Puzzle Plugged by Protein Pore Plasticity

Daniel L. Minor, Jr.[1,2,3,4,*]
[1] Cardiovascular Research Institute
[2] Department of Biochemistry and Biophysics
[3] Department of Cellular and Molecular Pharmacology
[4] California Institute for Quantitative Biomedical Research
University of California, San Francisco, San Francisco, CA 94158-2330, USA
*Correspondence: daniel.minor@ucsf.edu
DOI 10.1016/j.molcel.2007.05.003

In this issue of *Molecular Cell*, Li et al. (2007) report a set of remarkable structural adaptations by the protein translocon SecY that permit it to function in the face of seemingly major deletions of an important component known as "the plug."

Proteins are marvelous molecules. One of their amazing properties is an ability to tolerate many types of insults, such as those inflicted by point mutations or insertions and deletions, without losing their three-dimensional structure or activity. This robustness is thought to arise from some degree of redundancy in the information encoding the final structure of the protein. In the presence of point mutations or insertions, proteins usually maintain their native folds by absorbing sequence changes through alteration of the structural elements near the mutation site. Thus, changing any particular interaction is not necessarily a complete disaster.

One of the best-studied examples of structural robustness and plasticity in response to mutations and insertions is found in the landmark work on T4 lysozyme mutants (Matthews, 1995). One notable T4 lysozyme mutant bearing a sequence insertion into a β sheet loop shows an extreme form of plasticity (Sagermann et al., 2006). X-ray structural analysis of this mutant shows that the inserted sequence is recruited to replace the native sequence, yet the native fold of the protein is maintained. Such dramatic protein chain shape-shifting is not commonplace; however, there are enough examples of context-dependent structural plasticity, such as that demonstrated by the Chameleon sequence in which a designed sequence folded as an α helix or β sheet, depending on its tertiary environment (Minor and Kim, 1996), and the serpins, where proteolytic cleavage causes a loop to insert itself into the middle of a pre-existing β sheet (Huntington, 2006), to give one pause. In this issue of *Molecular Cell*, the Rapoport laboratory (Li et al., 2007) reports a remarkable example of protein plasticity. They find that, in response to deletions designed to remove an α-helical element of the SecY translocation channel known as "the plug," the protein rearranges the loop bearing the deletions to create a new α-helical plug that seals the channel pore.

Lipid membranes demarcate the innards of a cell from the external environment and form a barrier to the passage of ions, metabolites, and proteins. Cells use a host of membrane protein channels, transporters, and pumps to facilitate and regulate the transport of matter across the cell membrane. Secretory and transmembrane proteins pass through a special protein conduction channel, known as SecY in prokaryotes and Sec61 in eukaryotes, that is conserved across the kingdoms of life. X-ray crystallographic studies of the SecY complex from the hyperthermophile *Methanococcus jannaschii* gave the first three-dimensional view of this important translocation passage (Van den Berg et al., 2004). The structure revealed an hourglass-shaped transmembrane channel that forms the likely transit path for a polypeptide to cross the membrane. The narrowest point of the channel is ~5–8 Å across and is lined by a set of hydrophobic amino acids. This constriction could act as a significant barrier to ions and small molecules. Preventing such leakage is critical, as the unregulated flow of ions through a leaky translocation pore could dissipate the transmembrane electrochemical gradient that is essential for prokaryotic life. The immediate consequence of leaky translocons in the endoplasmic reticulum of eukaryotes is less clear. The transmembrane passageway is capped on the extracellular side by a short α helix, called the plug, formed from a portion of the loop that bridges the first two transmembrane helices: TM1 and TM2b. The observation of two elements that could act as barriers, the narrow hydrophobic constriction and the plug, highlighted the importance of keeping the channel closed in the absence of a translocating substrate but left open the question about whether both elements formed the seal together or whether the ring was sufficient and the plug served as some type of gating element.

A typical way for investigators to test the importance of a protein domain or structural element with respect to a particular function is to disable or amputate it and assay for changes in function. From such studies, one might expect that the absence of a dramatic effect indicates that the altered element is unimportant for the function in question. The puzzle over the importance of the translocon plug for blocking the pore arose from seemingly conflicting results from different types of alteration experiments. Deletions of the yeast Sec61 plug suggested

that the plug was unlikely to be critical for sealing the pore as the mutants produced viable cells, did not inactivate the channel, and had modest functional effects (Junne et al., 2006). In *E. coli*, immobilization of the plug by a disulfide bond that fixed the channel in a permanently open conformation was lethal (Harris and Silhavy, 1999) and suggested a critical role for the plug in keeping the channel closed. In contrast, *E. coli* plug deletions were found to produce viable cells (Li et al., 2007; Maillard et al., 2007) and functional translocons in vitro (Li et al., 2007). This result seems to oppose the expectations set by the immobilization experiment and seems more in line with the yeast Sec61 experiments that indicate little role for the plug in sealing the pore.

How can the plug appear simultaneously necessary and unnecessary for the sealing function? Li et al. (2007) make an important observation that answers the puzzle. X-ray crystallographic analysis of two different plug deletion mutants, Δ60–65 and Δ57–67, reveals a change in protein conformation that molds a new plug from remaining portions of the TM1-TM2 loop. Thus, the deletion mutants are not plug deletions at all. Remarkably, this result does not depend on the specific deletion and suggests that the sequence requirements for making a plug are not strict. Even though the new plugs work, they do not appear to be as robust as the wild-type. Nevertheless, the combination of functional and structural data clearly indicates that a poor plug is preferable to an open pore.

The ability of SecY to rearrange the TM1-TM2 loop into new plugs is a remarkable instance of context-dependent folding. Other, larger plug deletion mutants have been shown to make functional translocons (Junne et al., 2006; Maillard et al., 2007). Determining whether these more dramatic deletions also lead to alternative plug elements and what the structural constraints are for making a functional plug are interesting directions for future studies. The protein conformational plasticity manifested by the SecY deletion mutants serves as an excellent lesson in protein shape-shifting potential and as a potent reminder that interpreting mutational data in the absence of structures should always be done with caution.

REFERENCES

Harris, C.R., and Silhavy, T.J. (1999). J. Bacteriol. *181*, 3438–3444.

Huntington, J.A. (2006). Trends Biochem. Sci. *31*, 427–435.

Junne, T., Schwede, T., Goder, V., and Spiess, M. (2006). Mol. Biol. Cell *17*, 4063–4068.

Li, W., Schulman, S., Boyd, D., Erlandson, K., Beckwith, J., and Rapoport, T.A. (2007). Mol. Cell *26*, this issue, 511–521.

Maillard, A.P., Lalani, S., Silva, F., Belin, D., and Duong, F. (2007). J. Biol. Chem. *282*, 1281–1287.

Matthews, B.W. (1995). Adv. Protein Chem. *46*, 249–278.

Minor, D.L., Jr., and Kim, P.S. (1996). Nature *380*, 730–734.

Sagermann, M., Baase, W.A., and Matthews, B.W. (2006). Protein Sci. *15*, 1085–1092.

Van den Berg, B., Clemons, W.M., Jr., Collinson, I., Modis, Y., Hartmann, E., Harrison, S.C., and Rapoport, T.A. (2004). Nature *427*, 36–44.

The Oxazolidinone Class of Drugs Find Their Orientation on the Ribosome

Daniel N. Wilson[1,*] and Knud H. Nierhaus[2,*]
[1] Gene Center and Department for Chemistry and Biochemistry, University of Munich, Feodor-Lynen-Strasse 25, D-81377 Munich, Germany
[2] Max-Planck-Institut für Molekulare Genetik, Ihnestrasse 73, D-14195 Berlin, Germany
*Correspondence: wilson@lmb.uni-muenchen.de (D.N.W.), nierhaus@molgen.mpg.de (K.H.N.)
DOI 10.1016/j.molcel.2007.05.008

Recent studies by Leach et al. (2007) in *Molecular Cell* identify the orientation of the oxazolidinone antibiotic linezolid at the peptidyltransferase center of bacterial and mitochondrial ribosomes, providing a structural basis for the mechanism of drug action and observed side effects.

An ever-growing problem in the fight against bacterial infection is the increasing resistance to antibiotics. In order to reduce the probability of resistance developing to a particular drug, combination drug therapies are common practice. In these cases, drugs exhibiting different modes of inhibition against specific pathogens are employed. The rationale is that the probability of developing resistance against two drugs is dramatically lower than against a single drug; for example, if one in 10^{-6} pathogens are resistant against individual drugs, then the usage of two drugs with a different inhibition mode will improve the probability to 10^{-12}. This is, of course, not the