

P001 Biochemical analysis of TorD: a chaperone involved in protein targeting and cofactor loading

Kostas Hatzixanthis, David J. Richardson and Frank Sargent
*Centre for Metalloprotein Spectroscopy and Biology,
School of Biological sciences, University of East Anglia,
Norwich NR4 7TJ, UK*

In *Escherichia coli* a subset of periplasmic proteins are synthesised with specialised N-terminal signal peptides containing a distinctive SRRxFLK 'twin-arginine' amino acid sequence motif. Precursor proteins bearing twin-arginine signal peptides are targeted post-translationally to the twin-arginine translocation (Tat) system. The majority of proteins targeted to the Tat pathway in *E. coli* contain redox-active cofactors that must be inserted prior to export of the *fully folded* proteins. It is likely that cellular mechanisms exist that prevent either wasteful export of immature substrates or competition between immature and mature proteins for the transporter. In this work, this 'proofreading' activity has been investigated using the molybdoprotein trimethylamine *N*-oxide reductase (TorA) and its private chaperone TorD as a model system. TorD and the immature form of TorA interact during loading of the molybdenum cofactor prior to the transport event. Here, we show that TorD recognises at least two independent binding-sites on the TorA precursor – one of which is the twin-arginine signal peptide itself. The kinetics of Tat signal peptide binding to TorD have been explored. A proofreading mechanism in which TorD prevents premature targeting of TorA during cofactor-loading is proposed.