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 May 1995, **2**:335–337

▶ Protein Folding: **An unfolding story**
TE Creighton (1995). *Curr. Biol.* **5**, 353–356.

Most studies of protein folding have been hampered by the complexity and cooperativity of the process, as well as the complications introduced by slow *cis–trans* isomerization of proline residues. Despite this, work on the proteins barnase and chymotrypsin inhibitor 2 by Alan Fersht and his colleagues has made significant progress in the elucidation of a mechanism of folding that may well be general to most small globular proteins. As reviewed here, these studies show that the cooperativity of multiple interactions responsible for the stability of the native state is generated throughout the folded structure in the transition state for folding. Within the unfolded state, individual interactions are rapidly formed and rapidly disrupted. Rare species that have multiple interactions will be stabilized only if these interactions can form cooperative structures; accretion of structures that stabilize each other eventually leads to a state in which completion of folding is energetically favored, and can occur rapidly.
1 April 1995, Dispatch, *Current Biology*

▶ Chromosome Structure: **Coiling up chromosomes**
Susan M Gasser (1995). *Curr. Biol.* **5**, 357–360.

The mechanism by which eukaryotic chromosomes condense as cells enter mitosis has long been inaccessible to molecular biologists. As reviewed here, an important clue has now been provided by the identification of a ubiquitous non-histone protein family, the SMCs. When the genes encoding these proteins are mutated in yeast, chromosomes do not condense. The SMC family members have strong structural similarity; they consist of an amino-terminal nucleotide-binding globular head domain, followed by two extended α-helical stretches that are likely to form a coiled-coil structure in the (putative) dimer. The carboxy-terminal domain shows homology to the 'Walker B' motif, which correlates with NTP hydrolysis. This structure is reminiscent of mechanochemical motor proteins, suggesting that these proteins may 'reel in' or coil up DNA before mitosis.
1 April 1995, Dispatch, *Current Biology*

▶ SH3 Domains: **Minding your p's and q's**
Bruce J Mayer and Michael J Eck (1995). *Curr. Biol.* **5**, 364–367.

SH3 domains are protein–protein interaction domains that are found in a wide variety of proteins ranging from cytoskeletal components to signal transduction enzymes, and which are thought to bind to short proline-rich peptides in their target proteins. The three-dimensional structures of no less than ten different SH3 domains have been solved by X-ray crystallography or NMR spectroscopy, with and without a variety of specific ligands. This wealth of structural data provides a solid foundation for building general models of SH3 domain–ligand interactions. Beyond binding specificity, the most pressing questions about SH3 domains concern the consequences of binding and how binding is regulated. Binding of SH3 domains to the target protein frequently changes the activity of the target protein. SH3 domains may also regulate the activity of the protein they are part of, and intramolecular interactions may be important in regulating the activity of the SH3 domains themselves.
1 April 1995, Dispatch, *Current Biology*

▶ HIV Integration: **Ini1 for integration?**
Michael D Miller and Frederic D Bushman (1995). *Curr. Biol.* **5**, 368–370.

The HIV-1 integrase has just been found to bind the newly-discovered cellular protein, integrase-interacting protein 1 (Ini1). Binding to Ini1 appears to enhance integration efficiency, and is probably involved in the mechanism of integrase action, perhaps forming a 'bridge' between the integration machinery and host DNA. Ini1 has a high degree of homology to the yeast transcriptional activator Snf5, part of the Swi/Snf complex that was recently reported to alter the structure of nucleosomal DNA *in vitro*. It is therefore also possible that some similar activity of Ini1, resulting in changes in nucleosomal structure and DNA topology, is involved in promoting integration. These findings, together with recent advances towards directing HIV-1 integration to specific DNA sequences using the DNA-binding domain of the bacteriophage λ repressor, may have consequences for gene therapy. It may now be possible to influence integration-site selection during retroviral infection for the safer delivery of DNA sequences, or for the insertional inactivation of a harmful gene.
1 April 1995, Dispatch, *Current Biology*

▶ Ion-Channel Gating: **Twist to open**
Mark SP Sansom (1995). *Curr. Biol.* **5**, 373–375.

The nicotinic acetylcholine receptor (nAChR), responsible for synaptic transmission at vertebrate neuromuscular junctions, is one of the best characterized ion channel proteins. The 9 Å structure of the closed conformation of the nAChR, determined two years ago, has recently been complemented by a structure for the open conformation of the channel obtained using cryo-electron microscopy. The comparison of these two structures provides us with our first real picture of a channel gating mechanism, and reveals that acetylcholine binding leads to channel opening via a series of subunit rotations propagated from the extracellular acetylcholine binding sites to the membrane-spanning zone. This structural-gating model permits a more detailed molecular interpretation of mutational data. In particular, the key role of a transmembrane kink region, which is shown to act as a 'molecular swivel' in the closed-to-open transition, has refocused attention on the leucine residue which sits at the apex of the kink and partially occludes the channel pore in the closed conformation.
1 April 1995, Dispatch, *Current Biology*

▶ **A conserved amino-terminal Shc domain binds to phosphotyrosine motifs in activated receptors and phosphopeptides**
Peter van der Geer, Sandra Wiley, Venus Ka-Man Lai, Jean Paul Olivier, Gerald D Gish, Robert Stephens, David Kaplan, Steven Shoelson and Tony Pawson (1995). *Curr. Biol.* **5**, 404–412.

Signal transduction by growth factor receptor protein-tyrosine kinases is generally initiated by autophosphorylation on tyrosine residues following ligand binding. Phosphotyrosines within activated receptors form binding sites for the Src homology 2 (SH2) domains of cytoplasmic signaling proteins. One such protein, Shc, is tyrosine-phosphorylated in response to a large number of growth factors and cytokines, becoming a target for the Grb2

adaptor protein, thus coupling normal and oncogenic protein-tyrosine kinases to Ras activation. Shc contains an SH2 domain at its carboxyl terminus, but the function of the amino-terminal half of the protein is unknown. The authors report that the Shc amino-terminal region binds to a number of tyrosine-phosphorylated proteins in v-*src*-transformed cells. The domain also binds directly to the activated epidermal growth factor (EGF) receptor and to the autophosphorylated nerve growth factor receptor (Trk). Binding appears to be specific for phosphorylated tyrosine residues within the sequence NPXpY, which is conserved in many Shc-binding sites. The Shc amino-terminal region has little sequence similarity to known SH2 domains, suggesting that it represents a new class of phosphotyrosine-binding modules. Shc may thus bind to tyrosine-phosphorylated proteins at both of its ends, acting as an 'adaptor' to link the proteins together.
1 April 1995, Research Paper, *Current Biology*

► **Regulation of pre-mRNA processing by *src***
Henry Neel, Pierre Gondran, Dominique Weil and François Dautry (1995). *Curr. Biol.* **5**, 413–422.
Little is known of the contribution of pre-mRNA nuclear processing to the regulation of gene expression. The authors report that, in co-transfection experiments, an activated *src* gene can modify the splicing rates of TNFβ and β globin transcripts, whereas an activated *ras* gene cannot. The *src* gene appears to have at least two distinct effects on pre-mRNA processing; it both slows down splicing and allows the export of partially spliced transcripts. This implies that multiple levels of regulation exist. These observations establish that specific signal transduction pathways have the capacity to regulate gene expression at a post-transcriptional level within the nucleus. This regulation could reflect a modulation by Src of the activity of components of the splicing and transport machineries, but could also involve RNA-binding proteins, which have been shown to interact with Src.
1 April 1995, Research Paper, *Current Biology*

► **Elevated levels of wild-type p53 induced by radiolabeling of cells leads to apoptosis or sustained growth arrest**
Jo Yeargin and Martin Haas (1995). *Curr. Biol.* **5**, 423–431.
The tumor suppressor protein p53 regulates progression through the checkpoint between G1 and S phases of the cell cycle in response to radiation- or drug-induced DNA damage. The authors report that wild-type p53 protein concentrations rapidly increased to high levels after metabolic radiolabeling of cells with [^{35}S]methionine or [^{3}H]thymidine. The increase was dependent on dose and time, and resulted in apoptosis of normal human peripheral blood lymphocytes and of murine T-cell acute lymphoblastic leukemia cells. Radiolabeling of human diploid fibroblasts resulted in prolonged growth arrest. These changes did not occur in radiolabeled cells containing no p53 or only mutant *p53* alleles. The results of experiments employing these radiolabeling techniques may be seriously influenced by the induction of aberrant cell-cycle arrest and/or apoptosis mediated by wild-type p53. The results suggest that p53's function as a checkpoint to allow DNA to be repaired may be secondary to its function in causing arrest or death of damaged cells.
1 April 1995, Research Paper, *Current Biology*

► **Regulation of *Caenorhabditis elegans* degenerin proteins by a putative extracellular domain**
Jaime García-Añoveros, Charles Ma and Martin Chalfie (1995). *Curr. Biol.* **5**, 441–448.
Dominant, gain-of-function mutants of members of the degenerin gene family of *Caenorhabditis elegans* (*deg-1*, *mec-4* and *mec-10*) cause neurons to become vacuolated, swell, and die. The degenerins are homologous to ion channels; *mec-4* and *mec-10* are needed for the reception of gentle touch stimuli, and may therefore contribute to a mechanosensory ion channel. All of the dominant degeneration-causing mutations in degenerin genes affect equivalent residues in a hydrophobic region structurally similar to the H5 domain of several ion channels, which could form the channel lining. The authors show that a missense change in a predicted extracellular region of the proteins encoded by *deg-1* and *mec-4* degenerin mutants causes cell death. Mutations in either the predicted pore-lining or the predicted extracellular regions of *deg-1* are suppressed by additional, dominantly acting mutations that substitute larger for smaller residues in the channel lining which may block the pore of a multisubunit channel. These findings suggest that the putative extracellular domain negatively regulates degenerin activity, perhaps by gating the channel. This region is not found in the homologous mammalian genes, and may be required for more rapid regulation of the nematode channels to allow mechanosensation.
1 April 1995, Research Paper, *Current Biology*

► **The cell cycle and suc1: from structure to function?**
JA Endicott and P Nurse (1995). *Structure* **3**, 321–325.
The product of the yeast *Schizosaccharomyces pombe* CKS/suc1 gene, p13^{suc1}, is essential for the function of the cell cycle regulatory protein kinase p34^{cdc2} *in vivo*. But nearly a decade after their original characterization, p13^{suc1} and its relatives, the CKS proteins (for CDC28 *k*inase *s*ubunit) still have no known catalytic activity. This minireview focuses on the recently determined structures of p13^{suc1}, and its human equivalent, p9^{CKS2}. The structures provide some long-awaited clues about the role of CKS/suc1 in cell cycle control. Both structures reveal a potential phosphotyrosine-binding site and a second 'hydrophobic and aromatic patch' site. An association of p13^{suc1} with tyrosine-phosphorylated p34^{cdc2} may block access of the p80^{cdc25}-encoded tyrosine phosphatase, thus preventing dephosphorylation and activation of the protein kinase. An additional binding interaction known to occur with active p34^{cdc2} kinase may be mediated by the hydrophobic and aromatic patch.
15 April 1995, Minireview, *Structure*

► **Problems in simulating macromolecular movements**
Donald LD Caspar (1995). *Structure* **3**, 327–329.
Although it may be appealing to think of protein molecules as little pieces of biological machinery that function in a mechanistically predictable way, Caspar suggests that recent structural studies show that protein molecules may behave more like organisms, carrying out their specific functions with some stochastic individuality. At issue are the idiosyncratic behavior of a protein and the problems encountered in tracking its movements. How are the atomic movements in protein molecules correlated with each other and how well does a molecular dynamics simulation sample these correlations? Protein molecules have sharply defined average structures only in well ordered lattices. But even in a crystal, the accuracy with which the average structure can be determined appears less certain than generally assumed by protein cyrstallographers. Thus, insufficient sampling of conformational sub-states by current molecular dynamics simulations, due to the constraints imposed by computational resources, produces deficient representations of the fluctuating separations between pairs of atoms in macromolecules.
15 April 1995, Minireview, *Structure*

► **The structural basis for seryl-adenylate and Ap_4A synthesis by seryl-tRNA synthetase**
Hassan Belrhali, Anya Yaremchuk, Michael Tukalo, Carmen Berthet-Colominas, Bjarne Rasmussen, Peter Bösecke, Olivier Diat and Stephen Cusack (1995). *Structure* **3**, 341–352.
Seryl-tRNA synthetase (SerRS) is a homodimeric class II aminoacyl-tRNA synthetase that specifically charges cognate tRNAs

with serine. In the first step of this two-step reaction, Mg•ATP and serine react to form the activated intermediate, seryl-adenylate. The serine is subsequently transferred to the tRNA. In common with most other synthetases, SerRS can also synthesize the dinucleotide diadenosine tetraphosphate (Ap_4A) by attack of a second ATP molecule on the enzyme-bound adenylate. Ap_4A has been implicated in a number of metabolic processes and stress-related phenomena. The authors describe three new crystallographic structures of SerRS at 2.3–2.6 Å resolution complexed with ATP, seryl-adenylate and Ap_4A, each in the presence of Mn^{2+}. Superposition of these structures allows the authors to propose a common reaction mechanism in which both Ap_4A and seryl-adenylate are formed by in-line displacement reactions involving a pentavalent transition state at the α-phosphate position of the adenylate, but with the displacement going in opposite directions. The divalent cation is essential for both reactions and may be directly involved in stabilizing the transition state.
15 April 1995, Research Article, *Structure*

▶ **Structure of HIV-1 reverse transcriptase in a complex with the non-nucleoside inhibitor α-APA R 95845 at 2.8 Å resolution**

J Ding, K Das, C Tantillo, W Zhang, AD Clark, Jr, S Jessen, X Lu, Y Hsiou, A Jacobo-Molina, K Andries, R Pauwels, H Moereels, L Koymans, PAJ Janssen, RH Smith, Jr, M Kroeger Koepke, CJ Michejda, SH Hughes and E Arnold (1995). *Structure* **3**, 365–379.

HIV-1 reverse transcriptase (RT) is a multifunctional enzyme that copies the RNA genome of HIV-1 into DNA. It is a crucial target for structure-based drug design, and potent inhibitors have been identified, whose efficacy, however, is limited by drug resistance. The authors report the 2.8 Å resolution crystal structure of HIV-1 RT in complex with a non-nucleoside inhibitor, α-anilinophenylacetamide (α-APA) R95845. The inhibitor binds in a hydrophobic pocket lined by a number of aromatic side chains that are likely to stabilize the binding of a non-nucleoside inhibitor, explaining the observation that non-nucleoside inhibitors invariably contain at least one aromatic ring. The mutations that cause high-level resistance to non-nucleoside inhibitors are located close to the bound inhibitor, and presumably affect the stability of inhibitor binding. Comparison of the structure with that of HIV-1 RT bound to a double-stranded DNA template-primer and the Fab of a monoclonal antibody shows significant changes in the local environment of the inhibitor-binding pocket which may explain how α-APA inhibits HIV-1 RT.
15 April 1995, Research Article, *Structure*

▶ **Comparative analysis of the X-ray structures of HIV-1 and HIV-2 proteases in complex with CGP 53820, a novel pseudosymmetric inhibitor**

JP Priestle, A Fässler, J Rösel, M Tintelnot-Blomley, P Strop and MG Grütter (1995). *Structure* **3**, 381–389.

The less pathogenic subtype of the human immunodeficiency virus (HIV), HIV-2, has recently been shown to be responsible for the accelerated spread of AIDS in India and East Asia. The protease enzymes from HIV-1 and HIV-2 are aspartic acid proteases which share about 50 % sequence identity and are essential for maturation of the infectious particle. The authors report the X-ray crystal structures of both proteases, each in complex with the pseudosymmetric inhibitor, CGP 53820, to 2.2 Å and 2.3 Å resolution, respectively. They find that minor sequence changes in subsites at the active site can explain some of the observed differences in substrate and inhibitor binding between the two enzymes. As some of the point mutations that confer drug resistance on HIV-1 protease change the HIV-1 sequence to that of HIV-2 protease, this structural analysis may help in the design of inhibitors that do not easily trigger the development of drug-resistant mutants.
15 April 1995, Research Article, *Structure*