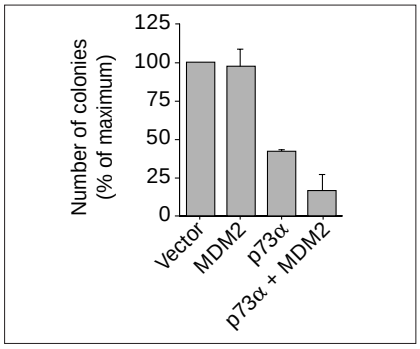
Elsewhere in biology

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

Chemistry & Biology October 1999, 6:R286–R289

☐ **MDM2 and MDMX bind and stabilize the p53-related protein p73**
Weg M Ongkeko, Xiao Qi Wang, Wai Yi Siu, Anita WS Lau, Katsumi Yamashita, Adrian L Harris, Lynne S Cox, Randy YC Poon (1999). *Curr. Biol.* 9, 829–832.

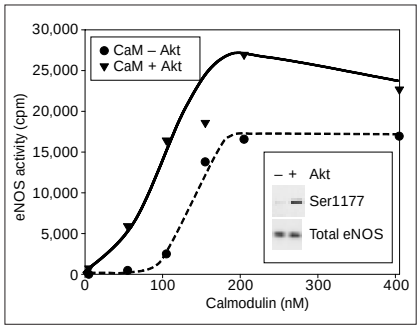
The p53 gene encodes one of the most important tumor suppressors in human cells and undergoes frequent mutational inactivation in cancers. MDM2,



a transcriptional target of p53, binds p53 and can both inhibit p53-mediated transcription and target p53 for proteasome-mediated proteolysis. A close relative of p53, p73, has recently been identified. The authors report that, like p53, p73α and the alternative transcription product p73β also bind MDM2. Interaction between MDM2 and p53 represents a key step in the regulation of p53, as MDM2 promotes the degradation of p53. In striking contrast to p53, the half-life of p73 was found to be increased by binding to MDM2. Like MDM2, the MDM2-related protein MDMX also bound p73 and stabilized the level of p73. Moreover, the growth suppression functions of p73 and the induction of endogenous p21, a major mediator of the p53-dependent growth arrest pathway, were enhanced in the presence of MDM2. These differences between the regulation of p53 and p73 by MDM2/MDMX may highlight a physiological difference in their action.
26 July 1999, Brief Communication, *Current Biology*.

☐ **The Akt kinase signals directly to endothelial nitric oxide synthase**
BJ Michell, JE Griffiths, KI Mitchelhill, I Rodriguez-Crespo, T Tiganis, S Bozinovski, PR Ortiz de Montellano, BE Kemp, RB Pearson (1999). *Curr. Biol.* 9, 845–848.

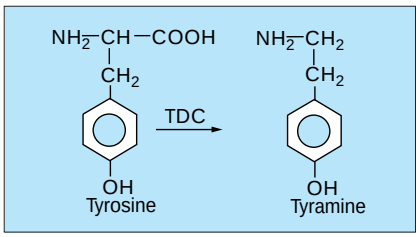
Endothelial nitric oxide synthase (eNOS) is an important modulator of angiogenesis and vascular tone. It is stimulated by treatment of endothelial cells in a phosphatidylinositol 3-kinase (PI 3-kinase)-dependent fashion by insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) and is activated by phosphorylation at Ser1177 in the sequence RIRTQS^{1177}F. The protein



kinase Akt is an important downstream target of PI 3-kinase, regulating VEGF-stimulated endothelial cell survival. Akt phosphorylates substrates within a defined motif, which is present in the sequence surrounding Ser1177 in eNOS. Both Akt and eNOS are localized to, and activated at, the plasma membrane. The authors found that purified Akt phosphorylated cardiac eNOS at Ser1177, resulting in activation of eNOS. Phosphorylation at this site was stimulated by treatment of bovine aortic endothelial cells (BAECs) with VEGF or IGF-1, and Akt was activated in parallel. Preincubation with wortmannin, an inhibitor of Akt signalling, reduced VEGF- or IGF-1-induced Akt activity and eNOS phosphorylation. Akt was detected in immunoprecipitates of eNOS from BAECs, and eNOS in immunoprecipitates of Akt, indicating that the two enzymes become associated *in vivo*. These results strongly suggest that Akt has an important role in the regulation of normal angiogenesis and raise the possibility that the enhanced activity of this kinase that occurs in carcinomas might contribute to tumor vascularization and survival.
26 July 1999, Brief Communication, *Current Biology*.

☐ **The trace amine tyrosine is essential for sensitization to cocaine in *Drosophila***
Colleen McClung, Jay Hirsch (1999). *Curr. Biol.* 9, 853-860.

Sensitization to psychostimulant drugs of abuse is thought to be an important aspect of human addiction, yet how it develops is still unclear. How sensitization to cocaine develops in the the fruit fly *Drosophila melanogaster* is strikingly similar to that observed in vertebrates. By taking advantage of the powerful genetic approaches that are possible in Drosophila, the authors were able to identify and characterize mutants that fail to develop sensitization. They found that the Drosophila mutant *inactive* (*iav*) failed to become sensitized to cocaine. Mutant

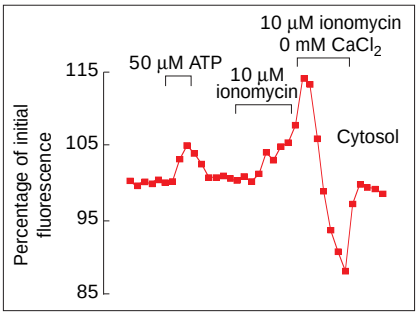


flies had reduced amounts of the trace amine tyramine in the brain because of reduced activity of the enzyme tyrosine decarboxylase (TDC), which converts tyrosine to tyramine. Furthermore, cocaine exposure induced TDC enzyme

activity in a time-dependent manner that paralleled the development of behavioral sensitization. The sensitization failure of *iav* flies could be rescued by feeding the flies with tyramine; other biogenic amines or amine precursors did not have the same effect. These results indicate an essential role for tyramine in cocaine sensitization in Drosophila.
30 July 1999, Research Paper, *Current Biology*.

☐ **Imaging Ca^{2+} concentration changes at the secretory vesicle surface with a recombinant targeted cameleon**
Evaggelia Emmanouilidou, Anja G Teschemacher, Aristea E Pouli, Linda I Nicholls, Elizabeth P Seward, Guy A Rutter (1999). *Curr. Biol.* 9, 915–918.

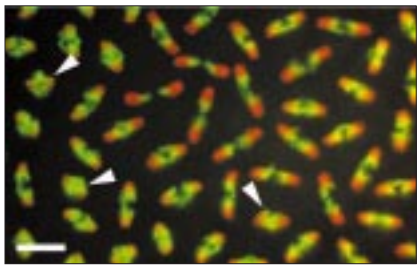
Regulated exocytosis involves the Ca^{2+}-triggered fusion of secretory vesicles with the plasma membrane, by activation of vesicle membrane Ca^{2+}-binding proteins. The Ca^{2+}-binding sites of these proteins are likely to lie within 30 nm of the vesicle surface, a domain in which changes in Ca^{2+} concentration cannot be resolved by conventional fluorescence microscopy. A fluorescent indicator for Ca^{2+} called a yellow 'cameleon' (Ycam2) — comprised of a fusion between a cyan-emitting mutant of the



green fluorescent protein (GFP), calmodulin, the calmodulin-binding peptide M13 and an enhanced yellow-emitting GFP — which can be targetted to specific intracellular locations, has been described previously. Here, the authors generated a fusion between phogrin, a protein that is localised to secretory granule membranes, and Ycam2 (phogrin–Ycam2) to monitor changes in Ca^{2+} concentration ($[Ca^{2+}]$) at the secretory vesicle surface ($[Ca^{2+}]_{gd}$) through alterations in fluorescence resonance energy transfer (FRET) between the linked cyan and yellow fluorescent proteins (CFP and YFP, respectively) in Ycam2. In both neuroendocrine PC12 and MIN6 pancreatic β cells, apparent resting values of cytosolic $[Ca^{2+}]$ and $[Ca^{2+}]_{gd}$ were similar throughout the cell. In MIN6 cells following the activation of Ca^{2+} influx, the minority of vesicles that were within ~1 µm of the plasma membrane underwent increases in $[Ca^{2+}]_{gd}$ that were significantly greater than those experienced by deeper vesicles, and greater than the apparent cytosolic $[Ca^{2+}]$ change. The ability to image both global and compartmentalised $[Ca^{2+}]$ changes with recombinant targeted cameleons should extend the usefulness of these new Ca^{2+} probes.
16 August 1999, Brief Communication, *Current Biology*.

☐ ***Drosophila grapes*/CHK1 mutants are defective in cyclin proteolysis and coordination of mitotic events**
Tin Tin Su, Shelagh D Campbell, Patrick H O'Farrell (1999). *Curr. Biol.* 9, 919–922.

The *Drosophila grapes* (*grp*) gene, which encodes a homolog of the *Schizosaccharomyces pombe* Chk1 kinase, provides a cell-cycle checkpoint that delays mitosis in response to inhibition of DNA replication. Grp is also required in the undisturbed early embryonic cycles: in its absence, mitotic abnormalities appear in cycle 12 and chromosomes fail to fully separate in subsequent cycles. In other systems, Chk1 kinase phosphorylates and suppresses the activity of Cdc25 phosphatase: the resulting failure to remove inhibitory phosphate from cyclin-dependent kinase 1 (Cdk1) prevents entry into mitosis. Because in *Drosophila* embryos Cdk1 lacks inhibitory phosphate during cycles 11–13, it is not clear that known actions of Grp/Chk1 suffice in these cycles. The authors found that the loss of *grp*

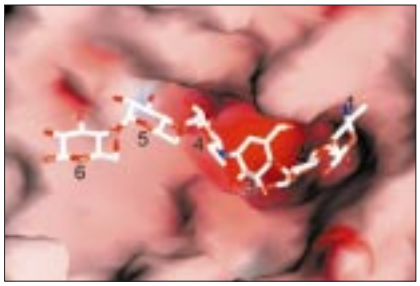


compromised cyclin A proteolysis and delayed mitotic disjunction of sister chromosomes. These defects occurred before previously reported *grp* phenotypes. The authors conclude that Grp activates cyclin A degradation, and functions to time the disjunction of chromosomes in the early embryo. As cyclin A destruction is required for sister chromosome separation, a failure in Grp-promoted cyclin destruction can also explain the mitotic phenotype. The mitotic failure described previously for cycle 12 *grp* embryos might be a more severe form of the phenotypes that we describe in earlier embryos and we suggest that the underlying defect is reduced degradation of cyclin A.
16 August 1999, Brief Communication, *Current Biology*.

☐ **Specific inhibition of insect α-amylases: yellow meal worm α-amylase in complex with the *Amaranth* α-amylase inhibitor at 2.0 Å resolution**
Pedro José Barbosa Pereira, Valentin Lozanov, András Patthy, Robert Huber, Wolfram Bode, Sándor Pongor, Stefan Strobl (1999). *Structure* 7, 1079–1088.

α-Amylases constitute a family of enzymes that catalyze the hydrolysis of α-D-(1,4)-glucan linkages in starch and related polysaccharides. The *Amaranth* α-amylase inhibitor (AAI) specifically inhibits insect α-amylases. AAI is the smallest proteinaceous α-amylase inhibitor described so far and has no known homologs in the sequence databases. Its mode of inhibition of α-amylases was unknown until now. The crystal structure of yellow meal worm α-amylase (TMA) in complex with AAI was determined. The overall fold of AAI identifies it as a knottin-like protein. The inhibitor binds to the

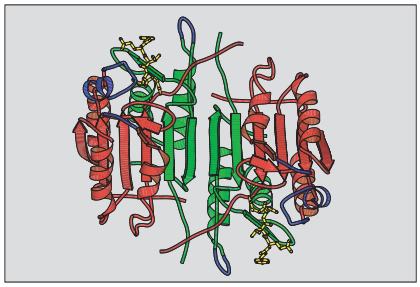
active-site groove of TMA, blocking the central four sugar-binding subsites. The binding of AAI to TMA presents a new inhibition mode for α-amylases. Because of its unique specificity towards insect α-amylases, AAI could be a valuable tool for protecting crop plants against predatory insects. The



close structural homology between AAI and 'knottins' opens new perspectives for the engineering of various novel activities onto the small scaffold of this group of proteins.
26 August 1999, Research Paper, *Structure*.

☐ **The three-dimensional structure of caspase-8: an initiator enzyme in apoptosis**
Helen Blanchard, Lalitha Kodandapani, Peer RE Mittl, Stefania Di Marco, Joseph F Krebs, Joe C Wu, Kevin J Tomaselli, Markus G Grütter (1999). *Structure* 7, 1125–1133.

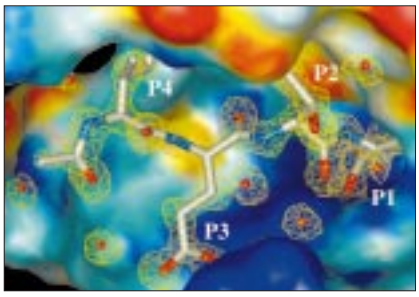
In the initial stages of Fas-mediated apoptosis the cysteine protease caspase-8 is recruited to the cell receptor as a zymogen (procaspase-8) and is incorporated into the death-signalling complex. Procaspase-8 is subsequently activated leading to a cascade of proteolytic events, ultimately resulting in cell death. Variations in the substrate specificity of different caspases have been reported. The authors report here the crystal structure of a complex of the activated human caspase-8 (proteolytic domain) with the irreversible peptidic inhibitor Z-Glu–Val–Asp-dichloromethylketone. This is the first structure of a representative of the long prodomain initiator caspases and of the group III substrate specificity class. The overall protein architecture resembles the caspase-1 and caspase-3 folds, but shows distinct structural differences in regions forming the active site. In particular, differences observed in subsites S_3, S_4 and the loops involved in inhibitor interactions explain the preference of caspase-8 for substrates with the sequence (Leu/Val)–Glu–X–Asp. The structural differences were correlated with the observed substrate specificities of caspase-1, caspase-3 and caspase-8, as determined from kinetic experiments. This information will help us to



understand the role of the various caspases in the propagation of the apoptotic signal, and should be useful for the design of specific inhibitors.
27 August 1999, Research Paper, *Structure*.

☐ **The atomic-resolution structure of human caspase-8, a key activator of apoptosis**
William Watt, Kenneth A Koeplinger, Ana M Mildner, Robert L Heinrikson, Alfredo G Tomasselli, Keith D Watenpaugh (1999). *Structure* 7, 1135–1143.

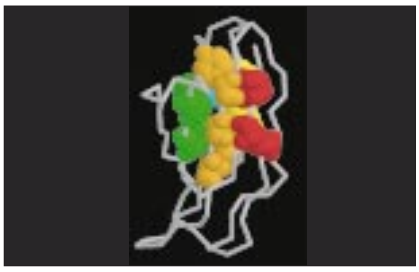
Caspases are a family of cysteine proteases that play important intracellular roles in inflammation and apoptosis. Caspase-8 activates downstream caspases that are unable to carry out autocatalytic processing and activation. Caspase-8 is designated an initiator caspase and is believed to sit at the apex of the Fas- or TNF-mediated apoptotic cascade. In light of this role, the enzyme is an attractive target for the design of inhibitors aimed at blocking the undesirable cell death associated with a range of degenerative disorders. The structure of recombinant human caspase-8, covalently modified with the



inhibitor acetyl-Ile–Glu–Thr–Asp-aldehyde, has been determined. The asymmetric unit contains the p18–p11 heterodimer; the biologically important molecule contains two dimers. The overall fold is very similar to that of caspase-1 and caspase-3, but significant differences exist in the substrate-binding region. The structure answers questions about the enzyme–inhibitor complex that could not be explained from earlier caspase structures solved at lower resolution. The caspase-8–inhibitor structure provides the basis for understanding structure/function relationships in this important initiator of the proteolytic cascade that leads to programmed cell death.
27 August 1999, Research Paper, *Structure*.

☐ **Folding studies of immunoglobulin-like β-sandwich proteins suggest that they share a common folding pathway**
Jane Clarke, Ernesto Cota, Susan B Fowler, Stefan J Hamill (1999). *Structure* 7, 1145–1153.

Are folding pathways conserved in protein families? To test this explicitly and ask to what extent structure



specifies folding pathways requires comparison of proteins with a common fold. The authors chose to examine members of a highly diverse protein family with no conservation of function

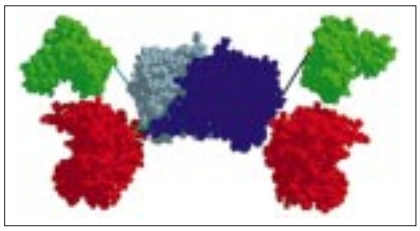
and little or no sequence identity, but with structures that are essentially the same. The immunoglobulin-like fold is one of the most common structural families, and is subdivided into superfamilies with no detectable evolutionary or functional relationship. The folding of a number of immunoglobulin-like proteins that have a common structural core were compared and a strong correlation between folding rate and stability was found. The results suggest that the folding pathways of these immunoglobulin-like proteins share common features. This study is the first to compare the folding of structurally related proteins that are members of different superfamilies. The most likely explanation for the results is that interactions that are important in defining the structure of immunoglobulin-like proteins are also used to guide folding.

31 August 1999, Research Paper, *Structure*.

☐ **X-ray crystal structure of aminoimidazole ribonucleotide synthetase (PurM), from the *Escherichia coli* purine biosynthetic pathway at 2.5 Å resolution**

Chenglong Li, T Joseph Kappock, JoAnne Stubbe, Todd M Weaver, Steven E Ealick (1999). *Structure* 7, 1155–1166.

Eleven enzymes are involved in the procaryotic purine biosynthetic pathway, six of which use ATP. Enzymes 5 and 6 of this pathway, formylglycinamide ribonucleotide (FGAR) amidotransferase (PurL) and aminoimidazole ribonucleotide (AIR) synthetase (PurM) use ATP to activate the oxygen of an amide within their substrate toward nucleophilic attack by a nitrogen. AIR synthetase uses the product of PurL, formylglycinamidine ribonucleotide (FGAM) and ATP to make AIR, ADP and P_i. The structure of a hexahistidine-tagged PurM has been solved, and the final model of PurM consists of two crystallographically independent dimers and four sulfates. The active site,



identified in part by conserved residues, is proposed to be a long groove generated by the interaction of two monomers. A search of the sequence databases suggests that the ATP-binding sites between PurM and PurL may be structurally conserved. Sequence searches suggest that two successive enzymes in the purine biosynthetic pathway, proposed to use similar chemistries, will have similar ATP-binding domains.

1 September 1999, Research Paper, *Structure*.