Commentary

Subcellular targeting of proteins and pathways during evolution

'It appears logical, however, that under certain circumstances, [...] bacterial organisms may develop an absolute symbiosis with a higher organism and in some way or another impress a new character on the factors of heredity. The simplest and most readily conceivable mechanism by which the alteration takes place would be the addition of new genes to the chromosomes from the bacterial symbiont.'

(Wallin, 1925, p. 144)

The origin of mitochondria and chloroplasts were two of evolution's all-time greatest hits, because both eukaryotes (including us) and plants (our food chain) owe their beginnings to those fateful endosymbiotic encounters. One of the more interesting aspects about chloroplast and mitochondrial symbioses is the process of gene transfer that relocated genes from those endosymbionts to the chromosomes of the host. Gene transfer and the reimport of encoded products is what welds endosymbionts and their host into a new evolutionary unit. Gene transfer from endosymbionts is hardly a new concept, as the introductory quote from Wallin (1925), who was writing about mitochondria, points out. Ninety years later, we are still in the process of more fully understanding how the transfer of genes from organelles to the nucleus and the subsequent (re-)routing of those gene products to various possible destinations within the cytosol impacts cell evolution. In this issue of *New Phytologist*, Baudisch *et al.* (pp. 80–90) deliver an important contribution on the topic.

Baudisch *et al.* investigated the targeting of *Arabidopsis thaliana* mitochondrial and plastidal proteins encoded by nuclear genes that stem from cyanobacteria (plastids) and α-proteobacteria (mitochondria) and that are predicted by current targeting prediction software to be specifically imported into the respective organelles. Among 16 well-chosen proteins tested, 10 are targeted to both organelles simultaneously, as localization studies in transformed plants and *in vitro* organelle import assays show. Although dual targeting has been known for some time (Peeters & Small, 2001), systematic studies to assess its prevalence have been lacking. The new findings indicate that dual targeting is much more widespread than previously assumed. But the converse formulation, namely that targeting is ostensibly much less specific than most of us currently think, might turn out to be more significant.

Why should we think that proteins should be specifically targeted to plastids and mitochondria in the first place? Theory on the topic probably takes root in a paper by Weeden (1981) who was considering nuclear encoded chloroplast-cytosol isoenzymes involved in sugar phosphate metabolism, like phosphoglucoisomerase. The gene for the cytosolic enzyme should reflect the evolution of the host lineage that acquired the chloroplast, while the gene for the chloroplast isoenzyme should be an acquisition from cyanobacteria, with the product being specifically targeted back to the organelle in which it originally operated. This reasoning, often implicit in evolutionary studies, is called the 'product specificity corollary' to endosymbiotic theory. Work on a variety of chloroplast cytosol isoenzymes later showed that there is no strict correlation between gene origin and protein targeting (Martin & Schnarrenberger, 1997): once a copy of an organelle gene becomes established in the nucleus, its product is, over evolutionary time, free to explore various targeting possibilities within the cell and thus contribute to natural variation. A gene for an organelle-encoded protein, if copied to the nucleus (Allen, 1993) in such a way as to generate a useful promoter, will first tend to encode a cytosolic protein, for lack of the N-terminal targeting sequences that are typically thought to direct proteins to the TIC/TOC translocon of the plastid (Strittmatter *et al.*, 2010) or the TIM/TOM translocon of the mitochondrion (Dolezal *et al.*, 2006). However, as many as 30% of yeast mitochondrial proteins now seem to have other targeting signals than the canonical N-terminal presequences (Chacinska *et al.*, 2009), so we are still a far cry from understanding which signals, exactly, direct the sub-cellular compartmentation of proteins. The work by Baudisch *et al.* points the way towards large-scale, perhaps genome-wide, investigations that would provide the needed data.

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The new findings suggest that, as far as dual targeting goes, we are just seeing the tip of an iceberg. But at the same time, we should not forget that chloroplasts and mitochondria are distinct organelles; whatever degree of dual targeting is ultimately found among various species, the biochemical differences that are defining for chloroplasts and mitochondria (photosynthesis and respiration) remain. This brings up one aspect that was not covered by Baudisch *et al.*, namely residence time; getting in is not the same as staying in. It remains possible that many dual targeted proteins have different

long-term stabilities in one organelle over the other. This is something for future exploration and might provide information on the role of differential degradation in maintaining organelle identity. It also raises another point, namely the specificity of targeting for proteins of the TIM/TOM and TIC/TOC translocons themselves, because if those components end up in the 'wrong' membranes, the consequences could be dramatic, albeit not sufficient to transform one organelle into another – the DNA retained in chloroplasts and mitochondria safeguards against that.

Dual targeting might be helpful in an evolutionary context when it comes to the transfer of whole pathways from one compartment to another. This has always been a bit of a puzzle. If subcellular targeting is specific, relocating an entire pathway from one subcellular compartment to another during evolution would be very difficult, because each gene for the enzymes of the pathway would need to acquire the new (specific) targeting signal. This would necessarily occur by chance mutation, hence on a gene-by-gene basis, so that retargeting could only proceed one enzyme at a time, at best, yet to what avail? One enzyme of a pathway in a new compartment is useless (and possibly deleterious), hence is hardly selectable, hence cannot become fixed. But, recalling that a few percent of wild-type activity can confer a wild-type phenotype, if small amounts of an entire pathway are constantly in the 'wrong' compartment, this would provide a unit of function that could easily be selected and increased bit-by-bit under suitable conditions. Whether or not whole genome duplications, which are very common in eukaryote evolution, figure into the issue of retargeting is an open question.

One biotechnologically relevant example of pathway retargeting during evolution that has attracted a good bit of attention of late is hydrogen production in *Chlamydomonas reinhardtii*. The ability of this green alga to generate molecular H_2 under various conditions has put it high on the list of biofuel priorities (Hemschemeier *et al.*, 2009). Under standard photoautotrophic growth conditions, *Chlamydomonas* of course produces O_2 as a byproduct of photosynthesis. But if transferred to anaerobic, heterotrophic conditions in the dark, the alga switches to a fermentative metabolism within *c.* 30 min, producing as byproducts H_2, acetate, CO_2, formate, ethanol, and traces of lactate and glycerol (Mus *et al.*, 2007). This by itself should not be so surprising, because many eukaryotes are known that can produce this spectrum of metabolic endproducts. However, in eukaryotes, H_2 is typically generated by metabolic pathways that are localized in hydrogenosomes, which are anaerobic forms of mitochondria, although in some eukaryotic lineages the hydrogenosomal pathway has been transferred to the cytosol (Müller *et al.*, 2012). The key enzymes for eukaryotic H_2 production are pyruvate:ferredoxin oxidoreductase (PFO), which generates reduced ferredoxin that can pass the electrons along to an iron-only hydrogenase (Fe-Hyd) that in turn deposits them on protons to generate the endproduct H_2.

Chlamydomonas PFO was recently shown to localize to the chloroplast (Van Lis *et al.*, 2013). The two Fe-Hyds of *Chlamydomonas*, HydA1 and HydA2, also localize to the chloroplast (Happe *et al.*, 1994). The hydrogenase maturases, HydG, HydE and HydF (Posewitz *et al.*, 2004), which are required to assemble small organic ligands to the H_2-generating active site of the enzyme (Mulder *et al.*, 2011) would also be required in the plastid accordingly, but their localization has not so far been shown. This example of a small pathway that has moved between mitochondria, the cytosol and plastids during evolution shows that pathways are indeed still on the move in different eukaryotic lineages, in line with the new findings of Baudisch *et al.* A better understanding of the mechanisms through which proteins reach their proper destination(s) within the cell should also improve our understanding of how evolution decides which of those destinations work(s) best in the organism's given niche.

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Key words: chloroplasts, dual targeting, endosymbiotic gene transfer, mistargeting, mitochondria, protein import, recompartmentation.