LETTER

Domain Stealing by Receptors in a Protein Transport Complex

Joanne M. Hulett, Peter Walsh, and Trevor Lithgow

Department of Biochemistry and Molecular Biology, and Bio21 Molecular Sciences and Biotechnology Institute, University of Melbourne, Parkville 3010, Australia

The mitochondrion is an essential cellular compartment in eukaryotes. The mitochondrial proteins Tom20 and Tom22 are receptors that ensure recognition and binding of proteins imported for mitochondrial biogenesis. Comparison of the sequence for the Tom20 and Tom22 subunits in the yeasts Saccharomyces cerevisiae and Saccharomyces castellii, show a rare case of domain stealing, where in Saccharomyces castellii Tom22 has lost an acidic domain, and Tom20 has gained one. This example of domain stealing is a snapshot of evolution in action and provides excellent evidence that Tom20 and Tom22 are subunits of a single, composite receptor that binds precursor proteins for import into mitochondria.

The comparison of genome sequences from closely related species provides a unique and powerful means of understanding the progress and mechanisms of speciation (Pääkkö and Langkjaer 2004; Scannell et al. 2006). In eukaryotic cells, mitochondria are an essential compartment maintained through a continual process of protein import (Bohnert et al. 2007; Neupert and Herrmann 2007; Hoogenraad et al. 2007). The mitochondrial proteins Tom20 and Tom22 are receptor subunits that collaborate to ensure the import of proteins required for mitochondrial biogenesis (Endo and Kohda 2002; Bohnert et al. 2007; Neupert and Herrmann 2007). Tom22 is an unusually acidic protein and the structure of Tom20 reveals a hydrophobic binding groove; the mitochondrial targeting sequences bound by Tom22 and Tom20 are basic and amphipathic and can be accommodated by the combined action of the two receptor subunits (Mayer et al. 1995; Bolliger et al. 1995; Abe et al. 2000). Unexpectedly, comparatively analyses with the genomes of various species of yeasts from the genus Saccharomyces revealed a crucial segment from Tom22 was “stolen” by Tom20 some time after the divergence of Saccharomyces cerevisiae and Saccharomyces castellii. This example of domain stealing, i.e. the theft of a domain from one subunit of a multi-subunit protein by another, is a remarkable example of evolution, and provides excellent support to the proposition that Tom20 and Tom22 each contribute domains to a single, composite receptor in the TOM complex.

The highly acidic receptor domain of Tom22 is shorter at the N-terminus in S. castellii than in the other Saccharomyces species analysed (fig. 1a). This 22-residue region is functionally important, since deletion of the corresponding residues from Tom22 of S. cerevisiae yields a dramatic defect in cell viability and the relative rate of growth (fig. 1b).

How does S. castellii cope with this truncated Tom22? The answer seems to lie in gene duplication and domain stealing. One of the key events in the recent history of the Saccharomyces group of yeasts was a genome duplication followed by rapid loss of many of the duplicated genes; however, in some species some of the duplicated genes were retained because modifying them allowed adaptation to adverse events or new environments (Byrne and Wolfe 2005; Scannell et al. 2006). As detailed in figure 2, Wolfe and co-workers showed that one of the blocks of duplicated genes in Saccharomyces contains the TOM20 gene (Scannell et al. 2006). In both Candida glabrata and S. cerevisiae, one of these redundant genes has been lost altogether while in S. castellii one of the TOM20 genes has been modified to code for a protein with an extension of acidic residues at the C-terminus (fig. 3a). The other, shorter TOM20 gene (716.41) has been truncated such that it no longer contains a transmembrane segment. Analysis of Tom20 sequences from a range of yeasts, other fungi and animals has never before revealed an extended form of Tom20 (Likić et al. 2005), and examination of mitochondrial proteins from S. castellii shows that the extended form of Tom20 is expressed, while the smaller truncated protein is not expressed, at least at levels we can detect by immunoblotting (fig. 3b). We have so far been unable to express the extended form of Tom20 from S. castellii to complement function in strains of S. cerevisiae with the short form of Tom22, which would provide the ultimate proof that the extension of Tom20 is required to compensate for the truncation in Tom22.

The Tom20 of S. castellii has acquired an acidic domain while the Tom22 has lost one. At the protein level, this is one of the few documented cases of domain stealing. Domain stealing was previously proposed to explain the

---

1 These authors contributed equally.

Key words: mitochondria, protein import, protein targeting, domain stealing, yeast, comparative genomics.

E-mail: t.lithgow@unimelb.edu.au.

doi:10.1093/molbev/msn126
Advance Access publication June 22, 2007

© The Author 2007. Published by Oxford University Press on behalf of the Society for Molecular Biology and Evolution. All rights reserved.
For permissions, please e-mail: journals.permissions@oxfordjournals.org
The evolution of Class I MHC receptors from the structurally-related Class II MHC. The functional Class II MHC receptor is a dimeric, integral membrane protein, anchored to the plasma membrane. Each subunit consists of a C-terminal transmembrane segment, followed by an IgG-like domain and then an N-terminal antigen-binding domain (fig 4a). In the course of evolving a more sophisticated immune system (a domain stealing event) that might have been mediated through chromosomal recombination, gave rise to the distinct Class I MHC receptors in which the alpha-subunit carries both antigen-binding domains at its N-terminus, and where the beta-subunit consists only of an IgG-like domain. These rearrangements in domain architecture of the individual subunits through domain stealing do not disturb the overall quaternary structure of the functional receptor (Heringa and Taylor 1997). Being a relatively recent event, we are in the privileged position to trace the ancestry of the domain ‘‘stolen’’ by Tom20 from Tom22 in S. castellii (fig 4b) The process appears not to have required a chromosomal recombination event. Instead, two adenine residues inserted immediately before the original stop codon, change the reading frame and extend the Tom20 protein. The region encoding the extension to Tom20 in S. castellii shares 38% nucleotide sequence identity with the syntenic downstream, untranslated region of the TOM20 locus in S. cerevisiae (data not shown; Cliften et al. 2001). It seems that evolution has acted on this segment to convert its coding potential to a more acidic form, to compensate for the loss of function associated with loss of the acidic region of Tom22.

Fig. 2.—Duplication and modification of the chromosomal region surrounding the TOM20 gene in species of Saccharomyces. Schematic of the gene synten in S. cerevisiae and S. castellii, which evolved after the gene duplication event, and S. kluveri, a related species that diverged before the gene duplication event and maintains what may be a more ancestral arrangement of yeast genes. The data were compiled using the Yeast Gene Order Browser (http://wolfe.gen.tcd.ie/ygob/) (Byrne and Wolfe 2005). In S. cerevisiae, the duplicated genes have been mapped to blocks on Chromosomes VII and XVI (Scannell et al. 2006). Arrows indicate the direction of transcription for each gene. The region corresponding to the TOM20 gene has been lost from the sequence on chromosome XVI, a common phenomenon unless maintaining the gene provides a selective advantage (Scannell et al. 2006).

Fig. 3.—Long and short forms of Tom20 in yeast. (a) Tom20 alignment (for details, see Supplementary fig. S1): black designates the acidic region of the receptor, white boxes designate the TM domain. The S. castellii (long) corresponds to gene 704.37 (see fig. 2), and the S. castellii (short) corresponds to gene 716.41. This shortened gene has lost most of the sequence that could code for a transmembrane segment and may or may not be a functional gene. The extended region of S. castellii Tom20 (long) contains ~35% acidic residues with several lysine and glutamine residues, all characteristic of natively-disordered structures like that predicted for Tom22 (Perry & Lithgow 2005). (b) Western blot of yeast mitochondrial proteins. Mitochondria were purified and proteins were separated by SDS-PAGE and analyzed with antibodies that recognize either Tom20, Tom22 or, as a control, Tom40.

Fig. 4.—Domain stealing in multi-subunit complexes. (a) Class II MHC receptor is a dimeric, integral membrane protein, anchored to the plasma membrane, with an IgG-like domain and an N-terminal antigen-binding domain that binds peptide substrates in a cleft (shaded). The alpha-subunit of the Class I MHC receptors carries both antigen-binding domains at its N-terminus, having “stolen” one from the beta-subunit (Heringa and Taylor 1997). (b) Depiction of how domain-stealing has added a C-terminal acidic extension (light grey) to Tom20 in S. castellii with the N-terminal domain of Tom22 concomitantly shortened. Mitochondrial targeting sequence peptides bind a site on Tom20 (shaded), with the N-terminal acidic domain of Tom22 contributing to binding (Bolliger et al. 1995).
Methods
Yeast strains

One chromosomal copy of \textit{S. cerevisiae TOM22} was truncated in diploid strain MH272 by homologous recombination with a PCR product encoding the \textit{HIS3} gene. The \(\Delta 2-23\) haploid strain and the corresponding “wild-type” strain were obtained by sporulation and dissection of this diploid. \textit{Saccharomyces castellii} was obtained from Jim Dover (Department of Genetics, Washington University School of Medicine) (Cliften et al. 2001).

Miscellaneous

For western blots, cultures grown on rich (YP) medium with glucose as a carbon source were harvested at mid-log phase and mitochondria prepared as previously described (Daum et al. 1982). After separation on SDS-PAGE, proteins were analyzed by immunoblotting using antisera raised to \textit{S. cerevisiae} Tom20, Tom22, or Tom40.

Supplementary Material

Supplementary figure S1 is available at Molecular Biology and Evolution online (http://www.mbe.oxfordjournals.org/).

Acknowledgments

JMH and PW contributed equally to this work, supported by a grant from the Australian Research Council (to TL). We thank Alana Mitchell for comments on the manuscript and Jim Dover for the \textit{Saccharomyces castellii}.

Literature Cited


Geoffrey McFadden, Associate Editor

Accepted June 18, 2007