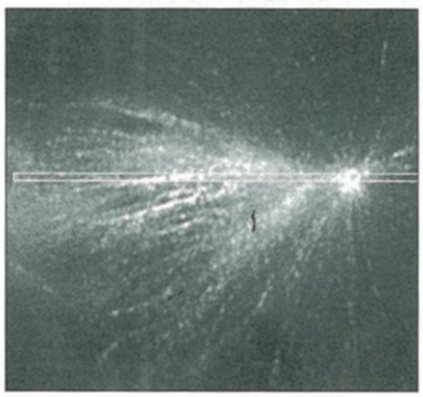
Elsewhere in biology

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

Chemistry & Biology December 1998, 5:R340–R343

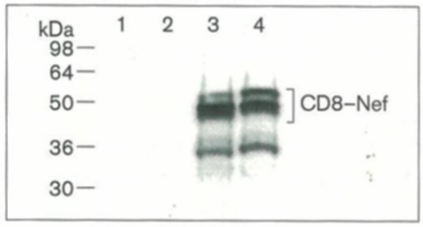
☐ **Fluorescent speckle microscopy, a method to visualize the dynamics of protein assemblies in living cells.**
Clare M Waterman-Storer, Arshad Desai, J Chloe Bulinski and ED Salmon (1998). *Curr. Biol.* **8**, 1227–1230.

Fluorescence microscopic visualization of fluorophore-conjugated proteins that have been microinjected or expressed in living cells and have been incorporated into cellular structures has yielded much information about protein localization and dynamics. This approach has, however, been limited by high background fluorescence and the difficulty of detecting movement of fluorescent structures because of uniform labeling. These problems have been partially alleviated by the use of more cumbersome methods such as three-dimensional confocal microscopy, laser photobleaching and photoactivation of fluorescence. The authors report here a method called fluorescent speckle microscopy (FSM) that uses a very low concentration of fluorescent subunits, conventional wide-field fluorescence light microscopy and digital imaging with a low-noise, cooled charged coupled device (CCD) camera. A unique feature of this method is that it reveals the assembly dynamics, movement and turnover of protein assemblies throughout the image field of view at diffraction-limited resolution. FSM also significantly reduces out-of-focus fluorescence and greatly improves visibility of fluorescently labeled structures and their dynamics in thick regions of living cells. Initial applications explored in this paper include the measurement of microtubule movements in mitotic spindles and actin retrograde flow in migrating cells.
26 October 1998, Brief Communication, *Current Biology*.



☐ **A dileucine motif in HIV-1 Nef acts as an internalization signal for CD4 downregulation and binds the AP-1 clathrin adaptor.**
Patricia A Bresnahan, Wes Yonemoto, Sharon Ferrell, Debora Williams-Herman, Romas Geleziunas and Warner C Greene (1998). *Curr. Biol.* **8**, 1235–1238.

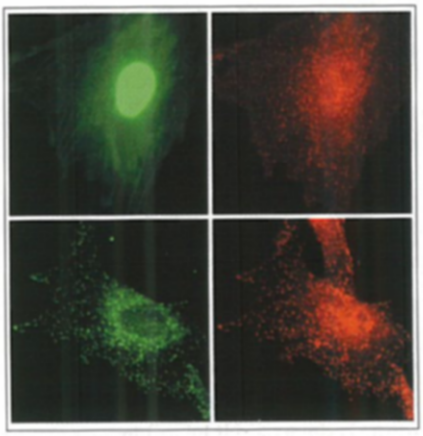
Human immunodeficiency virus 1 (HIV-1) Nef downregulates surface expression of CD4, an integral component of the functional HIV receptor complex, through accelerated endocytosis of surface receptors and diminished transport of CD4 from the Golgi network to the plasma membrane. HIV-1 Nef also diminishes surface expression of major histocompatibility complex (MHC) class I antigens. In the case of HIV-2 and simian immunodeficiency virus 1 (SIV-1) Nef, amino-terminal tyrosine-based motifs mediate the binding of Nef to the AP-1 and AP-2 adaptors and this interaction appears to be required for CD4 downregulation. As these tyrosine motifs are not present in the HIV-1 Nef protein, the molecular basis for the presumed interaction of Nef with components of the endocytic machinery is unknown. Here, a highly conserved dileucine motif in HIV-1 Nef



that is required for downregulation of CD4 has been identified. This motif acts as an internalization signal in the context of a CD8-Nef chimera or in a fusion of the interleukin-2 receptor a with an 11-amino-acid region from Nef containing the dileucine motif. Finally, HIV-1 Nef binds to the AP-1 adaptor, both *in vitro* and *in vivo*, in a dileucine-dependent manner. The authors conclude that this conserved dileucine motif in HIV-1 Nef serves as a key interface for interaction with components of the host protein trafficking machinery. These findings also reveal an evolutionary difference between HIV-1 and HIV-2/SIV in which the Nef proteins utilize structurally distinct motifs for binding cellular adaptors.
5 November 1998, Brief Communication, *Current Biology*.

☐ **A dileucine motif in HIV-1 Nef is essential for sorting into clathrin-coated pits and for downregulation of CD4.**
Michael Greenberg, Louis DeTulleo, Iris Rapoport, Jacek Skowronski and Tomas Kirchhausen (1998). *Curr. Biol.* **8**,1239–1242.

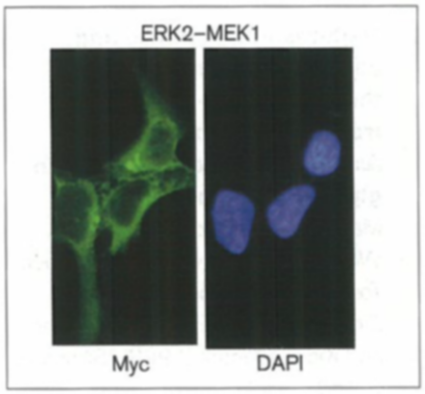
Nef, a 200 residue multifunctional regulatory protein of human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV), interacts with components of host cell signal transduction and clathrin-dependent protein sorting pathways. The downregulation of surface CD4 molecules and major histocompatibility complex (MHC) class I antigens by Nef is believed to be important in AIDS pathogenesis. Nef contains a globular core domain and two disordered segments — a myristylated arm at the amino terminus and a carboxy-terminal loop projecting from the globular core. Here, the authors aimed to determine



the sorting signals in HIV-1 Nef responsible for its involvement in the clathrin-mediated pathway. A sequence in the carboxy-terminal disordered loop of Nef was found to be essential for downregulation of CD4. This sequence resembles the dileucine motif, one of two well-characterized sorting signals that target membrane proteins to clathrin-coated vesicles. The dileucine-motif-containing segment of Nef bound directly and specifically to the β-adaptin subunit of the clathrin adaptor complexes AP-1 and AP-2, which are responsible for recruiting sorted proteins into coated pits. Unlike wild-type Nef, a mutant form of Nef that lacked the dileucine motif did not localize to clathrin-coated pits and did not downregulate CD4 expression, although it was able to downregulate MHC class I surface expression. Thus, the dileucine motif in HIV-1 is required for CD4 downregulation and for interaction with clathrin adaptor complexes.
5 November 1998, Brief Communication *Current Biology*.

☐ **A constitutively active and nuclear form of the MAP kinase ERK2 is sufficient for neurite outgrowth and cell transformation.**
Megan J Robinson, Stephen A Stippec, Elizabeth Goldsmith, Michael A White and Melanie H Cobb (1998). *Curr. Biol.* **8**, 1141–1150.

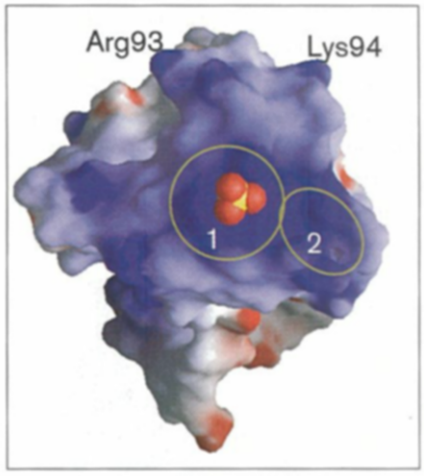
Mitogen-activated protein (MAP) kinases are ubiquitous components of many signal transduction pathways. Constitutively active variants have been isolated for every component of the extracellular-signal-regulated kinase 1 (ERK1) and ERK2 MAP kinase pathway except for the ERK itself. To create an activated ERK2 variant, the authors fused ERK2 to the low activity form of its upstream regulator, the MAP kinase kinase MEK1. The ERK2 in this fusion protein was active in the absence of extracellular signals. Expression of the fusion protein in mammalian cells did not activate endogenous ERK1 or ERK2. It was sufficient, however, to



induce activation of the transcription factors Elk-1 and AP-1, neurite extension in PC12 cells in the absence of nerve growth factor, and foci of morphologically and growth-transformed NIH3T3 cells, if the fusion protein was localized to the nucleus. A cytoplasmic fusion protein had no effect. Activation of ERK2 is sufficient to cause several transcriptional and phenotypic responses in mammalian cells. Nuclear localization of activated ERK2 is required to induce these events.
22 October 1998, Research Paper, *Current Biology*.

☐ **Crystal structure of the NK1 fragment of human hepatocyte growth factor at 2.0 Å resolution.**
Mark Ultsch, Nathalie A Lokker, Paul J Godowski and Abraham M de Vos (1998). *Structure* **6**, 1383–1393.

Hepatocyte growth factor (HGF) is a mitogen for hepatocytes and has also been implicated as an epithelial morphogen in tumor invasion. HGF activates its specific cellular receptor, c-*met*, through an aggregation mechanism potentiated by heparan sulfate glycosaminoglycans. HGF consists of an amino-terminal (N) domain, four kringle domains (the first of which carries receptor-binding determinants), and an inactive serine-protease-like domain. NK1, a naturally occurring fragment of HGF, acts as an antagonist of HGF in the absence of heparin. The N domain of NK1 consists of a central five-stranded antiparallel β sheet flanked by an α helix and a two-stranded β ribbon. The overall N domain structure in the context of the NK1 fragment is similar to the structure of the isolated domain; two lysines and an arginine residue coordinate a bound sulfate ion. The NK1 kringle domain is homologous to kringle 4 from plasminogen, except that the lysine-

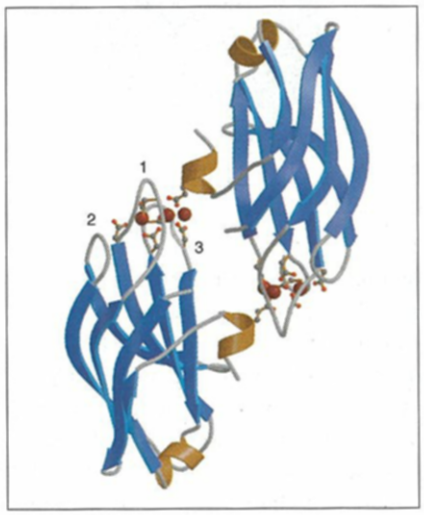


binding pocket is altered by the insertion of a glycine residue. In the structure described here, a HEPES molecule is bound in the pocket. The asymmetric unit of the crystal contains a 'head-to-tail' NK1 dimer. This dimer was used to propose a model of the NK2 fragment of HGF. A cluster of exposed lysine and arginine residues in or near the hairpin-loop region of the N domain might form part of the NK1 heparin-binding site. In the author's NK2 model, both kringle domains pack loosely against the N domain, and a long, positively charged groove lines the interface. This groove might be

involved in glycosaminoglycan binding. The HGF receptor-binding determinants are clustered near the binding pocket of the first kringle domain, opposite the N domain.
15 November 1998, Research Paper, *Structure*.

☐ **Structure of the protein kinase Cβ phospholipid-binding C2 domain complexed with Ca^{2+}.**
R Bryan Sutton and Stephen R Sprang (1998). *Structure* **6**, 1395–1405.

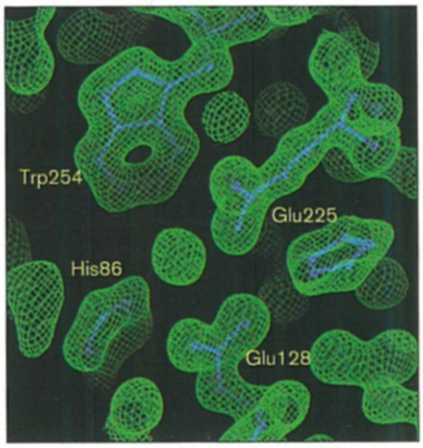
Conventional isoforms (α, β and γ) of protein kinase C (PKC) are synergistically activated by phosphatidylserine and Ca^{2+}; both bind to C2 domains located within the PKC amino-terminal regulatory regions. C2 domains contain a bipartite or tripartite Ca^{2+}-binding site formed by opposing loops at one end of the protein. Neither the structural basis for cooperativity between phosphatidylserine and Ca^{2+}, nor the binding site for phosphatidylserine is known. The structure of the C2 domain from PKCβ complexed with Ca^{2+} and o-phospho-L-serine has been determined. The eight-stranded, Greek key β-sandwich



fold of PKCβ-C2 is similar to that of the synaptotagmin I type I C2 domain. Three Ca^{2+} ions, one at a novel site, were located, each sharing common aspartate ligands. One of these ligands is donated by a dyad-related C2 molecule. A phosphoserine molecule binds to a lysine-rich cluster in C2. Shared ligation among the three Ca^{2+} ions suggests that they bind cooperatively to PKCβ-C2. Cooperativity may be compromised by the accumulation of positive charge in the binding site as successive ions are bound. Model building shows that the C1 domain could provide carboxylate and carbonyl ligands for two of the three Ca^{2+} sites. Ca^{2+}-mediated interactions between the two domains could contribute to enzyme activation as well as to the creation of a positively charged phosphatidylserine-binding site.
15 November 1998, Research Paper, *Structure*.

☐ **High-resolution native and complex structures of thermostable β-mannanase from *Thermomonospora fusca* – substrate specificity in glycosyl hydrolase family 5.**
Mark Hilge, Sergio M Gloor, Wojciech Rypniewski, Oliver Sauer, Tom D Heightman, Wolfgang Zimmermann, Kaspar Winterhalter and Klaus Piontek (1998). *Structure* **6**, 1433–1444.

β-Mannanases hydrolyse the O-glycosidic bonds in mannan, a hemicellulose constituent of plants. These enzymes have potential use in pulp and paper production and are of significant biotechnological interest. Thermostable β-mannanases would be particularly useful due to their high temperature optimum and broad pH tolerance. The thermophilic actinomycete *Thermomonospora fusca* secretes at least one β-mannanase

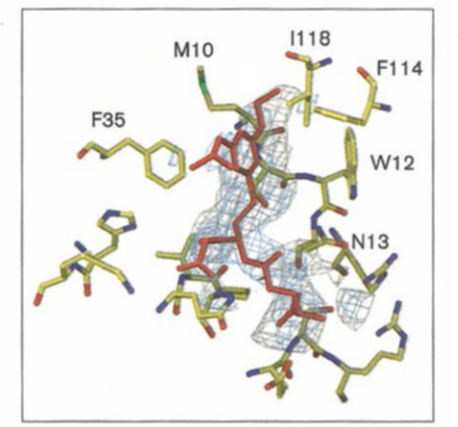


(molecular mass 38 kDa) with a temperature optimum of 80°C. No three-dimensional structure of a mannan-degrading enzyme has been reported until now. The crystal structure of the thermostable β-mannanase from *T. fusca* has been determined. In addition to the native enzyme, the structures of the mannotriose- and mannohexaose-bound forms of the enzyme have been determined. Analysis of the –1 subsite of *T. fusca* mannanase reveals neither a favourable interaction towards the axial HO–C(2) nor a discrimination against the equatorial hydroxyl group of gluco-configurated substrates. The authors propose that selectivity arises from two possible mechanisms: a hydrophobic interaction of the substrate with Val263, conserved in family 5 bacterial mannanases, which discriminates between the different conformations of the hydroxymethyl group in native mannan and cellulose; and/or a specific interaction between Asp259 and the axial hydroxyl group at the C(2) of the substrate in the –2 subsite. Compared with the catalytic clefts of family 5 cellulases, the groove of *T. fusca* mannanase has a strongly reduced number of aromatic residues providing platforms for stacking with the substrate. This deletion of every second platform is in good agreement with the orientation of the axial hydroxyl groups in mannan.
15 November 1998, Research Paper, *Structure*.

☐ **Structures of herbicides in complex with their detoxifying enzyme glutathione S-transferase – explanations for the selectivity of the enzyme in plants.**
Lars Prade, Robert Huber and Babara Bieseler (1998). *Structure* **6**, 1445–1452.

Glutathione S-transferases (GSTs) are detoxifying enzymes present in all aerobic organisms. These enzymes catalyse the conjugation of glutathione with a variety of electrophilic compounds. In plants, GSTs catalyse the first step in the degradation of several herbicides, such as triazines and

acetamides, thus playing an important role in herbicide tolerance. We have solved the structures of GST-I from maize in complex with an atrazine–glutathione conjugate and GST from *Arabidopsis thaliana* (araGST) in complex with an FOE-4053–glutathione conjugate. These ligands are products of the detoxifying reaction and are have well-defined electron density. The herbicide-binding site (H site) is different in the two structures. The architecture of the glutathione-binding site (G site) of araGST is different to that of the previously described



structure of GST in complex with two S-hexylglutathione molecules, but is homologous to that of GST-I. Three features are responsible for the differences in the H site of the two GSTs described here: the exchange of hydrophobic residues of different degrees of bulkiness; a slight difference in the location of the H site; and a difference in the degree of flexibility of the upper side of the H site, which is built up by the loop between helices α4 and α5. Taking these two structures as a model, the different substrate specificities of other plant GSTs may be explained. The structures reported here provide a basis for the design of new, more selective herbicides.

15 November 1998, Research Paper
Structure.