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The yeast nascent polypeptide-associated complex initiates protein targeting to mitochondria *in vivo*

REBECCA GEORGE, TRAVIS BEDDOE, KARINA LANDL, AND TREVOR LITHGOW*

School of Biochemistry, La Trobe University, Bundoora 3083, Australia

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ABSTRACT **The yeast nascent polypeptide-associated complex (NAC) is encoded by two genes, *EGD1* and *EGD2*, and is associated with cytoplasmic ribosomes. Yeast mutants lacking NAC (Δ*egd2*) are viable but suffer slight defects in the targeting of nascent polypeptides to several locations including the endoplasmic reticulum and mitochondria. If both NAC and Mft52p are missing from yeast cells, inefficient targeting of mitochondrial precursor proteins leads to defects in both mitochondrial function and morphology. We suggest that NAC provides a ribosomal environment for nascent mitochondrial targeting sequences to achieve secondary structure, thereby enhancing the efficiency of protein targeting.**

Proteins do not arrive at their final subcellular location by chance. Maintenance of correct protein targeting is crucial for cell function, and nascent polypeptides are ushered from ribosomes in the cytosol to their specific subcellular location by soluble targeting factors (1, 2). In the case of secreted proteins, it is clear that translation, targeting, and translocation across the membrane of the endoplasmic reticulum are intimately related processes (3). A soluble targeting factor, the signal recognition particle (SRP), binds the amino-terminal signal sequence on nascent secretory proteins as they emerge from the ribosome to initiate targeting to the endoplasmic reticulum and translocation across the membrane (4–7). The initial binding of SRP to ribosomes bearing nascent polypeptides is a regulated event. The nascent polypeptide-associated complex (NAC) sitting on the surface of mammalian ribosomes at the site of the emerging polypeptide chain can prevent binding of SRP to nascent chains without signal sequences and can hinder binding of vacant ribosomes to the endoplasmic reticulum because of its position on the surface of the ribosome (8–10).

SRP binds exclusively to nascent polypeptides bearing secretion signals, but NAC initially contacts nascent polypeptides destined for many (and probably all) subcellular compartments. Both the α and β subunits of NAC can be crosslinked to nascent polypeptides destined for the cytosol, peroxisome, endoplasmic reticulum, and mitochondria (11). These *in vitro* assays suggest that NAC binds to nascent polypeptides before the specific factors that would regulate protein distribution and delivery. The sequence of events that determines specific delivery of secretory proteins to the endoplasmic reticulum has been well studied (3, 12), and we have begun to analyze the steps involved in specifically delivering precursor proteins from ribosomes to the mitochondria, including the role of the cytosolic targeting factor Mft52p (13).

Here, we identify yeast NAC and find it associated with cytosolic ribosomes. Disruption of the *EGD2* gene, encoding the α subunit of the NAC heterodimer, leads to decreased targeting of proteins to the endoplasmic reticulum and mitochondria *in vivo*. In terms of mitochondrial targeting, yeast mutants lacking both NAC and Mft52p show synthetic mitochondrial defects: reduced targeting of reporter proteins, tendency to lose mitochondrial DNA, and changes to organelle morphology. We conclude that NAC is involved in protein targeting to many (if not all) subcellular locations and that NAC and Mft52p cooperate in initiating mitochondrial protein targeting *in vivo*.

MATERIALS AND METHODS

Expression of Egd2p and Antibody Production. The ORF encoding *EGD2* was amplified from yeast genomic DNA by PCR and ligated into pQE31 (Qiagen, Chatsworth, CA), and hexahistidine-tagged Egd2p was expressed in *Escherichia coli* for antibody production in rabbits.

Disruption of the *EGD2* Gene. The DNA fragment corresponding to the ORF was digested with *Bgl*II, into which a *Bgl*II fragment of the *ADE2* gene was inserted, disrupting *EGD2* after the codon corresponding to Phe_{56}. The *egd2*::*ADE2* fragment was transformed into the yeast strain MH272–3fa/α to generate the strain YRLG1 (*Mat*a/α, *ura3*/*ura3*, *leu2*/*leu2*, *his3*/*his3*, *trp1*/*trp1*, *ade2*/*ade2*, *egd2*::*ADE2*/*EGD2*), and disruption of the *EGD2* gene was confirmed by Southern analysis (not shown). The Ade^+-isogenic strain JK9–3d (*Mat*a, *ura3*, *leu2*, *his4*, *trp1*) was used as a wild-type control. To check the respiratory competence of Δ*egd2* cells, a culture of cells (YRLG3) was grown overnight on rich medium with Glc as a carbon source. Cells were counted, and ≈100 cells were plated onto yeast extract/peptone/dextrose, incubated overnight, and replica-plated onto yeast extract/peptone/lactate and yeast extract/peptone/dextrose. Colonies formed were counted after incubation at 30°C. Mutants deficient in both Egd2p and Mft52p were formed from the strains YRLG3 and YTHB2 (*Mat*a, *leu2*, *ura3*, *his4*, *trp1*, *mft1*Δ*LEU2*; ref. 14). The diploid cells were sporulated, and tetrads were dissected to obtain haploid double mutants (YRLG5: *Mat*a, *leu2*, *ura3*, *trp1*, *ade2*, *egd2*::*ADE2, mft1*::*LEU2*).

Subcellular Fractionation. Yeast cytosol and partially purified ribosomes were prepared as described (13–15). For routine analysis, mitochondria were isolated as described (16), and when indicated, EDTA-washed and $MgCl_2$-treated mitochondria were prepared in modified buffers (17). To quantitate the relative amounts of Egd2p and ribosomal protein L3 on free ribosomes and ribosomes bound to mitochondria, replica samples were separated by SDS/PAGE and blotted to nitrocellulose, and then strips corresponding to the position of Egd2p or L3 were marked. Ribosomal samples containing 0.5, 1, 2, 5, 10, and 20 μg ribosomes were included. After processing the filters with primary antibodies and secondary alkaline phosphatase-conjugated antibodies, one filter was developed as an immunoblot, while the bands corresponding to Egd2p

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Abbreviations: NAC, nascent chain-associated complex; SRP, signal recognition particle; CoxIV, cytochrome oxidase subunit IV; GFP, green-fluorescent protein; DHFR, dihydrofolate reductase.
*To whom reprint request should be addressed. e-mail: T.Lithgow@latrobe.edu.au.

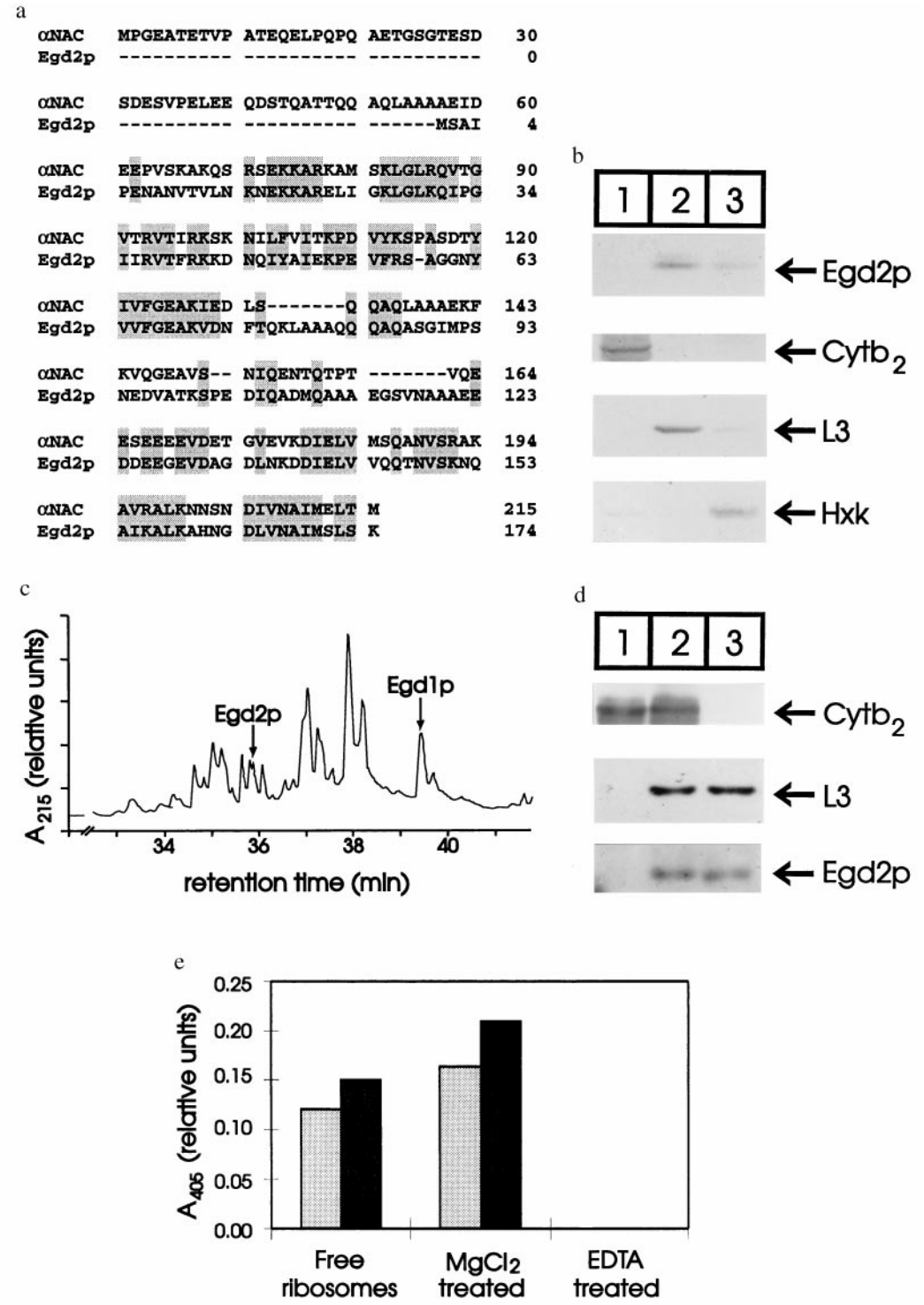

FIG. 1. The yeast αNAC homolog Egd2p is associated with free ribosomes. (*a*) Amino acid sequence alignment of bovine αNAC (11) with the *Saccharomyces cerevisiae* homolog Egd2p (39). Residues conserved between the two sequences are shaded. (*b*) Wild-type yeast cells were separated into mitochondria (lane 1), ribosomes (lane 2), and cytosol (lane 3) and were analyzed by SDS/PAGE and immunoblotting against Egd2p, cytochrome b_2 (cyt b_2), ribosomal protein L3, and the cytosolic enzyme hexokinase (Hxk). (*c*) Yeast ribosomes were extracted with high salt, and the extracted proteins were separated by reversed phase HPLC. (*d*) Mitochondria purified in buffers containing 10 mM EDTA (100 μg of total protein; lane 1) or mitochondria purified in buffer containing 2 mM $MgCl_2$ (100 μg of total protein; lane 2) and free ribosomes (≈2 μg of total protein; lane 3) were analyzed by SDS/PAGE and immunoblotting. (*e*) The presence of Egd2p (black bars) relative to ribosomal protein L3 (gray bars) was analyzed in samples of free ribosomes (≈2 μg total of protein), mitochondria purified in buffers containing 2 mM $MgCl_2$ (100 μg total of protein), or mitochondria purified in buffer containing 10 mM EDTA (100 μg total of protein). Proteins were separated by SDS/PAGE and blotted to nitrocellulose, and then strips corresponding to the position of Egd2p or L3 were excised, processed, and incubated with *p*-nitrophenolphosphate.

and L3 were excised from the replica filter and incubated with 0.5 ml of *p*-nitrophenolphosphate (Sigma Fast *p*-NPP) in microfuge tubes. Alkaline phosphatase activity was measured spectrophotometrically at 405 nm.

Purification of Proteins Associated with Yeast Ribosomes. Salt-extracted ribosome-associated proteins (11) were injected into a VyDac C_8 column (Alltech Associates) equilibrated with 0.1% (vol/vol) trifluoroacetic acid in H_2O, with the second pump delivering 0.09% trifluoroacetic acid in 70% acetonitrile (buffer B). Proteins were eluted from the column at 1 ml/min with a gradient of 0–50% buffer B in 30 min, then with a rapid phase from 50 to 100% buffer B. Elution was monitored at 215 nm, and 1-ml fractions were collected for amino-terminal sequence analysis. The sequence identified in the peak corresponding to Egd1p was PIDQEKLAKLQKLSANNKVG.

Fluorescence Microscopy. Haploid Δegd2 ($resp^-$) and Δegd2 ($resp^+$) were grown in yeast extract/peptone/dextrose at 30°C until midlog phase, stained with 4′,6-diamidino-2-phenylindole, dihydrochloride, and observed in the Olympus BX50 microscope (18). To visualize mitochondrial morphology, homozygous diploid yeast cells were transformed with the centromeric plasmid p416MET25COXIV encoding a fusion between the first 21 amino acids of cytochrome oxidase subunit IV and the jellyfish green-fluorescent protein (CoxIV-GFP). Freshly transformed cells were grown on synthetic media without Ura at 30°C until midlog phase (OD_{600} 0.5–0.7) in preparation for microscopy. To visualize protein secretion, diploid cells were transformed with the plasmid p416MET25sec (a kind gift from Traude Beilharz, La Trobe University) encoding GFP with a secretory signal sequence.

RESULTS

Egd2p Is a Cytoplasmic Protein Associated with Ribosomes. In mammalian cells, NAC exists as a heterodimer and both the α and β subunits have been sequenced (19). Sequencing of the yeast genome revealed that a single yeast gene, YHR193C (*EGD2*), encodes a protein homologous to the α subunit of NAC (Fig. 1*a*). To determine the subcellular location of yeast NAC, yeast cells were separated into cytosolic, ribosomal, and mitochondrial fractions and analyzed by immunoblotting with antibodies recognizing Egd2p. Egd2p was found to be associated mainly with cytoplasmic ribosomes (Fig. 1*b*, lane 2) with a small pool also present in the cytosolic fraction (lane 3). From analysis of the yeast proteome, there is an approximate stoichiometry of the two subunits of NAC (Egd1p and Egd2p) with the core ribosomal proteins RPL1 and RPS5 (20, 21). Whereas in extracts from mammalian cells there is a larger soluble pool of NAC in addition to that located on ribosomes (19), the yeast cytoplasm appears to have one NAC heterodimer per ribosome.

Mammalian NAC can be stripped from ribosomes by treatment with high salt levels (11). To assess whether NAC was similarly associated with yeast ribosomes, cytosolic ribosomes were purified and extracted with 0.5 M potassium acetate, and the stripped proteins were separated by reversed-phase HPLC (Fig. 1*c*). Amino acid sequencing identified Egd1p (the β subunit of NAC) as a ribosome-associated protein. Immunoblotting revealed that Egd2p also is extracted from the ribosomes with salt treatment, but Egd2p is amino terminally blocked.

In the course of translating mitochondrial precursor proteins, some ribosomes become associated with the mitochondrial surface (22–25). These membrane-associated ribosomes can be recovered by isolating mitochondria in the presence of $MgCl_2$ (Fig. 1*d*, lane 2). At this stage, polypeptide translocation must have proceeded at least as far as binding of the amino-terminal targeting sequence to receptors in the mitochondrial membranes. NAC was found on both ribosomes isolated from the cytosol (Fig. 1*d*, lane 3) and ribosomes associated with mitochondria (lane 2). The internal control for these experiments was the presence of the core ribosomal protein L3. Quantitative analysis confirmed that NAC was associated with ribosomes prepared from the soluble phase of the cell and that it did not leave the ribosomes even after they had become associated with the mitochondrial surface (Fig. 1*e*).

Loss of Egd2p Impairs Normal Growth and Respiratory Function. To understand the function of NAC *in vivo*, we disrupted the *EGD2* gene. Yeast mutants lacking Egd2p (Δegd2) were viable and grew at wild-type rates on rich media with Glc as a carbon source but displayed a slow growth phenotype on rich lactate media as a result of a progressive loss of respiratory function (data not shown). To measure this loss of mitochondrial function, Δ*egd2* cells were grown overnight on rich Glc media. Cells were counted, and replica were plated on rich media with either Glc or lactate as a carbon source. Under these conditions, <1% of wild-type cells (0–1 of 250 colonies per experiment) loose the ability to form colonies on lactate media, whereas one-third of the Δ*egd2* cells failed to form colonies on the nonfermentable carbon source.

The respiratory-deficient Δ*egd2* cells became ρ^o, eventually losing all of their mtDNA. Mitochondria prepared from respiration-deficient Δegd2 cells were pink-colored, a characteristic of cytoplasmic petite strains (26), and 4′,6-diamidino-2-phenylindole, dihydrochloride staining revealed a progressive loss of DNA associated with the mitochondria in the respiration-deficient Δ*egd2* cells (data not shown). In addition, these cells could not complement either of the paired ρ^- tester strains aM9–3-6C (*Mat***a**, *oxi2–1*, *ade1*) or aM28–82-11A (*Mat***a**, *oli2–1*, *ade1*) and could no longer respire if the wild-type *EGD2* gene is returned to them on a plasmid (data not shown).

Loss of Egd2p Leads to a Defect in Mitochondrial Protein Targeting. Whereas loss of mtDNA can be an indirect result of chronic growth defects such as those seen in yeast mutants lacking SRP (27, 28), the Δ*egd2* cells grow like wild-type cells on rich Glc medium and show no obvious morphological defects. Because NAC is the first protein to bind the amino-terminal targeting sequences of $F_1\beta$ and CoxIV *in vitro* (11),

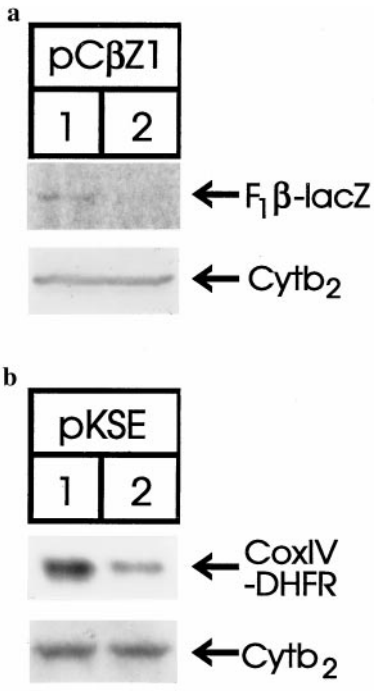

FIG. 2. Egd2p is required for efficient delivery of mitochondrial-directed fusion proteins. (*a*) Mitochondria were prepared from wild-type (lane 1) and Δ*egd2* (lane 2) cells expressing $F_1\beta$-LacZ (encoded by the plasmid pCβZ1). (*b*) Mitochondria were prepared from wild-type (lane 1) and Δ*egd2* (lane 2) cells expressing CoxIV-DHFR (encoded by the plasmid pKSE). LacZ or DHFR targeted to the mitochondria was measured by immunoblotting after SDS/PAGE. As a control, blots were reprobed with antisera recognizing cytochrome b_2.

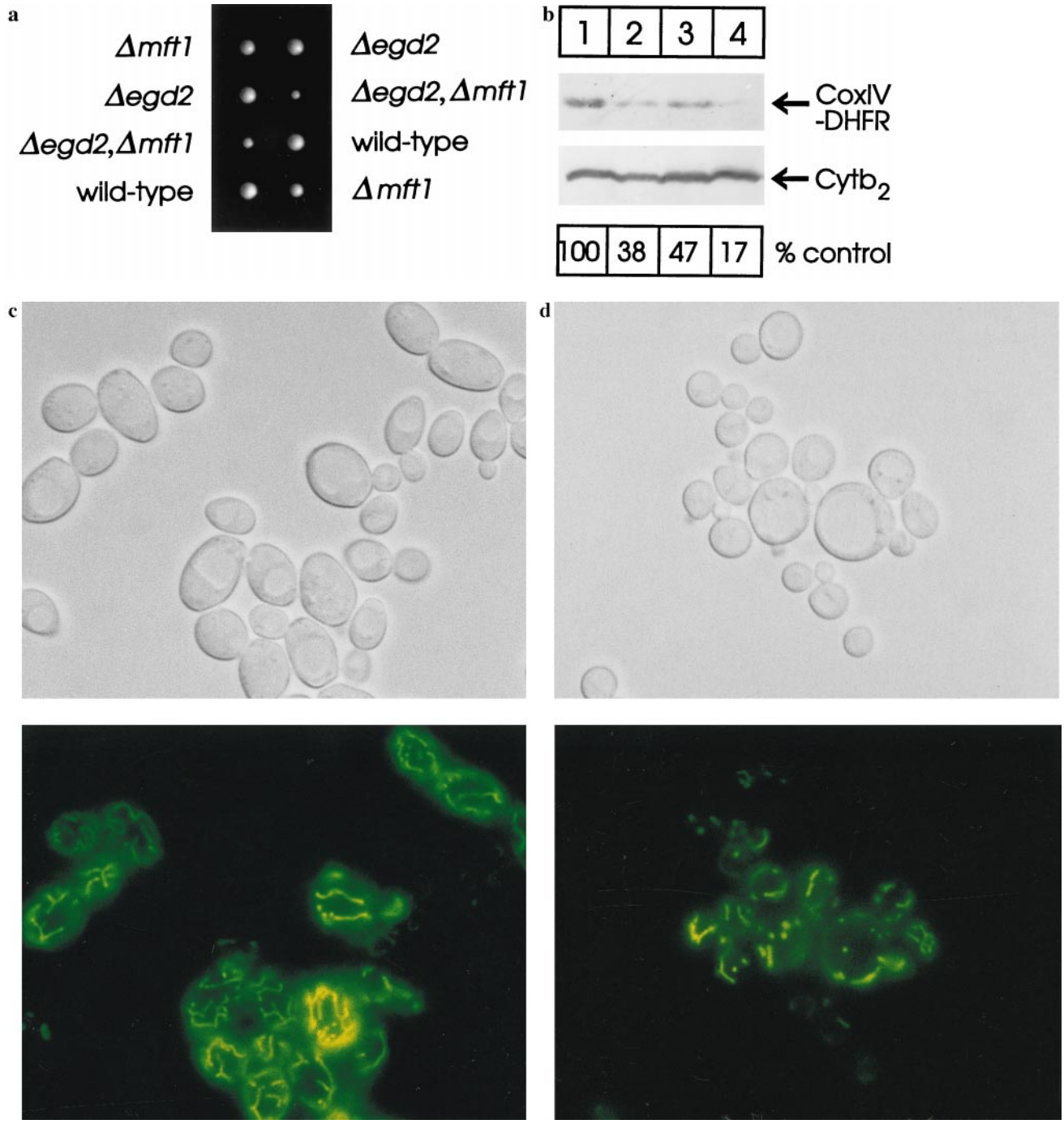

FIG. 3. Both Egd2p and Mft52p are involved in mitochondrial biogenesis. (*a*) Heterozygous (*EGD2*/Δ*egd2*, *MFT1*/Δ*mft1*) diploid yeast cells were sporulated, tetrads were dissected, and cells were incubated at 30°C on rich Glc medium for 2 days. (*b*) Mitochondria were prepared from wild-type (lane 1), Δ*egd2* (lane 2), Δ*mft1* (lane 3), and Δ*egd2*, Δ*mft1* (lane 4) yeast cells expressing CoxIV-DHFR. DHFR delivered to the mitochondria was measured by immunoblotting after SDS/PAGE. The blots were reprobed with antisera recognizing cytochrome b_2. DHFR delivered to mitochondria was quantitated by densitometry and is expressed as a percentage of the wild-type level of DHFR:cytochrome b_2. (*c*) Wild-type (diploid) yeast cells were transformed to express CoxIV-GFP. The cells were viewed with Nomarski optics and analyzed by fluorescence microscopy. (*d*) The homozygous diploid Δ*egd2*, Δ*mft1* cells.

we asked whether the defect in mitochondrial biogenesis is due to a protein targeting defect *in vivo*. Wild-type and Δ*egd2* yeast cells were transformed with each of two reporter plasmids. The first encodes the amino-terminal targeting sequence of CoxIV fused to the reporter dihydrofolate reductase (CoxIV-DHFR), and the second encodes a fusion between an amino terminal fragment of $F_1\beta$ and β-galactosidase ($F_1\beta$-LacZ). Note that, in all cases, cells were grown and maintained on lactate media to select against the loss of mtDNA from the Δ*egd2* cells. The amounts of $F_1\beta$-LacZ (Fig. 2*a*) and CoxIV-DHFR (Fig. 2*b*) targeted to the mitochondria of Δegd2 cells are reduced substantially relative to mitochondria in wild-type cells.

Simultaneous Disruption of EGD2 and MFT1 Leads to a Synthetic Growth Defect. Loss of Mft52p reduces the efficiency of protein targeting to mitochondria (13), and the partial defect in delivery of model precursors to the mitochondria of Δegd2 cells predicts a synthetic effect on protein targeting in double Δegd2, Δmft1 mutants. Fig. 3*a* shows tetrads derived from sporulation of *egd2*::*ADE2*/*EGD2*, *mft1*::*LEU2*/*MFT1* diploid cells: One spore of each tetrad does

exhibit markedly slow growth, which cosegregates with the Ade$^+$,Leu$^+$ phenotype of the Δegd2, Δmft1 cells.

Immunoblotting of mitochondria isolated from Δegd2, Δmft1 cells expressing CoxIV-DHFR revealed a synthetic defect suffered by these cells (Fig. 3*b*). The mitochondrial defects of Δegd2, Δmft1 cells also are manifested in an aberrant mitochondrial morphology. Whereas mitochondria of wild-type cells form a tubular reticulum around the cell cortex (Fig. 3*c*), the Δegd2, Δmft1 cells tend to have relatively few, discrete mitochondria per cell. Few cells in the population of Δegd2, Δmft1 mutants accumulate wild-type levels of CoxIV-GFP (Fig. 3*d*).

DISCUSSION

Mitochondrial Protein Targeting Starts on the Ribosome. Loss of NAC from the ribosomes seriously effects protein targeting to the mitochondria *in vivo*. These results provide the first evidence that a targeting decision is made at the level of the ribosome for mitochondrial precursor proteins. Because NAC is required largely for the efficient targeting of CoxIV-DHFR, in which all of the mitochondrial-targeting information is contained within the first 14 amino acids of the polypeptide's sequence, the decision is made at an early stage of translation.

Fusion proteins such as CoxIV-DHFR and CoxIV-GFP serve as exquisitely sensitive probes to uncover the protein targeting defects in Δ*egd2* and Δ*mft1* cells. The effect on targeting of "natural" precursor proteins is less severe because the overall protein composition of proteins in mitochondria is largely unaltered. Many precursor proteins are assisted in their delivery to the mitochondria by molecular chaperones such as mitochondrial import stimulating factor and HSP70, and a spectrum of precursor protein dependency on these factors exists (29). COXIV-DHFR and similar fusion proteins sit at the extreme end of this spectrum; being targeted to the mitochondria with minimal assistance from other chaperones, the fusion proteins appear largely reliant on the action of NAC and Mft52.

Translation of a mitochondrial precursor protein and its import into the mitochondria are independent processes. There is no evidence to suggest that the import receptors on the mitochondrial surface can discriminate a partly synthesized precursor protein emerging from a ribosome as being different from a fully synthesized polypeptide. We suggest that the role of NAC and Mft52p is to commit the precursor protein for delivery to the mitochondria and suspect that, in intact yeast cells, additional cytosolic factors like Hsp70 (15, 30, 31) and DnaJ (32) then assist the efficient transfer of the precursor protein through the cytosol (Fig. 4). In mammalian cells, even more cytosolic chaperones involved in the targeting and delivery process have been identified. At least one of these, mitochondrial import stimulating factor, is selective for mitochondrial precursor proteins (33) and remains bound to the precursor until it has associated productively with the import receptor in the mitochondrial outer membrane (34).

Targeting to Other Organelles. Although we have focused on the role of NAC in initiating protein targeting to the mitochondria, *in vitro* assays have detailed a role for NAC in protein targeting to several different subcellular locations including the endoplasmic reticulum (11). We anticipate that Δ*egd2* cells will suffer subtle defects in each of these targeting pathways as well. Although *srp* mutants (Δ*srp54*, Δ*scr1*, *sec65*) have severe growth defects, tend to become sterile, and suffer the effects of reduced secretion (refs. 27 and 28; R.G. and T.L., unpublished work), the secretory defects of Δ*egd2* cells are more subtle: The cells are more rounded, and haploid Δ*egd2* cells show abnormal budding that might reflect defects in cell wall biogenesis and remodeling (data not shown). We also find that the efficiency of GFP targeting into the endoplasmic reticulum is reduced in Δ*egd2* cells (data not shown), and this fusion protein might serve as a useful probe for the further characterization of NAC function *in vivo*.

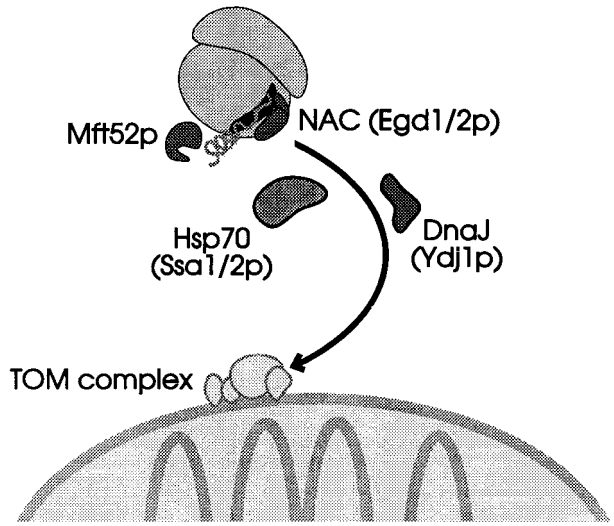

FIG. 4. Cytoplasmic targeting factors for yeast mitochondrial precursor proteins. For most mitochondrial precursor proteins synthesized in the cytosol, the amino terminus has the potential to form a basic, amphipathic helix. NAC (Egd1p and Egd2p) protects the nascent chain from all cytosolic proteins until 30–50 amino acids have been translated. Subsequently, the targeting sequence becomes accessible to Mft52p and the molecular chaperones Hsp70 (Ssa1p and Ssa2p) and DnaJ (Ydj1p). Other yeast factors, including Mft2p and a homolog of the mammalian protein mitochondrial import stimulating factor (MSF), also may be involved in delivering the precursor protein to the outer membrane translocase (TOM) complex (29) on the mitochondrial surface.

The EGD Genes. Like several ribosomal proteins, NAC appears to have a second function in the nucleus (35). The Egd1p/Egd2p heterodimer initially was identified in bandshift assays as an Enhancer of *G*AL4 *D*NA binding (36). Because ribosomal biogenesis occurs in the nucleus, the regulation of mRNA synthesis by "free" ribosomal proteins might be an important means of controlling gene expression, integrating the processes of transcription and translation.

What Is the Precise Role of NAC on the Ribosome? By controlling the length of nascent preprolactin chains synthesized on isolated ribosomes, Wiedmann *et al.* (11) used elegant cross-linking assays to show that NAC is in contact with the growing polypeptide chain until it achieves a length of 30–60 residues and that only longer nascent chains become available for SRP to bind. Similarly, growing $F_1\beta$ or CoxIV polypeptides destined for the mitochondria are first in contact with NAC and subsequently with a specific factor of ≈50 kDa, which we suspect to be the mammalian homolog of Mft52.

We envisage the loss of NAC might have one of two consequences. The first possibility is that the nascent polypeptide might be recognized inappropriately by targeting factors and mistargeted; for example, some mitochondrial proteins might be bound by SRP and mistargeted to the endoplasmic reticulum. This result can occur to some extent in *in vitro* assays (8, 11), and we have tested whether this result occurs *in vivo*. If incorrectly targeted to microsomes *in vitro*, the abundant mitochondrial porin is inserted into the membrane and glycosylated (37). However, immunoblots of Δ*egd2* cell extracts showed no evidence of glycosylated porin, suggesting that, if any, <0.1% of porin could have been mistargeted because of the loss of NAC (data not shown). Similarly, jellyfish GFP is folded and fluoresces within the lumen of the endoplasmic reticulum (38), but expression of CoxIV-GFP in Δ*egd2* produced no detectable fluorescence in any organelle other than

in the mitochondria (data not shown; see Fig. 3*c*). Although these are negative results only, they suggest that, in the absence of NAC, any mistargeting of mitochondrial precursor proteins that might occur *in vivo* is insignificant.

The second possibility, which we now favor, is that, without NAC, nascent polypeptides are not bound efficiently by any specific targeting factors and nascent targeting sequences assume their active structures with reduced efficiency. As translation continues, the unattended precursor protein might be prone to aggregation and/or proteolytic destruction in the cytosol and therefore reach its destination at reduced efficiency. This outcome would occur to some extent for any mitochondrial precursor protein but is revealed most easily by fusion proteins containing only amino-terminal targeting information. If both NAC and Mft52p are missing, the targeting process becomes so inefficient that pleiotropic mitochondrial defects result.

In this model, NAC provides a cradle for the targeting sequence to achieve secondary structure and a context for the targeting sequence to be recognized efficiently by specific targeting factors like Mft52p. It is already clear that protein targeting to the endoplasmic reticulum is initiated at an early stage of translation. We suggest that the surface of the ribosome is also the site for the first stage in targeting proteins to mitochondria.

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1. Rassow, J. & Pfanner, N. (1996) *Curr. Biol.* **6,** 115–118.
2. Schatz, G. & Dobberstein, B. (1996) *Science* **271,** 1519–1526.
3. Martoglio, B. & Dobberstein, B. (1996) *Trends Cell Biol.* **6,** 142–147.
4. Krieg, U. C., Walter, P. & Johnston, A. E. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8604–8608.
5. Kurzchalia, T. V., Wiedmann, M., Girshovich, A. S., Bochkareva, E. S., Bielka, H. & Rapoport, T. A. (1986) *Nature (London)* **320,** 634–636.
6. Lütcke, H., High, S., Römisch, K., Ashford, A. J. & Dobberstein, B. (1992) *EMBO J.* **11,** 1543–1551.
7. Miller, J. D., Wilhelm, H., Gierasch, L., Gilmore, R. & Walter, P. (1993) *Nature (London)* **366,** 351–354.
8. Lauring, B., Sakai, H., Kreibich, G. & Wiedmann, M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 5411–5415.
9. Lauring, B., Kreibich, G. & Wiedmann, M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 9435–9439.
10. Powers, T. & Walter, P. (1996) *Curr. Biol.* **6,** 331–338.
11. Wiedmann, B., Sakai, H., Davis, T. A. & Wiedmann, M. (1994) *Nature (London)* **370,** 434–437.
12. Rapoport, T. A. (1992) *Science* **258,** 931–936.
13. Cartwright, P., Beilharz, T., Hansen, P., Garrett, J. & Lithgow, T. (1997) *J. Biol. Chem.* **272,** 5320–5325.
14. Beilharz, T., Beddoe, T., Landl, K., Cartwright, P. & Lithgow, T. (1997) *FEBS Lett.* **407,** 220–224.
15. Nelson, R. J., Ziegelhoffer, T., Nicolet, C., Werner-Washburne, M. & Craig, E. A. (1992) *Cell* **71,** 97–103.
16. Glick, B. S., Brandt, A., Cunningham, K., Müller, S., Hallberg, R. L. & Schatz, G. (1992) *Cell* **69,** 809–822.
17. Pon, L., Moll, T., Vestweber, D., Marshallsay, B. & Schatz, G. (1989) *J. Cell Biol.* **109,** 2603–2616.
18. Pringle, J. R., Preston, R. A., Adams, A. E. M., Stearns, T., Drubin, D. G., Haarer, B. K. & Jones, E. H. (1989) *Methods Cell Biol.* **31,** 357–435.
19. Wang, S., Sakai, H. & Wiedmann, M. (1995) *J. Cell Biol.* **130,** 519–528.
20. Sagliocco, F., Guillemot, J., Monribot, C., Capdevielle, J., Perrot, M., Ferran, E., Ferrara, P. & Boucherie, H. (1996) *Yeast* **12,** 1519–1533.
21. Sagliocco, F., Perrot, M., Monribot, C. & Boucherie, H. (1997) *Yeast* **13,** S264 (abstr.).
22. Verner, K. (1993) *Trends Biochem. Sci.* **18,** 366–370.
23. Kellems, R. E., Allison, V. F. & Butow, R. A. (1975) *J. Cell Biol.* **65,** 1–14.
24. Ades, I. Z. & Butow, R. A. (1980) *J. Biol. Chem.* **255,** 9918–9924.
25. Suissa, M. & Schatz, G. (1982) *J. Biol. Chem.* **257,** 13048–13055.
26. Leighton, J. & Schatz, G. (1995) *EMBO J.* **14,** 188–195.
27. Felici F., Cesareni, G. & Hughes, J. M. X. (1989) *Mol. Cell. Biol.* **9,** 3260–3268.
28. Hann, B. C. & Walter, P. (1991) *Cell* **67,** 131–144.
29. Lithgow, T., Glick, B. S. & Schatz, G. (1995) *Trends Biochem. Sci.* **20,** 98–101.
30. Chirico, W. J., Waters, M. G. & Blobel, G. (1988) *Nature (London)* **332,** 805–810.
31. Deshaies, R. J., Koch, B. D., Werner-Washburne, M., Craig, E. A. & Schekman, R. (1988) *Nature (London)* **332,** 800–805.
32. Cyr, D. M., Langer, T. & Douglas, M. G. (1994) *Trends Biochem. Sci.* **19,** 176–181.
33. Hachiya, N., Komiya, T., Alam, R., Iwahashi, J., Sakaguchi, M., Omura, T. & Mihara, K. (1994) *EMBO J.* **13,** 5146–5154.
34. Hachiya, N., Mihara, K., Suda, K., Horst, M., Schatz, G. & Lithgow, T. (1995) *Nature (London)* **376,** 705–709.
35. Wool, I. G. (1996) *Trends Biochem.* **21,** 164–165.
36. Parthun, M. R., Mangus, D. A. & Jaehning, J. A. (1992) *Mol. Cell. Biol.* **12,** 5683–5689.
37. Sakaguchi, M., Hachiya, N., Mihara, K. & Omura, T. (1992) *J. Biochem.* **112,** 243–248.
38. Niedenthal, R. K., Riles, L., Johkston, M. & Hegemann, J. H. (1996) *Yeast* **12,** 773–786.
39. Johnston, M., Andrews, S., Brinkman, R., Cooper, J., Ding, H., Dover, J., Du, Z., Favello, A., Fulton, L., Gattung, S., *et al.* (1994) *Science* **265,** 2077–2082.