Elsewhere in 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. Volume 2 Issue 1 covers Interaction, assembly and processing (edited by Peter S Kim and Alanna Schepartz) and Biocatalysis and biotransformation (edited by J Bryan Jones and Chi-Huey Wong) and is published in February.

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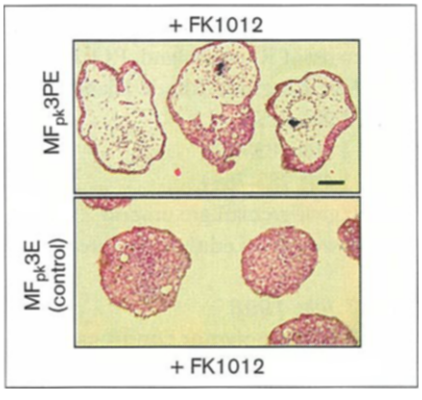
A selection of interesting papers published last month in *Chemistry & Biology*'s sister journals, *Current Biology*, *Folding & Design* and *Structure*, chosen and summarized by the staff of *Chemistry & Biology*.

Chemistry & Biology February 1998, 5:R44–R48

☐ **Small-molecule control of insulin and PDGF receptor signaling and the role of membrane attachment.**
Jian-xin Yang, Karen Symes, Mark Mercola and Stuart L Schreiber (1997). *Curr. Biol.* **8**, 11–18.

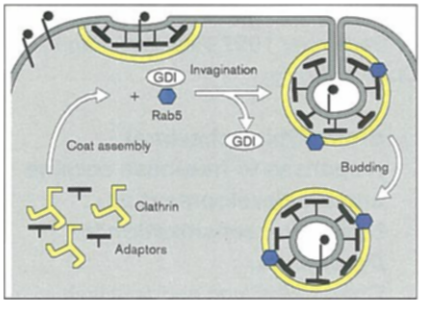
Receptor tyrosine kinases (RTKs) regulate the proliferation, differentiation and metabolism of cells, and play key roles in tissue repair, tumorigenesis and development. To facilitate the study of RTKs, the authors have made conditional alleles that encode monomeric forms of the normally



heterotetrameric insulin receptor and monomeric platelet-derived growth factor (PDGF) β receptors fused to the FK506-binding protein 12 (FKBP12). The chimeric receptors can be induced to undergo dimerization or oligomerization by a small synthetic molecule called FK1012, and the consequences were studied in cells and embryonic tissues. When equipped with an amino-terminal plasma membrane localization sequence and expressed in HEK293 cells, these chimeric receptors could signal to downstream targets as indicated by the FK1012-dependent activation of p70 S6 kinase (p70S6k) and mitogen-activated protein (MAP) kinase. In *Xenopus* embryos, the engineered PDGF receptor protein induced the formation of mesoderm from animal-pole explants in an FK1012-dependent manner. A cytosolic variant of the protein underwent efficient transphosphorylation, yet failed to activate appreciably either $p70^{S6k}$ or MAP kinase following treatment with FK1012. These results provide evidence of a requirement for membrane localization of RTKs. This system should allow the further dissection of RTK-mediated pathways.
20 November 1997, Research Paper, *Current Biology*.

☐ **A novel role for Rab5-GDI in ligand sequestration into clathrin-coated pits.**
Hilary McLauchlan, Jane Newell, Nick Morrice, Andrew Osborne, Michele West and Elizabeth Smythes (1997). *Curr. Biol.* **8**, 34–45.

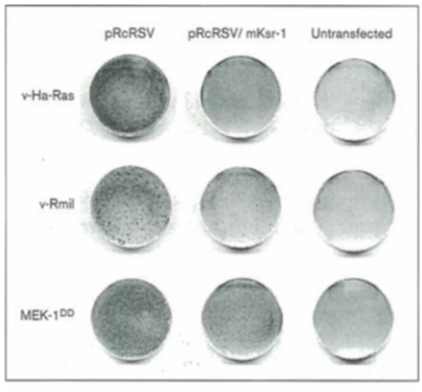
Clathrin-coated pits are formed at the plasma membrane by the assembly of the coat components: clathrin and adaptors from the cytosol. Little is known about the regulation and mechanism of this assembly process. The authors have used



an *in vitro* assay for clathrin-coated pit assembly to identify a novel component required for the invagination of newly formed coated pits. They identified it as a complex of Rab5 and GDI (guanine-nucleotide dissociation inhibitor) that was previously demonstrated to be involved in downstream processing of endocytic vesicles. They demonstrated that the Rab5–GDI complex is required for ligand sequestration into clathrin-coated pits. Given the well-established role of Rab5 in the fusion of endocytic vesicles with endosomes, the results suggest that recruitment of essential components of the targeting and fusion machinery is coupled to the formation of functional transport vesicles.
4 December 1997, Research Paper, *Current Biology*.

☐ **Murine Ksr interacts with MEK and inhibits Ras-induced transformation.**
A Denouel-Galy, EM Douville, PH Warne, C Papin, D Laugier, G Calothy, J Downward and A Eychènes (1997). *Curr. Biol.* **8**, 45–55.

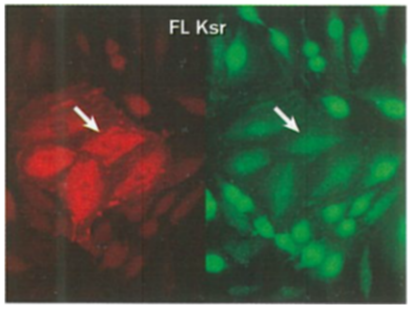
Ksr (kinase supressor of Ras) was identified as a regulator of the Ras–MAP kinase (mitogen-activated protein kinase) pathway by genetic screens in *Drosophila* and *Caenorhabditis elegans*. Ksr is a kinase with similarities to the three conserved regions of Raf kinases, especially within the kinase domain. To investigate whether these structural similarities correlated with common functional properties, the authors examined the ability of mKsr-1, the murine homolog of Ksr, to interact with components of the vertebrate MAP kinase pathway. In the yeast two-hybrid interaction assay, mKsr-1 did not bind to either Ras, B-Raf or Raf-1, but interacted strongly with both MEK-1 and MEK-2, activators of MAP kinase. Ectopically expressed mKsr-1 co-precipitated with endogenous MEK-1 in COS-1 cells, and endogenous Ksr and MEK co-precipitated from PC12 cells.



Phosphorylation of MEK by mKsr-1 was not detected, however. In contrast, the MEK subpopulation complexed with mKsr-1 in COS-1 cells or PC12 cells did not display kinase activity. The ability of Ksr to block MEK in an inactive form correlated with a biological response: mKsr-1 did not transform NIH3T3 cells, and, furthermore, mKsr-1 reduced Ras-induced transformation. Similarly, mKsr-1 inhibited the proliferation of embryonic neuroretina cells induced by Ras and B-Raf but not that induced by MEK. The results suggest a novel mechanism for Ksr in regulating the MAP kinase pathway, at least in part through an ability to interact with MEK.
4 December 1997, Research Paper, *Current Biology*.

☐ **Regulation of the MAP kinase pathway by mammalian Ksr through direct interaction with MEK and ERK.**
Wei Yu, Wendy J Fantl, Greg Harrowe and Lewis T Williams (1997). *Curr. Biol.* **8**, 56–64.

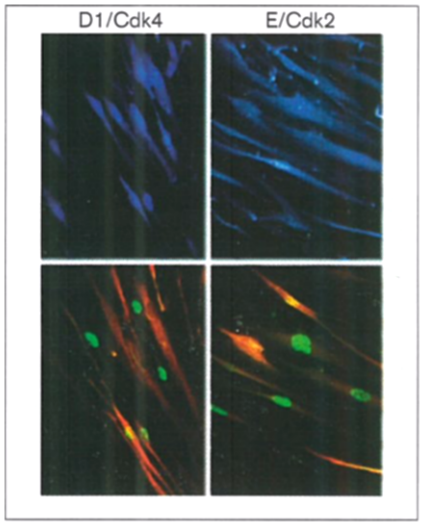
Genetic screens in *Drosophila melanogaster* and *Caenorhabditis elegans* identified the kinase suppressor of Ras, Ksr, as a new component in the Ras intracellular signaling pathway. In these organisms, mutations in Ksr resulted in attenuation of Ras-mediated signaling. Homologs of Ksr have also been isolated from mice and humans; their precise role in Ras signaling is not well defined. The authors present data showing interactions between the murine form of Ksr (mKsr-1) and other components of the Ras pathway. They found an interaction



between the carboxy-terminal region of mKsr-1 and mitogen-activated protein (MAP) kinase kinase 1 (MAPKK-1 or MEK-1). An interaction was also detected between MAP kinase (also called extracellular signal-regulated kinase; ERK), and the amino-terminal region of mKsr-1. When COS-7 cells were transfected with either full-length mKsr-1 or only its carboxy-terminal region, an inhibition of serum-stimulated MAP kinase activation was observed. Microinjection of full-length mKsr-1 or its carboxy-terminal blocked serum-induced DNA synthesis in rat embryo fibroblasts. Co-injection of mKsr-1 with MEK-1 reversed the blockade. The authors propose that mKsr-1 may control MAP kinase signaling by serving as a scaffold protein that links MEK and its substrate ERK.
4 December 1997, Research Paper, *Current Biology*.

☐ **G1 cyclin-dependent kinases are sufficient to initiate DNA synthesis in quiescent human fibroblast.**
Lisa Connell-Crowley, Stephen J Elledge and J Wade Harper (1997). *Curr. Biol.* 8, 65–68.

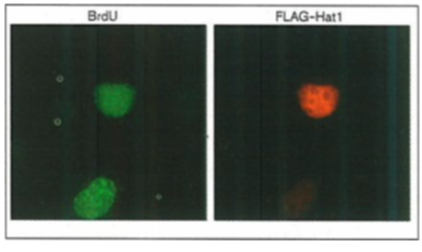
Mammalian fibroblasts require mitogens in order to exit from G0 (quiescence) and progress through the G1 phase of the cell cycle, although once they pass the restriction point late in G1 they can enter S phase and complete the cell cycle without mitogens. Mitogenic signals are integrated through the



GTPase Ras, which regulates the levels of cyclin D1, a component of the cell cycle machinery that operates during G1 phase by activating cyclin-dependent kinase 4 (Cdk4). The accumulation of active cyclin E-Cdk2 complexes also requires Ras. These two G1 cyclin-Cdk complexes act on a family of E2F-associated transcriptional repressors typified by the retinoblastoma protein (Rb) to bring about a transcriptional program that promotes passage through S phase, but can also activate DNA replication independently of Rb-E2F. Although G1 cyclin-Cdk complexes are required for S-phase entry and can shorten G1 phase when overexpressed, it is not known whether they are sufficient for this transition. Here, the authors report that serum-starved (G0) diploid human fibroblasts initiate DNA synthesis upon microinjection of active G1 cyclin-Cdk complexes, but not upon microinjection of an S-phase cyclin-Cdk complex. These data indicate that G1 Cdk activation is rate-limiting for S-phase entry, and that Cdk activation is likely to be the primary function of growth factor signalling pathways that lead to DNA synthesis.
1 January 1998, Brief Communication, *Current Biology*.

☐ **Nucleosomal DNA regulates the core-histone-binding subunit of the human Hat1 acetyltransferase.**
Alain Verreault, Paul D Kaufman, Ryuji Kobayashi and Bruce Stillman (1998). *Curr. Biol.* **8**, 96–108.

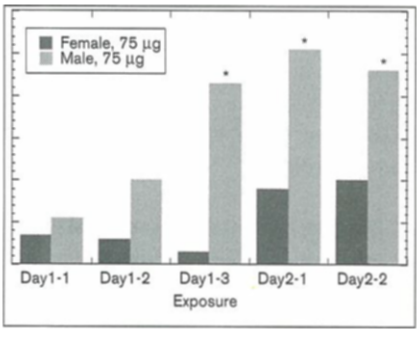
In eukaryotic cells, newly synthesized histone H4 is acetylated at residues Lys5 and Lys12, a transient modification erased by deacetylases shortly after deposition of histones into chromosomes. Genetic studies in *Saccharomyces cerevisiae* revealed that acetylation of newly synthesized histones H3 and H4 is likely to be important for maintaining cell viability; the precise biochemical function of this acetylation is not known, however. The identification of enzymes mediating site-specific acetylation of H4



at Lys5 and Lys12 may help explain the function of the acetylation of newly synthesized histones. A cDNA encoding the catalytic subunit of the human Hat1 acetyltransferase was cloned and the Hat1 holoenzyme was purified from human 293 cells. The human enzyme acetylates soluble but not nucleosomal H4 at Lys5 and Lys12 and acetylates histone H2A at Lys5. Unexpectedly, Hat1 was found in the nucleus of S-phase cells. Like its yeast counterpart, the human holoenzyme consists of two subunits: a catalytic subunit, Hat1, and a subunit that binds core histones, p46, which greatly stimulates the acetyltransferase activity of Hat1. Both p46 and the highly related p48 polypeptide (the small subunit of human chromatin assembly factor 1; CAF-1) bind directly to helix 1 of histone H4, a region that is not accessible when H4 is in chromatin. The authors suggest that p46 and p48 are core-histone-binding subunits that target chromatin assembly factors, chromatin remodeling factors, histone acetyltransferases and histone deacetylases to their histone substrates in a manner that is regulated by nucleosomal DNA.
17 December 1997, Research Paper, *Current Biology*.

☐ **Stereotypic behavioral responses to free-base cocaine and the development of behavioral sensitization in *Drosophila*.**
Colleen McClung and Jay Hirsh (1998). *Curr. Biol.* 8, 109–112.

Cocaine abuse is a large social and economic problem that has received much public and scientific attention in recent years. Rodent and primate models have been used to study the behavioral and neurological effects of cocaine. Repeated intermittent doses of cocaine lead to progressive increases in both locomotor activity and stereotyped behaviors known as 'reverse tolerance' or behavioral sensitization, which may model the behavioral and neurochemical processes occurring in cocaine-addicted

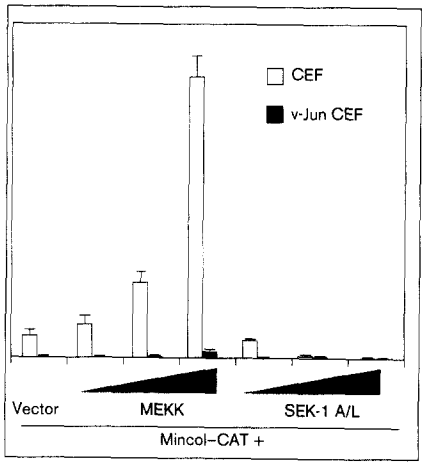


humans. The biological basis of sensitization is poorly understood. The authors report that free-base cocaine administered in volatile form to the fruit fly *Drosophila melanogaster* induces

multiple reflexive motor responses that resemble cocaine-induced behaviors in rodents. These behaviors are both dose dependent and sexually dimorphic. Furthermore, *Drosophila* develops a behavioral sensitization to intermittent doses of cocaine. These results suggest that the pathways leading to cocaine-induced responses and sensitization are evolutionarily conserved between *Drosophila* and higher vertebrates, and that this genetically tractable animal can be used as a new model system to help determine the biological mechanisms underlying these processes.
15 January 1998, Brief Communication, *Current Biology*.

☐ **An oncogenic mutation uncouples the v-Jun oncoprotein from positive regulation by the SAPK/JNK pathway *in vivo*.**
GHW May, M Funk, E J Black, W Clark, S Hussain, JR Woodgett and DAF Gillespie (1998). *Curr. Biol.* **8**, 117–120.

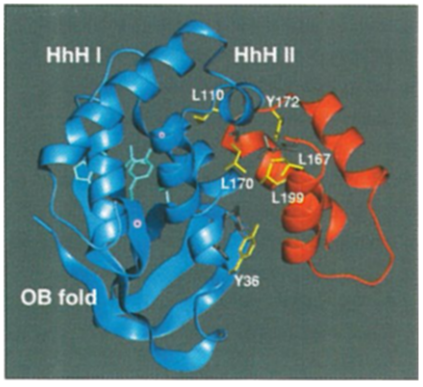
Stimulation of c-Jun transcriptional activity via phosphorylation mediated by the stress-activated or c-Jun amino-terminal (SAPK/JNK) subgroup of mitogen-activated protein kinases (MAP



kinases) is thought to depend on a kinase-docking site (the delta region) within the amino-terminal activation domain, which is deleted from the oncogenic derivative, v-Jun. This mutation markedly enhances v-Jun oncogenicity but its transcriptional consequences have not been resolved. Using a two-hybrid approach, the authors show that SAPK/JNK stimulates c-Jun transactivation in yeast and that this depends on both catalytic activity and physical interaction between the kinase and its substrate. Furthermore, c-Jun is active when tethered to DNA via SAPK/JNK, demonstrating that kinase binding does not preclude transactivation. Taken together, these results suggest that SAPK/JNK acts primarily as a positive regulator of c-Jun transactivation *in situ*, and that loss of the docking site physically uncouples v-Jun from this control. This loss-of-function model accounts for the deficit of v-Jun regulatory phosphorylation and repression of TPA response element (TRE)-dependent transcription observed in v-Jun-transformed cells and predicts that an important property of the oncoprotein is to antagonise SAPK/JNK-dependent gene expression.
15 January 1998, Brief Communication, *Current Biology*.

☐ **Functional analyses of the domain structure in the Holliday junction binding protein RuvA.**
Tatsuya Nishino, Mariko Ariyoshi, Hiroshi Iwasaki, Hideo Shinagawa and Kosuke Morikawas (1997). *Structure* **6**, 11–22.

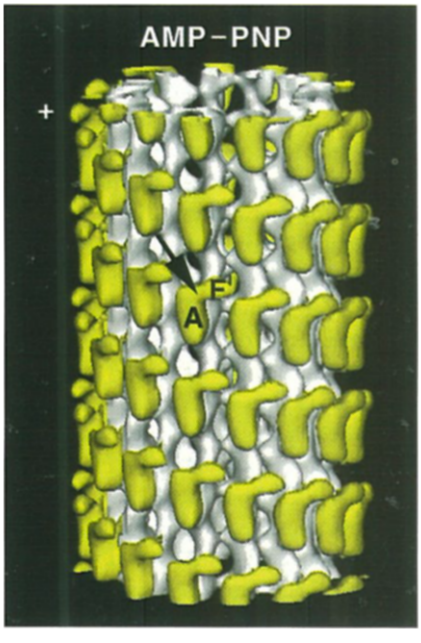
Homologous recombination is essential for genetic diversity and repairing damaged chromosomes. In *Escherichia coli* cells, the RuvA, RuvB and RuvC proteins participate in the processing of an important intermediate, the Holliday junction. The RuvA–RuvB protein complex facilitates branch migration of the junction, depending on ATP hydrolysis. The authors report the crystal structure of RuvA. The RuvA molecule is formed by four identical subunits, each with three domains, I, II and III. The locations of the putative DNA-binding motifs define an interface between the DNA and the Holliday junction. Domain III is weakly attached to the core region, comprising domains I and II; the core domains can form a tetramer in the absence of domain III. Functional analyses of the mutant proteins and the partial digestion products, including Holliday junction binding and branch-



migration assays, revealed that domain III and the preceding loop are essential for RuvB binding and branch migration, although this region is not required for the junction–DNA binding.
15 January 1998, Research Paper, *Structure*.

☐ **Nucleotide-dependent conformations of the kinesin dimer interacting with microtubules.**
Isabelle Arnal and Richard H Wade (1998). *Structure* **6**, 33–38.

Kinesins are a superfamily of motor proteins that use ATP hydrolysis to move along microtubules. Many of these motors are heterotetramers with two

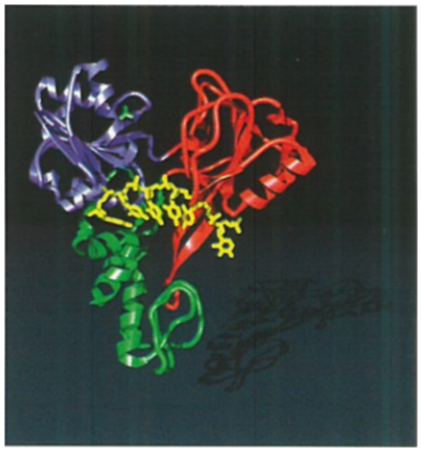


heavy and two light chains. The heavy chain has a globular motor domain that interacts with microtubules and shows a

similar sequence throughout the family. Compared with myosin and dynein, kinesin provides a 'simple' model for understanding molecular motors. Electron cryomicroscopy and three-dimensional reconstruction methods have been used to investigate microtubule–kinesin dimer complexes in different nucleotide states. Three-dimensional maps were obtained in the presence of AMP–PNP, ADP–AlF_4, ADP and apyrase. In all cases, kinesin has one attached and one free head per tubulin heterodimer. The attached heads appear very similar whereas the free heads show distinct conformations and orientations depending on their nucleotide states. The kinesin dimer probably undergoes considerable conformational changes during its ATP hydrolysis cycle. In all nucleotide states, the kinesin dimer attaches to a microtubule using one motor domain with the other motor domain hanging free. Only the free domain changes conformation in the presence of different nucleotides, suggesting that it, or the region linking both motor domains to the coiled coil, is the determinant of directionality. These results give some structural clues as to how kinesin moves along microtubules.
15 January 1998, Research Paper, *Structure*.

☐ **Hepatitis C virus NS3 RNA helicase domain with a bound oligonucleotide: the crystal structure provides insights into the mode of unwinding.**
Joseph L Kim, Kurt A Morgenstern, James P Griffith, Maureen D Dwyer, John A Thomson, Mark A Murcko, Chao Lin and Paul R Caron (1998). *Structure* **6**, 89–100.

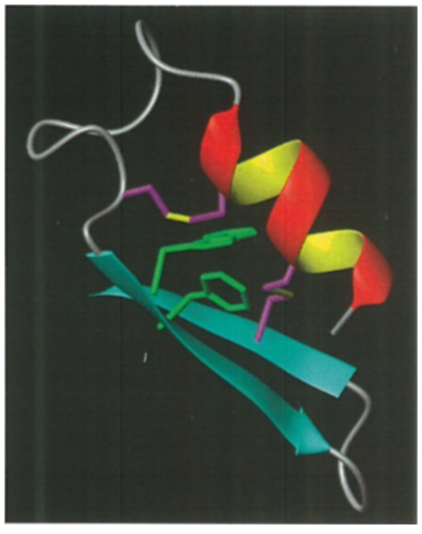
Hepatitis C virus (HCV) represents a major health concern as it is responsible for a significant number of hepatitis cases worldwide. Much research has focused on the replicative enzymes of HCV as possible targets for more effective therapeutic agents. HCV NS3 helicase may provide one such suitable target. Helicases are enzymes that can unwind double-stranded regions of DNA or RNA in an ATP-dependent reaction. The structures of several helicases have been published but the structural details as to how ATP binding and hydrolysis are coupled to DNA unwinding are



unknown. In this paper, the structure of the HCV NS3 RNA helicase domain complexed with a single-stranded DNA oligonucleotide has been solved. The protein consists of three structural domains with the oligonucleotide lying in a groove between the first two domains and the third. The first two domains have an adenylate kinase like fold, including a phosphate-binding loop in the first domain. HCV NS3 helicase is a member of a superfamily of helicases, termed superfamily II. Residues of NS3 helicase which are conserved among superfamily II helicases line an interdomain cleft between the first two domains. The oligonucleotide binds in an orthogonal binding site and contacts relatively few conserved residues. There are no strong sequence-specific interactions with the oligonucleotide bases.
15 January 1998, Research Paper, *Structure*.

☐ **The solution structure of the N-terminal domain of hepatocyte growth factor reveals a potential heparin-binding site.**
Hongjun Zhou, Marie J Mazzulla, Joshua D Kaufman, Stephen J Stahl, Paul T Wingfield, Jeffrey S Rubin, Donald P Bottaro and R Andrew Byrd (1997). *Structure* **6**, 109–116.

Hepatocyte growth factor (HGF) is a multipotent growth factor that transduces a wide range of biological signals, including mitogenesis, motogenesis, and morphogenesis. The amino-terminal (N) domain of HGF, containing a hairpin–loop region, is important for receptor binding and the potent biological activities of HGF. The N domain is also the primary binding site for heparin or heparan sulfate, which enhances receptor/ligand oligomerization and modulates receptor-dependent mitogenesis. The rational design of artificial modulators of HGF signaling requires a detailed understanding of the



structures of HGF and its receptor, as well as the role of heparin proteoglycan. The authors report a high-resolution solution structure of the N domain of HGF. This first structure of HGF reveals a novel folding topology with a distinct pattern of charge distribution and indicates a possible heparin-binding site. The hairpin-loop region of the N domain plays a major role in stabilizing the structure and contributes to a putative heparin-binding site, which explains why it is required for biological functions. These results suggest several basic and/or polar residues that may be important for use in further mutational studies of heparin binding.
15 January 1998, Research Paper, *Structure*.