

# Review of: "A bipartite chromatophore transit peptide and N-terminal processing of protein in the *Paulinella* chromatophore"

Luis Delaye<sup>1</sup>

<sup>1</sup> Center for Research and Advanced Studies of the National Polytechnic Institute

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According to the *selfish gene* theory, natural selection will favor integration of symbionts over evolutionary time once their genes are inherited together (Dawkins, 1976). Integration of the mergers includes the movement of genes from the endosymbiont to the genome of the host; as well as extensive gene loss in the endosymbiont. In addition, the integration is enhanced by the evolution of nuclear-encoded proteins targeted into the endosymbiont. The apotheosis of this process are mitochondria and plastids, which evolved from free-living bacteria over a billion years ago (Betts et al. 2018). These organelles are so integrated that it is impossible to understand the origin, physiology and evolution of eukaryotes without them.

A central question in this process is: *what are the genetic changes that allow different cell lineages to merge into a new one?* Unfortunately, the evolutionary events that led to mitochondria and plastids are so ancient that the early steps that led to these organelles are lost. Here is where *Paulinella chromatophore* comes to the rescue. *P. chromatophore* is a cercozoan amoeba that evolved an endosymbiosis with a cyanobacterium approximately 100 Mya (Delaye et al. 2016). The endosymbiotic cyanobacterium evolved into a *chromatophore* which has been described as a *plastid in the making* (Marin et al. 2005). It represents a unique opportunity to study a relatively recent primary endosymbiosis. In this amoeba, one third of the proteins found in the chromatophore are encoded in the nucleus and the mechanism to import these proteins to the 'plastid in the making' is not known.

In the manuscript "A bipartite chromatophore targeting peptide and N-terminal processing of proteins in the *Paulinella* chromatophore" by Oberleitner et. al, the authors use a mass spectrometry-based approach to study the N-termini of chromatophore localized proteins. Depending on the size, proteins targeted to the chromatophore can be classified into two: large proteins (> 250 amino acids) carry a conserved N-terminal extension named 'chromatophore transit peptide' (crTP); and short peptides (< 90 amino acids) that lack crTP.

As mentioned by the authors, their results imply that 'crTP mediates trafficking through the Golgi' and

propose a hypothetical model for crTP-mediated protein import into the chromatophore; they also found that crTP becomes partially cleaved upon import to the chromatophore (surprisingly, only the N-terminal third (part 1) of the crTP is processed); while short peptides (< 90 amino acids) '*remain largely unprocessed*'.

The results described in this manuscript shed light on the mechanism used by *P. chromatophora* to target proteins to the chromatophore; but also pave the way for new questions. The acquisition of a chromatophore by the ancestor of *P. chromatophora* must have required several evolutionary innovations as well as fine adjustments to the pre-existing cell machinery. For instance, as mention by the authors the crTP "*has no similarity to other known proteins or protein domains*" and must have evolved *the novo*. It is likely that other cell components (genes/proteins) also originated concomitantly with the chromatophore. A genome comparison with non-photosynthetic close relatives to *P. chromatophora* would help to identify such gene/protein innovations. In addition, components of the cell machinery co-opted to target proteins to the chromatophore, very likely evolved by positive selection for fine adjustment to its new function. Again, a comparative analysis with non-photosynthetic close relatives to *P. chromatophora* would help to identify cell components that suffered from positive selection at the onset of the symbiosis. A similar analysis realized to study adaptive evolution in a very different cell lineage, suggested adaptive evolution in components of the secretory machinery of the cell (Delaye et al. 2018).

This manuscript adds important pieces of information to better understand how a novel plastid *is being make*.

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