ELSEWHERE IN BIOLOGY

A selection of interesting papers and minireviews published last month in *Chemistry & Biology*'s sister journals, *Current Biology* and *Structure*.

Chemistry & Biology March 1995, **2**:185–186

► **Swapping DNA strands and sensing homology without branch migration in λ site-specific recombination**
Simone E. Nunes-Düby, Marco A. Azaro and Arthur Landy (1995). *Curr. Biol.* **5**, 139–148.

Many site-specific recombinases act by forming and resolving branched Holliday junction intermediates. Current models for this process hold that the crossover between two strands that results in the Holliday junction forms at one position, then migrates at least three base pairs away from the initial site along the 'overlap region', a region in which homology between the two strands is essential, allowing a second strand exchange which resolves the Holliday junction. Lack of homology between the two strands in the overlap region would inhibit the migration of the crossover point, and would therefore be expected to inhibit recombination. The authors constructed synthetic Holliday junctions in which the mobility of the crossover point is restricted to the central region. Surprisingly, recombination does occur in these artificial junctions, but not in other artificial junctions in which the crossover point is frozen at the position predicted to give resolution. The authors therefore propose a model in which the crossover point is close to the center of the overlap region in both strand-exchange events.
1 February 1995, *Current Biology*

► **Accessory proteins function as matchmakers in the assembly of the T4 DNA polymerase holoenzyme**
Barbara Fenn Kaboord and Stephen J. Benkovic (1995). *Curr. Biol.* **5**, 149–157.

Two accessory proteins are required for bacteriophage T4 DNA replication, the 44/62 ATPase and the 45 protein. These proteins are required to form a processive DNA polymerase holoenzyme. The authors show that the 44/62 protein is required for holoenzyme assembly; the 44/62 protein can act catalytically to load the 45 protein onto the DNA primer/template. The complex of DNA primer/template and 45 protein then combines with the DNA polymerase. The 44/62 protein thus acts as a molecular matchmaker, but does not form an essential part of the processive complex.
1 February 1995, *Current Biology*

► **Overlap in the repertoires of peptides bound *in vivo* by a group of related class I HLA-B allotypes**
Linda D. Barber, Beth Gillece-Castro, Lucy Percival, Xiaobin Li, Carol Clayberger and Peter Parham (1995). *Curr. Biol.* **5**, 179–190.

The polymorphic class I molecules of the major histocompatibility complex (MHC) bind peptides and present them to cytotoxic T lymphocytes. The fact that class I molecules vary in sequence results in differences in the sets of peptides that bind to the different molecules; this is believed to have implications for disease susceptibility in people who carry particular MHC genes. New class I alleles are generally produced by gene-recombination events. To assess the importance of the differences in peptide-binding specificities resulting from such events, the authors compare the endogenous peptide-binding specificities of six related HLA-B allotypes. Although their peptide-binding sites differed by one to nine amino acids, all six MHC molecules retained a strong preference for Pro at position 2, and four peptides were isolated that were bound by two or more allotypes. Cross-reactive cytotoxic T cells were also found. Thus, peptide-based vaccines designed to elicit cytotoxic T cells, or small-molecule peptide mimics designed to block peptide–MHC binding, may not need to be designed individually for every MHC type.
1 February 1995, *Current Biology*

► **Hot numbers in signal transduction**
Peter D. Burbelo and Alan Hall (1995). *Curr. Biol.* **5**, 95–96.

Burbelo and Hall review the resurgence of interest in 14-3-3 proteins, whose functions have been a puzzle for more than 25 years. Proteins of this family have recently been implicated in the regulation of the Ras/Raf signaling pathway (which links cell-surface receptors to changes in gene expression via the mitogen-activated protein (MAP) kinase pathway). They also appear to regulate protein kinase C and bind to the serine-threonine kinase Bcr. Experiments in yeast have now identified two 14-3-3 isoforms, rad24 and rad25, which may be important in cell-cycle control. It is thus becoming clear that the class of 14-3-3-dependent protein kinases is important in many signaling pathways.
1 February 1995, *Current Biology*

► **Palmitoylation in G-protein signaling pathways**
Elliott M. Ross (1995). *Curr. Biol.* **5**, 107–109.

Where a signaling protein resides in the cell can have marked effects on what it does and to whom it does it. This review focuses on the reversible palmitoylation of G-protein α subunits and G-protein-coupled receptors via thioester linkages to Cys residues. Palmitoylation has been shown to affect the association of the Gα subunit with the plasma membrane. Lipid addition increases overall hydrophobicity, increasing the association with the plasma membrane and, presumably, enhancing binding to other membrane-bound proteins. The function of palmitoylation in the G-protein-coupled receptors, which are integral membrane proteins, is not so clear; it appears to affect receptor desensitization, perhaps by enhancing receptor endocytosis. Difficulties in measuring receptor palmitoylation and in distinguishing between palmitoylated and de-palmitoylated receptors have made this phenomenon hard to study.
1 February 1995, *Current Biology*

► **Describing the elephant**
Scott Baskerville and Andrew D. Ellington (1995). *Curr. Biol.* **5**, 120–123.

The three-dimensional structures of RNAs are notoriously difficult to determine. The new non-crystallographic techniques reviewed here have made some headway with this difficult problem. Using *in vitro* selection of functional RNA molecules, analyzing the co-variance between these partially-randomized molecules to locate potential structural interactions, and deriving a single three-dimensional structure from the possibilities using energy minimization, a model of the structure of the Rev-binding element (RBE) of HIV-1 was obtained that closely matched a structure determined by NMR. In cases where such 'phylogenetic' analysis is insufficient to provide tertiary structural information, photo-crosslinking using an azidophenacyl-tagged 5' end, or fluorescence resonance energy transfer (FRET) using fluorophore-tagged residues can help to determine which secondary structural elements are near to one another. The demonstration that a variety of such techniques can together produce structural information holds promise for an area where very little crystallographic information is available.
1 February 1995, *Current Biology*

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► **Single-stranded DNA–protein interactions in canine parvovirus**
Michael S. Chapman and Michael G Rossmann (1995). *Structure* **3**,151–162.
The mechanism for specific encapsidation of viral genomes into a virus particle is poorly understood. In some systems, specific recognition of the viral nucleic acid is required for virus assembly, while in others non-viral oligonucleotides suffice. The authors have determined the structure of an 11-nucleotide loop of the single-stranded DNA genome of canine parvovirus (CPV) bound to the inside of the CPV capsid. The conformation of the loop is unusual, with the bases pointing outwards (or 'flipped out') towards the capsid protein and the phosphates surrounding metal ions on the inside. Most of the contacts between the DNA and the protein involve the bases, rather than the ribose-phosphate backbone, indicating that some DNA sequences are preferred.
15 February 1995, *Structure*

► **The crystal structure of the lysyl-tRNA synthetase (LysU) from *Escherichia coli***
Silvia Onesti, Andrew D. Miller and Peter Brick (1995). *Structure* **3**, 163–176.
Lysyl-tRNA synthetase catalyzes the attachment of the amino acid lysine to the cognate tRNA. The enzyme is a member of the class II amino-acyl-tRNA synthetases; the crystal structures of the seryl- and aspartyl-tRNA synthetases from this class are already known. In *E. coli*, there are two forms of this enzyme; LysU, the subject of this study, is only expressed under extreme physiological conditions such as heat shock. This enzyme can synthesize adenyl dinucleotide oligophosphates (in particular AppppA) , which are proposed to act as cellular signals of stress conditions. The authors report the crystal structure of LysU to 2.8 Å resolution and compare it to the seryl- and aspartyl-tRNA synthetases, identifying a conserved core. A number of catalytically-important residues are conserved in the three structures, and the amino-acid binding pockets of all three enzymes show a similar extended network of hydrogen bonds.
15 February 1995, *Structure*

► **The refined structure of the quinoprotein methanol dehydrogenase from *Methylobacterium extorquens* at 1.94 Å**
Minakshi Ghosh, Chris Anthony, Karl Harlos, Matthew G. Goodwin and Colin Blake (1995). *Structure* **3**, 177–187.
Methanol dehydrogenase (MDH) is a soluble quinoprotein which oxidizes methanol to formaldehyde in methylotrophic bacteria. Its prosthetic group is pyrroloquinoline quinone (PQQ), from which it passes electrons to cytochrome c_L. The authors have determined the structure of MDH to 1.94 Å. The α-subunit of MDH has eight-fold radial symmetry, with eight β sheets stabilized by a novel tryptophan-docking motif. The PQQ in the active site is held in place by a coplanar tryptophan and by a novel disulfide ring formed between adjacent cysteines which are bonded by an unusual non-planar *trans* peptide bond. One of the carbonyl oxygens of PQQ is bonded to Ca^{2+}, probably facilitating attack on the substrate, and the other carbonyl oxygen is out of the plane of the ring, confirming the presence of the predicted free-radical semiquinone form of the prosthetic group. The tryptophan-docking and calcium sites are probably similar in alcohol dehydrogenase and glucose dehydrogenase, as the sequence similarity in these regions is strong.
15 February 1995, *Structure*

► **High-resolution crystal structure of the non-specific lipid-transfer protein from maize seedlings**
Dong Hae Shin, Jae Young Lee, Kwang Yeon Hwang, Kyeong Kyu Kim and Se Won Suh (1995). *Structure* **3**, 189–199.
Plant non-specific lipid-transfer proteins (ns-LTPs) aid in the movement of a broad range of lipids between membranes, but are also thought to be important in other biological functions, such as regulating lipid degradation in peroxisomes, in which they bind to non-phospholipid molecules that contain acyl groups. The authors describe the first three-dimensional structure of a plant ns-LTP, finding a tunnel-like hydrophobic cavity capable of accommodating an acyl chain of a long fatty acid. This arrangement is confirmed by the authors' determination of the structure of the complex between maize ns-LTP and palmitate. The study provides a structural basis for understanding the functions of ns-LTPs in the transfer and binding of acyl chains.
15 February 1995, *Structure*

► **The oestrogen receptor recognizes an imperfectly palindromic response element through an alternative side-chain conformation**
John W.R. Schwabe, Lynda Chapman and Daniela Rhodes (1995). *Structure* **3**, 201–213.
Many proteins recognize DNA targets that differ from the sequence thought to be their ideal target sequence. For example, consensus DNA targets for steroid hormone receptors are rare. The authors describe the structure of a complex between a dimer of the DNA-binding domain from the human oestrogen receptor and a non-consensus DNA target site in which there is a single base substitution in one half of the palindromic binding site. The protein adapts to the DNA site by rearranging a lysine side chain to make an alternative base contact. Rearrangement of side chains at the protein–DNA interface could be a general method by which proteins adapt their binding sites to recognize different DNA sequences. In this case, the rearrangement results in a significant loss of DNA-binding affinity.
15 February 1995, *Structure*

► **Structural basis for the specific interaction of lysine-containing proline-rich peptides with the N-terminal SH3 domain of c-Crk**
Xiaodong Wu, Beatrice Knudsen, Stephan M. Feller, Jie Zheng, Andrej Sali, David Cowburn, Hidesaburo Hanafusa and John Kuriyan (1995). *Structure* **3**, 215–226.
The general mechanisms by which proline-rich peptides bind to SH3 domains are known, but cannot explain the preferential binding of certain peptides by certain SH3 domains. The viral oncogene product Crk, the first of the family of 'adapter proteins' to be discovered, is capable of transforming cells by using its SH2 and SH3 domains to modulate tyrosine kinase pathways. The authors show how the specific binding of a peptide from the C3G guanine nucleotide exchange factor to the N-terminal SH3 domain of the cellular form of Crk (c-Crk) is mediated by an electrostatic interaction with a rare lysine residue in a position in the traditional polyproline helix more commonly held by an arginine. This interaction is thought to be responsible for the fact that the C3G peptide binds to c-Crk with one of the highest affinities reported for an SH3 domain (K_d = 1.9μM).
15 February 1995, *Structure*