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Review

A reevaluation of dual-targeting of proteins to mitochondria and chloroplasts ☆

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ABSTRACT

Over 100 proteins are found in both mitochondria and chloroplasts, via a variety of processes known generally as 'dual-targeting'. Dual-targeting has attracted interest from many different research groups because of its profound implications concerning the mechanisms of protein import into these organelles and the evolution of both the protein import machinery and the targeting sequences within the imported proteins. Beyond these aspects, dual-targeting is also interesting for its implications concerning shared functions between mitochondria and chloroplasts, and especially the control of the activities of these two very different energy organelles. We discuss each of these points in the light of the latest relevant research findings and make some suggestions for where research might be most illuminating in the near future. This article is part of a Special Issue entitled: Protein Import and Quality Control in Mitochondria and Plastids.

1. Introduction

Mitochondria and chloroplasts have many parallels in their evolutionary history; both are derived from bacterial endosymbionts, both are crucial contributors to the energy metabolism of the cell, both retain their own reduced genome and some components of the gene expression machinery, and both import a large proportion (over 95%) of the proteins they need to function. During this parallel evolutionary history, the two organelles have co-existed and multiplied in the same cells for around a billion years. Given the many similar metabolic and genetic functions required in mitochondria and chloroplasts, it is not that surprising that many similar proteins are found in both organelles. In most cases, these similar proteins are paralogues encoded by different genes (e.g. the 450 pentatricopeptide repeat proteins targeted to mitochondria or chloroplasts and involved in similar post-transcriptional processes in both [1]). However, there are a steadily increasing number of examples where proteins with identical sequence are found in both organelles, clearly translated from the same gene. These instances are referred to as 'dual-targeting', a term first coined in this context by Ref. [2] to describe this phenomenon.

Dual-targeting is interesting for many different reasons, and has attracted a number of reviews over the last decade and more [3–8]. From an evolutionary point of view, the loss of previous duplicated genes to concentrate two functions in a single gene is relatively unusual, as the more common tendency is neo-specialization via gene duplications. This implies that dual-targeting may confer advantages that are not immediately apparent. Dual-targeting is also interesting from a mechanistic point of view, as the protein import machineries of mitochondria and chloroplasts arose independently, are non-homologous, and therefore would not normally be expected to recognize the same proteins [9,10]. Finally, dual-targeting is interesting for its implications concerning the control of mitochondrial and chloroplast biogenesis and function. How can the activities of the two organelles be independently controlled if many of their critical components are shared?

In this short review, we treat each of these questions in turn, and attempt to explain current thinking in this field in the light of the latest discoveries. Many questions remain, however, and we also suggest the types of experiments likely to be most informative in the near future.

2. Which proteins are dual-targeted?

Over 100 proteins have been proposed to be dual-targeted, based on individual analysis, generally by tagging with fluorescent markers such as GFP [11]. It is important to note that such experiments cannot formally prove that an identical protein is imported into both compartments, as a single DNA construct can produce 2 or more proteins of distinct sequence via alternative transcription or translation starts, or alternative splicing [8]. These can still be considered examples of functional dual-targeting [12], but if the peptide signals recognized by the import machinery vary between isoforms, then this does not pose the same mechanistic conundrum as when an identical protein is transported into both organelles. A smaller number of putatively dual-targeted examples have been investigated in more detail, using

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Table 1
Over 100 proteins have been reported to be dual-targeted to mitochondria and chloroplasts.

Accession	Description	GO biological process	Solubility	Method	Ref.
At1g13900	Purple acid phosphatase 2 (AtPAP2)	Biological process	Membrane	Fluorescent tagging/Western blotting	[40]
At5g06810	Mitochondrial transcription termination factor	Biological process	Soluble	Fluorescent tagging	[41]
At5g08710	Regulator of chromosome condensation (RCC1)	Biological process	Soluble	Fluorescent tagging	[11]
At5g16200	50 S ribosomal protein-related	Biological process	Soluble	Fluorescent tagging	[42]
At4g30490	AFG1-Like ATPase protein	Biological process	Soluble	Fluorescent tagging	[42]
At5g23060	Calcium sensing receptor (CAS)	Cellular response to calcium ion	Membrane	Fluorescent tagging	[11]
At5g26940	Mg2 + dependent DNA exonuclease (DPD1)	DNA catabolic process, exonucleolytic	Soluble	Fluorescent tagging	[43]
At5g24850	Flavin adenine dinucleotide (Cry3)	DNA repair	Soluble	Fluorescent tagging/in vitro import	[44]
At1g30680	Toprim domain containing DNA helicase	DNA replication	Soluble	Fluorescent tagging	[11]
At1g50840	DNA Polymerase gamma 2	DNA replication	Soluble	Fluorescent tagging	[45]
AB174899	DNA-directed DNA polymerase 2	DNA replication	Soluble	Fluorescent tagging	[46]
At3g10690	DNA gyrase A	DNA replication	Soluble	Fluorescent tagging	[47]
AY351386	DNA gyrase A	DNA replication	Soluble	Fluorescent tagging	[48]
AY351387	DNA gyrase B	DNA replication	Soluble	Fluorescent tagging	[48]
At3g20540	DNA Polymerase gamma 1	DNA replication	Soluble	Fluorescent tagging	[11]
AB174898	DNA-directed DNA polymerase 1	DNA replication	Soluble	Fluorescent tagging	[46]
At4g31210	DNA topoisomerase	DNA replication	Soluble	Fluorescent tagging	[11]
At5g40810	Cytochrome C1 protein	Electron transporter	Membrane	Fluorescent tagging/in vitro import	[49]
CAA44055	Cytochrome C1	Electron transporter	Membrane	Fluorescent tagging/in vitro import	[49]
At3g54660	Glutathione reductase 2 (AtGR2)	Glutathione metabolic process	Soluble	Fluorescent tagging/in vitro import	[50]
P27456	Glutathione reductase	Glutathione metabolic process	Soluble	Enzyme assay/in vitro import	[51]
At4g26500	Sulfur acceptor (AtSufE1)	Iron–sulfur cluster assembly	Soluble	Fluorescent tagging	[52]
At1g21400	Thiamin diphosphate binding fold protein	Metabolic process	Soluble	Fluorescent tagging	[42]
At5g35630	Glutamine synthetase 2 (GLN2)	Metabolic process	Soluble	Fluorescent tagging/Western blotting	[53]
Nicotiana tabacum	NADP + dependant isocitrate dehydrogenase	Metabolic process	Soluble	Fluorescent tagging	[19]
Phypa_180964	Early response to dehydration 4 homolog (ERD4)	Metabolic process	Soluble	Fluorescent tagging	[21]
Phypa_202996	Phosphatidylinositol dependent phospholipase (PLC)	Metabolic process	Soluble	Fluorescent tagging	[21]
5915312	Hexokinase 8	Metabolic process	Membrane	Fluorescent tagging	[54]
5947550	Hexokinase 3	Metabolic process	Membrane	Fluorescent tagging	[54]
5947828	Hexokinase 2	Metabolic process	Membrane	Fluorescent tagging	[54]
5917393	Hexokinase 7	Metabolic process	Membrane	Fluorescent tagging	[54]
5933371	Hexokinase 9	Metabolic process	Membrane	Fluorescent tagging	[54]
5939952	Hexokinase 10	Metabolic process	Membrane	Fluorescent tagging	[54]
5942660	Hexokinase 11	Metabolic process	Membrane	Fluorescent tagging	[54]
At1g74600	PPR protein (OTP87)	mRNA modification	Soluble	Fluorescent tagging	[55]
At4g21170	PPR protein	mRNA modification	Soluble	Fluorescent tagging	[41]
At4g32400	Nucleotide carrier protein (Brittle 1)	Nucleotide transport	Membrane	Fluorescent tagging	[17]
AAA33438	Brittle 1	Nucleotide transport	Membrane	Fluorescent tagging/immuno gold labeling	[17]
At3g05790	Lon protease 4 (Lon4)	Oxidation-dependent protein catabolic process	Soluble	Fluorescent tagging/Western blotting	[56]
At5g26860	Lon protease 1 (Lon1)	Oxidation-dependent protein catabolic process	Soluble	Fluorescent tagging	[57]
At4g08390	Stromal ascorbate peroxidase (sAPX)	Oxidation-reduction process	Soluble	Fluorescent tagging/in vitro import	[50]
At5g08740	NAD(P)H dehydrogenase C1 (NDC1)	Oxidation-reduction process	Membrane	Fluorescent tagging/in vitro import	[58]
At2g39290	Phosphatidylglycerolphosphate synthase 1 (PGPS1)	Phospholipid biosynthetic process	Soluble	Fluorescent tagging	[59]
At5g04140	Ferredoxin-dependent glutamate synthase (GLS1)	Photorespiration	Soluble	Fluorescent tagging	[60]
At5g38710	Methylenetetrahydrofolate reductase family protein	Proline catabolic process	Soluble	Fluorescent tagging	[42]
At5g55200	Co-chaperone grpE protein (MGE1)	Protein folding	Soluble	Fluorescent tagging	[42]
Phypa_187670	FtsZ family protein	Protein polymerization	Soluble	Fluorescent tagging	[21]
At1g49630	Zinc metalloprotease (AtPrep2)	Protein processing	Soluble	Fluorescent tagging/in vitro import	[61]
At3g16480	Mitochondrial processing peptidase alpha 2 (MPPalpha2)	Protein processing	Membrane	Fluorescent tagging/in vitro import	[62]
At3g19170	Zinc metalloprotease (AtPrep1)	Protein processing	Soluble	Fluorescent tagging/in vitro import/ Western blotting	[61]
At3g25740	Methionine aminopeptidase 1B (MAP1B)	Protein processing	Soluble	Fluorescent tagging	[63]
Os02g52420	Methionine aminopeptidase	Protein processing	Soluble	Fluorescent tagging	[31]
At4g37040	Methionine aminopeptidase 1D (MAP1D)	Protein processing	Soluble	Fluorescent tagging	[63]
At1g11870	Seryl-tRNA synthetase	Protein translation	Soluble	Fluorescent tagging/in vitro import	[64]
BG462707	Seryl-tRNA synthetase	Protein translation	Soluble	Fluorescent tagging/Western blotting	[65]
At1g48520	glutamyl-tRNA(Gln) amidotransferase subunit B	Protein translation	Soluble	In vitro import/Western blotting	[66]
At1g50200	Alanyl-tRNA synthetase	Protein translation	Soluble	Fluorescent tagging/in vitro import	[64]
At2g04842	Threonyl-tRNA synthetase	Protein translation	Soluble	In vitro import	[64]
At2g25840	Tryptophan-tRNA synthetase	Protein translation	Soluble	Fluorescent tagging	[64]
At2g31170	Cysteinyl-tRNA synthetase	Protein translation	Soluble	Fluorescent tagging	[67]
At3g02660	Tyrosine-tRNA synthetase	Protein translation	Soluble	In vitro import	[64]
Os01g31610	Tyrosine-tRNA synthetase	Protein translation	Soluble	Fluorescent tagging	[31]
At3g12370	Ribosomal protein L10	Protein translation	Soluble	Fluorescent tagging	[68]
Os05g03030	Ribosomal protein L10	Protein translation	Soluble	Fluorescent tagging	[68]
At3g13490	Lysyl-tRNA synthetase	Protein translation	Soluble	Fluorescent tagging/in vitro import/ Western blotting	[64]
At3g25660	glutamyl-tRNA(Gln) amidotransferase subunit A	Protein translation	Soluble	In vitro import/Western blotting	[66]
At3g46100	Histidyl-tRNA synthetase	Protein translation	Soluble	Fluorescent tagging	[69]

Table 1 (*continued*)

Accession	Description	GO biological process	Solubility	Method	Ref.
At3g48110	Glycine-tRNA synthetase	Protein translation	Soluble	Fluorescent tagging	[64]
At3g55400	Methionyl-tRNA synthetase	Protein translation	Soluble	Fluorescent tagging/in vitro import	[70]
At3g58140	Phenylalanyl-tRNA synthetase	Protein translation	Soluble	In vitro import	[64]
At4g10320	Isoleucine-tRNA synthetase	Protein translation	Soluble	Fluorescent tagging	[64]
At4g17300	Asparaginyl-tRNA synthetase	Protein translation	Soluble	Fluorescent tagging	[67]
At4g32915	glutamyl-tRNA(Gln) amidotransferase subunit C	Protein translation	Soluble	In vitro import	[66]
At4g33760	Aspartyl-tRNA synthetase	Protein translation	Soluble	In vitro import	[64]
At5g14660	Peptide deformylase 1B (PDF1B)	Protein translation	Soluble	Fluorescent tagging	[63]
Os01g45070	Peptide deformylase 1B	Protein translation	Soluble	Fluorescent tagging	[71]
At5g16715	Valine-tRNA synthetase	Protein translation	Soluble	Fluorescent tagging/in vitro import	[64]
At5g22800	Alanyl-tRNA synthetase	Protein translation	Soluble	In vitro import	[64]
At5g52520	Proline-tRNA synthetase	Protein translation	Soluble	In vitro import	[64]
At5g56940	Ribosomal protein S16-2	Protein translation	Soluble	Fluorescent tagging	[72]
Os09g32274	Ribosomal protein S16-1	Protein translation	Soluble	Fluorescent tagging	[72]
Os08g40610	Ribosomal protein S16-2	Protein translation	Soluble	Fluorescent tagging	[72]
AB365529	Ribosomal protein S16-1	Protein translation	Soluble	Fluorescent tagging	[72]
AB365530	Ribosomal protein S16-2	Protein translation	Soluble	Fluorescent tagging	[72]
B365527	Ribosomal protein S16-2	Protein translation	Soluble	Fluorescent tagging	[72]
AB365528	Ribosomal protein S16	Protein translation	Soluble	Fluorescent tagging	[72]
At5g64050	Glutamate-tRNA synthetase	Protein translation	Soluble	Fluorescent tagging	[64]
Os02g02860	Glutamate-tRNA synthetase	Protein translation	Soluble	Fluorescent tagging	[31]
At1g12520	Copper-zinc superoxide dismutase copper chaperone (AtCcs)	Removal of superoxide radicals	Soluble	In vitro import	[73]
At1g63940	Monodehydroascorbate reductase 6 (MDAR6)	Response to cadmium ion	Soluble	Fluorescent tagging/in vitro import	[50]
Os08g05570	Monodehydroascorbate reductase	Response to cadmium ion	Soluble	Fluorescent tagging	[31]
At4g25200	AtHsp23.6	Response to heat	Soluble	Fluorescent tagging	[42]
At5g51440	AtHsp23.5	Response to heat	Soluble	Fluorescent tagging	[42]
At1g31170	Cysteine-sulfinic acid reductase (Sulfiredoxin, AtSrx)	Response to oxidative stress	Soluble	Immuno gold labeling/in vitro import/ Western blotting	[74]
At3g23830	glycine-rich RNA binding protein	Response to salt stress	Soluble	Fluorescent tagging	[5]
At5g63980	3′(2′),5′bisphosphate nucleotidase and inositol polyphosphate 1 phosphatase(AtSAL1)	RNA catabolic process	Soluble	Fluorescent tagging/Western blotting	[75]
At2g30320	Pseudouridine synthase	RNA modification	Soluble	Fluorescent tagging	[5]
At1g22660	Polynucleotide adenylyltransferase protein	RNA processing	Soluble	Fluorescent tagging	[76]
At5g44785	Organellar single-stranded DNA binding protein (OSB3)	Single-stranded DNA binding	Soluble	Fluorescent tagging	[77]
At1g79230	Mercaptopyruvate sulfurtransferase 1 (MST1)	Sulfate transport	Soluble	Fluorescent tagging/Western blotting	[78]
At5g54770	Thiamine biosynthetic gene (THI1)	Thiamine biosynthetic process	Soluble	Fluorescent tagging	[79]
At5g15700	DNA-directed RNA polymerase 2	Transcription	Soluble	Fluorescent tagging	[80]
AJ302019	DNA-directed RNA polymerase 2	Transcription	Soluble	Fluorescent tagging	[80]
Q8VWF8	DNA-directed RNA polymerase Tpm	Transcription	Soluble	Fluorescent tagging	[81]
CAC95163	DNA-directed RNA polymerase 1	Transcription	Soluble	Fluorescent tagging	[82]
CAC95164	DNA-directed RNA polymerase 2	Transcription	Soluble	Fluorescent tagging	[82]
At4g36580	AAA-type ATPase family protein	Zinc ion binding	Soluble	Fluorescent tagging	[42]

import assays to formally demonstrate that a single protein is a substrate for both import machineries [11].

These known examples are probably still only a minority of the truly dual-targeted proteins; 277 proteins have been experimentally observed in mitochondrial and plastids by various groups using different approaches on different occasions (data from SUBA [13]). Although it is probable that some of these observations are false-positives (e.g. due to cross-contamination of organellar fractions) many are likely to be true examples of dual-targeting that have yet to be analyzed in any detail.

Dual-targeted proteins show a tendency to fall within a few specific functional groupings [3] (Table 1). This biased distribution may to some extent reflect the interests of the researchers who have taken a particular interest in dual-targeting, but could also reflect mechanistic constraints on dual-targeting and functional advantages/disadvantages that may drive selection pressure for or against dual targeting. Of the known dual-targeted proteins, most are soluble matrix/stromal proteins. Only a few are membrane-bound, most commonly outer envelope proteins (Table 1). Of course, mitochondria do not contain a compartment analogous to the thylakoids, so a lack of thylakoid proteins in mitochondria is hardly surprising. However, the relative lack of dual-targeted membrane proteins extends to the inner envelope membrane and to a lesser extent, the outer envelope of both organelles too. These membranes do share many molecular functions across the two organelles, including transport of similar or identical metabolites, protein import and cytoskeletal attachments, but share very few proteins. The comparative lack of dual-targeted membrane proteins may therefore be a result of differences in membrane protein insertion/import pathways between mitochondria and chloroplasts rather than functional constraints.

Within the large group of dual-targeted soluble proteins, it is obvious that there is a strong bias towards proteins involved in a small set of central processes, namely nucleotide metabolism; DNA replication, recombination and repair; tRNA biogenesis and translation. In these cases, it is hard to imagine what mechanistic features of organelle import systems would produce such an apparent bias, and therefore we assume that the bias is linked to the biological functions of these proteins. These functions give us clues for considering the selective pressures that might favor dual-targeting over evolutionary timescales, a topic that we shall return to in a later section.

3. How are proteins dual-targeted?

It appears that at least for dual-targeting between mitochondria and chloroplasts, the majority of examples do indeed involve an identical precursor protein being recognized by receptors on both organelles [3]. These events have been termed ‘ambiguous’ targeting, as a single signal peptide carries a dual function [5]. Dual-targeting between other compartments (e.g. plastids and cytosol, or mitochondria and nucleus) is much more likely to involve alternative gene expression processes leading to generation of distinct proteins from the same gene [8].

Given the non-homologous origin of the two import systems, how can a single protein be efficiently recognized by both systems? In theory, three alternative routes can be imagined:

- the mitochondrial and chloroplast import machineries contain a shared receptor specific for dual-targeted proteins;
- dual-targeted proteins contain a multipartite transit peptide in which different segments are recognized by different receptors in each of the mitochondrial and plastid import machineries;
- dual-targeted proteins contain a truly ambiguous transit peptide in which the same sequences/structures are recognized by disparate receptors in each of the mitochondrial and plastid import machineries.

The first of these routes seems rather unlikely, given that no shared components have been identified, and that the transit peptides of dual-targeted proteins show no obviously specific features that would distinguish them from singly targeted proteins [14,15]. Furthermore, if this was true, mutations in the transit peptide sequences of dual-targeted proteins would abolish targeting to both organelles, and that is not what is always observed. For example, deletion of the N-terminal segment of the targeting sequences of AspRS and LysRS affected import into mitochondria much more than import into plastids, whereas the contrary was observed for ProRS [16]. Furthermore, the targeting peptide of the dual-targeted ThrRS acts as an inhibitor to mitochondrial and plastid protein import of singly-targeted proteins, strongly suggesting that it is interacting with the same receptors [14].

The second route would perhaps seem the easiest to imagine mechanistically. Here, the prediction would be that localized alterations to the transit peptide should only affect targeting to one organelle, but not the other. However, again this does not fit well with many published observations. For three of the aminoacyl-tRNA synthetases (TyrRS, ValRS, ThrRS) tested by Berglund et al. [14,16] deletion of the extreme N-terminus affected import into both organelles equally. These deletions ranged from 20 to 25 amino acids, so if the N-terminus of these proteins contains two distinct domains required for targeting to each organelle, they must be relatively short. For three other aminoacyl-tRNA synthetases that were investigated in the same study, deletion of similar N-terminal regions preferentially affected import into one organelle, strongly suggesting that the targeting information is to some extent separated [16]. In other examples, this spatial separation of the targeting information is clear-cut. Dual-targeting of the carrier protein BT1 requires an N-terminal plastid targeting sequence but an internal mitochondrial targeting sequence, and thus almost certainly involves completely different receptors on both organelles [17]. Another rather unusual example is the cucumber necrosis virus coat protein which, via distinct domains, associates with both organelles [18].

The third route seems the most difficult to conceive, given the requirement for a single protein surface to bind to two different non-homologous ligands (and probably multiple non-homologous ligands, given the number of probable protein–protein contacts required during protein import in both organelles). The prediction in this case would be that mutations/deletions in the transit peptide might affect import into either organelle or both, depending on the nature of the mutations and their location. The complex effects of the deletions that have been tested are compatible with this hypothesis, particularly those cases where targeting to both organelles is lost with a single deletion [14,16]. However, deletion analysis is a fairly blunt instrument and further testing of this explanation will require more precise studies that examine the effects of single amino acid changes, and identification of exactly which receptors are used by each protein during import [20].

Studies of the transit peptides of dual-targeted proteins have shown that they share features of both mitochondrial and plastid transit peptides, often appearing intermediate between the two [14,15]. Bioinformatics predictions of targeting based on transit peptide sequences have always proved extremely problematic for dual-targeted proteins [11,21]. If indeed the same sequence is being recognized by different receptors, then these difficulties become understandable.

It is interesting to consider the evolutionary implications of the plant cell being 'forced' (by the existence of dual-targeted proteins) to maintain import machineries in both organelles that can recognize the same proteins. In the absence of dual-targeting, one would expect selection pressure to drive both transit peptides and the import machinery to diverge to become as distinct as possible to avoid mistargeting. With the existence of dual-targeting via ambiguous transit peptides, on the contrary, there must also be strong balancing selection preventing the two import systems becoming too distinct. Comparison of plant mitochondrial transit peptides with those from animals and fungal showed convincingly that the plant sequences are more highly constrained in terms of sequence diversity [22]. The authors of this work took this to indicate selection against mistargeting, but the observations could also be partly explained by the constraining effects of essential dual-targeting on the import machinery. In fact, there is evidence to suggest that plastid transit peptides have evolved to be more similar to mitochondrial targeting signals [23]. Either way, it implies that low levels of mistargeting are likely to persist, allowing selection of new localizations over evolutionary timescales [24]. This has led to the suggestion that if the cost of having a pool of dual targeted proteins is low, it can be an evolutionary advantage to allow a subset of proteins to explore a different compartment of the cell [25].

4. Why are proteins dual-targeted?

We consider this to be one of the most interesting questions that remain to be answered. The fact that so many proteins are dual-targeted implies that either there is an advantage for the individual, or there is an ineluctable process that is driving gene loss. As an example of the latter process, gene transfer/loss from the organelle genomes is almost certainly driven by a one-way 'ratchet' quite apart from any selective advantage that might be accrued—whereas the nuclear genome incorporates organelle DNA at relatively high frequency, nuclear DNA insertions into organelle genomes are extremely unusual [26–28]. Therefore loss of an organellar gene is almost irreversible.

Imagine a pair of nuclear genes encoding proteins of identical molecular function, one a mitochondrial isoform, the other a plastid isoform. If one of the genes acquires mutations rendering the encoded protein dual-targeted, the second gene is now potentially redundant [29]. Loss of this redundant gene by deletion or mutational drift might appear to resemble the case of loss of organellar genes described above. However, plant genomes undergo repeated duplications, both local duplications of individual genes and whole genome duplications via hybridization and polyploidy [30]. This recurrent generation of duplicated genes provides plenty of raw material to re-establish the initial situation of two genes encoding singly-targeted proteins (Fig. 1), and yet such 'reversions' are rarely if ever observed [31].

The apparent discrepancy between the rates at which new dual-targeted proteins arise compared with the rate at which they are lost implies that there may be general advantages to dual-targeting acting to preserve the dual-targeting state once it has arisen. What might these advantages be? An obvious benefit is the apparent economy of only needing to maintain a single gene instead of two, but this appears to be trivial; even the total extra gene load of duplicating all known genes encoding dual-targeted proteins would be fairly insignificant, and the load of a single extra gene is surely negligible. On the other hand, there are clear disadvantages in that the freshly diverted protein is unlikely to be immediately functionally optimal in its new organelle; differences in ion and metabolite concentrations, pH and macromolecular interactions are all likely to negatively impact function. Nevertheless, these changes in targeting have occurred repeatedly and successfully.

Some insight is provided by considering the types of proteins that are dual-targeted, i.e. predominantly those involved in DNA synthesis

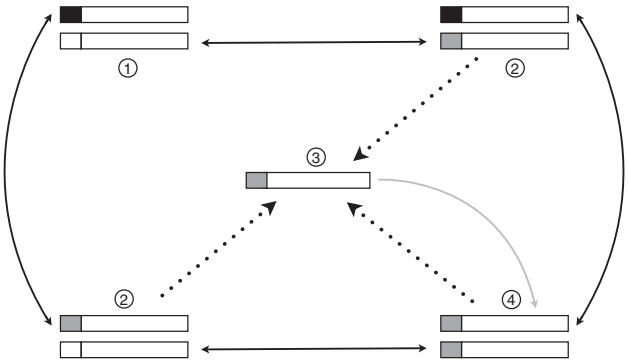

Fig. 1. Evolutionary pathways to and from the dual-targeting state. A pair of essential genes (1) encoding transit peptides for mitochondria (black) or plastids (white) may be subject to mutations (solid arrows) giving rise to ambiguous targeting sequences (gray) (2). The gene encoding the singly-targeted protein is now redundant, and can be lost via deletion (dashed arrows), leaving a single gene encoding the dual-targeted protein (3). In theory, the process can be reversed by gene duplication (gray arrow), giving a pair of genes encoding dual-targeted proteins (4), which via further mutations could restore any of the prior states. In practice, this reversal appears to be infrequent and to our knowledge has never been shown to have occurred.

and maintenance, and translation (Table 1) [3]. These are fundamental and essential processes, and because of this, these proteins tend to retain highly conserved functions. This conservation facilitates dual-targeting by ensuring that the proteins from the two organelles are functionally interchangeable. Indeed, it may be that there is a (cryptic) general advantage to dual-targeting for all proteins, and that the only reason that there is an observed preference for dual-targeting within these classes of proteins is that they are the most easily shared because of conserved function. However, there is a more interesting possibility, even if it remains rather speculative. Nuclear genes involved in organellar DNA replication and translation share two other important characteristics; they are under strict control within the cell, being coordinately expressed at particular phases of development [32,33], and they genetically interact closely with the organellar genes, which are inherited in a non-Mendelian fashion and do not segregate independently during meiosis. Nuclear genes involved in organellar DNA replication and translation are physically clustered [34] and thus tend to be inherited as a block, as are the organelle genomes that they act on. This will tend to limit disadvantageous (in this case) genetic variability. Dual-targeting can be considered as another process that limits genetic variability, both by limiting the number of genetic loci and by exposing the gene sequence to a double selection pressure for function in two distinct environments.

5. Implications for control

Dual-targeting of proteins has considerable implications for control of their expression and function. It seems unlikely that both mitochondria and chloroplasts have an equal need for all dual-targeted proteins in all cells at all stages of the life-cycle. Clearly the requirements for mitochondrial and plastid function are very different in a leaf mesophyll cell compared to a root cortex cell, and although very few, if any, of the proteins showing massive cell-type specific changes in gene expression (e.g. proteins involved in photosynthesis) are dual-targeted (Table 1), one would still expect significant differential changes in processes (such as translation) in which large numbers of dual-targeted proteins are involved. So how are expression and targeting of dual-targeted proteins regulated when the biogenesis of mitochondrial and plastids is temporally and quantitatively different?

To a large extent, broad, coordinate regulation may be achieved by maintaining appropriate sinks—by expressing more mitochondrial than plastid import components, targeting is diverted to mitochondria in some cells [35], whereas with the opposite ratio in other cells, targeting would be preferentially to plastids. Although this hypothesis has not been thoroughly investigated to our knowledge, it seems a reasonable supposition, and could explain appropriate targeting for most dual-targeted proteins in most cells. However, more specific mechanisms may also be at work, including cell-type specific expression of particular import receptors [36], targeting of mRNA to the vicinity of specific organelles prior to translation [37], phosphorylation of targeting peptides [38], or redox regulation of the two sets of import machineries [39].

6. Conclusions and future research directions

The importance of dual-targeting is now widely recognized, both in terms of the sheer number of proteins that are involved and the importance of the processes these proteins are involved in. Many of the initial questions about the mechanisms of dual-targeting have been answered, although detailed examinations of exactly which outer-membrane receptors are involved in each organelle remains to be carried out.

In the near future, research is likely to switch to dynamic and cell-specific studies of targeting in order to look for how targeting is regulated under physiologically relevant conditions, i.e. in whole plants. The evolutionary questions posed by dual-targeting are starting to be appreciated, and it would be an interesting case to look at using cost-benefit models, if reasonable estimates for all the necessary parameters can be obtained. First, though, some systematic studies of the evolutionary stability of the dual-targeted state are needed.

Acknowledgments

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