Elsewhere in Biology

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

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

Chemistry & Biology July 1996, **3**:591–594

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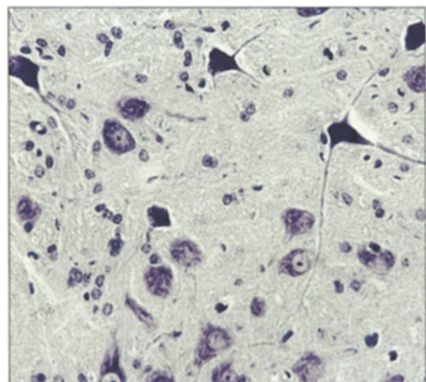
☐ **Cryptic physiological trophic support of motoneurons by LIF revealed by double gene targeting of CNTF and LIF.** M. Sendtner, R. Götz, B. Holtmann, J-L. Escary, Y. Masu, P. Carroll, E. Wolf, G. Brem, P. Brûlet and H. Thoenen (1996). *Curr. Biol.* **6**, 686–694.

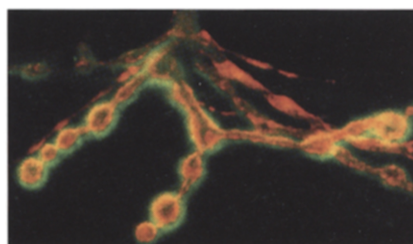
Ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF) have no sequence homology, but share a similar tertiary structure, common receptor components, and the ability to support the survival of motoneurons. The inactivation of the gene encoding LIF in mice has no apparent effect on the number, structure, or functioning of motoneurons, however, and the inactivation of the gene encoding CNTF has only mild degenerative effects. The inactivation of both genes results in significant defects, however; in such mice, motoneurons are severely atrophic and reduced in number from an early age, and there is a marked reduction in grip strength. Significantly greater losses of motoneurons are also seen in the double mutant after nerve lesion. These results are indicative of the redundancy in the nervous system, and suggest that inactivating mutations in the CNTF gene, which are present in a high proportion of individuals in certain populations, may be a predisposing factor for degenerative disorders of motoneurons.



1 June 1996, Research Paper, *Current Biology*

☐ **The *Drosophila* tumor suppressor gene, *dlg*, is involved in structural plasticity at a glutamatergic synapse.** Bo Guan, Beate Hartmann, Young-Ho Kho, Michael Gorczyca and Vivian Budnik (1996). *Curr. Biol.* **6**, 695–706.

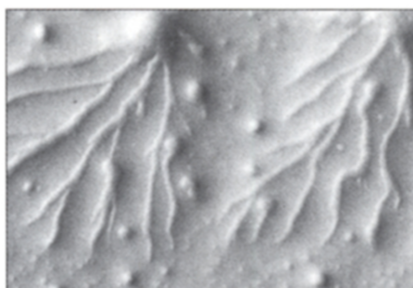
Mutation of the *discs-large* (*dlg*) gene in *Drosophila* leads to the formation of neoplastic tumors in epithelial and neural tissues and the abnormal development of septate and synaptic junctions, implicating DLG in the contact-dependent development of cells and cell junctions. DLG has been shown to be important for the normal development of a glutamatergic synapse, and this study shows that DLG function is required at two stages of *Drosophila* development. At late embryogenesis, DLG may determine synapse structure in the initial stages of synapse formation as the type of motoneuron is defined, and during the larval stages DLG is required for the expansion of synaptic contacts with the rapidly growing muscle target. It is possible that this type of plasticity may have some parallels with that seen in mammals, as a family of *dlg* homologs expressed at mammalian synapses are known to interact with the N-methyl-D-aspartate receptor, which has been implicated in various forms of synaptic plasticity.



1 June 1996, Research Paper, *Current Biology*

☐ **The *aimless* RasGEF is required for processing of chemotactic signals through G-protein-coupled receptors in *Dictyostelium*.** Robert H. Insall, Jane Borleis and Peter N. Devreotes (1996). *Curr. Biol.* **6**, 719–729.

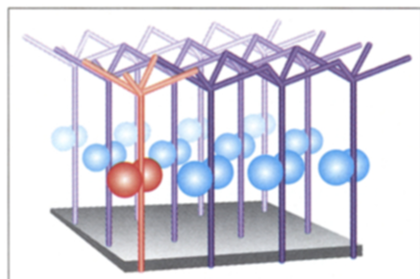
Dictyostelium discoideum amoebae respond to starvation by secreting cAMP in pulses, which causes the cells to move chemotactically towards each other and form slugs and, subsequently, fruiting bodies. The cAMP is detected by a seven transmembrane domain receptor, which is coupled to a single G protein. In the genetic screen described here, mutants that cannot aggregate are isolated and one, defective in the *aimless* gene, is characterised. *Aimless* null mutants have no defects in signal perception, or in receptor coupling to or activation of G proteins, but they cannot activate adenylyl cyclase or respond to chemotactic signals because of a defect downstream of the G protein. The sequence of *aimless* suggests that it encodes a guanine nucleotide exchange factor for a Ras-like small GTPase. The exact target of its activity is not known, but the discovery of *aimless* strongly suggests that a Ras-related protein is involved downstream of the cAMP-responsive G-protein-coupled receptor; until now, little has been known about Ras-related pathways that are controlled by molecules other than receptor tyrosine kinases.



1 June 1996, Research Paper, *Current Biology*

☐ **A hyperthermostable protease of the subtilisin family bound to the surface layer of the Archeon *Staphylothermus marinus*.** Jutta Mayr, Andrei Lupas, Josef Kellermann, Christoph Eckerskorn, Wolfgang Baumeister and Jürgen Peters (1996). *Curr. Biol.* **6**, 739–749.

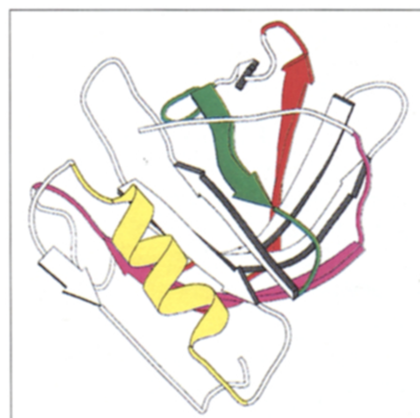
Staphylothermus marinus, an archeon isolated from a geothermally heated marine environment, is a peptide-fermenting, sulfur-dependent organism with an optimum growth temperature of 92 °C. It forms grape-shaped clusters of cells, which adhere to each other and to sulfur granules via their surface layer. This glycoprotein layer, the archeon's equivalent of a cell wall, forms a canopy which is held at a distance of 70 nm from the cell membrane by membrane-anchoring stalks. Two copies of a globular protease, which probably generates the peptides required in the energy metabolism of the cell, are attached near the middle of each stalk. The purification and characterization of this protease reveals that it has a domain with clear homology to the subtilisins, and has broad substrate specificity and a pH optimum of 9.0. It is fully stable from pH 3.2 to 12.7, and is resistant to heat-inactivation to 95 °C in the free form and to 125 °C in the stalk-bound form. At around 130 °C proteins become unstable because of spontaneous hydrolysis of the polypeptide backbone, so the stability of this protease may approach the limit for any protein.



1 June 1996, Research Paper, *Current Biology*

☐ **High helicity of peptide fragments corresponding to β-strand regions of β-lactoglobulin observed by 2D-NMR spectroscopy.** Yutaka Kuroda, Daizo Hamada, Toshiki Tanaka and Yuji Goto (1996). *Folding & Design* **1**, 255–263.

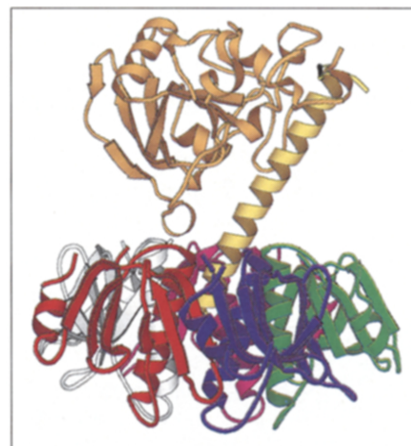
The current consensus is that peptides, if they form any secondary structure in solution, will exhibit structures consistent with those observed in the native structure of the full length protein. This is consistent with the framework model of protein folding, in which the structured fragments correspond to regions of the native structure that are especially stable and reflect conformations that prevail in the early stages of protein folding, when local interactions prevail. The protein analysed here, β-lactoglobulin, is predominantly composed of β-sheets, but its individual fragments have high helical propensity and are shown to form helices that can be further stabilized by solvents other than water. This suggests that protein folding can be non-hierarchical, with non-native structures involved in the early stages, and that fragments designed in isolation may not have the same secondary structure when integrated into a protein.



17 Jun 1996*, Research Paper, *Folding & Design*

☐ **Crystal structure of a new heat-labile enterotoxin, LT-IIb.** Focco van den Akker, Steve Sarfaty, Edda M Twiddy, Terry D Connell, Randall K Holmes and Wim GJ Hol (1996). *Structure* **4**, 665–678.

Cholera toxin (CT) from *Vibrio cholerae* and the two types of heat-labile enterotoxin (LT-Is and LT-IIs) from *Escherichia coli* all share the oligomeric structure AB_5. CT and LT-Is are known to act on intestinal epithelial cells, where recognition of specific ganglioside receptors by the B subunits leads to internalisation of the A subunit, which then ADP-ribosylates the G-protein subunit $G_s\alpha$. The LT-IIs have been isolated more recently, and their role in pathogenesis remains to be established. The structure of the LT-II determined here reveals that it is strikingly similar to LT-I, despite the dramatically different sequences of the respective B subunits. The B subunit pentamer forms a central pore with a diameter of 10–18 Å, and a common hydrophobic ring is observed at the A–B_5 interface, which may be important for assembly of the heterohexamer. The receptor binding sites are clustered in similar positions in LT-I and LT-IIb.



15 June 1996, Research Paper, *Structure*

☐ **Crystal structure of a phosphatase-resistant mutant of sporulation response regulator Spo0F from *Bacillus subtilis*.** Madhusudan, James Zapf, John M Whiteley, James A Hoch, Nguyen H Xuong and Kottayil I Varughese (1996). *Structure* **4**, 679–690.

Bacteria recognize and respond to changes in their environment through the action of two-component signal

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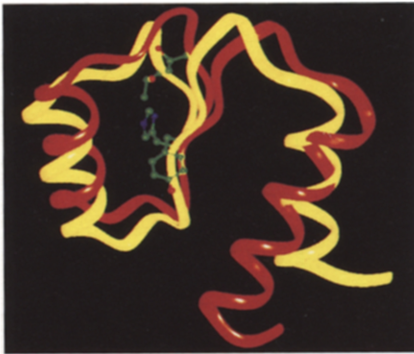
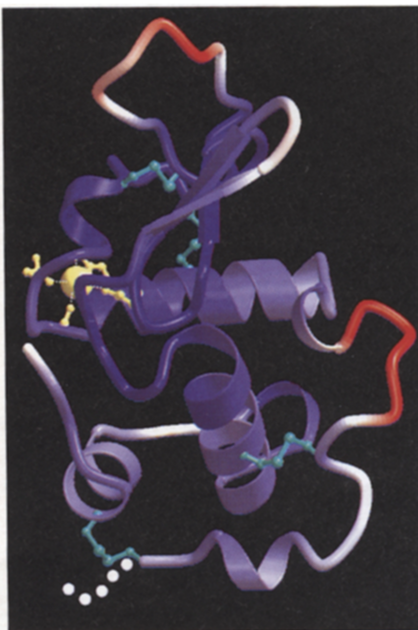


transduction systems, in which one component is phosphorylated on an invariant aspartate residue. The phosphorelay that triggers sporulation in *Bacillus subtilus* has a greater number of components but is essentially a variation on this system. Two sensor kinases phosphorylate the response regulator Spo0F, and the phosphate is then transferred by Spo0B to the transcriptional activator Spo0A. The structure of Spo0F determined here is similar in its overall fold and the placement of certain critical active-site residues to another response regulator CheY, which in its phosphorylated form activates flagellar motors in the bacterial chemotactic system. The activated state of CheY is stable for a matter of seconds, whereas that of Spo0F is stable for five hours. This difference is reflected in the active site: in Spo0F Lys56 caps the carboxylate group of Asp54 and makes it inaccessible to phosphatases, whereas in CheY the equivalent residue, Asn59, has a shorter side chain that cannot shield the phosphate.

15 June 1996, Research Paper, *Structure*

☐ **Crystal structures of guinea-pig, goat and bovine α-lactalbumins highlight the enhanced conformational flexibility of regions that are significant for its action in lactose synthase.** Ashley CW Pike, Keith Brew and K Ravi Acharya (1996). *Structure* **4**, 691–703.

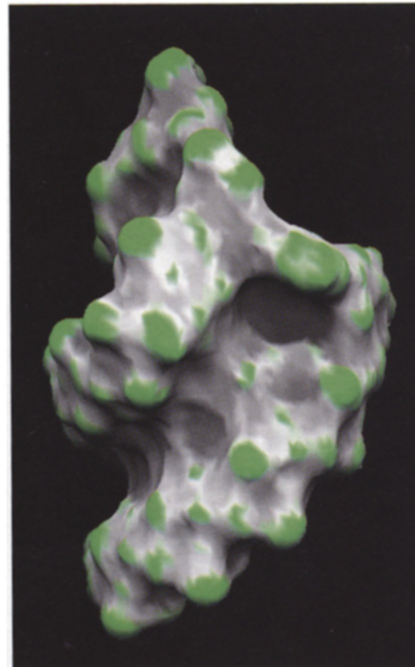
In the *trans*-Golgi membranes of most cells, galactosyltransferase (GT) participates in the biosynthesis of oligosaccharide chains on secretory and membrane-bound proteins. But in the lactating mammary gland α-lactalbumin binds to GT and changes the substrate specificity of GT from oligosaccharides to glucose, thus converting it into lactose synthase. To gain further insight into the mechanism by which α-lactalbumin increases the affinity of GT for glucose 1000-fold, the authors determine the structure of three species variants of α-lactalbumin by molecular replacement. A region adjacent to two residues that have been implicated in monosaccharide binding by lactose synthase exhibits a variety of distinct conformers. This implies that conformational adjustments may be important for the formation and function of lactose synthase, and that the development of conformational flexibility may have been one prerequisite in the evolution of α-lactalbumin from its close relative, lysozyme.



15 June 1996, Research Paper, *Structure*

☐ **Solution structure of the donor site of a *trans*-splicing RNA.** Nancy L Greenbaum, Ishwar Radhakrishnan, Dinshaw J Patel and David Hirsh (1996). *Structure* **4**, 725–733.

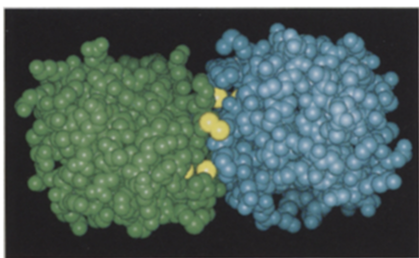
In *Caenorhabditis elegans*, the 22-nucleotide spliced leader (SL) of the SL1 RNA is transferred to ~70 % of pre-mRNA molecules. The SL may well be a translational enhancer or a signal for RNA transport, but in any case the mechanism of this *trans*-splicing event is believed to be almost identical to that of the more common *cis*-splicing of pre-mRNA. The first stem–loop of SL1 RNA contains both the splice-donor site and a complementary base-paired region, mimicking the pairing of the 5' splice site of pre-mRNA with the U1 small nuclear (sn) RNA in the spliceosome prior to *cis*-splicing. Thus, the SL1 RNA resembles a chimaera of the U1 snRNA and the 5' exon, lending further support to the idea that the SL1 RNA may be an evolutionary intermediate between the self-splicing introns and snRNAs of the spliceosome. Here the solution structure of a domain of the first stem loop of SL1 RNA is solved using homonuclear and heteronuclear NMR. The loop is highly structured, and contains a deep pocket formed by a bulged adenine at its base. The splice-donor site resides nearby, in the A-helical stem. The proximity of the pocket to the splice-donor site, combined with the observation that the nucleotides in this motif are conserved among all nematode SL RNAs, suggests that it may provide a recognition site for a protein or RNA molecule in the *trans*-splicing process.



15 June 1996, Research Paper, *Structure*

☐ **Crystal structure of reduced, oxidized, and mutated human thioredoxins: evidence for a regulatory homodimer.** Andrzej Weichsel, John R Gasdaska, Garth Powis and William R Montfort (1996). *Structure* **4**, 735–751.

Human thioredoxin reduces the disulfide bonds of numerous proteins *in vitro*, and can activate transcription factors such as NF-κB *in vivo*. Thioredoxin can also act as a growth factor, and is overexpressed and secreted in certain tumor cells. All thioredoxin-like proteins contain the active-site sequence Cys-Xaa-Xaa-Cys, and all apparently have depressed pK_a values for the first cysteine in this sequence; the degree to which the pK_a is depressed is variable, and this is thought to be the main factor affecting the redox potential of the enzyme. Here, the crystal structures for reduced and oxidized wild-type human thioredoxin, and for the reduced mutant proteins Cys73→Ser and Cys32→Ser/Cys35→Ser are reported. Thioredoxin is normally a disulfide-linked dimer, with the intersubunit dimer formed by the two Cys73 residues. It is therefore surprising that the Cys73→Ser mutant crystallizes as a dimer. Most of the dimerization energy is presumably provided by the formation of the dimer interface. Dimer formation buries the active site, however, and the physiological role, if any, of dimerization is unknown. Conformational changes in the active site accompany oxidation of the active-site cysteines, Cys32 and Cys 35. A hydrogen bond between the sulfhydryls of these residues may reduce the pK_a of Cys32.



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