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DOI: 10.1016/j.cub.2012.03.034

Organelle Evolution: *Paulinella* Breaks a Paradigm

It is commonly assumed that transformations of endosymbionts into organelles are exceptionally rare evolutionary events because of hypothetical difficulties in the origin of an import apparatus for nuclear-encoded, organelle-targeted proteins along with their targeting signals. A challenge to this view comes from recent studies of protein import into the cyanobacterial endosymbionts/organelles of *Paulinella chromatophora*.

Andrzej Bodył[1,*], Paweł Mackiewicz[2], and Przemysław Gagat[2]

It is beyond doubt that mitochondria and plastids (chloroplasts) evolved from free-living organisms enslaved by other cells. All mitochondria trace directly to a single endosymbiosis involving an α-proteobacterium [1], but plastid evolution has been more complex. Plastids evolved from a so-called primary endosymbiosis of a cyanobacterium taken up by a eukaryotic heterotroph [2]. This endosymbiosis resulted in plastids surrounded by two membranes, which are found in glaucophytes, red algae, and green plants. Primary plastid-containing algae, both green and red, later were incorporated via secondary endosymbiosis into other heterotrophic eukaryotes resulting in complex plastids [2]. 'Green' secondary plastids are found in three algal groups (e.g., euglenids), whereas those of 'red' origin occur in as many as six algal lineages (e.g., cryptophytes, haptophytes, dinoflagellates). We also know of tertiary plastids that are derived from algae with secondary plastids (e.g., from haptophytes) in a few dinoflagellates [2].

How can we explain the occurrence of distinct kinds of plastids in so many eukaryotic lineages? The prevailing view, representing a paradigm of cellular evolution, posits that this distribution resulted from only a few endosymbiotic events in the ancestors of large eukaryotic clades. For example, originally it was suggested that all secondary plastids trace back to one 'green' and one 'red' endosymbiosis [3]. This was based on the assumption that the transformation of an endosymbiont into an organelle is very difficult and is, therefore, an exceptionally rare event [3]. A hallmark of mitochondrial and plastid evolution was massive gene transfer from the endosymbiont genome to the host nucleus [4], which necessitated the evolution of an import apparatus for protein products of transferred genes along with their amino-terminal targeting signals. However, despite the perceived difficulty of 'inventing' such protein transport, available data clearly indicate that 'green' secondary plastids evolved on three separate occasions [2]. Evidence also suggests that, among 'red-derived' plastids, only those in cryptophytes have a secondary origin, whereas all others evolved by tertiary (or serial) endosymbioses, as has been demonstrated for unusual dinoflagellate plastids [2].

While eukaryotic alga-derived plastids clearly have been acquired by distinct eukaryotic hosts on multiple occasions, classical primary plastids appear to be derived from a single cyanobacterial endosymbiosis [2] (but see [5]). This suggests that transformations of prokaryotic endosymbionts into cell organelles were exceptionally rare events (consider also the single origin of mitochondria). A new challenge to this view comes from recent protein import studies by Nowack and Grossman [6] in *Paulinella chromatophora*. The amoeba harbors photosynthetic cyanobacterial endosymbionts with two envelope membranes, which were acquired independently of classical primary plastids from distinct cyanobacteria, 60 Mya *versus* 1,500 Mya, respectively [7] (Figure 1A). Interestingly, *Paulinella* endosymbiont genomes have lost ~75% of their ancestral coding capacities and at least 30 endosymbiont genes have been identified as being expressed in the host nucleus [8], indicating the existence of a protein import mechanism.

Most proteins imported into classical primary plastids carry amino-terminal transit peptides that target them across the plastid envelope post-translationally via Toc and Tic translocons [9]. Because some of the *Paulinella* endosymbiont genes transferred to the host nucleus appear to encode signal peptides, it was hypothesized that these endosymbionts/organelles import their nuclear-encoded proteins via the endomembrane system [7]. Nowack and Grossman provided experimental evidence for this by demonstrating that three photosynthetic proteins, PsaE, PsaK1, and PsaK2, are imported into *Paulinella* organelles via the Golgi apparatus [6] (Figure 1B). However, despite their Golgi-mediated targeting, these three proteins are devoid of cleavable amino-terminal signal peptides [6] usually responsible for co-translational protein entrance into the endomembrane system. Nevertheless, both PsaK1 and PsaK2 have strongly hydrophobic amino-terminal domains that resemble those of signal peptides [10]. The case of PsaE is more controversial. Although PsaE from *Paulinella* strain CCAC 0185, investigated by Nowack and Grossman, is devoid of any amino-terminal hydrophobic domains [10], its homolog from strain FK01carries an identifiable signal peptide [7]. It is possible that other proteins imported into *Paulinella* organelles (e.g., PsbN) also evolved signal peptides [10], making

endomembrane-mediated targeting a universal feature of this endosymbiosis.

A group of plastid proteins with signal peptides and imported via the endomembrane system was found in higher plants [11,12]. Based on this finding, it was hypothesized that such targeting was the original import mechanism for classical primary plastids [11]. However, this scenario encounters several obstacles. For example, the putative signal peptide-carrying proteins represent only 0.6% of plastid-targeted proteins, which is similar to the level of falsely predicted signal peptides in cytosolic and mitochondrial proteins [12]. Moreover, they appear to constitute a specific group of non-photosynthetic proteins targeted to higher plant plastids via the endomembrane system, probably because they require glycosylation in the Golgi apparatus and/or are targeted dually to the plastid and the cell wall [12]. In accordance with this hypothesis, no known photosynthetic proteins in the eukaryotes with classical primary plastids carry signal peptides, which is in clear contrast to the situation found in *P. chromatophora*.

When considering the significant differences in the import of photosynthetic proteins between classical primary plastids and *Paulinella* organelles, it is possible that their cyanobacterial ancestors were differentially established as organelles. The key to the import differences could be the respective properties of their outer membranes. The outer membranes of classical primary plastids have a chimeric nature with eukaryotic phosphatidylcholine and bacterial galactolipids along with the porin-like Toc75 channel [12]. In contrast, the absence the *toc75* gene from the endosymbiont and nuclear genomes of *P. chromatophora* [7,8] suggests that its outer membrane may be derived solely from the host phagosomal membrane, possibly preventing Toc75 insertion and necessitating import via the endomembrane system.

Bacterial endosymbionts are widespread in protists, animals and plants [13,14] and are even found in other bacteria [15]. Interestingly, in most cases, their genomes are drastically reduced [16], suggesting that at least some of them also may import proteins. One example is

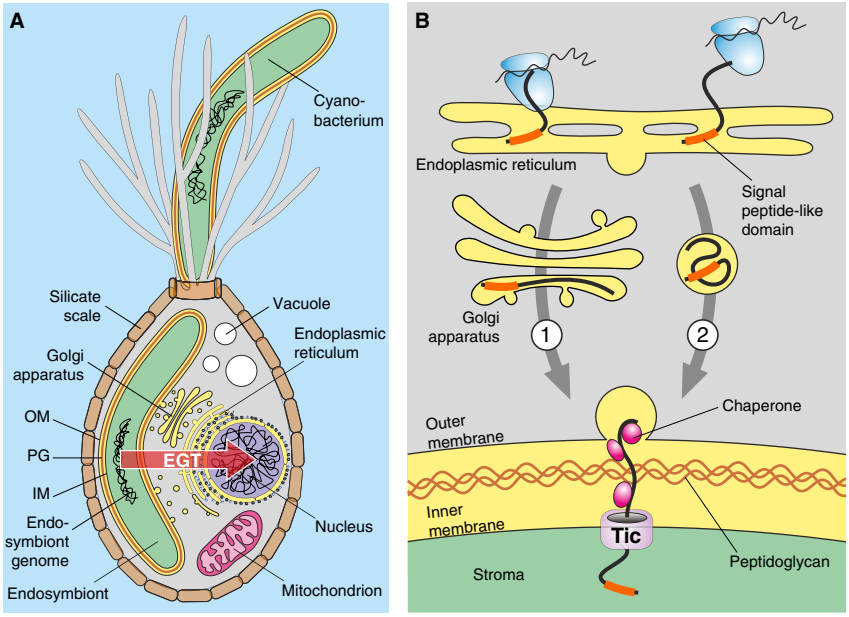

Figure 1. *Paulinella chromatophora* and its cyanobacterial endosymbionts.

(A) These endosymbionts are surrounded by outer (OM) and inner (IM) membranes with the peptidoglycan wall (PG) located between them. Some of their genes already have moved to the host nucleus via endosymbiotic gene transfer (EGT). (B) Proteins encoded by these genes are targeted into the new organelles via the endomembrane system using signal peptide-like sequences [6,7]. The first step in their import could either be co- (left) or post-translational (right) transport into the endoplasmic reticulum. It was demonstrated that their further import involves the Golgi apparatus (1), but some proteins also could bypass that compartment (2). After fusion of a transport vesicle with the outer organelle membrane, imported proteins are released into the intermembrane space and must cross the peptidoglycan wall. They all have low molecular weights and nearly neutral charges, which probably represent adaptations to facilitate this passage [7,10]. A final import step is probably Tic-dependent translocation across the inner membrane [7].

Rhizobium bacteria residing in root nodules of legume plants. Although not transmitted generation-to-generation, they are surrounded by phagosomal/symbiosome membranes and import plant NCR peptides carrying signal peptides via the endomembrane system [13]. This indicates that evolution of a protein import pathway can predate establishment of permanent endosymbioses. A further elaboration is found in *Buchnera* bacteria, which are egg-transmitted permanent endosymbionts of aphids. The aphid nucleus contains five genes transferred from bacteria that encode eukaryotic signal peptides [14]. Because *Buchnera* endosymbionts are surrounded by phagosomal/symbiosome membranes, it is reasonable to assume that proteins encoded by the transferred genes also are imported into these bacteria via the endomembrane system.

It was previously believed that mitochondrial and plastid endosymbionts could be acquired only by eukaryotic cells with a fully developed endomembrane system, cytoskeleton and an ability to carry out endocytosis [17]; however, bioenergetic considerations suggest that the origin of mitochondria (as an energy source) must have preceded evolution of all these features and the origin of the eukaryotic cell itself [18]. It also demonstrates that a single origin of mitochondria cannot be considered evidence that prokaryotic origins of eukaryotic cell organelles are rare because they probably predated the appearance of eukaryotic cells. Moreover, central components of mitochondrial and primary plastid import systems were derived from proteins pre-existing in their bacterial ancestors and did not require *de novo* evolution [19]. If future studies continue to demonstrate that other bacterial endosymbionts import

nuclear-encoded proteins, as shown for *P. chromatophora*, we must abandon the paradigm that organelle births are exceptionally rare in eukaryote evolution.

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DOI: 10.1016/j.cub.2012.03.020

Brain Oscillations: Phase-Locked EEG Alpha Controls Perception

New research suggests that auditory stimuli can reset human oscillatory activity in visual cortex. This change in rhythmical brain activity leads to modulation of visual perception.

Paul Sauseng

When visual stimuli are presented very briefly, we tend to consciously perceive some of them and miss others. Whether we perceive such stimuli or not seems to be a matter of chance, but could there be fluctuations in perceptual threshold based on spontaneous brain activation patterns in the visual cortex? If this is the case, how are these patterns influenced or modulated by neural or environmental events? In this issue of Current Biology, Romei *et al.* [1] report that the instantaneous phase of rhythmical brain activity around 10 Hz — known as alpha activity, 100 ms wavelength — in the human primary visual cortex is reset by brief auditory stimuli. This was probed by delivering transcranial magnetic stimulation (TMS) to the primary visual cortex with different latencies after auditory stimulation. When the visual cortex is stimulated with TMS, single pulses, at a high enough intensity, elicit action potentials in V1 leading to the perception of a light flash, known as a phosphene. An increased likelihood of perceiving a phosphene was reported when a TMS pulse had been delivered around 100 or 200 ms after onset of a brief auditory stimulus. Phosphene perception was thus modulated at a timescale equal to two periods of phase-locked alpha waves (see Figure 1 for schematic results).

In a second experiment, Romei *et al.* [1] combined electroencephalographic (EEG) recordings with TMS to investigate more directly the association between auditory-evoked alpha oscillations (alpha waves time- and phase-locked to the brief auditory stimuli) and phosphene perception. Auditory stimuli not only led to significant phase-locking of alpha waves at recording sites over the auditory cortex, but significant phase-locking was also observed over visual cortex. The instantaneous phase of parieto-occipital alpha oscillations was significantly correlated to phosphene perception for at least two complete alpha periods (200 ms). Furthermore, reactivity of visual alpha amplitude to magnetic stimulation of occipital cortex (amplitude decrease as a marker of cortical excitability) was modulated in the same cyclic way as alpha waves evoked by auditory stimuli (see Figure 1 for an illustration of main findings). Cross-modal phase-resetting of alpha oscillations has been reported previously in monkeys [2], but Romei *et al.* [1] demonstrate similar effects of evoked alpha waves on visual perceptual processing in humans.

These exciting results suggest that stimulation of one sensory modality can lead to a strictly timed activation pattern in primary sensory cortex of another modality. The strict timing is possibly indicative of the temporal structure of cross-modal processing in the brain. Moreover, the new findings of Romei *et al.* [1] have important implications for the functional interpretation of oscillatory alpha activity. They provide evidence that evoked alpha waves at brain areas not associated with the primary sensory processing of a stimulus, for example,