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 June 1995, **2**:419–421

▶ Centromeric Chromatin: **Histone deviants**
Alan P Wolffe (1995). *Curr. Biol.* **5**, 452–454.

The packaging of DNA into nucleosomes by core histones has long been thought of as an invariant feature of the eukaryotic chromosome. This view has been challenged, however, by the recent discovery of highly specialized histone proteins that localize uniquely to the centromere — the region of a chromosome at which the kinetochore assembles to mediate sister-chromatid segregation at mitosis. The specialized histones assemble into specific nucleosomal structures that are essential for centromeric functions. Another dynamic feature of histones was revealed by genetic experiments in which the sequences of the amino-terminal tails of core histones were altered indicating that histone tails can regulate the transcriptional activation or repression of specific genes, perhaps by influencing transcription-factor access to nucleosomal DNA. Thus, the nucleosome is not as inert as previously thought. Studies on the assembly of centromeric chromatin have now shown that two histone variants, one from primate (CENP-A) and one from yeast (CSE-4), can substitute for histone H3 in nucleosome cores, and may selectively recognize centromeric DNA. The specialized nucleosomes containing these histone variants might then act as the structural foundation for formation of a higher-order complex.
1 May 1995, Dispatch, *Current Biology*

▶ Molecular Chaperones: **Resurrection or destruction?**
Arthur L Horwich (1995). *Curr. Biol.* **5**, 455–458.

The chaperones of the heat-shock protein (Hsp) 70 and Hsp60 families have been well studied, and are generally understood to prevent protein aggregation. Recent work on the Hsp100 family of chaperones has now excitingly expanded our view of what chaperones can do. Hsp104, from *Saccharomyces cerevisiae*, has been shown to resolubilize protein aggregates, returning the proteins to their native state. But the proteins of the related Clp family in *Escherichia coli*, ClpA, ClpB and ClpX, so far seem to have more to do with directing aggregated proteins toward proteolysis than with rescuing them. These proteins act as regulatory elements for the ATP-dependent ClpP protease. So do these very similar families of proteins have completely different functions? Recent results have now made more sense of the role of ClpA; when ClpP is absent, ClpA can reactivate dimers of the protein RepA, releasing it in a functional monomeric form. Although this ATP-dependent dissociation of a dimer to a monomer is not the same as the disaggregation caused by Hsp104, it still qualifies ClpA as a chaperone. ClpA and ClpX may well act to unfold target proteins, increasing the rate of proteolysis when the ClpP protease is present, but increasing the likelihood of re-folding when it is not. ClpB has not yet been directly shown to be involved in proteolysis, however, and its role may thus be closer to that of Hsp104.
1 May 1995, Dispatch, *Current Biology*

▶ Haemoglobin Engineering: **For fun and money**
M Brunori, F Cutruzzolà and B Vallone (1995). *Curr. Biol.* **5**, 462–465.

Haemoglobin has proven to be an ideal molecular guinea pig for the development of new biophysical techniques, such as protein crystallography, and the testing of new ideas about the structural basis of biological regulation. Molecular oxygen binds cooperatively to the haemoglobin tetramer, with an affinity that can be modulated by a number of small effector molecules, such as H^+, diphosphoglyceride, CO_2 and Cl^-. One extreme example of this kind of regulation, which enables the efficient delivery of O_2 to sites in the body where it is needed, can be found in the crocodile. In order to hold their breath long enough to drown their prey, crocodiles make use of a unique allosteric effect which drastically lowers their haemoglobin's affinity for O_2 upon binding bicarbonate, the anion resulting from hydration of the CO_2 that accumulates as the final product of respiration. The recent transplantation of this effect from crocodile to human haemoglobin has implications for both molecular evolution and the engineering of artificial blood substitutes. The finding that at most twelve residues are responsible for the bicarbonate effect strongly argues that adaptive changes can be brought about by few substitutions of key residues, rather than by the gradual accumulation of mutations that each produce a small shift in chemical affinity.
1 May 1995, Dispatch, *Current Biology*

▶ AIDS: **Viruses, cytokines and Kaposi's sarcoma**
Don Ganem (1995). *Curr. Biol.* **5**, 469–471.

Kaposi's sarcoma (KS) is one of the great enigmas of human oncology. A complex proliferative lesion, the disease has surfaced as a major complication of acquired immunodeficiency syndrome (AIDS); HIV-positive patients are at 20 000-fold increased risk of developing KS compared to the general population. It has previously been suggested that virus-activated growth factors may be involved in the initiation of KS. This notion rationalizes many of the known facts, but does not wholly explain the epidemiology of the disease. New evidence now suggests that a novel virus may be important in the initiation of KS lesions. PCR products from a KS sample reveal open reading frames whose predicted products have homology to known proteins of two lymphotropic herpesviruses, herpesvirus saimiri and the human Epstein–Barr virus. Sequences corresponding to these clones have been identified by PCR in 25 out of 27 cases of HIV-related KS. Although it remains possible that the agent in question is simply a marker of KS risk, preliminary studies have identified similar viral sequences in KS specimens from several different HIV-negative cohorts as well, greatly strengthening the association of the agent with KS and supporting the view that all clinical forms of the disease may be related.
1 May 1995, Dispatch, *Current Biology*

▶ Microtubule Dynamics: **Kinetochores get a grip**
AA Hyman (1995). *Curr. Biol.* **5**, 483–484.

During mitosis, specialized chromosomal structures, the kinetochores, bind to the microtubules of the mitotic spindle — an interaction that is required for chromosome segregation. It has been known for some time that kinetochores bind to dynamic microtubules *in vitro*, and can move along them using plus-end directed ATP-dependent motors. If the microtubule is shrinking, the kinetochores maintain attachment and follow the shrinking end, without using ATP. The molecular nature of the interaction between kinetochores and shrinking microtubules has now been probed in some detail. Kinesin, a plus-end directed motor, is sufficient to allow latex beads to follow the end of a shrinking microtubule. Furthermore,

 ISSN 1074-5521

antibodies to kinesin (especially the kinetochore-associated Cenp-E protein, a member of the kinesin superfamily) inhibit the ability of chromosomes to remain attached to shinking microtubules. Thus, the kinetochore uses motors to move toward the plus end as microtubules grow, and towards the minus end as they shrink, so that the kinetochore stays at the end of the microtubule. The question of how microtubule dynamics are regulated to generate chromosome movement thus lies at the heart of the mechanism of mitosis.
1 May 1995, Dispatch, *Current Biology*

▶ Structure Prediction: **How good are we?**
RB Russell and MJE Sternberg (1995). *Curr. Biol.* **5**, 488–490.
In certain circumstances, the secondary structures of proteins can be predicted with high accuracy. The availability of large numbers of homologous sequences for pleckstrin homology (PH) domains has allowed multiple sequence alignments to be carried out, so that the sequence variation within alignments could be assessed. Two labs have used this information to predict the number, type and location of core secondary structure elements in PH domains. Several recently determined PH domain structures show that these predictions were remarkably accurate, with residue-by-residue accuracies of 75–94 %. Similar levels of accuracy have been seen in secondary structure predictions for many, but not all, of the protein families recently examined. The fact that multiple sequence alignment often allows accurate predictions of secondary structure has implications for our ability to predict tertiary structure. Currently, tertiary structure prediction methods attempt to assess whether the protein might form a known fold. Combining fold recognition with alignment-based secondary structure prediction may greatly reduce the number of possible folds to be examined. General strategies for accurate and credible predictions of protein folds may be just around the corner.
1 May 1995, Dispatch, *Current Biology*

▶ **A role for activator-mediated TFIIB recruitment in diverse aspects of transcriptional regulation**
Stefan GE Roberts, Bob Choy, Scott S Walker, Young-Sun Lin and Michael R Green (1995). *Curr. Biol.* **5**, 508–516.
Transcription by RNA polymerase II in eukaryotic cells requires the ordered assembly of general transcription factors on the promoter to form a preinitiation complex. Transcriptional activator proteins (activators) stimulate transcription by increasing the rate and/or extent of preinitiation complex assembly. The authors previously showed that acidic activators increase the stable association of the transcription factor TFIIB with the promoter. Here they show that diverse classes of activators recruit TFIIB to the promoter by a similar mechanism, and relate this common function of activators to transcriptional regulation. The decrease in transcriptional activity that results when the distance between the bound activator and the promoter is increased correlates with a decrease in TFIIB recruitment. They further find that, after transcription has been initiated, TFIIB dissociates from the promoter, so that activators are required to reassemble the preinitiation complex for each new round of transcription. Thus, activator-mediated recruitment of TFIIB appears to be important in the regulation of transcription.
1 May 1995, Research Paper, *Current Biology*

▶ **Density-dependent regulation of cell growth by contactinhibin and the contactinhibin receptor**
G. Gradl, D Faust, F Oesch and RJ Wieser (1995). *Curr. Biol.* **5**, 526–535.
The number of cells within mammalian tissues is maintained by growth-stimulating and growth-inhibiting mechanisms. This is reflected, in the culture of normal adherent cells, by the phenomenon of density-dependent inhibition of growth; cells cease proliferation after becoming a confluent monolayer. A plasma membrane glycoprotein, contactinhibin, has been shown to be a major effector of this negative growth regulation. The authors show that a 92kD plasma membrane protein binds specifically to the N-linked glycans of contactinhibin, and mediates the contact-dependent inhibition of growth of cultured human fibroblasts. The binding of this contactinhibin receptor (CiR) to contactinhibin is dependent on the presence of terminal β-linked glactose residues on contactinhibin and is modulated by phosphorylation. CiR purified from SV40 transformed fibroblasts has drastically reduced ability to bind contactinhibin. Impaired CiR function may result in loss of growth control, which may eventually lead to tumorigenesis.
1 May 1995, Research Paper, *Current Biology*

▶ **Coincidence detection at the level of phospholipase C activation mediated by the m4 muscarinic acetylcholine receptor**
Reed C Carroll, Anthony D Morielli and Ernest G Peralta (1995). *Curr. Biol.* **5**, 536–544.
One of the principal mechanisms by which G-protein-coupled receptors evoke cellular responses is by activating phospholipase C (PLC), releasing Ca^{2+} from intracellular stores. Receptors that couple to pertussis toxin (PTX)-insensitive G proteins typically evoke large increases in PLC activity and Ca^{2+} release, whereas receptors that use PTX-sensitive G proteins usually give only weak PLC-dependent responses, instead regulating adenylate cyclase. The authors show that PTX-sensitive Ca^{2+} release mediated by the m4 muscarinic acetylcholine receptor in transfected Chinese hamster ovary cells is greatly enhanced when endogenous purigenic receptors simultaneously activate a PTX-insensitive signaling pathway. The m4 receptor may thus participate in a system that amplifies simultaneous PLC-activating signals generated by PTX-sensitive and PTX-insensitive G-protein pathways. Cells presumably receive multiple hormonal and neurotransmitter signals *in vivo*; these findings suggest a way in which the information encoded in varying signals can be integrated to give distinct cellular responses.
1 May 1995, Research Paper, *Current Biology*

▶ **Proteasome: a complex protease with a new fold and a distinct mechanism**
Alexander Wlodawer (1995). *Structure* **3**, 417–420.
The recently determined crystal structure of the 20S proteasome, coupled with mutational analysis, has shed considerable light on a new and fascinating class of proteases. Proteasomes degrade misfolded and denatured proteins in a rather non-specific manner. They are multicatalytic complexes found in all eukaryotic cells and some Archaebacteria, with each molecule consisting of at least 28 subunits arranged in a cylinder. The central cavity of the cylinder has now been found to contain 14 independent active sites, newly characterized as threonine proteases, which appear to be a tetrad version of the classic catalytic triad of serine proteases. The closest distance between these sites is about 28 Å, corresponding to the length of a 7–8 amino-acid peptide chain. It is possible that this explains the ability of the proteasome to cut proteins into 7–8 amino acid fragments, the size most appropriate for presentation by MHC class I molecules. The constricted access to the central cavity also explains the fact that proteasomes do not degrade native proteins.
15 May 1995, Minireview, *Structure*

▶ **A new flavor in phosphotyrosine recognition**
Michael J Eck (1995). *Structure* **3**, 421–424.
Because of its central role in phosphotyrosine-mediated signaling, the SH2 domain has held center stage in the unfolding signal transduction drama. But it must now share the spotlight with a new

modular signaling motif, dubbed the PTB domain for its phosphotyrosine-binding function. Although both SH2 and PTB bind phosphotyrosine in the context of specific amino-acid sequences, they appear to be structurally unrelated. Initially found in the amino-terminal region of Shc, an adaptor protein that couples growth factor receptor signaling to the Ras activation pathway, PTB domains have been identified by sequence homology in several other signaling proteins. The sequence motif recognized by the PTB domain, NPXpY, has been found in a number of activated growth factor receptors. The divergent binding specificities that have evolved for the SH2 domain have allowed it to be used in many signaling pathways. It remains to be seen whether PTB domains are similarly versatile.
15 May 1995, Minireview, *Structure*

► **Cadherin structure: a revealing zipper**
William I Weis (1995). *Structure* **3**, 425–427.
The development and maintenance of tissues depends critically upon cells of the same type adhering to one another. The cadherin cell-adhesion molecules mediate Ca^{2+}-dependent, homophilic interactions: cells expressing the same cadherin adhere to one another, and mixed cells expressing different cadherins segregate according to cadherin type. Cadherins contain one or more copies of the cadherin domain (CAD). Recently, structures of the amino-terminal CAD from neural and epithelial cadherins have provided a framework for understanding how CADs assemble into larger structures, why the cadherins are Ca^{2+} dependent, and how cadherin interactions contribute to cell–cell adhesion. Acidic residues at the amino- and carboxy-terminal ends of linked CADs appear to form a Ca^{2+}-binding site that stabilizes the interface between successive domains, producing a stiff, elongated molecule in the presence of Ca^{2+}. Head-to-head cadherin dimer formation is seen in cystaline neural CAD, strongly suggesting that such interactions also form when opposing cells interact. The regions that form the dimer interface vary in such a way as to preserve the homodimeric contact in different cadherins, plausibly explaining homophilic binding. The neural CAD crystal lattices form an infinite repeat in which the domains interlock like a 'zipper', perhaps reflecting an oligomeric interaction at the cell surface in which many weakly interacting cadherins interlock to form a strong junction.
15 May 1995, Minireview, *Structure*

► **Atomic structure of GTP cyclohydrolase I**
Herbert Nar, Robert Huber, Winfried Meining, Cornelia Schmid, Sevil Weinkauf and Adelbert Bacher (1995). *Structure* **3**, 459–466.
GTP cyclohydrolase I (GTP-CH-I) catalyzes the initial step in the biosynthesis of pteridines such as methanopterin, folic acid and tetrahydrobiopterin (BH_4). Disorders associated with altered levels of BH_4 include Parkinson's disease and Alzheimer's disease. The authors report the crystal structure of the *E. coli* GTP-CH-I, a homodecameric complex, at 3.0 Å resolution. They find that the active site is positioned at the interface of three subunits and contains a novel GTP-binding site, distinct from that found in G proteins. The structure indicates that three histidines and possibly a cystine are involved in catalysis. Despite a lack of significant sequence homology, the enzyme catalyzing the second step in BH_4 biosynthesis shares a common subunit fold and oligomerization mode with GTP-CH-I. These studies provide a basis for understanding the reaction mechanism of GTP-CH-I.
15 May 1995, Research Article, *Structure*

► **Two structures of the catalytic domain of phosphorylase kinase: an active protein kinase complexed with substrate analogue and product**
DJ Owen, MEM Noble, EF Garman, AC Papageorgiou and LN Johnson (1995). *Structure* **3**, 467–482.
All known protein kinases possess a common structure termed the 'kinase catalytic core'. Kinases are often regulated by phosphorylation on a segment of peptide chain, termed the activation loop. Alternatively, they may be regulated through intrasteric inhibition by a pseudosubstrate. Phosphorylase kinase (Phk) is thought to fall into the latter group. The authors report the crystal structures of the kinase catalytic core of the Phk γ-subunit complexed with either Mn^{2+}/AMPPNP (a non-hydrolyzable analog of the substrate) or the Mg^{2+}/ADP reaction product. Instead of containing a phosphorylatable residue, the activation loop contains a glutamate which traps the loop in an active conformation. Intersubunit regulation is still possible, however, since the glutamate can interact with a conserved arginine adjacent to the catalytic residue.
15 May 1995, Research Article, *Structure*

► **Movie of the structural changes during a catalytic cycle of nucleoside monophosphate kinases**
Clemens Vonrhein, Gerd J Schlauderer and Georg E Schulz (1995). *Structure* **3**, 483–490.
The 17 known crystal structures of nucleoside monophosphate kinases show large conformational changes upon binding of substrates. The structures exhibit various intermediate conformational states caused by crystal-packing forces, allowing the authors to follow a range of protein motion between a 'closed' conformation and a less well defined 'open' conformation. The authors have aligned the structures, worked out the conformational changes, sorted the static pictures along trajectories and combined them into a movie. Such a movie should approximate the action of a protein much more effectively than a single structure; it represents a novel way of visualizing how proteins move at the atomic level.
15 May 1995, Research Article, *Structure*

► **Structure of human estrogenic 17β-hydroxysteroid dehydrogenase at 2.20 Å resolution**
Debashis Ghosh, Vladimir Z Pletnev, Dao-Wei Zhu, Zdislaw Wawrzak, William L Duax, Walter Pangborn, Fernand Labrie and Sheng-Xiang Lin(1995). *Structure* **3**, 503–513.
The principal human estrogen, 17β-estradiol, is a potent stimulator of certain endocrine-dependent forms of breast cancer. Because human estrogenic 17β-hydroxysteroid dehydrogenase (type I 17β-HSD) catalyzes the last step in the biosynthesis of 17β-estradiol, it is an attractive target for the design of inhibitors of estrogen production and tumor growth. The authors report that the structure of type I 17β-HSD, the first mammalian steroidogenic enzyme studied by X-ray crystallography, reveals features that are conserved in short-chain dehydrogenases. The structure also contains three α-helices and a helix-turn-helix motif that restrict access to the active site and appear to influence substrate specificity. One or more of these helices may also be involved in the reported association of the enzyme with membranes. Modeling the position of estradiol in the active site suggests that a histidine side chain may be critical in substrate recognition. The structure of the active site provides a rational basis for designing more specific inhibitors of this enzyme.
15 May 1995, Research Article, *Structure*