Just follow the acid chain

Gottfried Schatz

Mitochondria have to import almost all of the proteins they need for their function as the cell's energy producers. A combination of biochemistry and genetics now reveals a tiny but crucial cogwheel in the import machinery.

A typical eukaryotic cell contains roughly 10,000 different proteins. About ten per cent of these are in the mitochondria — the double-walled organelles in which respiration occurs. Because mitochondria synthesize only about a dozen proteins, they must import 99 per cent of their proteins from the cytoplasm. But how do they recognize these proteins, and how are they transported across the two membrane barriers? On page 195 of this issue, Dietmeier *et al.*¹ describe a yeast mitochondrial protein that seems to work in both processes and thereby links them. This protein, which they call Tom5, is an integral protein of the mitochondrial outer membrane, and it is so small that it would be missed by most commonly used analytical methods.

There is, however, nothing small about what Tom5 does. It seems to be an important cogwheel in the dauntingly complex mitochondrial protein-import machine², which works as follows. A protein that is destined to be imported to the interior of a mitochondrion is usually synthesized as a precursor, having a transient amino-terminal targeting signal that can form a strongly basic amphiphilic helix. As the precursor emerges from the ribosome, it binds to one of the cytosolic chaperone proteins, and it is then transferred to proteins that act as 'protein-import receptors' at the mitochondrial outer membrane. These receptors deliver the precursor, amino terminus first, to a protein-conducting channel in the outer membrane. Once the amino-terminal targeting signal has negotiated this barrier, it inserts into a protein-conducting channel in the inner membrane. This presumably occurs in response to the pull of the electric potential (negative inside) across that membrane. The precursor chain — which at this stage may span both membranes — is then pulled completely into the matrix space by an ATP-driven translocation motor that is attached to the inner side of the inner-membrane transport channel.

Mitochondria therefore have two separate protein-transport systems — the 'Tom' system in the outer membrane (for transport across the outer membrane), and the 'Tim' system in the inner membrane (Fig. 1). The Tom system consists of a hetero-oligomeric

Figure 1 Dietmeier *et al.*¹ have identified Tom5, a small protein that plays a crucial part in a 'binding relay' (black arrows) for the import of proteins into mitochondria. The Tom system in the outer mitochondrial membrane consists of four receptors (Tom37, Tom70, Tom20 and Tom22) and a transmembrane protein channel composed of Tom40, Tom6, Tom7 and probably also Tom5. The basic targeting signal of some precursor proteins binds to Tom37/70 and then, through electrostatic interactions, to the 'acid bristle' receptors Tom20 and Tom22. (Other precursors bind directly to Tom20/22.) The receptor-bound precursor is then transferred to Tom5 and, after passage through the Tom channel, to several different binding sites on the inner face of the outer membrane. These sites include an internal Tom22 domain and possibly Tom7, as well as one or more unidentified sites. Transport to the Tim system may be facilitated by binding of the targeting signal to an acidic domain of Tim23. Acidic domains constituting the binding relay ('acid chain') are in red and are bordered by minus signs.

Tom channel, and at least four receptor proteins. Although the Tom and Tim systems are not firmly linked to each other, they interact dynamically to form a molecular pontoon bridge. This helps a targeting signal emerging from the Tom channel to enter the Tim channel, through which it is then moved by an electric potential. But the outer membrane lacks a transmembrane potential. So how is a receptor-bound precursor threaded into and across the Tom channel? Enter Tom5.

Dietmeier *et al.*¹ found that when the outer membrane is solubilized with the detergent digitonin, Tom40 — the core component of the outer-membrane protein-transport channel — co-fractionates with

three small proteins termed Tom5, Tom6 and Tom7. Their earlier work had already indicated that Tom6 and Tom7 are subunits of the Tom channel, which modulate its protein conductance³. But what about Tom5?

Microsequencing of the protein (M_r 5,980) revealed it to be the product of a previously unidentified nuclear yeast gene. Inspection of the amino-acid sequence, along with biochemical studies, led the authors to conclude that Tom5 is an integral outer-membrane protein with a carboxy-terminal transmembrane anchor and an acidic amino-terminal region that is exposed to the cytosol. When they deleted the *TOM5* gene, yeast cells became temperature-sensitive for growth. Moreover, isolated mitochondria that lacked Tom5 imported precursor proteins with greatly reduced efficiency, even though they were normal in other respects. Clearly, a hitherto undiscovered component of the mitochondrial protein-import machine had been found.

Where in this machine does Tom5 work? To answer this question, Dietmeier *et al.* first used all the tricks of the trade to check which of the previously characterized translocation intermediates were no longer formed efficiently in mitochondria lacking Tom5, or in wild-type mitochondria that had been treated with an antibody to Tom5. The Tom5-deficient mitochondria bound precursor proteins as efficiently as wild-type mitochondria, but they transferred bound precursors into the import channel of the outer membrane only very slowly. A similar message came from chemical crosslinking experiments — Tom5 was most effectively crosslinked to an early import intermediate that was still exposed on the outer membrane and had not yet inserted its amino terminus across the inner membrane. These results identified Tom5 as a link between the known receptors and the outer-membrane import channel.

Tom5 could be regarded as yet another import receptor on the mitochondrial surface, but it differs from the four known receptors in at least two ways: it acts after them, and it seems to be firmly associated with Tom40. Tom40 is efficiently co-immunoprecipitated by an antibody against Tom5, and Tom5 protects an amino-terminal region of Tom40 from proteolytic attack. So Tom5 may be an acidic subunit on the outer mouth of the Tom channel that accepts precursors with their basic targeting signals from the acidic receptors Tom20 and Tom22. In this way, Tom5 could facilitate the insertion of the signal into the channel. Being both a receptor and a subunit of the Tom channel, Tom5 may be part of a 'binding relay' of acidic binding sites. This relay could capture the precursors that are destined for import and feed them into the protein-transport channel of the outer membrane.

But the binding relay mechanism does

not seem to end there. The basic targeting signals can also bind to the inner face of the outer membrane⁴, and the authors cite their unpublished data which confirm that part of this binding is to an acidic Tom22 domain that protrudes into the intermembrane space⁵. Tom7 may contribute yet another *trans*-binding site. So, as sketched by the black arrows in Fig. 1, protein transport across the outer membrane may be driven by binding of the targeting signal to a series of strategically placed acidic binding sites of increasing avidity. A binding relay mechanism could also mediate transfer of the precursor to the inner membrane — the awaiting outer mouth of the Tim channel has exposed acidic domains on Tim23, which bind targeting signals⁶. Even the cytosol may be part of this proposed binding array, as the acidic cytosolic protein Mft2 (whose 'acid bristles' resemble those of Tom20) controls the import of some artificial mitochondrial precursors *in vivo*, and binds mitochondrial targeting signals *in vitro*⁷.

The binding relay model begs two questions. First, why do precursors not short-circuit this array by binding immediately to Tom5? Some short-circuiting may, in fact, be tolerated, because yeast cells lacking either Tom5 or Tom20 can still grow slowly at room

temperature. In other words, they can probably still import proteins into their mitochondria. But massive short-circuit on the mitochondrial surface may be prevented by the cytosolic chaperone to which the precursor is bound⁸. Ordered binding to the intramitochondrial sites might be safeguarded by the topological arrangement of these sites. Second, what clears the bound precursor from the last member in the array? Logical candidates are the potential across the inner membrane and the ATP-driven protein-import motor on the matrix side of the inner membrane. Viewed in this light, Tom5 is the newest and smallest link in an 'acid chain' that guides basic mitochondrial-targeting signals to their final destination. □

Gottfried Schatz is in the Biozentrum der Universität Basel, Klingenbergstrasse 70, CH-4056 Basel, Switzerland.

1. Dietmeier, K. *et al.* *Nature* **388**, 195–200 (1997).
2. Schatz, G. *J. Biol. Chem.* **271**, 31763–31766 (1996).
3. Hönlinger, A. *et al.* *EMBO J.* **15**, 2125–2137 (1996).
4. Mayer, A., Neupert, W. & Lill, R. *Cell* **80**, 127–137 (1995).
5. Bolliger, L., Junne, T., Schatz, G. & Lithgow, T. *EMBO J.* **14**, 6318–6326 (1995).
6. Bauer, M. F., Sirrenberg, C., Neupert, W. & Brunner, M. *Cell* **87**, 33–41 (1996).
7. Cartwright, P., Beilharz, T., Hansen, P., Garrett, J. & Lithgow, T. *J. Biol. Chem.* **272**, 5320–5325 (1997).
8. Komiya, T., Rospert, S., Schatz, G. & Mihara, K. *EMBO J.* (in the press).

strain resulting from fault slip, following years of slow strain accumulation. Elastic rebound remains a central tenet of modern theories of earthquake generation.

The triangulation measurements central to Reid's analysis remain invaluable, because advances in our understanding of earthquake mechanics and vastly improved computational capabilities allow use of the data in modelling of the earthquake in ways that were not imaginable in his day. Thatcher and his colleagues at the US Geological Survey have been at the forefront of this research, and have now refined¹ earlier modelling of the slip that occurred on the San Andreas on 18 April 1906.

This study adds previously unanalysed data at the northern end of the 1906 rupture near Cape Mendocino (Fig. 1). The San Andreas is located offshore between Point Arena and Shelter Cove, and the interpretation of the fault geometry near Shelter Cove has been controversial⁵. Assuming the main trace of the fault runs through Shelter Cove, the triangulation data suggest 8.6 m of fault slip in this region, the maximum slip anywhere along the 1906 rupture.

But it is data from the southeastern end of the rupture that yield the most significant results, for this is the location of the magnitude-6.9 1989 Loma Prieta earthquake. In this region it is possible to compare the strain released in the 1906 earthquake with strain accumulation in the years between 1906 and 1989. Geological and geodetic measurements suggest that the San Andreas fault in this region slips 19 ± 4 mm yr⁻¹ when averaged over many earthquake cycles⁶. Mapping indicated 1.5 m of slip in this area during the 1906 event, leading to some forecasts of a high probability of a moderate earthquake on this segment of the fault^{7–9}.

The reasoning was quite simple: if the San Andreas slips 19 mm yr⁻¹ on average, and if the 1906 slip in this region was 1.5 m, then the strain released by the 1906 earthquake should have recovered by 1985. However, there is, of course, considerable uncertainty associated with both the long-term slip-rate and the 1906 co-seismic slip. Furthermore, our understanding of earthquake physics does not require the earthquake to occur when the earthquake strain-drop recovers. The so-called time-predictable model¹⁰ is nonetheless widely used in long-term forecasting, and deserves careful testing. It is for this reason that the slip in 1906 near Loma Prieta is so important.

Although the surface mapping indicated 1.5 m of slip, the fault trace in this area is complex and it is possible that additional slip was distributed over a fault zone a few hundred metres wide, and was thus missed. Here is where the triangulation measurements are crucial. These data show that the Loma Prieta moved 1.2 m to the southeast in 1906, consistent with 2.5 ± 0.4 m of slip on a

Seismology

New insights into old earthquakes

Paul Segall

Geodetic survey measurements conducted in the late nineteenth and early twentieth centuries continue to improve our understanding of the great San Francisco earthquake of 18 April 1906. These data are crucial to understanding the seismic strain history of the greater San Francisco Bay region, as exemplified in the latest work from Wayne Thatcher and colleagues, published in the *Journal of Geophysical Research*¹.

The 1906 earthquake was a milestone in the history of seismology. To many Californians it is the archetypal Big One, although amongst twentieth-century earthquakes it is not even close to being the biggest — the 1960 Chilean earthquake, the largest instrumentally recorded earthquake, had a seismic moment 350 times greater. Nevertheless, the 1906 earthquake was critical for what it revealed about the physics of earthquakes. Geologists quickly recognized that over 300 km of the San Andreas fault had slipped up to 6 m during the quake² (see map, Fig. 1). This observation helped to establish that fault slip caused earthquakes, rather than being a manifestation of secondary ground failure.

The transient seismic waves unleashed by the slip in 1906 devastated San Francisco. Permanent displacements of the Earth's crust, which remained after the shaking

stopped, caused other, albeit less dramatic, problems. For example, roads were beheaded by the fault. Permanent ground displacement distorted precise survey networks which had been painstakingly measured to map the coastline of California and San Francisco Bay. These surveys, begun in the 1850s, determined the relative latitude and longitude of landmarks with a precision of a few parts in a million (a few decimetres over a distance of 100 km). With earthquake displacements of many metres occurring in the quake, it was 'decided to repair the old triangulation, damaged by the earthquake, by doing new triangulation'³.

The 1906 epicentral region was resurveyed by triangulation in 1906 and 1907 by the US Coast and Geodetic Survey. When the data were analysed they revealed that fault-parallel motions extended tens of kilometres from the San Andreas. The horizontal gradient in these displacements showed a decrease in shear strain acting on the fault. Furthermore, the measurements made between the 1850s and 1880s showed that shear strain had been building up on the fault in the decades before the earthquake. From these data, Harry Fielding Reid formulated the elastic rebound theory⁴, in which earthquakes are caused by sudden release of shear