



## BIOLOGICAL CHEMISTRY

### Protein Structure and Function

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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 explore the chemistry that governs the specificity and strength of interactions of proteins with substrates, inhibitors, nucleic acids, and other proteins.

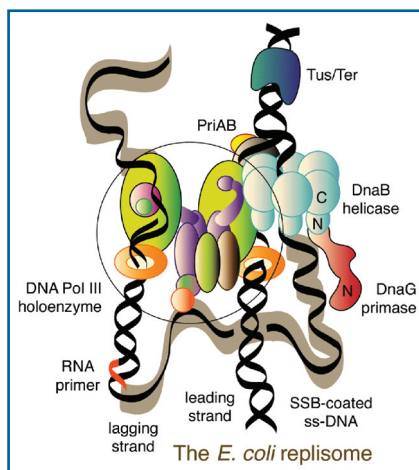
The first program concerns the thirty or so different proteins that collaborate to replicate the DNA of the bacterial chromosome prior to cell division. DNA replication represents a good model system to study general aspects of protein–protein and protein–nucleic acid interactions because the proteins 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 structures of the individual proteins, and to relate their structures to how they work and interact with each other and with DNA. This year, we have focused our efforts on how the interaction of the replicative helicase (DnaB) with the replication terminator protein arrests progress of the replisome. We have also looked at how DnaB interacts with its loading partners, the DnaG primase, and subunits of DNA polymerase III (Pol III) holoenzyme.

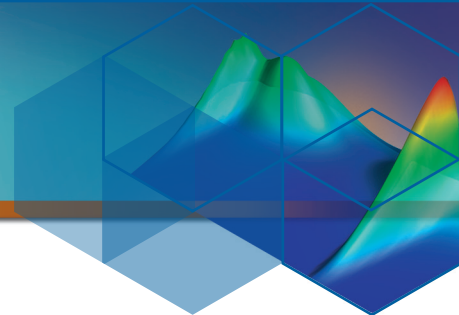
Our other research program has complementary objectives. A suite of new techniques in protein chemistry is being developed, including methods for *in vitro* evolution of new protein functions, *in vitro* synthesis of proteins on a preparative scale, library methods for precise location of boundaries 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 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 the fundamental chemistry that underpins the relationship between the structure, folding, stability and functions of proteins.

### *Molecular Motors in the Bacterial Replisome*

The replisome contains two molecular motors that interact with each other. One is Pol III holoenzyme, the machine that actually synthesises new DNA chains during DNA replication, and the other is the ring-shaped hexameric DnaB helicase that drives the replisome and separates the two strands of DNA at the apex of the replication fork. The Pol III holoenzyme contains a catalytic core ( $\alpha$ ,  $\epsilon$  and  $\theta$  subunits), a sliding clamp ( $\beta_2$ ), and the six subunits of the clamp loader ( $\delta$ ,  $\delta'$ ,  $\gamma$ ,  $\tau$ ,  $\chi$  and  $\psi$ ). We now have methods for the





isolation of large quantities of all ten subunits, and have isolated many sub-complexes of them for studies of their structures. Work on the structure and function of the  $\epsilon$  proofreading exonuclease subunit is being used to model the chemical mechanism of related binuclear metallohydrolases. The structure of  $\epsilon$  has been determined at 1.15 Å resolution, and several structures with metals other than the natural Mn(II) ions and inhibitors have been solved. Structural studies are proceeding with regions of the  $\tau$  subunit that interact with the  $\alpha$  subunit and DnaB, and methods are being devised to study these interactions in detail. In work on DnaB this year, we have focused on understanding how the helicase interacts with other proteins, including its loading partner DnaI (from *Bacillus subtilis*), the primase DnaG, and the replication terminator protein, Tus. The structures of the DnaB interaction domains of primase and DnaI have been determined using combinations of X-ray crystallographic and NMR methods. (With B Bancia, P D Carr, S Jergic, M A Keniry, P E Lilley, K V Loscha, A J Oakley, D L Ollis, G Otting, K Ozawa, A Y Park, G Pintacuda, P Prosselkov, P M Schaeffer, X-C Su, N K Williams, and J M Carazo, Y Robledo [Centro Nacional de Biotecnología, Madrid], J M Guss [U Sydney], E Liepinsh [Karolinska Institute, Stockholm], B Hankamer, G Schenk [U Old])

### *A Molecular Mousetrap in Replication Termination*

The mechanism of termination of DNA replication in *Escherichia coli* has been debated for nearly twenty years. The terminator protein Tus binds *Ter*-site DNA as a monomer, yet replication fork arrest is a polar (unidirectional) process. A replisome approaching from the 'permissive' face of the Tus-*Ter* complex can continue its progress, apparently by displacing Tus, whereas one approaching from the 'non-permissive' face is arrested. How can this be? This year we have shown that this process works like a molecular mousetrap that is set by DNA strand separation by the DnaB helicase, and sprung to create an intermediate species that is kinetically trapped by its unusually stable binding to Tus. (With M Mulcair, A J Oakley, P M Schaeffer, and T M Hill [U North Dakota])

### *New Protein Technologies*

Substantial progress is being made in development of new methods for *in vitro*-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 paramagnetic lanthanide ions into proteins, for the use of library methods for protein domain identification, and for use of mass spectrometry in studies of protein-ligand complexes. (With M Headlam, P E Lilley, M Mulcair, G Otting, K Ozawa, A Y Park, P Prosselkov, P M Schaeffer, N K Williams, and G Coia [EvoGenix, Melbourne], R Dean [U Canberra], M Ehrenberg [U Uppsala], J L Beck, M M Sheil [U Wollongong], J M Matthews [U Sydney], D Spencer, H-X Zhou [Florida State U], K Alexandrov, A Rak [Max-Planck Institute for Molecular Physiology, Dortmund, Germany])

<http://rsc.anu.edu.au/research/dixon.php>



Penny Lilley retired in 2004 after more than 17 years as a research assistant in the Dixon group. She was honoured for her service to the group and the School at a dinner in December, at which we presented her with a glass rose fabricated by Chris Tompkins, RSC glass blower.