# Elsewhere in Biology

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

**Current Biology Ltd has launched a new journal entitled *Current Opinion in Chemical Biology*, which will be of particular interest to readers of *Chemistry & Biology*. The editors of the journal are Donald Hilvert and Steven V Ley. The fourth issue, on model systems (edited by Julius Rebek Jr and David N Reinhoudt) and biopolymers (edited by Anne Dell, Barbara Imperiali and Larry McLaughlin) is published in December.**

Current Opinion in Chemical Biology
**Model systems/Biopolymers**
Contents for December 1997 issue



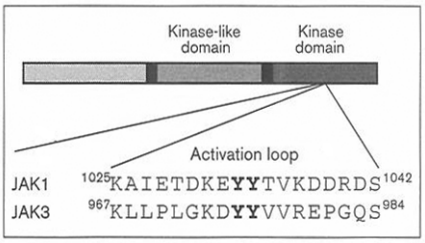
**A selection of interesting papers published last month in *Chemistry & Biology*'s sister journals, *Current Biology*, *Folding & Design* and *Structure*.**

**Chemistry & Biology** December 1997, 4:979–982



☐ **Janus kinases in interleukin-2-mediated signaling: JAK1 and JAK3 are differentially regulated by tyrosine phosphorylation.** Kathleen D Liu, Sarah L Gaffen, Mark A Goldsmith and Warner C Greene (1997). *Curr. Biol.* **7**, 817–826.

Cytokines mediate a variety of effector cell functions, including cellular proliferation, differentiation, and modulation of the immune response. Many cytokines activate receptor-associated Janus kinases (JAKs) that promote tyrosine phosphorylation of signal transducers and activators of transcription (STAT) factors. Although JAK activation has been correlated with



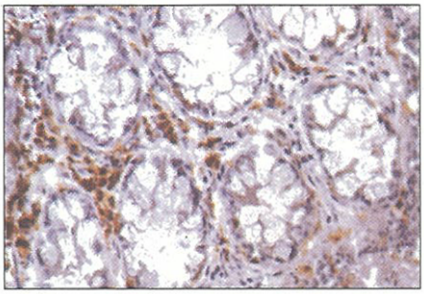

phosphorylation, the role of this tyrosine phosphorylation in the regulation of JAK1 and JAK3 remains unclear. Furthermore, the relative roles of JAK1 and JAK3 in the activation of STAT5 by interleukin-2 (IL-2) remain poorly understood. The authors targeted two conserved tyrosine residues within the activation loop of the JAK1 and JAK3 kinase domains for substitution with phenylalanines. In an overexpression system, the catalytic function of JAK1 strictly required the presence of the first of

these tyrosines, Tyr1033. In contrast, JAK3 retained catalytic activity when either or both of the tyrosines were mutated. Analysis of JAK1/3 chimeras showed that JAK activity was also controlled by intramolecular interactions involving the amino-terminal domain of the JAK as well as by the inherent signaling properties of the kinase domain. The authors reconstituted IL-2-dependent STAT5 induction in a cell line lacking detectable expression of JAK1 and JAK3. Catalytically active versions of both JAK1 and JAK3 must be present for effective induction of STAT5. JAK1 and JAK3 are differentially regulated by specific tyrosines within their respective activation loops and, the amino-terminal domain of JAK3 appears to contain regulatory sequences that modify the function of the kinase domain. Finally, both JAK1 and JAK3 must retain catalytic function for IL-2-induced STAT5 activation.

6 October 1997, Research Paper, *Current Biology**

☐ **Functional expression of the eotaxin receptor CCR3 in T lymphocytes co-localizing with eosinophils.** B O Gerber, M P Zanni, M Uguccioni, M Loetscher, C R Mackay, W J Pichler, N Yawalkar, M Baggiolini and B Moser (1997). *Curr. Biol.* **7**, 836–843.

The chemokine eotaxin is produced at sites of allergic inflammation, binds selectively to the chemokine receptor CCR3 and attracts eosinophil and basophil leukocytes.

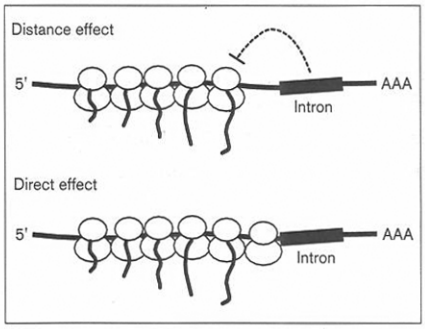


Responses of T lymphocytes to eotaxin have not been reported so far. The authors have investigated the expression of CCR3 in T lymphocytes and analysed the properties and *in vivo* distribution of T lymphocytes expressing this receptor. They found that T lymphocytes co-localizing with eosinophils at sites of allergic inflammation express CCR3, suggesting that eotaxin/CCR3 represents a novel mechanism of T-lymphocyte recruitment. These cells are essential in allergic inflammation, as mice lacking mature T lymphocytes were insensitive to allergen challenge. Surface CCR3 may mark a subset of T lymphocytes that induce eosinophil mobilization and activation through local production of Th2-type cytokines.

14 October 1997, Research Paper, *Current Biology*

☐ **Translational attenuation mediated by an mRNA intron.** Rowan E Chapman and Peter Walter (1997). *Curr. Biol.* **7**, 850–859.

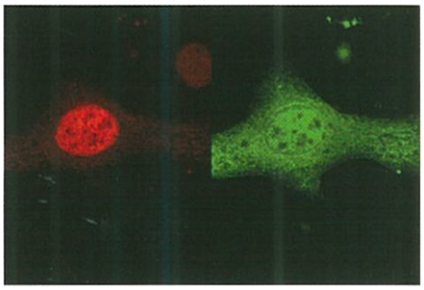
The unfolded protein response (UPR) is an intracellular signaling pathway that is activated by the accumulation of unfolded proteins in the endoplasmic reticulum (ER). The UPR results in an increase in transcription of ER-resident proteins that facilitate protein folding in the ER. A key regulatory step in UPR activation is the regulated splicing of *HAC1* mRNA, which encodes Hac1p, a transcription factor dedicated to this pathway. Hac1p can be detected only when the spliced form of *HAC1* mRNA is produced; this was surprising because the unspliced *HAC1* mRNA is equally stable in cells. The authors show that, in contrast to most other unspliced pre-mRNAs, the unspliced *HAC1* mRNA is transported from the nucleus into the cytosol. Although the unspliced *HAC1* mRNA is associated with polyribosomes, no detectable protein is produced unless the intron is removed, indicating that the presence of the intron prevents mRNA translation. In this paper, the authors have shown that the *HAC1* mRNA intron is both necessary and sufficient to prevent complete translation of polyribosome-associated mRNAs. This, to the knowledge of the authors, identifies a new way by which translation of a mRNA can be attenuated.




17 October 1997, Research Paper, *Current Biology*

☐ **Design of a synthetic Mdm2-binding mini protein that activates the p53 response *in vivo*.** Angelika Böttger, Volker Böttger, Alison Sparks, Wei-Li Liu, Stephanie F. Howard and David P Lane (1997). *Curr. Biol.* **7**, 860–869.

The transcriptional activation function of the p53 tumour suppressor protein is induced by DNA damage and results in growth arrest and/or apoptotic responses. A key component of this response is the dramatic rise in p53 protein concentration resulting from an increase in the protein's stability. Very recently, it has been suggested that interaction with the Mdm2 protein may target p53 for rapid degradation. The authors have designed a gene encoding a small protein that binds tightly to the p53-binding pocket on the Mdm2 protein. When introduced into cells containing low levels of wild-type p53, the protein causes a striking accumulation of the endogenous p53 protein, activation of a p53-responsive reporter gene and cell-cycle arrest, mimicking the effects seen in these cells after exposure to ultraviolet or ionising radiation. Microinjection of a



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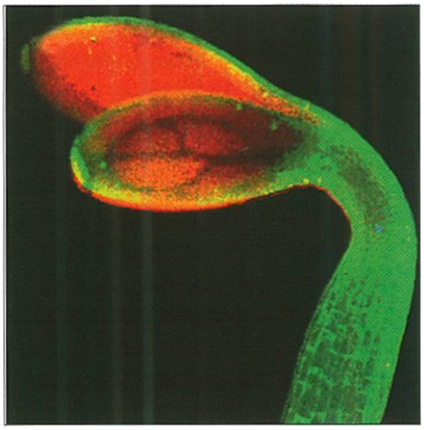
monoclonal antibody to the p53-binding site on Mdm2 has a similar effect. The results demonstrate that the p53 response is constitutively regulated in normal cells by Mdm2 and that disruption of the interaction alone is sufficient to stabilise the p53 protein and activate the p53 response. The approach provides a powerful new method to activate p53 without causing DNA damage. More broadly, it establishes a general method for determining the biological consequences of the specific disruption of protein–protein interactions in cells.
17 October 1997, Research Paper, *Current Biology*

☐ **The phytofluors: a new class of fluorescent protein probes.** John T Murphy and J Clark Lagarias (1997). *Curr. Biol.* **7**, 870–876.

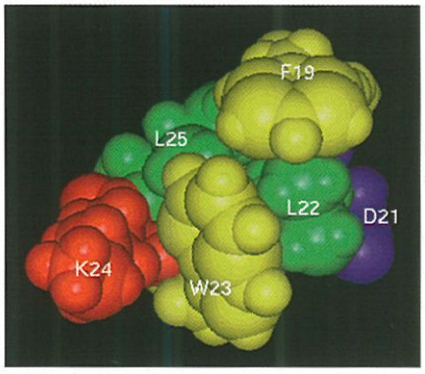
Biologically compatible fluorescent protein probes, particularly the self-assembling green fluorescent protein (GFP) from the jellyfish *Aequorea victoria*, have revolutionized research in cell, molecular and developmental biology because they allow visualization of biochemical events in living cells. Additional fluorescent proteins that could be reconstituted *in vivo* while extending the useful wavelength range towards the orange and red regions of the light spectrum would increase the range of applications for fluorescent protein probes. Intensely orange fluorescent adducts, phytofluors, are spontaneously formed upon incubation of recombinant plant phytochrome apoproteins with phycoerythrobilin, the linear tetrapyrrole precursor of the phycoerythrin chromophore. Phytofluors have large molar absorption coefficients, fluorescence quantum yields greater than 0.7, excellent photostability, stability over a wide range of pH, and can be reconstituted in living plant cells. The phytofluors are a new class of fluorophore that can potentially be produced upon bilin uptake by any living cell expressing an apophytochrome cDNA. Mutagenesis of the phytochrome apoprotein and/or alteration of the linear tetrapyrrole precursor by chemical synthesis are expected to yield new phytofluors that have fluorescence excitation and emission spectra spanning the visible to near-infrared light spectrum.



17 October 1997, Research Paper, *Current Biology*

☐ **Solution conformation of an essential region of the p53 transactivation domain.** Maria Victoria E Botuyan, Jamil Momand and Yuan Chen (1997). *Fold. Des.* **2**, 331–342.

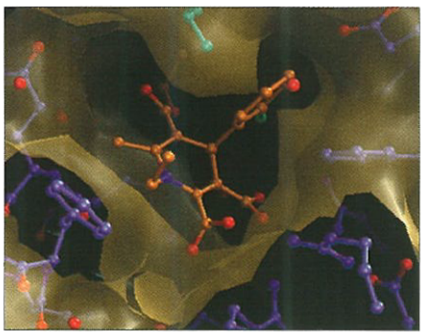
The p53 tumor supressor protein responds to cellular stress, such as DNA damage. The peptide segment surrounding residues Leu22 and Trp23 of the p53 transactivation domain plays a critical role in the transactivation activity of p53. This region binds basal transcriptional components such as the TATA-box binding protein associated factors $TAF_{II}40$ and $TAF_{II}60$ as well as the Mdm2 and adenovirus type 5 E1B 55 kDa oncoproteins. The structure of residues 14–28 of p53 was studied by nuclear magnetic resonance spectroscopy and found to prefer a two-ß-turn structure stabilized by a hydrophobic cluster consisting of residues known to be important for transactivation and binding to p53-binding proteins. The structural propensity observed in the wild-type p53 peptide is important for understanding the mechanism of transcriptional activation, because very few structural data are available on transactivation domains to date. The results should aid in the design of therapeutic agents that could competitively inhibit binding of p53 to the oncogene product Mdm2.




22 October 1997, Research Paper, *Folding & Design*

☐ **The structure of glycogen phosphorylase b with an alkyl-dihydropyridine-dicarboxylic acid compound, a novel and potent inhibitor.** Spyros E Zographos, Nikos G Oikonomakos, Katerina E Tsitsanou, Demetrios D Leonidas, Evangelia D Chrysina, Vicky T Skamnaki, Hilmar Bischoff, Siegfried Goldmann, Kimberly A Watson and Louise N Johnson (1997). *Structure* **5**, 1413–1425.

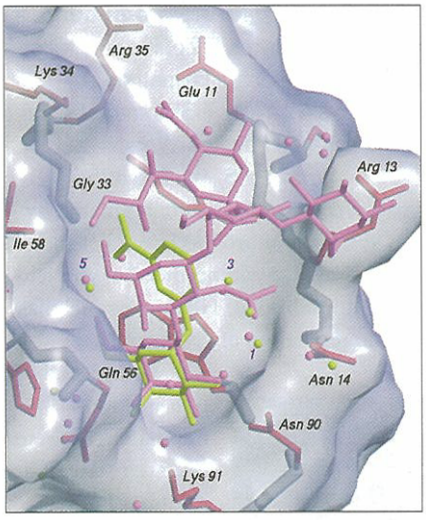
In muscle and liver, glycogen concentrations are regulated by the reciprocal activities of glycogen phosphorylase (GP) and glycogen synthase. An alkyl-dihydropyridine-dicarboxylic acid has been found to be a potent inhibitor of GP, and as such has potential to contribute to the regulation of glycogen metabolism in the non-insulin-dependent diabetes diseased state. The inhibitor has no structural similarity to the natural regulators of GP. The authors have carried out structural studies to elucidate the mechanism of inhibition. Kinetic studies with rabbit muscle glycogen phosphorylase b (GPb) show that the compound (–)(*S*)-3-isopropyl 4-(2-chlorophenyl)-1,4-dihydro-1-ethyl-2-methyl-pyridine-3,5,6-tricarboxylate (Bay W1807) has a $K_i = 1.6$ nM and is a competitive inhibitor with respect to AMP. The structure of the cocrystallised GPb–W1807 complex has



been determined. The high affinity of W1807 for GP appears to arise from the numerous nonpolar interactions made between the ligand and the protein. Its potency as an inhibitor results from the induced conformational changes that lock the enzyme in a conformation known as the T′ state. Allosteric enzymes, such as GP, offer a new strategy for structure-based drug design in which the allosteric site can be exploited. The results reported here may have important implications in the design of new therapeutic compounds.
15 November 1997, Research Paper, *Structure*

☐ **Structural foundation for the design of receptor antagonists targeting *Escherichia coli* heat-labile enterotoxin.** Ethan A Merritt, Steve Sarfaty, Ingeborg K Feil and Wim G J Hol (1997). *Structure* **5**, 1485–1499.

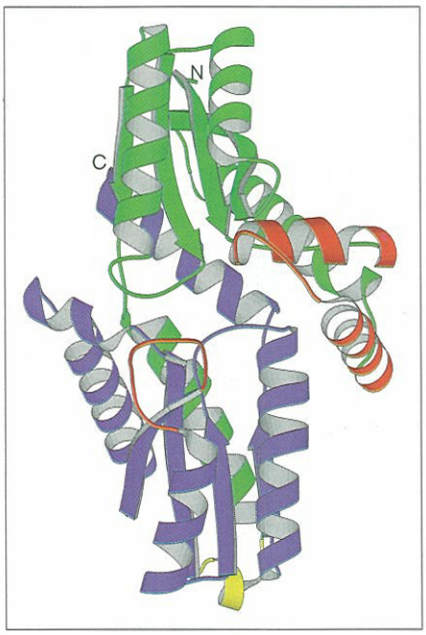
*Escherichia coli* heat-labile enterotoxin (LT) is the causative agent of traveller's diarrhoea, and it is also responsible for the deaths of hundreds of thousands of children per year in developing countries. LT is highly homologous in sequence, structure and function to cholera toxin (CT). Both toxins attack intestinal epithelial cells via specific binding to the branched pentasaccharide of ganglioside $G_{M1}$ at the cell surface. A receptor-binding antagonist that blocked this interaction would potentially constitute a prophylactic drug conferring protection both against the severe effects of cholera itself and against the milder but more common disease caused by LT. Four derivatives of the simple sugar galactose, members of a larger series of receptor antagonists identified by computer modeling and competitive binding studies, have been co-crystallized with either the full LT $AB_5$ holotoxin or the LT B pentamer. The crystal structures have provided detailed views of the toxin in complex with each of the four antagonists: melibionic acid, lactulose, metanitrophenylgalactoside (MNPG) and thiodigalactoside (TDG). The bound conformations of these receptor antagonist compounds preserve the toxin–galactose interactions previously observed for toxin–sugar complexes, but gain additional favorable interactions. The highest affinity compound, MNPG, is notable in that it displaces a water molecule that is observed to be well-ordered in all other previous and current crystal structures of toxin–sugar complexes. This could be a favorable entropic factor contributing to the increased affinity. The highest affinity members of the present set of antagonists (MNPG and TDG) bury roughly half of the binding-site surface covered by the full receptor $G_{M1}$ pentasaccharide, despite being considerably smaller. This provides an encouraging basis for the creation of subsequent generations of derived compounds that can compete effectively with the natural receptor.




15 November 1997, Research Paper *Structure*

☐ **Crystal structure of ferrochelatase: the terminal enzyme in heme biosynthesis.** Salam Al-Karadaghi, Mats Hansson, Stanislav Nikonov, Bodil Jönsson and Lars Hederstedt (1997). *Structure* **5**, 1501–1510.

The metallation of closed ring tetrapyrroles resulting in the formation of hemes, chlorophylls and vitamin $B_{12}$ is catalyzed by specific enzymes called chelatases. Ferrochelatase catalyzes the terminal step in heme biosynthesis by inserting ferrous ion into protoporphyrin IX by a mechanism that is poorly understood. Mutations in the human gene for ferrochelatase can result in the disease erythropoietic protoporphyria, and a further understanding of the mechanism of this enzyme is therefore of clinical interest. No three-dimensional structure of a tetrapyrrole metallation enzyme has been available until now. The authors have determined the three-dimensional structure of *Bacillus subtilis* ferrochelatase. The position of invariant residues, shown previously to be important for activity, reveals the location of the porphyrin-binding cleft and the metallation site of the enzyme. Comparison of amino acid sequences of ferrochelatases from different sources shows that ferrochelatase seems to have a structurally conserved core region that is common to the enzyme from bacteria, plants and mammals. The structure provides the basis for the exploration of the function of ferrochelatase and for understanding the mechanism of metal insertion into organic molecules in biological systems.

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