Elsewhere in Biology

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

Chosen and summarized by the staff of *Chemistry & Biology*.

Chemistry & Biology April 1995, **2**:241–3

▶ Cartilage Disorders: **The importance of being sulphated**
Gillian A Wallis (1995). *Curr. Biol.* **5**, 225–227.

The disease diastrophic dysplasia, a recessively inherited condition whose manifestations include alterations in the shape and form of the developing skeleton, is characterized by short-limbed dwarfism and problems such as curvature of the spine. It is rare in world-wide terms, but common in Finland, where the carrier frequency can approach 1 in 30. In this minireview, Wallis discusses the mapping of the gene responsible for the disorder by fine-structure linkage disequilibrium mapping. The gene has also been cloned and sequenced, and by sequence comparison is predicted to encode a novel sulphate transporter containing 12 transmembrane helices, with highly charged cytoplasmic domains at both ends. Aberrant sulphate uptake was indeed seen in one patient. It seems likely that the decreased intracellular sulphate levels affect the function of proteoglycans, including aggrecan, which is one of the major structural components of cartilage, and heparan sulphate, which is involved in many aspects of cell adhesion, growth and morphogenesis.
1 March 1995, *Current Biology*

▶ Superantigens: **Gazing into the crystal ball**
Ping-Ning Hsu and Brigitte T Huber (1995). *Curr. Biol.* **5**, 235–237.

Superantigens are microbial antigens that, once bound to molecules of the major histocompatibility complex (MHC), interact with an unusually large proportion of T cells. T-cell recognition of superantigen apparently requires only a single variable domain of the T-cell receptor, unlike recognition of normal antigen. The toxicity of bacterial superantigens, some of which are involved in food poisoning and toxic shock syndrome, may be mediated by their potent T-cell stimulatory activity. In this minireview, Hsu and Huber discuss the recent determination of the crystal structures of two bacterial toxin superantigens complexed with MHC class II molecules and the resulting insights into the interactions between superantigens and MHC molecules. The new structural information reveals that the TSST-1 superantigen binds across the peptide-binding groove of the MHC molecule, contacting the bound peptide, but the SEB superantigen binds on one side of the MHC molecule, away from the peptide-binding groove. The finding that different toxins bind to very different parts of the MHC molecule is unexpected; presumably binding of the T-cell receptor to the two complexes is also very different.
1 March 1995, *Current Biology*

▶ Chromatin: **Ga-ga over GAGA factor**
H Granok, BA Leibovitch, CD Shaffer and SCR Elgin (1995). *Curr. Biol.* **5**, 238–241.

It is well established that chromatin structure has an important role in the process of eukaryotic gene expression. It has been difficult to study the factors that affect chromatin structure *in vivo*, since organisms that are mutant for genes encoding critical chromatin proteins are hard to identify and maintain. In this minireview, Granok *et al.* discuss the recent discovery that the chromatin factor GAGA, originally identified as a transcriptional activator, is encoded by the *Trithorax-like* (*Trl*) gene in *Drosophila*. A large number of genes now appear to be targets of GAGA factor. *Trl* mutations enhance 'position effect variegation' *in vivo*; in this phenomenon, a specific pattern of gene inactivation is seen when inactive and active chromosomal domains are juxtaposed by chromosomal rearrangement. This pattern is clonally inherited. Together with the finding that GAGA factor causes local disruption of nucleosome assembly over a GAGA-factor binding site *in vitro*, this observation suggests that GAGA factor may help to establish and/or maintain nucleosome-free regions containing important gene regulatory sequences, making them accessible to RNA polymerase, for example.
1 March 1995, *Current Biology*

▶ RNA–Protein Interactions: **Diverse modes of recognition**
Dino Moras and Arnaud Poterszman (1995). *Curr. Biol.* **5**, 249–251.

When you think of regulation of gene expression, you often think first of transcriptional control. But after the primary RNA transcript is produced, there several steps that can be modulated before the final protein product is made, for example the splicing and transport of RNA. These processes involve numerous RNA-binding proteins, many of which carry an 'RNP motif' which is responsible for RNA binding. As discussed in this minireview, the recently determined crystal structure of the U1A small nuclear ribonucleoprotein (snRNP) in complex with its target sequence AUUGCAC has provided a considerable amount of information on the way that RNA is recognized by this protein. Together with what is already known about RNA recognition in aminoacyl tRNA synthetases, this structure may shed light on some common features of RNA–protein interactions.
1 March 1995, *Current Biology*

▶ **Structural characterization of viral fusion proteins**
Frederick M Hughson (1995). *Curr. Biol.* **5**, 265–274.

Eukaryotic cells need membrane fusion to allow the transport and import of proteins; gametes need to fuse for fertilization to occur; and viruses need fusion to allow them to enter cells. The process of membrane fusion is poorly understood. Influenza virus hemagglutinin is the best characterized protein with membrane fusion activity. In this full-length review, Hughson discusses recent structural results that provide insight into the molecular events of membrane fusion. The hemagglutinin protein undergoes enormous conformational changes upon exposure to the low pH of the intracellular compartment into which the membrane-bound virus is localized following endocytosis. These irreversible changes appear to propel the amino-terminal hydrophobic 'fusion peptide' into position to interact with the target membrane. Further changes modify the portion of the molecule that is close to the viral membrane, perhaps making it more flexible. This hypothetical flexibility has important implications for the possible mechanisms by which hemagglutinin might facilitate fusion.
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▶ **A small peptide inhibitor of DNA replication defines the site of interaction between the cyclin-dependent kinase inhibitor $p21^{WAF1}$ and proliferating cell nuclear antigen**
Emma Warbrick, David P Lane, David M Glover and Lynne S Cox (1995). *Curr. Biol.* **5**, 275–282.

$p21^{WAF1}$ is a potent inhibitor of the cell-cycle regulatory cyclin dependent kinases (Cdks). It binds to proliferating cell nuclear antigen (PCNA), a processivity factor for DNA polymerase δ which is essential in DNA replication and repair, and is induced by the tumor suppressor protein p53. $p21^{WAF1}$ may thus be important in coordinating cell-cycle progression with DNA replication and repair. Using the yeast two-hybrid screening method, the authors demonstrate a strong interaction between the central loop of PCNA and the carboxy-terminal region of $p21^{WAF1}$. A 20 amino acid peptide derived from this region binds PCNA strongly and specifically and can inhibit SV40 DNA replication *in vitro*, demonstrating that inhibition of SV40 DNA replication by $p21^{WAF1}$ is a function of PCNA binding. These results suggest that drugs based on the $p21^{WAF1}$-derived peptide may be therapeutically useful; they may be able to down-regulate the activity of PCNA in tumor cells, inhibiting their proliferation.
1 March 1995, *Current Biology*

▶ **Evidence that SH2 domains promote processive phosphorylation by protein-tyrosine kinases**
Bruce J Mayer, Hisamaru Hirai and Ryuichi Sakai (1995). *Curr. Biol.* **5**, 296–305.

The presence of SH2 domains, which bind to tyrosine-phosphorylated peptides with high affinity, in non-receptor protein-tyrosine kinases raises an interesting question: why would a kinase need a domain that cannot bind to the unphosphorylated substrate? One such kinase, Abl, requires its SH2 domain to malignantly transform cells. Exchanging the SH2 domain for an SH2 domain from another protein also alters the spectrum of proteins phosphorylated by Abl *in vivo*. Kinase substrates often contain several phosphorylation sites with similar sequences. One such substrate for Abl is $p130^{CAS}$. Here, the authors show that the SH2 domain is required for Abl to efficiently hyperphosphorylate $p130^{CAS}$ *in vitro*. The most effective SH2 domain tested is that from the adaptor molecule, Crk, which is also the SH2 domain that is predicted to bind most strongly to phosphorylated sites in $p130^{CAS}$. They therefore propose that the SH2 domain binds tightly to substrates that are phosphorylated at one site, facilitating the processive phosphorylation at additional sites. Substrate specificity can be broadened by an association between the kinase and proteins, such as Crk, that contain additional SH2 domains; this may be important in malignant transformation by Crk.
1 March 1995, *Current Biology*

▶ **Thioredoxin structure and mechanism: conformational changes on oxidation of the active-site sulfhydryls to a disulfide**
Arne Holmgren (1995). *Structure* **3**, 239–243.

Thioredoxin is present in all proteins from archaebacteria to man and has a large number of functions. In addition to its role in DNA synthesis as a hydrogen donor for the essential enzyme ribonucleotide reductase, thioredoxin provides general catalysis of thiol-disulfide exchange between a dithiol on one protein and a disulfide acceptor on another protein. The recent determination of high resolution solution structures of both the reduced and oxidized forms of human and *Escherichia coli* thioredoxins, as reviewed by Holmgren, has provided new opportunities for understanding the catalytic activity of thioredoxin as well as the increasingly important role of thiol-redox regulation of protein activity in different biological systems (see also the paper by Qin *et al.*, below). The results support a catalytic model of protein disulfide reduction involving nucleophilic attack by the active-site Cys32 thiolate to form a transition-state mixed disulfide. Thioredoxins are also of wide interest due to the number of proteins with a thioredoxin fold (also reviewed in this issue by J.L. Martin, *Structure* 245–250). It appears from these structures that thioredoxin uses a chaperone-like mechanism of conformational changes to bind a diverse group of proteins, and uses fast thiol-disulfide exchange chemistry in a hydrophobic environment to promote high rates of disulfide reduction.
15 March 1995, *Structure*

▶ **Hyperthermophiles: taking the heat and loving it**
Douglas C Rees and Michael WW Adams (1995). *Structure* **3**, 251–254.

This Ways & Means article focuses on the proteins from hyperthermophiles, a recently discovered group of microorganisms capable of growing in high temperature environments that in some cases exceed 100 °C. The highly thermostable enzymes isolated from these organisms have a number of technical applications including the polymerase chain reaction (made possible by hyperthermostable DNA polymerases), biocatalysis, and crystallization methods. Although the high resolution structures of only two hyperthermostable proteins have been determined so far, they provide some clues as to how such stability is achieved. Rather than relying on any one dominant type of interaction, these proteins appear to employ a number of subtle interactions involving surface energies, electrostatic interactions, increased stabilization of secondary structure, and packing effects.
15 March 1995, *Structure*

▶ **Crystal structure of the MS2 coat protein dimer: implications for RNA binding and virus assembly**
Chao-Zhou Ni, Rashid Syed, Ramadurgam Kodandapani, John Wickersham, David S Peabody and Kathryn R Ely (1995). *Structure* **3**, 255–263.

The coat protein from the MS2 bacteriophage self-aggregates to form an icosahedral shell which binds and encapsidates the single-stranded RNA genome. The protein also represses viral replicase synthesis by binding as a dimer to an RNA hairpin containing the translation-initiation region of the replicase gene. In addition to its genetic role, this binding event is an important first step in nucleation of virus assembly. The authors report the high resolution crystal structure of the unassembled dimer; to obtain this structure, a mutant MS2 coat protein that cannot assemble into a viral capsid was used. This is the first time that high resolution structures have been available for a viral protomer in both the isolated and assembled states. Comparison with the structure of the intact virus shows differences in the orientation of two of the RNA-recognition residues, suggesting a conformational adjustment on binding RNA during the first step in the assembly process. At the end of the β-strand that contains these two residues, a Trp→Arg substitution influences capsid assembly, apparently by imposing conformational restriction on an adjacent loop, preventing critical intersubunit contacts in the capsid.
15 March 1995, *Structure*

▶ **Structure of uncomplexed and linoleate-bound *Candida cylindracea* cholesterol esterase**
Debashis Ghosh, Zdzislaw Wawrzak, Vladimir Z Pletnev, Naiyin Li, Rudolf Kaiser, Walter Pangborn, Hans Jörnvall, Mary Erman and William L Duax (1995). *Structure* **3**, 279–288.

Cholesterol esterase reversibly hydrolyzes cholesteryl linoleate and oleate, esters of fatty acids that are the major components of arterial plaque. The enzyme has a hydrophobic active-site cavity,

and a flap that could cover the active site but is open in the structure as determined. The structure of the complex with cholesteryl linoleate reveals how the lipid and protein interact, and indicates that the carboxyl terminus of the protein may serve as a 'gate' for the release of product. The interactions of the substrate with the catalytic triad of the enzyme can also be seen. The protein crystallizes as a dimer, which may be a functionally active form of the enzyme. Comparison of the structure of cholesterol esterase to that of a triacylglycerol acyl hydrolase from *Candida rugosa* shows that 23 of the 55 residues that are different in the two proteins are located in the active site and dimer interface. These substitutions are responsible for the altered substrate specificity.
15 March 1995, *Structure*

► **Solution structure of human thioredoxin in a mixed disulfide intermediate complex with its target peptide from the transcription factor NFκB**
Jun Qin, G Marius Clore, WM Poindexter Kennedy, Jeffrey R Huth and Angela M Gronenborn (1995). *Structure* **3**, 289–297.
Human thioredoxin (h(TRX)) has a key role in maintaining the redox environment of the cell. It has recently been shown that h(TRX) is responsible for activating the DNA-binding properties of the cellular transcription factor, NFκB, by reducing a disulfide bond involving the Cys62 residue of the p50 subunit. A growing number of transcription factors are thought to be regulated in this way. To understand how h(TRX) recognizes the loop containing Cys62, the authors have determined the solution structure of h(TRX) covalently linked to a 13-residue peptide corresponding to residues 56–68 of p50, a complex that represents a kinetically stable mixed disulfide intermediate along the reaction pathway. In addition to the disulfide bond between Cys32 of h(TRX) and Cys62 of p50, the peptide is stabilized in a boot-shaped cleft of h(TRX) by hydrogen bonding and by electrostatic and van der Waals interactions. The structure allows predictions to be made about the specific requirements for h(TRX)-catalyzed disulfide bond reduction of proteins.
15 March 1995, *Structure*

► **Activity of the MAP kinase ERK2 is controlled by a flexible surface loop**
Jiandong Zhang, Faming Zhang, Douglas Ebert, Melanie H Cobb and Elizabeth J Goldsmith (1995). *Structure* **3**, 299–307.
The mitogen-activated protein (MAP) kinase ERK2, a tightly regulated enzyme in the Ras-activated protein kinase cascade, is activated by phosphorylation at two sites, Tyr185 and Thr183, that lie in the lip at the mouth of the catalytic site. To understand the roles of these sites in the activation of the kinase, the authors have determined the structures of four unphosphorylated ERK2 mutants that have substitutions at one or both of these sites. Disorder is observed throughout the lip when Tyr185 is mutated, indicating that the stability of the lip is low. Therefore, only modest amounts of binding energy should be required to dislodge the lip for phosphorylation, and the lip may undergo a conformational change in the active structure. The structure of the inactive form is dependent on Tyr185, but the structure of the active form requires both Tyr185 and Thr183, contributing to the tight control on the enzyme. The close proximity of the phosphorylation lip to the active site may also facilitate the tight regulation observed in MAP kinases.
15 March 1995, *Structure*