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. We aim to understand the chemistry that governs the specificity and strength of interactions of proteins with substrates, inhibitors, nucleic acids, and other proteins.

As a model system, we use the thirty different proteins that collaborate to replicate the DNA of the bacterial chromosome prior to cell division. DNA replication presents a good 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, which makes perfect copies of the chromosomes. 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, protein chemistry and biophysical techniques like surface plasmon resonance to study protein function and molecular interactions. This is complemented by structural and spectroscopic studies in collaborating laboratories, using techniques that include protein X-ray crystallography, ESR and high-field NMR spectroscopy, mass spectrometry, electron microscopy and computational methods. This enables us to relate the structures of proteins and complexes 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 studied interactions among the ten different subunits of the replicative DNA polymerase, DNA polymerase III holoenzyme (Pol III HE).

Many of the replication proteins are also being used for development of a suite of new techniques in protein chemistry, 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.

Intrinsically-unstructured Interaction Domains

The replisome contains two molecular motors that interact with each other. One is Pol III HE, the machine that synthesises the new DNA chains, and the other is the ring-shaped hexameric helicase (DnaB) that drives the replication machinery while separating the two strands of DNA at the apex of the replication fork. Pol III HE contains 10 subunits: a catalytic core (α, ε and θ), a sliding clamp (β_2), and the six subunits of the clamp loader (δ, δ', γ, τ, χ and ψ). We routinely prepare large quantities of all of the subunits, then mix them to isolate the many sub-complexes for structural studies. A few years ago we proposed that the replisome presents a new paradigm for such flexible, dynamic molecular machines, in particular that many of the protein–protein interactions occur through intrinsically-unstructured regions (domains) that attain their folded forms only when the interactions occur. In work this year, we have shown this to be true, at

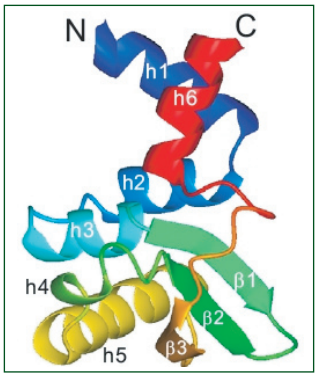

Figure 1: Structure of the folded core of the *C*-terminal domain of τ.

least in some cases. In collaborative work with the Biomolecular NMR group, we determined the molecular structure of the folded domain of the τ subunit that distinguishes it from γ (Figure 1). Although this domain contains the regions of τ that interact with the α subunit of Pol III and DnaB, the major sites of interaction are not in its structured region, but in the flexible regions that flank it. In other work, we showed that ψ interacts with γ through its intrinsically unstructured *N*-terminal region, and NMR and crystallographic studies of the structure and function of the ε proofreading exonuclease subunit have continued in collaboration with Professors Gottfried Otting and David Ollis, Drs Max Keniry and Gary Schenk. *(With P D Carr, M J Headlam, S Jergic, M John, M A Keniry, K V Loscha, A J Oakley, D L Ollis, G Otting, K Ozawa, A-Y Park, P Prosselkov, P M Schaeffer, X-C Su, N K Williams, P S C Wu, and E Liepinsh [Latvian U, Riga], G Schenk [U Queensland])*

Replication Termination

In the final stage of replication, forks encounter the terminator protein Tus in complex with *Ter*-site DNA, and are arrested in a polar manner – a replisome approaching from the one face of the Tus-*Ter* complex can progress, while another approaching from the other face is blocked. We showed that this process works like a molecular mousetrap that is set by DNA strand separation by DnaB, and sprung to create a structure that is kinetically trapped by unusually stable binding of the *Ter* DNA to Tus. This year, we confirmed the mechanism that determines polarity by solving the structure of the arrested "Tus-*Ter* Lock" complex (Figure 2). *(With M Mulcair, A J Oakley, P M Schaeffer, and T M Hill [U North Dakota], C Neylon [U Southampton])*

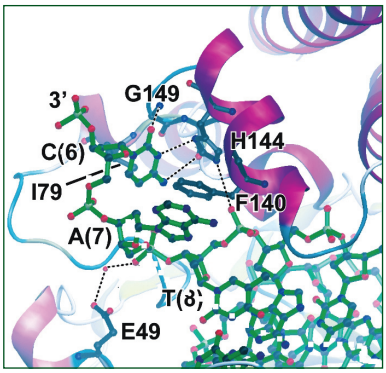

Figure 2: Structure of the arrested "Tus-*Ter* Lock".

New Protein Technologies

Figure 3: Synthetic resilin. See Elvin *et al.* Nature (2005) *437*, 999.

In collaboration with several other groups, new methods are being developed for *in vitro* molecular evolution of proteins with new functions, for intein-mediated end-to-end cyclisation of protein domains and peptides, for preparative *in vitro* protein synthesis and labelling for NMR studies, 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. A highlight this year was the publication in *Nature* of a method for preparation of a synthetic biopolymer that closely mimics the properties of resilin (Figure 3), the rubbery material that occurs in the wing hinges of insects, the legs of jumping fleas, and the tymbal organs of cicadas. *(With M J Headlam, M Mulcair, G Otting, K Ozawa, A-Y Park, P Prosselkov, P M Schaeffer, N K Williams, and K Alexandrov, A Rak [Max-Planck Institute for Molecular Physiology, Dortmund, Germany], J L Beck, M M Sheil [U Wollongong], G Coia [EvoGenix, Melbourne], C M Elvin [CSIRO Livestock Industries, Brisbane], D Spencer, H-X Zhou [Florida State U])*

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