news and views

can be obtained without falling into traps of illusions, as depicted by the blind man's view of the elephant (Fig. 1), adapted from an old Chinese fable. The results reported by Akiyama *et al.*[2] provide secondary structure information in the submillisecond time scale; they have added a new dimension to the current view of cytochrome *c* folding. Newly developed submillisecond techniques are opening new avenues leading to the understanding of the earliest events of folding in many other protein systems as well. They reveal that the development of the secondary structural elements early in the folding reaction varies from one protein to another. For example, a much larger fraction of the native secondary structure of apo-myoglobin[6,19] is established during the early phase of folding as compared to cytochrome *c*. To generate a general view of protein folding, it is important to investigate as many protein systems as possible by the new methods.

Syun-Ru Yeh and Denis L. Rousseau are in the Department of Physiology and Biophysics, Albert Einstein College of Medicine, Yeshiva University, Resnick Campus, 1300 Morris Park Avenue, Bronx, New York 10461, USA. Correspondence should be addressed to D.L.R. email: rousseau@aecom.yu.edu

1. Dobson, C. M., Sali, A. & Karplus, M. *Angew. Chem. Int. Ed.* **37**, 868–893 (1998).
2. Akiyama, S., Takahashi, S., Ishimori, K. & Morishima, I. *Nature Struct. Biol.* **7**, 514–520 (2000).
3. Jones, C. M. *et al.* *Proc. Nat. Acad. Sci. USA* **90**, 11860–11864 (1993).
4. Pascher, T., Chesick, J. P., Winkler, J. R. & Gray, B. H. *Science*, **271**, 1558–1560 (1996).
5. Gruebele, M., Sabelko, J., Ballew, R. & Ervin, J. *Acc. Chem. Res.* **31**, 699–707 (1998).
6. Dyer, R. B., Gai, F., Woodruff, W. H., Gilmanshin, R. & Callender, R. H. *Acc. Chem. Res.* **31**, 709–716 (1998).
7. Takahashi, S. *et al. Nature Struct. Biol.* **4**, 44–50 (1997).
8. Chan, C.-K. *et al. Proc. Nat. Acad. Sci. USA* **94**, 1779–1784 (1997).
9. Shastry, M. C. R., Luck, S. D. & Roder, H. *Biophys. J.* **74**, 2714–2721 (1998).
10. Yeh, S.-R., Takahashi, S., Fan, B. & Rousseau, D. L. *Nature Struct. Biol.* **4**, 51–56 (1997).
11. Yeh, S.-R. & Rousseau, D. L. *Nature Struct. Biol.* **5**, 222–228 (1998).
12. Yeh, S. R., Han, S., and Rousseau, D. L. *Acc. Chem. Res.* **31,** 727–736 (1998).
13. Pollack, L. *et al. Proc. Natl. Acad. Sci. USA* **96**, 10115–10117 (1999).
14. Shastry, M. C. R. & Roder, H. *Nature Struct. Biol.* **5**, 222–228 (1998).
15. Sauder, J. M. & Roder, H. *Folding Des.* **3**, 293–301 (1998).
16. Sosnick, T. R., Shtilerman, M. D., Mayne, L. & Englander, S. W. *Proc. Nat. Acad. Sci. USA* **94**, 8545–8550 (1997).
17. Babul, J., McGowan, E. B. & Stellwagen, E. *Arch. Biochem. Biophys.* **148**, 141–147 (1972).
18. Bryant, C. & Stellwagen, E. *J. Biol. Chem.* **260**, 332–336 (1985).
19. Callender, R. H., Gilmanshin, R. Dyer, R. B. & Woodruff, W. H. *Annu. Rev. Phys. Chem.* **49,** 173–202 (1998).
20. Colon, W., Wakem, L. P., Sherman, F. & Roder, H. *Biochemistry* **36**, 12535–12541 (1997).
21. Elove, G. A., Bhuyan, A. K. & Roder, H. *Biochemistry* **33**, 6925–6935 (1994).
22. Sosnick, T. R., Mayne, L., Hiller, R. & Englander, S. W. *Nature Struct. Biol.* **1**, 149–156 (1994).
23. Pierce, M. M. & Nall, B. T. *Protein Sci.* **6**, 618–627 (1997).

StARTing to understand cholesterol transfer

Douglas M. Stocco

The crystal structure of a lipid transfer START domain has been solved and shown to contain a hydrophobic tunnel that likely binds cholesterol. These results have implications for understanding the mechanism of action of the steroidogenic acute regulatory protein (StAR), which is indispensable in steroid biosynthesis.

The steroid hormones — which are required for the homeostatic maintenance of blood pressure, carbohydrate metabolism and reproductive function — represent some of the most important molecules found in the body. Their synthesis is regulated by signals from the anterior pituitary gland that act on specific steroidogenic cells found mainly in the adrenal glands and gonads. In response to these signals the steroidogenic acute regulatory protein (StAR) is synthesized. This protein is required for the rapid increase in steroid hormone production and mediates the delivery of cholesterol to the enzyme — cytochrome P450 side chain cleavage (P450 scc) enzyme — that converts cholesterol to pregnenolone, the first steroid formed[1] in the steroidogenic pathway. This enzyme resides in the inner mitochondrial membrane, and it has long been known that the transfer of choles-

terol from the outer mitochondrial membrane to this enzyme is the rate-limiting step in steroidogenesis[2].

To date, the details of how the biosynthesis of steroid hormones is regulated have remained obscure. Now, the paper by Tsujishita and Hurley[3] in the May issue of *Nature Structural Biology* reports a high resolution tertiary structure of a critical region, called the START domain (for StAR related lipid transfer domains), of the MLN64 protein, a 50 kDa protein of unknown function that is highly and specifically expressed in the malignant cells of breast carcinomas. This START domain contains a hydrophobic tunnel that appears capable of binding a single cholesterol molecule. Thus, this finding is a first step toward understanding cholesterol transfer.

START domains are found in a wide variety of proteins including the phos-

phatidylcholine transfer protein, acyl-CoA thioesterase, p122-RhoGAP, the Goodpasture antigen binding protein and, most importantly for the work described here, MLN64 and StAR[4]. They are ~200-residue lipid-binding motifs[4], and the importance of the new structure is that the START domain in MNL64 is highly homologous to the START domain in the StAR protein that is required for the regulation of steroid biosynthesis.

Many studies have demonstrated excellent correlations between StAR expression and steroid hormone biosynthesis. For example, expression of StAR through transfection results in increases in the transfer of cholesterol to the inner mitochondrial membrane and in steroid biosynthesis by both steroidogenic and nonsteroidogenic cells[5–7]. Therefore it is clear that StAR can somehow mediate cholesterol transfer to the inner mito-
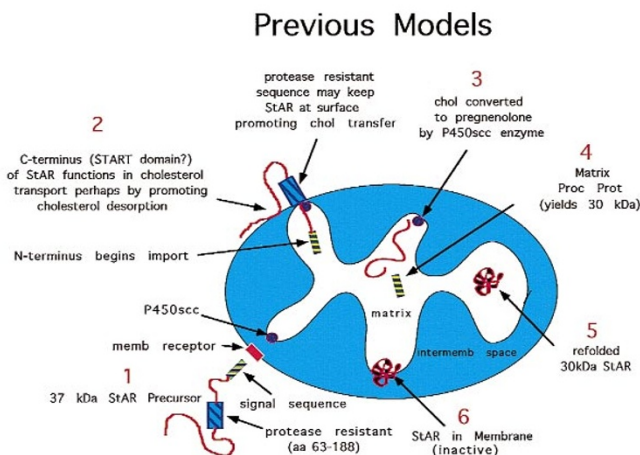
news and views

Previous Models

Fig. 1 Previously proposed models for StAR function.

chondrial membrane. Following the successful cloning of StAR in 1994 (ref. 8), one of the most important undertakings in this area became a quest for understanding how StAR mediates cholesterol transfer.

Early models (Fig. 1) speculated that StAR promotes cholesterol transfer by forming contact sites between the outer and inner mitochondrial membranes during the course of its import into the mitochondria[5]. However, this model required alteration when it was demonstrated that N-terminal truncations of the StAR protein, which removed as many as 62 amino acids and presumably could not be imported into mitochondria, supported full steroid production when transfected into COS-1 cells[9] or when analyzed in an *in vitro* system with isolated mitochondria[10].

In contrast, expression of StAR modified by removal of the C-terminal 28 amino acids resulted in a complete loss of steroid production[9,11]. Importantly, this is the location of the START domain in the protein. The importance of the C-terminal region in cholesterol transfer is suggested by the observation that MLN64, a protein that may be involved in cholesterol transfer in breast cancer tumors, has significant homology to the C-terminal region of StAR and can promote cholesterol transfer[12], as well as by the findings that virtually all of the mutations causing lipoid cogenital adrenal hyperplasia (CAH) are found in the C-terminus of StAR[13]. Nevertheless, the exact role of the START domains of StAR and MLN64 in promoting cholesterol transfer has been unclear.

Recent studies have demonstrated that StAR can act as a sterol transfer protein and can enhance sterol desorption from one membrane to another[14]. In this model, StAR is directed to the mitochondria *via* its N-terminus and then, utilizing C-terminal sequences, produces as yet unidentified alterations in the outer mitochondrial membrane that result in the transfer of cholesterol from the outer to the inner membrane. These studies suggested that transfer is specific for cholesterol; experiments employing phosphatidylcholine failed to show transfer of this phospholipid. Such desorption of cholesterol from the sterol-rich outer membrane to the sterol-poor inner membrane would serve to enhance steroid synthesis, explaining the requirement for StAR in this process[15].

Investigations of StAR structure-function relationships in cholesterol transfer have been conducted, to try to explain the exact role of StAR. Miller and colleagues[16]

subjected StAR to limited proteolysis at different pH values and found that the molecule is altered as the pH decreases. They showed that StAR can form a molten globule in the pH 3.5–4.0 range and proposed that if the mitochondrial micro-environment is acidic, the StAR molecule may undergo a conformational shift. They proposed that this shift could form an extended structure and increase the flexibility of the linker region located between the N-terminus and the biologically active C-terminus. They further hypothesized that as the transition to a molten globule occurred, this structural change would lower the energy required to open the StAR structure, possibly exposing a cholesterol channel and/or lengthening the time that StAR resides on the outer membrane, allowing increased transfer of cholesterol during this period (Fig. 2). While each of the models mentioned above offers features that explain the apparent activity of StAR, new information is required to distinguish these models and provide new insights into how StAR mediates cholesterol transfer.

One of the most sought after pieces of information in the study of StAR has been a high resolution structure — in particular, a structure of the cholesterol transferring START domain. However, purifying and crystallizing the StAR protein, or truncated versions, proved problematic. Thus, Tsujishita and Hurley[3] decided to focus instead on the START domain of MLN64, which is highly homologous to the StAR-START domain and was easier to analyze *in vitro*.

It was important that Tsujishita and Hurley[3] were able to show that MLN64-START is indeed functionally similar to StAR-START. They demonstrated that
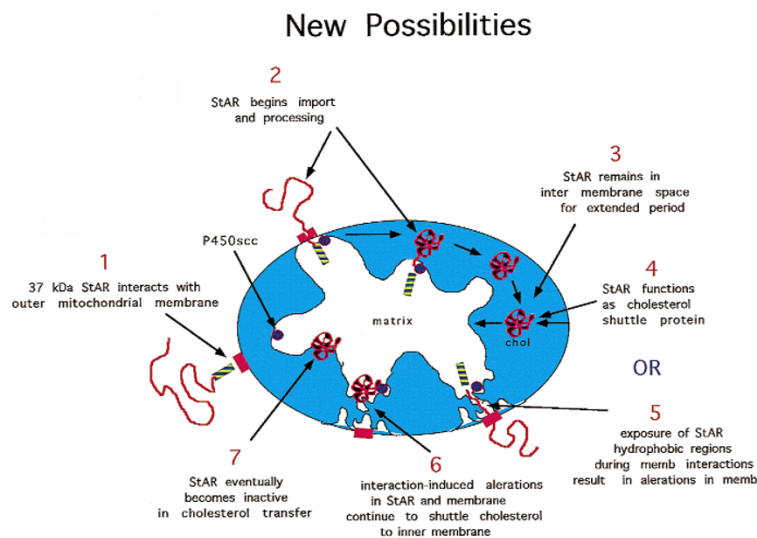
New Possibilities

Fig. 2 New possibilities for StAR function.

both StAR-START and MLN64-START could bind cholesterol and that the binding occurred in a ratio of 1:1. They also demonstrated that neither START domain could bind cholesteryl oleate, suggesting similar specificities and steric properties. Thus, it follows that structural information about MLN64-START should be helpful for understanding the function of StAR-START.

The crystal structure of MLN64-START at 2.2 Å shows an α+β fold built around a U-shaped incomplete β-barrel. Most importantly, the crystal structure of MLN64-START reveals a hydrophobic tunnel 26 Å × 12 Å × 11 Å in size that extends almost the entire length of the protein and is apparently large enough to bind a single molecule of cholesterol. Because of this, Tsujishita and Hurley[3] proposed that StAR functions in transferring cholesterol to the inner mitochondrial membrane, acting as a cholesterol shuttling protein (Fig. 2). Several mutations that result in lipoid CAH were mapped onto the MNL64-START structure. Three of these mutations (E169G, R182L, A218V) are quite close to each other in the tertiary structure, even though they are separated in sequence. In fact, these positions reside within the hydrophobic tunnel, and Tsujishita and Hurley[3] predict that the lipoid CAH causing mutations would destabilize the tunnel, supporting its importance in the function of the START domain.

There are, however, a number of previous observations that are not easily reconciled by the proposal that StAR acts as a cholesterol shuttling protein, moving cholesterol to the inner mitochondrial membrane one molecule at a time. For example, as mentioned above, some results have suggested that StAR can be active in steroidogenesis even without being imported into mitochondria[9–11]. In addition, once StAR is imported into a mitochondrion, it is processed from the 37 kDa precursor to the 30 kDa mature form that is no longer active in cholesterol transfer. Therefore, it appears that StAR would not be able to act as a carrier protein on a continuous basis[17] and because of this, one must consider whether enough cholesterol molecules could be delivered one at a time to P450scc, to account for the level of steroids synthesized, before the pool of StAR molecules becomes inactive. Moreover, mitochondrial proteins are required to be in an unfolded state before import can occur, and it is not clear how the refolding of StAR could occur in the intermembrane space, where it acts as a shuttling protein, because the refolding factors are thought to reside in the matrix.

Nevertheless, if StAR alone can indeed directly transfer cholesterol to the inner membrane, this may account for the inability of several investigators to identify StAR binding partners on the mitochondrial membrane. While many

questions about StAR remain to be answered, Tsujishita and Hurley's[3] accomplishment — determining the structure of a domain that is functional in cholesterol transfer — is a significant step toward solving the riddle of its mechanism of action.

Douglas M. Stocco is in the Department of Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, Texas 79430, USA. email: Doug.Stocco@ttmc.ttuhsc.edu

1. Simpson, E.R., & Boyd, G.S. *Biochem. Biophys. Res. Commun.* **24**, 10–17 (1966).
2. Privalle, C.T., Crivello, J., & Jefcoate, C.R. *Proc. Natl. Acad. Sci. USA* **80**, 702–706 (1983).
3. Tsujishita, Y. & Hurley, J.H. *Nature Struct. Biol.* **7**, 408–414 (2000).
4. Ponting, C.P. & Aravind, L. *Trends Biochem. Sci.* **24**, 130–132 (1999).
5. Stocco, D.M., & Clark, B.J. *Endocr. Rev.* **17**, 221–244 (1996).
6. Lin, D. *et al. Science* **267**, 1828–1831 (1995).
7. Sugawara, T. *et al. Biochemistry* **34**, 12506–12512 (1995).
8. Clark, B.J., Wells, J., King, S.R., & Stocco, D.M. *J. Biol. Chem.* **269**, 28314–28322 (1994).
9. Arakane, F. *et al. Proc. Natl. Acad. Sci. USA* **93**, 13731–13736 (1996).
10. Arakane, F. *et al. J. Biol. Chem.* **273**, 16339–16345 (1998).
11. Wang, X.J. *et al. Endocrinology* **139**, 3903–3912 (1998).
12. Watari, H. *et al. Proc. Natl. Acad. Sci. USA* **94**, 8462–8467 (1997).
13. Miller, W.L. *J. Mol. Endocrinol.* **19**, 227–240 (1997).
14. Kallen, C.B. *et al. J. Biol. Chem.* **273**, 26285–26288 (1998).
15. Martinez, F. & Strauss III, J.F. III. In *Cholesterol: Its functions and metabolism in biology and medicine.* (ed. Bittman, R.) 205–234 (Plenum Press, New York, New York; 1997).
16. Bose, H.S., Whitall, R.M., Baldwin, M.A., & Miller, W.L. *Proc. Natl. Acad. Sci. USA* **96**, 7250–7255 (1999).
17. Stocco, D.M., & Sodeman T.C. *J. Biol. Chem.* **266**, 19731–19738 (1991).

history

Fixing mistakes

The tRNA synthetase enzymes facilitate the attachment of particular amino acids to specific tRNAs in a two-part reaction. First, they activate the amino acid *via* condensation with ATP (forming an aminoacyl adenylate intermediate) and second, they transfer the amino acid moiety to a cognate tRNA that contains the anticodon specific for that amino acid. These enzymes must perform their tasks well or risk insertion of the wrong amino acids into growing polypeptide chains. The fidelity of protein synthesis is maintained in part because each tRNA synthetase primarily binds only its cognate amino acid. Nevertheless, errors do sometimes occur: a similarly shaped but

incorrect amino acid (such as valine instead of isoleucine) can get transferred to a tRNA molecule. Luckily, some tRNA synthetases can fix these infrequent mistakes. This proofreading ability — known as post-transfer editing — was discovered ~30 years ago.

Isoleucyl tRNA synthetase cannot discriminate sufficiently between valine and isoleucine — it can bind and activate both of these amino acids. In 1966, Baldwin and Berg[1] showed that the enzyme compensates for this poor initial discrimination with an additional proofreading step: in the presence of tRNA[Ile], the valyladenylate complex breaks down. They discovered that this reaction depends on an

intact tRNA[Ile] acceptor region (the site on the tRNA where the amino acid becomes attached). This suggested the possibility that valine was, in fact, being transferred to the tRNA[Ile], and that the enzyme was then catalyzing the hydrolysis of the valine–tRNA[Ile] bond to release free valine and tRNA[Ile]. However, this model was difficult to test because at the time it was not possible to make large quantities of tRNA[Ile] charged with valine, the incorrect amino acid.

In the early 1970s, it became possible to make such substrates, and Eldred and Schimmel[2] proceeded to test this prediction. They found that isoleucyl tRNA synthetase could indeed deacylate a valine–tRNA[Ile] substrate, resulting in the release of free valine and tRNA[Ile]. Moreover, they showed that this deacylation occurred