Protein Synthesis and Evolution

Dr Nicholas Dixon

Some proteins are enzymes that promote chemical reactions; others provide molecular switches that control metabolic and developmental processes through precise interactions with other proteins, nucleic acids and other ligands. In two distinct research programs, we are exploring the chemistry that governs the specificity and strength of interactions of proteins with other proteins, and ligands like substrates, inhibitors and nucleic acids.

The first program concerns the thirty or so different proteins that collaborate to replicate the DNA of the bacterial chromosome prior to cell division. DNA replication commends itself as a model system to study general aspects of protein–protein and protein–nucleic acid interactions because the proteins act together in a giant nucleoprotein assembly called the replisome, to make perfect copies of the chromosome. We use molecular genetics to engineer rich sources of the proteins and to produce mutant derivatives and segments of them, and conventional enzymology, DNA synthesis assays and protein chemistry to study protein function. Protein X-ray crystallography, ESR and high-field NMR spectroscopy, mass spectrometry, electron microscopy and computational methods are used with collaborating laboratories to further understand the structure of the individual proteins, and to relate their structures to how they work and interact with each other and DNA. This year, we have focussed our efforts on interactions between the replicative helicase DnaB and the DnaG primase, and on the ten different subunits of DNA polymerase III (Pol III) holoenzyme, the enzyme that actually synthesises new DNA chains during chromosomal DNA replication.

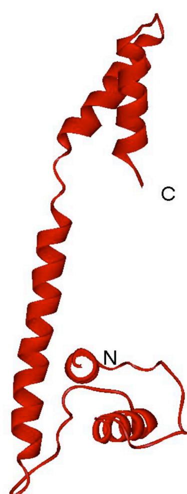
Our other research program has complementary objectives. A suite of new techniques in protein chemistry is being developed, including methods for *in vitro* evolution of new protein functions, *in vitro* synthesis of proteins on a preparative scale, library methods for precise location of boundaries between distinct folded domains in larger proteins, and stabilisation of small protein domains by end-to-end cyclisation of their polypeptide chains. Used together, these techniques are helping to overcome some of the major bottlenecks in rapid determination of protein structures and functions, thereby increasing the efficiency of worldwide efforts in structural and functional genomics. They are also being used to study fundamental aspects of the relationship between the structure, folding, stability and functions of proteins.

DNA Polymerase III Holoenzyme

The replisome is made up of several molecular machines that interact physically with each other. One is Pol III, the replicative DNA polymerase, which consists of the catalytic core (α, ε and θ subunits), the sliding clamp (β_2), and the six subunits of the clamp loader (δ, δ', γ, τ, χ and ψ). This year, methods have been developed for

purification of large quantities of the α (polymerase) subunit and the four largest subunits of the clamp loader, and complexes of them have been isolated for structural and functional studies. In collaboration with a group in CSIRO, these proteins have been used to further understand the way that the α and δ subunits compete for their interaction site on the β_2 sliding clamp. In 2002, we reported the high-resolution crystal structure of the ε (proofreading exonuclease) subunit, in a complex with a nucleotide inhibitor and two Mn(II) ions. This year, structures of complexes with other nucleotides and metal ions have been refined, and a method has been devised for making crystals of the apoenzyme. This sets the stage for further studies of the mechanism of action of this important binuclear metallohydrolase. Intein-mediated protein cyclisation has been used to produce circularised forms of ε that are more stable than the native form, and their structures have also been determined. The structure of the θ subunit in its complex with ε has been determined by NMR spectroscopy, and a new method using lanthanide derivatives of ε was used to produce a model for the structure of the ε-θ complex. *(with P.D. Carr, S. Jergic, M.A. Keniry, P.E. Lilley, D.L. Ollis, G. Otting, K. Ozawa, A.Y. Park, P. Prosselkov and C.M. Elvin, G. Wijffels [CSIRO, Brisbane], B. Hankamer [U. Qld], G. Pintacuda [Karolinska Institutet, Stockholm])*

The DnaB Helicase



The DnaB interaction domain

The molecular motor that drives the replisome and separates the two strands of DNA at the apex of the replication fork is the ring-shaped hexameric DnaB helicase. This year, we have concentrated on examining the defects in function of a series of designed mutants of DnaB, and on its interaction with the replicative priming enzyme, DnaG primase. We have shown that the small *C*-terminal domain of primase (DnaG-C) contains all of the determinants for interaction with DnaB, and in collaboration with a group at the University of Western Australia have solved the crystal structure of DnaG-C. The protein is unusual in that it is comprised of two small helical domains linked by a long naked helix. NMR studies were used to confirm that the structure in solution is the same as in the crystal. *(with B. Bancia, P.E. Lilley, K.V. Loscha, G. Otting, P.M. Schaeffer, N.K. Williams and J.M. Carazo, Y. Robledo [Centro Nacional de Biotecnología, Madrid], J.M. Guss [U. Sydney], E. Liepinsh [Karolinska Institutet, Stockholm], A.J. Oakley, M.C.J. Wilce [U. Western Australia])*

New Protein Technologies

Substantial progress has been made in development of new methods for directed molecular evolution of proteins with new binding specificities, for intein-mediated end-to-end cyclisation of protein domains and peptides, for preparative *in vitro* protein synthesis, for site-specific incorporation of unnatural amino acids and lanthanide chelates into proteins, and for the use of library methods for protein domain identification. *(with M. Headlam, P.E. Lilley, M. Mulcair, G. Otting, K. Ozawa, P. Prosselkov, P.M. Schaeffer, N.K. Williams and R. Dean [U. Canberra], M. Ehrenberg [U. Uppsala, Sweden], J.L. Beck, M.M. Sheil [U. Wollongong], J.M. Matthews [U. Sydney])*

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