synthesized in the cytosol before protein import into the organelle through sophisticated protein import machineries comprising the TOM/TIM and TOC/TIC (translocase of the outer/inner mitochondrial or chloroplast membrane) complexes [62–65,99].

The two organelles evolved unique features during their eukaryotic integration that are not paralleled in their prokaryotic relatives. One remarkable characteristic is their morphological and functional dynamism. Mitochondria rarely exist as individual rod-shaped organelles as depicted in text books; instead they usually form elongated tubules and develop networks by interconnecting with each other [5,100]. Such networks are dynamic and regulated by two fundamental events, fusion and fission, and are conserved in yeast, animals, and plants, playing crucial roles in respiration, mitochondrial DNA integrity, and apoptotic control. The balance of fusion and fission can be controlled to regulate mitochondrial morphology in response to different stimuli. By contrast, chloroplasts belong to a diverse group of organelles known as plastids. Other plastid types include: amyloplasts, which accumulate large quantities of starch and play important roles in energy storage and gravitropism; chromoplasts, which accumulate carotenoid pigments and act as attractants in flowers and fruits; and etioplasts, which are chloroplast precursors that develop in dark-grown plants [70]. Importantly, different plastid types are able to interconvert during specific phases of development, and these transitions are of critical importance for plant growth and development. In addition, both mitochondria and chloroplasts share a common feature in the context of dynamics, in that they can move and redistribute inside cells in response to environmental changes or intracellular signals [5,74]. The motility of these organelles is critical for their functions; for example, mitochondrial movement is important for neuron development, whereas the movement of chloroplasts and amyloplasts functions in strong-light avoidance and gravity sensing, respectively.

Box 2. The ubiquitin-proteasome system (UPS)

There are several systems for protein turnover in endosymbiotic organelles. Autophagy mediates the removal of whole or partial organelles through their delivery to lysosomes (or the vacuole in plants) for recycling [4]. Both organelles also contain internal protease systems inherited from their bacterial ancestors, and these mediate in-house degradation of damaged proteins [101,102]. Also, it recently became evident that the UPS directly controls the turnover of outer membrane proteins in both mitochondria and chloroplasts.

Substrates for ubiquitination are identified primarily by the E3 ligases, of which there are many (there are ~1400 and 617 E3s in *Arabidopsis* and humans, respectively, but only ~40 E2s and two E1s) [3,16]. The E3 ligases can be divided into four classes based on the presence of conserved domains or subunit compositions: HECT (homologous to the E6-AP carboxyl terminus), U-box, RING (really interesting new gene), and cullin-RING ligases (CRLs) [3]. In *Arabidopsis*, SCF (S-phase kinase-associated protein 1–cullin 1–F-box) E3s form the most diverse family of CRLs, with more than 700 F-box proteins that control substrate recognition. Such diversity of E3 ligases enables specific recognition (and regulation) of a multitude of substrates, with roles in processes as diverse as hormone signaling, environmental and pathogen responses, chromatin remodeling, and transcriptional regulation.

The proteasome is one of the biggest complexes in eukaryotic cells. It consists of the 19S regulatory particle and the 20S core particle, which are respectively responsible for the recognition and proteolysis of ubiquitinated substrates [103]. The UPS acts ubiquitously on different cellular compartments, from the nucleus and cytosol (where the proteasome and free ubiquitin reside) to membrane-restricted organelles such as the ER. Unlike the endosymbiotic organelles, the ER does not contain intrinsic proteases inherited from bacterial ancestors, and instead uses cytosolic UPS machinery for its proteomic control. However, because the UPS is physically obstructed by the ER membrane, the ER-associated degradation (ERAD) pathway employs special components (such as membrane-bound ubiquitination enzymes and the AAA^+ ATPase protein Cdc48) for the targeting and extraction of substrates for degradation by the cytosolic proteasome [104]. Recent findings have shed light on a new arena of UPS action, the endosymbiotic organelles, where the system is particularly concerned with the control of organellar dynamics.

fragmentation and overexpression producing elongated mitochondria [8,9]. By contrast, more recent studies showed that mutation of MARCH5 causes mitochondrial elongation [10,11]. The mechanisms underlying MARCH5 action are also complex and arguable. The earlier reports argued: that MARCH5 mediates ubiquitination of Fis1 and Drp1, which is likely to induce their turnover, thereby inhibiting mitochondrial fission [8,9]; and that MARCH5 binding to Mfn2 activates the function of Mfn2 [8]. By contrast, the later studies showed that Mfn1 (not the fission proteins) is the major ubiquitination substrate of MARCH5 [10]. They concluded that MARCH5 is responsible for the UPS-mediated degradation of Mfn1, and for activation of Drp1, thereby stimulating mitochondrial fission [10,11].

The above-mentioned discrepancies are possibly due to the use of differing experimental conditions and methods in different laboratories. Alternatively, it is possible that MARCH5 substrate preferences vary under different physiological conditions to achieve an optimal balance of fusion and fission. It was recently shown that Mfn1 ubiquitination by MARCH5 is elevated in G_2/M-phase cells, which is consistent with the fragmented mitochondrial morphology seen at this stage, and is likely regulated by the cyclin B1/Cdk1 kinase [12]. Moreover, a dominant-negative MARCH5 mutant prevented mitochondrial fragmentation under oxidative stress conditions [13]. Information on the physiological role of MARCH5 under other conditions linked to mitochondrial morphological changes should prove illuminating. Recent reports revealed that MARCH5 is also involved in mitochondrial quality control and nitrosative stress responses [14,15].

The MULAN/MAPL/GIDE/Mul1 protein was first identified as a mitochondrial E3 ligase in screens for mitochondrial morphology mutants [16,17]. It is localized in the OMM through two transmembrane domains, with its C-terminal RING domain facing the cytosol [16,17]. Overexpression of MULAN causes mitochondrial fragmentation, indicating that it stimulates mitochondrial fission [16,17], and was also reported to promote apoptosis [18]. Mitochondrial fission is well known to induce apoptosis [19], and so the effect of MULAN on apoptosis may be related to its role in fission. Although it has been argued that MULAN is a SUMO (small ubiquitin-like modifier)