Moreover, the binding of the extracellular domains of adhesins to host cell surface receptors remains incompletely characterized, as are the molecular interactions that govern processing by proteases.

Judging from their deep branching evolutionary position and present-day success, apicomplexans are likely to be with us for some time. Thus far, our glimpses into parasite motility have revealed a very different process than that used by mammalian cells. Although these differences may explain the tremendous success of apicomplexans, their understanding may also enable selective disruption of parasite motility. If we are to thwart these ancient and mysterious parasites, our attention should be focused on defining their unique biology.

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54. I thank colleagues who generously provided images, videos, unpublished data, and many helpful discussions and apologize for omissions due to space limitations. Support provided by the NIH and the Burroughs Wellcome Fund.

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REVIEW

Ancient Invasions: From Endosymbionts to Organelles

Sabrina D. Dyall, Mark T. Brown, Patricia J. Johnson*

The acquisitions of mitochondria and plastids were important events in the evolution of the eukaryotic cell, supplying it with compartmentalized bioenergetic and biosynthetic factories. Ancient invasions by eubacteria through symbiosis more than a billion years ago initiated these processes. Advances in geochemistry, molecular phylogeny, and cell biology have offered insight into complex molecular events that drove the evolution of endosymbionts into contemporary organelles. In losing their autonomy, endosymbionts lost the bulk of their genomes, necessitating the evolution of elaborate mechanisms for organelle biogenesis and metabolite exchange. In the process, symbionts acquired many host-derived properties, lost much of their eubacterial identity, and were transformed into extraordinarily diverse organelles that reveal complex histories that we are only beginning to decipher.

Analyses of mitochondrial genes and their genomic organization and distribution indicate that mitochondrial genomes are derived from an α-proteobacterium–like ancestor, probably due to a single ancient invasion (Fig. 1) of an Archea-type host that occurred >1.5 billion years ago (Ga) (*1*). Whether the host cell was already eukaryotic is unclear (Fig. 1), although all contemporary eukaryotes examined contain some genes contributed by this symbiont (*2*).

How the proto-mitochondrial ancestor invaded and avoided elimination by the host has generated many hypotheses since the symbiosis theory was revived by Margulis (*3*). Some account for the concurrent origin of eukaryotes and mitochondria (*4*, *5*). These hypotheses propose a metabolically driven symbiosis where the host is a methanogenic archaean that associated with a methanotrophic proteobacterium to obtain essential compounds, e.g., hydrogen (*4*). The hydrogen hypothesis accounts for both mitochondrial aerobic pathways and anaerobic pathways in organelles of possible mitochondrial ancestry, e.g., hydrogenosomes (*4*). Notably, these scenarios posit the invasion to have occurred under anoxic conditions because both host and symbiont were capable of anaerobic metabolism. In contrast, an "aerobic" origin theory hypothesizes that the symbiosis was driven by an aerobic proteobacterium relieving

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an anaerobic host from oxygen tension (*6*).

Fossils of red algae–like organisms confirm that multicellular plastid-bearing eukaryotes existed 1.2 Ga, and there is evidence of eukaryotic algaelike organisms around 1.5 Ga (Fig. 1). Mitochondria appear to predate the advent of plastids; therefore, the proto-mitochondrial invasion is believed to have occurred >1.5 Ga (*7*). Oxygenic photosynthesis is thought to have started 3.5 Ga, with oxygen levels becoming substantial by about 2.2 Ga, as intimated by the discovery of oxidized rocks (*8*). The sharp rise in oxygen 2.2 Ga supports the aerobically driven origin for mitochondrial endosymbiosis (*6*). However, carbon isotope signatures indicate that archaea and proteobacteria coexisted around 2.7 Ga, giving support to the anaerobic-driven hypotheses (*4*, *5*). Thus, it is not clear what evolutionary bottleneck forged the irreversible union of the endosymbiont and its host.

Of Mitochondria and Hydrogenosomes

Several microaerophilic protists, e.g., trichomonads, anaerobic fungi, and ciliates, do not have mitochondria but possess doublemembraned organelles called hydrogenosomes, which produce adenosine triphosphate (ATP) fermentatively. Unlike mitochondria, which use pyruvate dehydrogenase for pyruvate oxidation, *Trichomonas* hydrogenosomes decarboxylate pyruvate with pyruvate:ferredoxin oxidoreductase (PFOR), which transfers electrons to an [Fe]-hydrogenase, ultimately producing ATP, H_2, and CO_2 (*9*). PFOR and hydrogenase are typically found in anaerobic bacteria, and the origin of the eukaryotic homologs is unknown, although it appears that eukaryotic PFOR has a single origin (*2*). However, phylogenetic analyses of a few protein-coding genes have suggested a common ancestry for hydrogenosomes and mitochondria, as do similarities in organelle biogenesis (*10*).

Hydrogenosomes appear to lack a genome (*10*), and ultimately defining the proto-mitochondrion/hydrogenosome relationship will be circumstantial, because this must be proteome-based. For instance, phylogenetic analyses of >400 nucleus-encoded yeast mitochondrial proteins have revealed that 50% are of eukaryotic origin and 50% of prokaryotic origin. Of the latter, only 20% are α-proteobacteria-derived (*6*). In a reverse approach, where α-proteobacterial genomes were compared with eukaryotic genomes, it was found that only 14 to 16% of mitochondrial proteins were of α-proteobacterial origin (*11*), implying that most of the mitochondrial proteome is of nonendosymbiotic origin. Thus, the discovery of a few proteins of mitochondrial

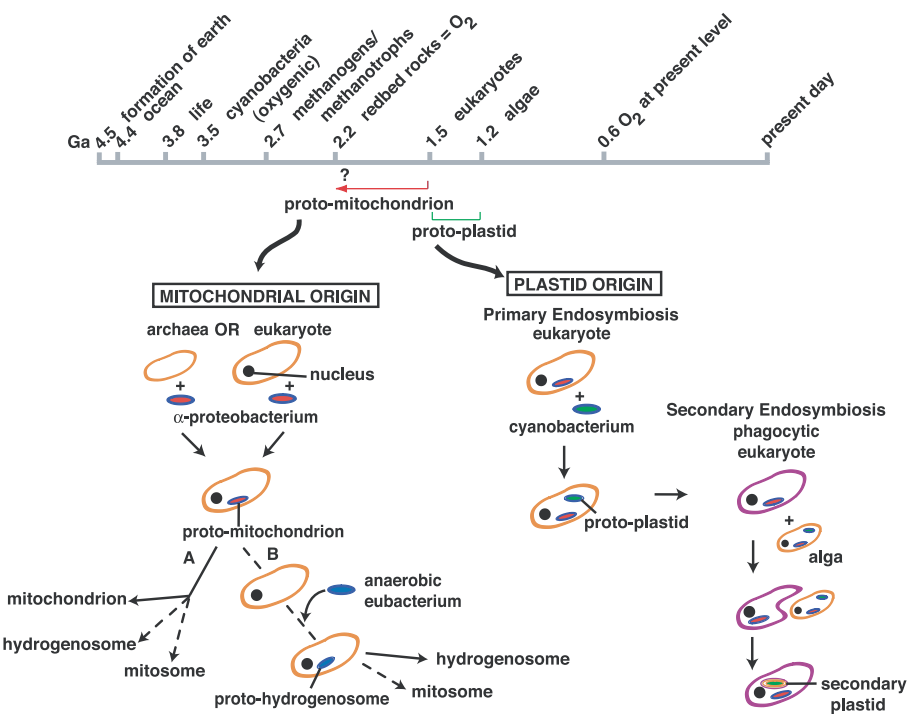

Fig. 1. Time line for the origin of life and major invasions giving rise to mitochondria and plastids. Mitochondria/α-proteobacteria are shown in red, hydrogenosomes/anaerobic eubacteria in dark blue, plastids/cyanobacteria in green, and endomembranes around secondary plastids in yellow. Broken lines indicate unresolved relationships. A, scenario A; B, scenario B; Ga, billion years ago. For an extensive discussion, see (*7*, *8*).

tein translocation machinery, the proto-mitochondrion was lost in some cells. A second invasion by an anaerobic eubacterium subsequently occurred, giving rise to a proto-hydrogenosome that acquired proteins generated by the prior endosymbiotic event. Such protein recruitment has been noted in chloroplasts for some Calvin cycle proteins that have a proteobacterial origin (*12*). Clearly, further appraisal of the hydrogenosomal proteome, particularly of eukaryotic-type proteins, is required before conclusively assigning an origin for this organelle. The origin of proposed mitochondrial remnants (*13*) found in three independent lineages—*Entamoeba* (mitosome), *Giardia* (mitosome), and a microsporidian (mitochondrial relic)—should likewise be viewed tentatively (Fig. 1, scenarios A and B). These structures have been defined by the presence of a single mitochondrial-like protein, which is different in each case (either cpn60, Hsp70, or IscS). Whether these structures are ultimately found to be directly derived from mitochondria will await proteomic analyses.

Invasion of the "Little Green Slaves"

Historically, the endosymbiotic theory of chloroplast evolution can be traced back to Mereschkowsky's hypothesis in 1905 that plastids are reduced forms of cyanobacteria acting as "little workers, green slaves" within the cell (*14*). Phylogenetic, structural, and biochemical analyses have now confirmed that a single symbiotic association between a cyanobacterium and a mitochondriate eukaryote between 1.2 and 1.5 Ga (Fig. 1) led to the birth of primary plastids of algae, plants, and glaucophytes (*7*, *15*). The type of cyanobacteria that gave rise to plastids is still being investigated. Remarkably, plastids have spread by secondary endosymbiosis, whereby photosynthetic eukaryotes were engulfed by nonphotosynthetic eukaryotes (Fig. 1). The resulting secondary plastids underwent genome reduction and in some cases even lost their photosynthetic functions, e.g., apicoplasts (*15*).

From Invaders to Captives: Genome Reduction

A critical step in the transition from autonomous endosymbiont to organelle was genome reduction. Contemporary mitochondrial genomes range from 3 to 67 protein-coding genes (*1*), and chloroplast genomes from 50 to 200 (*16*). Many endosymbiont genes have been lost (*11*), and most of the retained ones were transferred to the nucleus. Productive gene transfer would require serendipitous landing near active promoters or reacquiring promoters used by the host. Thus, genes would exist in duplicate until the system evolved a targeting machinery to relocate the gene product to the proto-organelle (Fig. 2).

A reduced, common subset of retained genes points toward a rapid ancient transfer and loss of the mitochondrial endosymbiont genome (*1*). Genetic transfer from endosymbiont genomes to the nucleus is, however, not limited to ancient events: Recent, frequent, and functional transfers have been demonstrated for mitochondrial (*17*) and chloroplast (*18*) genes within angiosperms. Although transfers seem to have reached a plateau in most eukaryotic groups (*1*, *17*, *19*), a selectable marker gene has been shown to move from the mitochondrial to the nuclear genome of transformed yeast at a surprisingly high frequency (*20*). Likewise, chloroplast to nucleus gene transfer has been observed at comparatively high frequencies (*21*, *22*). In this case, an intron within the marker gene was recovered following transfer, arguing against a cDNA-mediated mechanism (*21*). Large segments of mitochondrial and/or chloroplast ancestral genomes found in several nuclei similarly support DNA transfer en bloc (*23*). On the other hand, the presence of nuclear genes that appear to be derived from edited mitochondrial transcripts (*24*) indicates that both RNA- and DNA-mediated mechanisms drive genome transfer and reduction.

Why have organellar genomes retained a few genes, thus necessitating the retention of an entire machinery for genome replication, RNA expression, and translation? Analyses of 750 yeast mitochondrial proteins indicate that ~25% are involved in the maintenance of a genome encoding only eight highly hydrophobic membrane proteins (*25*) believed to be retained to avoid mistargeting. One of these, cytochrome c oxidase subunit 2 (Cox2), which is mitochondrion-encoded in most eukaryotes, is found in both nuclear and mitochondrial genomes of certain legumes, indicating a recent gene transfer. The nucleus-encoded Cox2 displays decreased local hydrophobicity relative to mitochondrial Cox2, a change demonstrated to be necessary for its import into mitochondria (*26*).

Organelle Biogenesis

Endosymbiotic organelle biogenesis involves two critical events: division and preprotein translocation. Both processes are driven by a combination of symbiont- and host-derived proteins. Two proteins, FtsZ and the dynamin-related protein (Drp1), play key roles in mitochondrial and plastid division. FtsZ, of endosymbiont origin, is a protein essential for eubacterial division and is found in most chloroplasts, but appears to be limited to the mitochondria of certain single-celled eukaryotes (*27*). Drp1, necessary for outer mitochondrial membrane fission, is closely related to dynamin, a eukaryotic-specific protein required to sever membranes during endocytosis (*28*). Importing proteins encoded by nuclear genes was a second prerequisite for organelle biogenesis. Extensive studies of mitochondrial (*29*) and plastidal (*30*, *31*) protein translocation machineries have revealed several common features (Fig. 3).

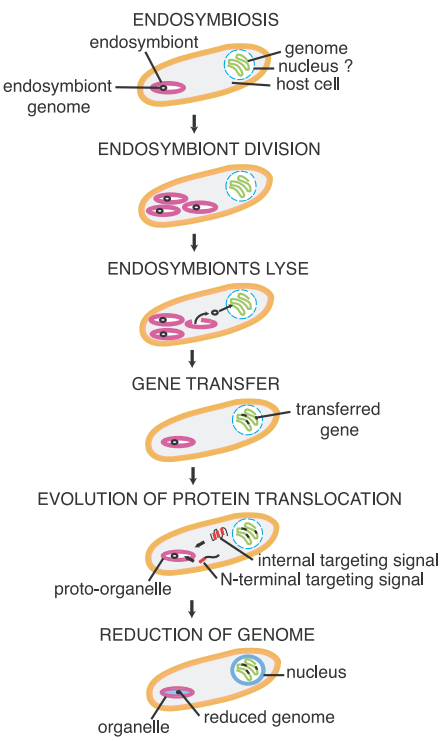

Fig. 2. Gene transfer to the nucleus. Bacterial endosymbionts probably existed as a replicating population (pink) within the host (orange), as observed in contemporary symbioses. The lysis of these bacteria, and/or DNA escape during division, could have provided the source for genetic transfer to the nucleus. The resulting genetic redundancy after the evolution of a protein translocation machinery and gene loss led to organellar genome reduction.

Mitochondrial proteins have four destinations: the outer membrane (OM), the intermembrane space (IMS), the inner membrane (IM), or the matrix (Fig. 3). Most proteins, including outer membrane proteins, are translocated by the TOM (translocase of the outer mitochondrial membrane) complex and then directed toward the inner membrane TIM23 (translocase of the inner mitochondrial membrane) translocon for insertion, or translocation into the IMS or the matrix. A number of inner membrane–spanning, eukaryotic-specific proteins with internal targeting signals, including the adenosine diphosphate (ADP)/ATP carrier (AAC) and some Tim proteins, are diverted toward the TIM22 translocon for insertion (*29*).

Within chloroplasts, proteins can be targeted to six compartments: the outer envelope (OE), the inner envelope (IE), the intermembrane space, the stroma, the thylakoid membrane, or the lumen (Fig. 3). Translocation through the outer envelope occurs via the TOC (translocase of the outer chloroplast envelope) complex, and the inner envelope via the TIC (translocase of the inner chloroplast envelope) complex (*30*). Thylakoid targeting occurs through four mechanisms (*31*).

The Origin of Targeting Peptides

Most nuclear-encoded mitochondrial, hydrogenosomal, and plastidal precursors have an N-terminal presequence that is necessary at multiple translocation steps (*10*, *29*–*32*). Thus, the presequence in these systems would have coevolved with the translocon. How did these presequences get appended to hundreds of genes? In plant mitochondria and chloroplasts, some presequences are partitioned on several exons, suggesting exon shuffling and alternative splicing to be mechanisms for presequence evolution (*33*). Alternatively, N-terminal presequences could have been created de novo by promoter-region duplication and mutation. Some recently transferred mitochondrial genes have been observed to scavenge mitochondrial presequence units from previously transferred genes encoding mitochondrial proteins (*17*).

Mitochondrial and plastidal presequences are loosely conserved and enriched in specific amino acid types (*29*, *32*). In contrast, hydrogenosomal targeting presequences, although shorter, show stronger primary sequence conservation (*10*). In secondary plastids, e.g., the apicoplast, the existence of two extra membranes necessitated the creation of a bipartite presequence, consisting of a signal peptide for entrance into the secretory pathway fused to a "traditional" plastid transit peptide for crossing the two inner plastid envelopes (*33*).

An important factor during the evolution of the proto-plastid was the presence of the mitochondrion. Coordinated evolution of both the mitochondrial and proto-plastid protein import machineries would be required for apparently conflicting reasons: to avoid mistargeting of potentially harmful proteins or to promote dual targeting of proteins shared by both organelles. Chloroplast and plant mitochondrial presequences share sim-

ilarities (*32*), yet specifically target proteins to their respective organelles. Interestingly, chloroplast transit peptides can target proteins to nonplant mitochondria (*34*), raising the possibility that the plastid transit peptide evolved from the mitochondrial presequence. Additionally, plant mitochondrial Tom receptors for presequence-bearing precursors differ markedly from their nonplant counterparts (*35*), presumably to prevent mistargeting. Nevertheless, dual-targeted proteins have been identified that use either tandem or ambiguous presequences. Such mechanisms would eliminate the need for several gene copies for shared biochemical functions.

The transit peptide may have allowed the delivery of novel functions into the evolving organelle. DNA encoding this peptide could land at the 5′ end of nonendosymbiotic-derived genes and would be retained if the encoded proteins conferred an advantage. Over millions of years, new pathways would evolve and "missing" elements, e.g., the enzymes in the mitochondrial Krebs cycle that are of nonproteobacterial origin (*36*), could have been replaced in old pathways.

Mitochondria have ribosome-binding sites to which some mRNAs have been localized. The majority of mitochondrion-bound messages are of prokaryotic origin, whereas mRNAs of eukaryotic origin are preferentially translated on cytosolic ribosomes (*37*). The preferential targeting of the prokaryotic-type mRNAs to mitochondria may reflect an early targeting mechanism prior to the advent of protein targeting signals.

Building the Protein Import Machine

Mitochondrial and plastid protein translocases have a dual origin. The emerging picture is that many translocases of the OM are of eukaryotic origin, those in the IM are of mixed origin, and soluble chaperones primarily bear prokaryotic traits (Fig. 3). Mitochondrial Tom40, which forms the channel of the OM protein import pore, has strongsecondary structure similarity to eubacterial OM beta-barrel porins but no notable primary sequence similarity (*38*). Two beta-barrel proteins, a porin from *Neisseria* (*39*) and a *Trichomonas* hydrogenosomal membrane protein, Hmp35 (*40*), can be targeted and inserted into mitochondrial membranes despite a lack of sequence similarity to any mitochondrial proteins. Furthermore, both proteins assemble into oligomers of similar size to those formed in their respective homologous systems (*39*, *40*). Thus, it appears that the targeting of beta-barrel proteins in the mitochondrial OM has an ancient origin and that beta-barrel proteins such as Tom40 and the mitochondrial-type porin may have arisen de novo by convergent evolution into pore-type proteins. A newly characterized mitochondrial OM protein, Sam50 (Fig. 3), is essential for the assembly of Tom40 and porin (*41*–*43*). Sam50 has a putative beta-barrel domain and is a member of the Omp85 family of proteins, which in *Neisseria* have been invoked in eubacterial outer envelope biogenesis (*44*, *45*). Phylogenetic analyses indicate a common ancestry for proteobacterial and mitochondrial members of the Omp85 family (*43*). It is possible that Sam50 was a primordial translocase that assisted the assembly of beta-barrel pores as they were being invented or recruited. In contrast to the eukaryotic-specific Tom40 protein, the chloroplast OE protein that forms the hydrophilic pore (*30*), Toc75, is of eubacterial origin (Fig. 3). Its homolog in the cyanobacterium *Synechocystis*, upon reconstitution in artificial bilayers, formed a voltage-gated peptide-sensitive channel (*46*). Interestingly, plastidal Toc75, like mitochondrial Sam50, forms part of the Omp85 family (*43*), thus showing that Omp85-like proteins were recruited for the biogenesis of two independent organelles, suggesting that their acquisition was critical.

The mitochondrial IM proteins Tim17, Tim22, and Tim23 are divergent homologs with domains distantly related to the bacterial LivH permease involved in translocating branched amino acids (*47*). A similar relationship has been shown between chloroplast Tic20 and LivH (*46*). The homology between Tim23 and Tim22, two channel proteins that specifically translocate either presequence-bearing precursors or eukaryotic-specific membrane proteins (Fig. 3), suggests the existence of a primordial channel that eventually duplicated after the advent of transit peptides and the invention of inner membrane proteins. Hmp31, a *Trichomonas* hydrogenosomal membrane protein related to mitochondrial AAC, can be imported into yeast mitochondria using the specific TIM22 pathway for AAC (*48*). Surprisingly, a basic local alignment search tool (BLAST) (*49*) search of the *Trichomonas vaginalis* genome (*50*, *51*) did not reveal homologs to any yeast translocases involved in this pathway (Fig. 3). Although it cannot be excluded that these genes are yet to be sequenced, it appears that despite our predictions (*10*, *40*, *48*), the hydrogenosomal and mito-

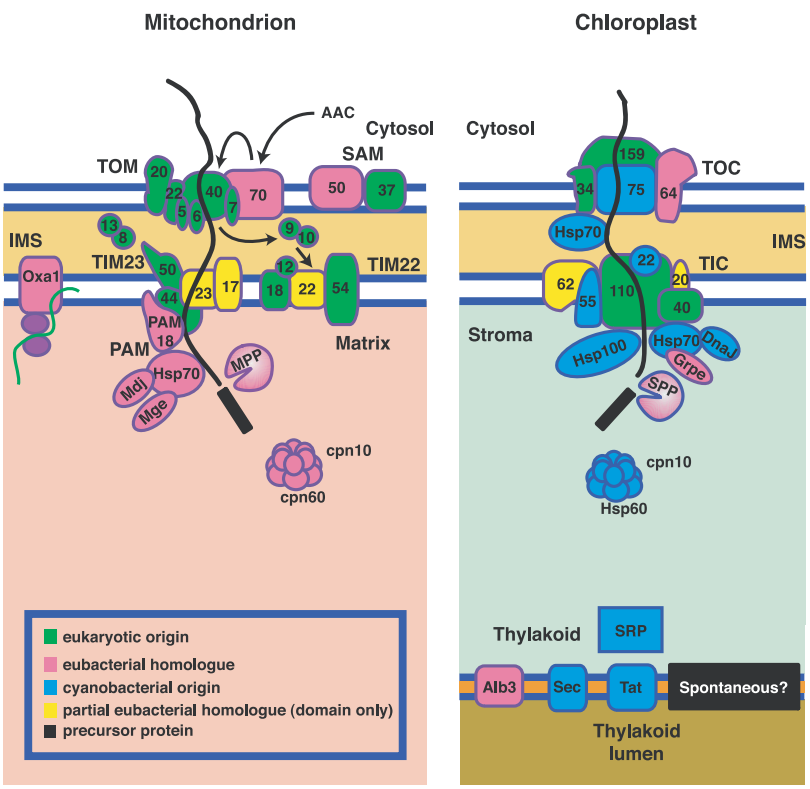

Fig. 3. Origins of mitochondrial and plastidal protein translocases. More than 25 mitochondrial translocases have been identified (**left**). TOM, translocase of the outer mitochondrial membrane; SAM, sorting and assembly machinery; TIM, translocase of the inner mitochondrial membrane; PAM, presequence translocase-associated motor; MPP, mitochondrial processing peptidase. Numbers correspond to component name and size (kD). The specific TIM22 pathway used by AAC and some eukaryotic-specific membrane proteins is indicated by black arrows. More than 15 translocases are identified in chloroplasts (**top right**) and thylakoids (**bottom right**). TOC, translocase of the outer chloroplast envelope; TIC, translocase of the inner chloroplast envelope; SPP, stromal processing peptidase; SRP, signal-recognition particle-dependent pathway; Sec, Sec-dependent pathway; Tat, Twin-arginine translocase; the black box depicts a spontaneous membrane protein insertion pathway. IMS, intermembrane space; colors indicate the possible origins of translocases determined by BLAST (*49*) searches with *Saccharomyces cerevisiae* mitochondrial translocases and *Pisum sativum* plastid translocases as input, except for cpn10 and SPP, where *Arabidopsis thaliana* homologs were used. Sources for mitochondrial translocases, (*29*, *41*, *47*, *52*, *53*); sources for plastid translocases, (*30*–*32*, *46*).

chondrial translocons are divergent or may have different origins (Fig. 1, scenario B).

Mitochondrial Oxa1 (Fig. 3) is involved in the post- or cotranslational insertion of certain inner membrane proteins of prokaryotic origin (*52*). Oxa1 has both a bacterial homolog, YidC, that is involved in Sec-independent membrane protein insertion and a thylakoidal homolog, Alb3 (Fig. 3), that functionally complements bacterial YidC (*52*). Thus, this family of membrane protein translocase is functionally conserved in bacteria, mitochondria, and plastids.

Within plastids, two membrane protein translocons of endosymbiotic origin coexist in thylakoids: a posttranslational SRP (signal-recognition particle)–dependent pathway for polytopic membrane proteins and a Tat (twin-arginine translocase) pathway for insertion of folded proteins (Fig. 3). A third Sec-dependent pathway of endosymbiotic origin and a fourth "spontaneous" pathway of possible eukaryotic origin that transports substrates of cyanobacterial origin (*31*) are also present.

Conclusion

Ancient eubacterial invasions gave rise to mitochondria and plastids and had an enormous impact on eukaryogenesis and the metabolism and homeostasis of eukaryotes. Although genomic analyses indicate that specific endosymbionts gave birth to these organelles, proteomics reveal a surprisingly large contribution from the host, multiple symbioses, and/or horizontal gene transfers. These studies attest to the flexibility of the eukaryotic cell while simultaneously revealing the conservation of mechanisms underlying the evolution of plastids, mitochondria, and derived organelles. Common mechanisms for protein translocation exist, yet specific targeting signals, translocation mechanisms, and retention of organellar-specific proteins have permitted the cohabitation of mitochondria and plastids. Despite considerable advances in our understanding of organelle evolution and biogenesis, future genomic and proteomic analyses promise to accelerate our understanding of these vital features of eukaryotic cells.

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54. We regret that space constraints prevent us from citing many relevant articles. We thank our colleagues for critical comments on the manuscript. This work was supported by NIH grant AI27857 (P.J.J.), the Burroughs-Wellcome Scholar Award for Molecular Parasitology (P.J.J.), NASA NAI02-0016-0023 through the Astrobiology Institute at the University of California–Los Angeles, and the Microbial Pathogenesis Training Grant (T32-AI07323) from NIH (M.T.B.).