# Elsewhere in biology

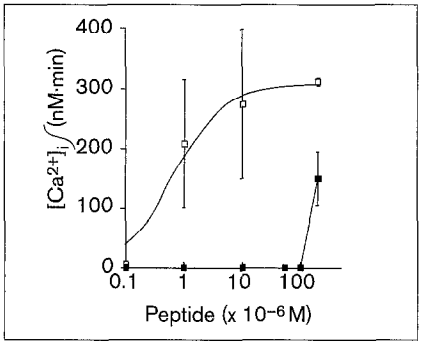
**A selection of interesting papers published last month in *Chemistry & Biology*'s sister journals, *Current Biology*, *Folding & Design* and *Structure*, chosen and summarized by the staff of *Chemistry & Biology*.**

**Chemistry & Biology** May 1998, 5:R120–R124



☐ **Dissociation of the signalling and antiviral properties of SDF-1-derived small peptides.**
Nikolaus Heveker, Mônica Montes, Lothar Germeroth, Ali Amara, Alain Trautmann, Marc Alizon and Jens Schneider-Mergener (1998). *Curr. Biol.* **8**, 369–376.

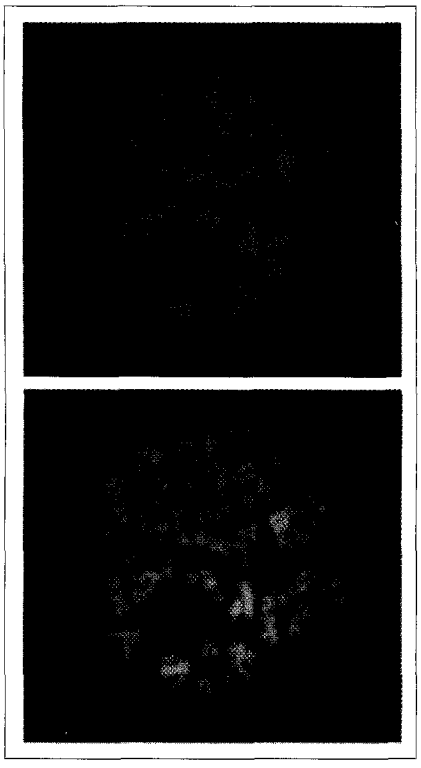
The chemokine receptor CXCR4 is a CD4-associated coreceptor for T-cell-tropic strains of human immunodeficiency virus 1 (HIV-1) and represents a target for antiviral therapy. Infection by T-tropic HIV-1 can be blocked by stromal-cell-derived factor-1 (SDF-1), the natural ligand of CXCR4. The broad variety of cells expressing CXCR4 and the perturbations observed in mice




deficient for SDF-1 suggest that antiviral compounds antagonizing the signalling activity of CXCR4 might have severe side effects *in vivo*. Compounds that interfere selectively with HIV entry and not with SDF-1 signalling would therefore be useful. A series of 13 residue peptides, spanning the entire SDF-1 sequence were tested for their ability to block HIV-1 infection. The antiviral and signalling properties of SDF-1 were retained by a peptide corresponding to its amino terminus. Removal of the first two residues resulted in an antiviral antagonist of the SDF-1–CXCR4 signalling pathway. The authors prepared 234 single-substitution analogues and identified one antiviral analogue that had drastically reduced agonistic or antagonistic properties. The antiviral peptides competed with the monoclonal antibody 12G5 for CXCR4 binding. Their antiviral activity seems to be due to receptor occupancy rather than induction of receptor endocytosis. The amino terminus of the SDF-1 chemokine is sufficient for signal transduction via CXCR4 and for inhibition of HIV-1 entry, but these activities could be dissociated in a peptide analogue. This peptide represents a lead molecule for the design of low molecular weight antiviral drugs.
10 March 1998, Research Paper, *Current Biology*.

☐ **Histone–GFP fusion protein enables sensitive analysis of chromosome dynamics in living mammalian cells.**
Teru Kanda, Kevin F Sullivan and Geoffrey M Wahl (1998). *Curr. Biol.* **8**, 377–385.

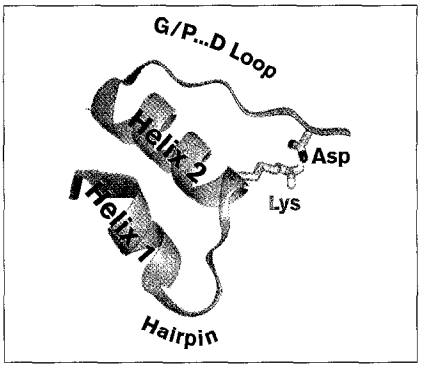
The amplification of oncogenes in cancer cells is often mediated by paired acentric chromatin bodies called double minute chromosomes (DMs), which can accumulate to a high copy number because of their autonomous replication during the DNA synthesis phase of the cell cycle and their subsequent uneven distribution to daughter cells during mitosis. The mechanisms that control DM segregation have been difficult to investigate, however, as direct visualization of DMs in living cells has been precluded because they are far smaller than normal chromosomes. The authors have visualized DMs by developing a highly sensitive method for observing chromosome dynamics in living cells. The human histone H2B



gene was fused to the gene encoding the green fluorescent protein (GFP) of *Aequorea victoria* and transfected into human HeLa cells to generate a stable line constitutively expressing H2B–GFP. The H2B-GFP fusion protein was incorporated into nucleosomes without affecting cell-cycle progression. Using confocal microscopy, H2B–GFP allowed high-resolution imaging of both mitotic chromosomes and interphase chromatin, and the latter revealed various chromatin condensation states in live cells. Using H2B–GFP, the authors could directly observe DMs in living cancer cells; DMs often clustered during anaphase, and could form chromosomal 'bridges' between segregating daughter chromosomes. Cytokinesis severed DM bridges, resulting in the uneven distribution of DMs to daughter cells. The H2B–GFP system allows the high-resolution imaging of chromosomes, including DMs, without compromising nuclear and chromosomal structures and has revealed the distinctive clustering behavior of DMs in mitotic cells.
10 March 1998, Research Paper, *Current Biology*.

☐ **Repair of oxidatively damaged guanine in *Saccharomyces cerevisiae* by an alternative pathway.**
Steven D Bruner, Huw M Nash, William S Lane and Gregory L Verdine (1998). *Curr. Biol.* **8**, 393–403.

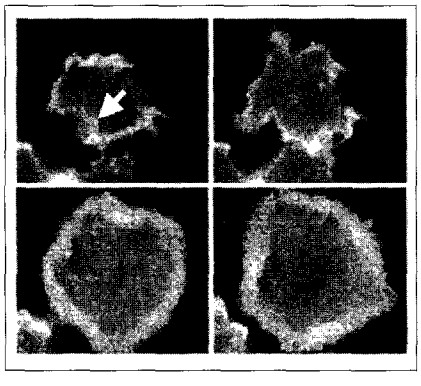
Transversion mutations are caused by 8-oxoguanine (OG), a DNA lesion produced by the spontaneous oxidation of guanine nucleotides, that mis-pairs with adenine during replication. Resistance to this mutagenic threat is mediated by the GO system, the components of which are functionally conserved in bacteria and mammals. To date, only one of three GO system components has been identified in the budding yeast *Saccharomyces cerevisiae*,




namely the OG:C-specific glycosylase/lyase yOgg1. Furthermore, *S. cerevisiae* has been reported to contain a unique glycosylase/lyase activity, yOgg2, which excises OG residues opposite adenines. Paradoxically, according to the currently accepted model, yOgg2 activity should increase the mutagenicity of OG lesions. The authors have isolated yOgg1 and a second protein, Ntg1, which had previously been shown to process oxidized pyrimidines in DNA. The authors demonstrate that Ntg1 has OG-specific glycosylase/lyase activity indistinguishable from that of yOgg2. Targeted disruption of the NTG1 gene resulted in complete loss of yOgg2 activity and yeast lacking NTG1 had an elevated rate of A:T to C:G transversions. The Ntg1 and yOgg2 activities are encoded by a single gene. The authors propose that yOgg2 has evolved to process OG:A mis-pairs that have arisen through mis-incorporation of 8-oxo-dGTP during replication. Thus, the GO system in *S. cerevisiae* is fundamentally distinct from that in bacteria and mammals.
11 March 1998, Research Paper, *Current Biology*.

☐ **Use of a fusion protein between GFP and an actin-binding domain to visualize transient filamentous-actin structures.**
Ka Ming Pang, Eunkyung Lee and David A Knecht (1998). *Curr. Biol.* **8**, 405–408.

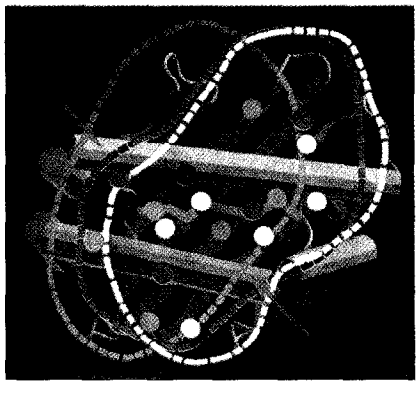
Many important processes in eukaryotic cells involve changes in the quantity, location and the organization of actin filaments. The authors have been able to visualize these changes in live cells using a fusion protein (GFP–ABD) comprising the green fluorescent protein (GFP) of *Aequorea victoria* and the 25 kDa highly conserved actin-binding domain (ABD) from the amino terminus of the actin cross-linking protein ABP–120. In live cells of the soil amoeba *Dictyostelium* expressing GFP–ABD, the three-dimensional architecture of the actin cortex was clearly visualized. The pattern of GFP–ABD fluorescence in these cells coincided with that of



rhodamine–phalloidin, indicating that GFP–ABD specifically binds filamentous (F) actin. On the ventral surface of non-polarized vegetative cells, a broad ring of F actin periodically assembled and contracted, whereas in polarized cells there were transient punctate F-actin structures; cells cycled between the polarized and non-polarized morphologies. During the formation of pseudopods, an increase in fluorescence intensity coincided with the initial outward deformation of the membrane. This is consistent with the models of pseudopod extension that predict an increase in the local density of actin filaments. In conclusion, GFP–ABD specifically binds F actin and allows the visualization of F-actin dynamics and cellular behavior simultaneously.
16 March 1998, Brief Communication, *Current Biology*.

☐ **Identification of a common docking topology with substantial variation among different TCR–peptide–MHC complexes.**
M-K Teng, A Smolyar, AGD Tse, J-H Liu, RE Hussey, SG Nathenson, H-C Chang, EL Reinherz and J-H Wang (1998). *Curr. Biol.* **8**, 409–412.

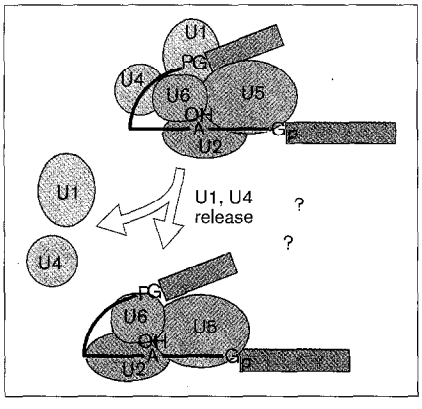
Whether T-cell receptors (TCRs) recognize antigenic peptides bound to major histocompatability complex (MHC) molecules through common or distinct docking modes is currently uncertain. The authors report the crystal structure of a complex between the murine N15 TCR and its peptide–MHC ligand, an octapeptide fragment representing amino acids 52–59 of the vesicular stomatitis virus nuclear capsid protein (VSV8) bound to the murine H-2K$^b$ class I MHC molecule. Comparison of the structure of the N15 TCR–VSV8–H-2Kb complex with the murine 2C TCR–dEV8-H-2K$^b$ and the human A6 TCR–Tax–HLA-A2 complexes revealed a common docking mode, regardless of TCR specificity or species origin, in



which the TCR variable V domain overlies the MHC α2 helix and the Vβ domain overlies the MHC α1 helix. As a consequence, the complementary determining regions CDR1 and CDR3 of the TCR V and Vβ domains make the major contacts with the peptide, while the CDR2 loops interact primarily with the MHC. Nonetheless, in terms of the details of the relative orientation and disposition of binding, there is substantial variation in TCR parameters, which the authors term twist, tilt and shift, and which define the variation of the V module of the TCR relative to the MHC antigen-binding groove.
16 March 1998, Brief Communication, *Current Biology.*

☐ **The DEAH-box splicing factor Prp16 unwinds RNA duplexes *in vitro*.**
Yan Wang, John DO Wagner and Christine Guthrie (1998). *Curr. Biol.* **8**, 441–451.

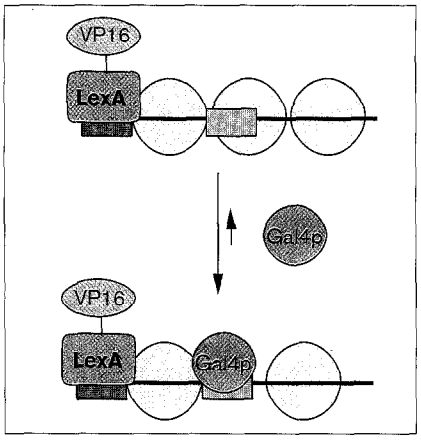
During pre-mRNA splicing, dynamic rearrangement of RNA secondary structure within the spliceosome is crucial for intron recognition and formation of the catalytic core. Splicing factors belonging to the DExD/DExH-box family of RNA-dependent ATPases are thought to have a central role in directing these rearrangements by




unwinding RNA helices. Proof of this hypothesis has, however, been conspicuously lacking. Prp16 is a DEAH-box protein that functions in the second step of splicing *in vitro*. Using various RNA duplexes as substrate, the authors have shown that Prp16 has an ATP-dependent RNA unwinding activity. This activity is independent of sequence in either the single-stranded or duplexed regions of the RNA substrate. A mutation (*prp16-1*) near the ATP-binding motif of Prp16 inhibits both the RNA-dependent ATPase activity and the ATP-dependent RNA-unwinding activity. These results suggest that Prp16 can disrupt a duplexed RNA structure on the spliceosome. Because the purified protein lacks sequence specificity in unwinding RNA duplexes, targeting of the unwinding activity of Prp16 in the spliceosome is likely to be determined by other interacting protein factors. The demonstration of unwinding activity will also help our understanding of how the fidelity of branchpoint recognition is controlled by Prp16.
24 March 1998, Research Paper, *Current Biology.*

☐ **Evidence for two modes of cooperative DNA binding *in vivo* that do not involve direct protein–protein interactions.**
Sanjay Vashee, Karsten Melcher, W Vivianne Ding, Stephen Albert Johnston and Thomas Kodadek (1998). *Curr. Biol.* **8**, 452–458.

The promoter regions of most eukaryotic genes contain binding sites for more than one transcriptional activator and these activators often bind cooperatively to promoters. The most common type of cooperativity is supported by direct protein–protein interactions. Recent studies have shown that proteins that do not specifically interact with one another can bind cooperatively to chromatin *in vitro*, probably by the localized destabilization of nucleosome structure by one factor, facilitating binding of another protein to a nearby site. This mechanism does not require that the transcription factors have activation domains. The authors have examined whether this phenomenon occurs *in vivo*. Unrelated non-interacting proteins can bind DNA cooperatively in yeast cells; this cooperative binding

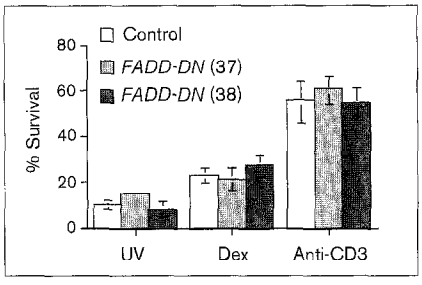



can contribute significantly to transcriptional activation, does not require that both factors have activation domains and is only operative over relatively short distances. In addition to this 'short-range' mechanism, unrelated non-interacting proteins can bind cooperatively to sites separated by hundreds of base pairs, so long as both have potent activation domains. Cooperative binding of transcription factors *in vivo* can occur by several mechanisms, some of which do not require direct protein–protein interactions and which cannot be detected *in vitro* using naked DNA templates. These findings must be taken into account when evaluating mechanisms for synergistic transcriptional activation.
24 March 1998, Research Paper, *Current Biology.*

☐ **p53-dependent impairment of T-cell proliferation in FADD dominant-negative transgenic mice.**
Martin Zörnig, Anne-Odile Hueber and Gerard Evan (1998). *Curr. Biol.* **8**, 467–470.

Members of the tumour necrosis factor (TNF) receptor family can trigger both apoptosis and proliferation in their cytoplasmic region. Some of these receptors share a conserved sequence motif — the 'death domain' — that is required for transduction of the apoptotic signal by recruiting other death-domain-containing adaptor molecules like the Fas-associated

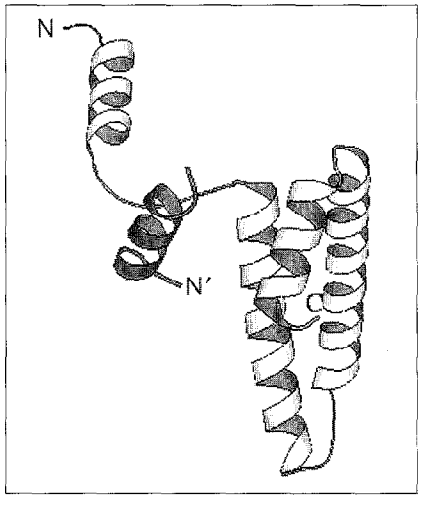
protein FADD/MORT1 or the TNF receptor-associated protein TRADD. FADD links the receptor signal to the activation of the caspase family of cysteine proteases. Functional inactivation of individual receptor




family members often fails to exhibit a distinctive phenotype, probably because of redundancy. To circumvent this problem, the authors used a dominant-negative mutant of FADD (FADD–DN) which should block all TNF receptor family members that use FADD as an adaptor. The authors established transgenic mice expressing *FADD–DN* under the influence of the *lck* promoter and investigated the consequences of its expression in T cells. As expected, *FADD–DN* thymocytes were protected from death induced by CD95 (Fas/Apo1), whereas apoptosis induced by ultraviolet irradiation, anti-CD3 antibody treatment or dexamethasone was unaffected, as was spontaneous cell death. Surprisingly, however, profound inhibition of thymocyte proliferation *in vivo* was observed and of activation-induced proliferation of thymocytes and mature T cells *in vitro*. This inhibition of proliferation was not due to increased cell death and appeared to be dependent on p53.
30 March 1998, Brief Communication, *Current Biology*.

☐ **Conformational variability of the N-terminal helix in the structure of ribosomal protein S15.**
William M Clemons Jr, Christopher Davies, Stephen W White and V Ramakrishnan (1998). *Structure* **6**, 429–438.

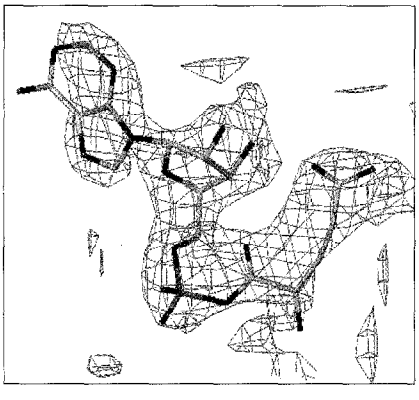
Ribosomal protein S15 is a primary RNA-binding protein that binds to the central domain of 16S rRNA. S15 also regulates its own synthesis by binding to its own mRNA. The binding sites for S15 on both mRNA and rRNA have been narrowed down to less than a hundred nucleotides each, making the protein an attractive candidate for the study of protein–RNA interactions. The crystal structure of S15 from *Bacillus stearothermophilus* has been solved and shown to consist of four α helices. Three of these helices form the core of the protein, whereas the amino-terminal helix protrudes out from the




body of the molecule to make contacts with a neighboring molecule in the crystal lattice. S15 contains a large conserved patch of basic residues that could provide a site for binding 16S rRNA. The conformation of the amino-terminal α helix is quite different from that reported in a recent NMR structure of S15 from *Thermus thermophilus*. The intermolecular contacts that this α helix makes with a neighboring molecule in the crystal, however, closely resemble the intramolecular contacts that occur in the NMR structure. The conformational variability of the amino-terminal helix has implications for the range of possible S15–RNA interactions. The conserved basic patch at one end of S15 and the cluster of conserved aromatic residues at the other end provide two possible RNA-binding sites on S15.
15 April 1998, Research Paper, *Structure*.

☐ **How glutaminyl-tRNA synthetase selects glutamine.**
Virginia L Rath, Laura F Silvian, Barbro Beijer, Brian S Sproat and Thomas A Steitz (1998). *Structure* **6**, 439–449.

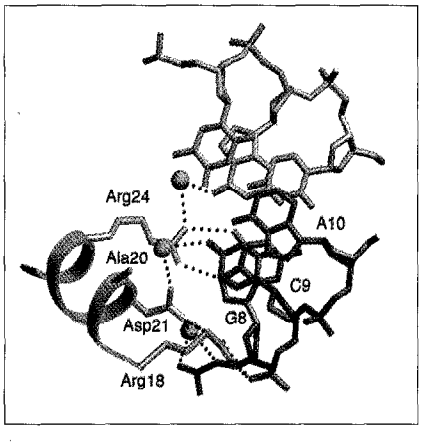
Aminoacyl-tRNA synthetases covalently link a specific amino acid to the correct tRNA. The fidelity of this reaction is essential for accurate protein synthesis. Each synthetase has a specific molecular mechanism to distinguish the correct pair of substrates from the pool of amino acids and isologous tRNA molecules. In the case of glutaminyl-tRNA synthetase (GlnRS) the prior binding of tRNA is required for activation of glutamine by ATP. A complete understanding of amino acid specificity in GlnRS requires the determination of the structure of the synthetase with both tRNA and substrates bound. A stable glutaminyl-adenylate analog was synthesized and cocrystallized with GlnRS and $tRNA_2^{Gln}$. The crystal structure of this ternary complex shows the interactions made between glutamine and its binding site. To select against glutamic acid or glutamate, both



hydrogen atoms of the nitrogen of the glutamine sidechain are recognized. The hydroxyl group of Tyr211 and a water molecule are responsible for this recognition. The prior binding of $tRNA^{Gln}$ that is required for amino-acid activation may result from the terminal nucleotide, A76, packing against and orienting Tyr211, which forms part of the amino acid binding site.
15 April 1998, Research Paper, *Structure*.

☐ **High-resolution structures of variant Zif268–DNA complexes: implications for understanding zinc finger–DNA recognition.**
Monicia Elrod-Erickson, Timothy E Benson and Carl O Pabo (1998). *Structure* **6**, 451–464.

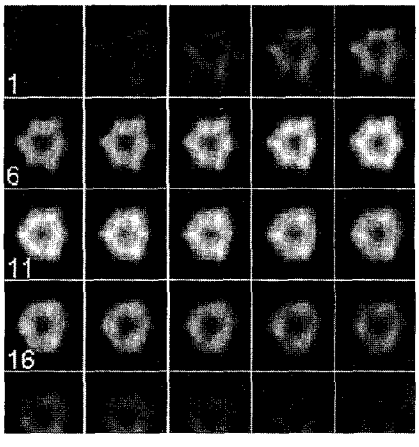
Zinc fingers of the $Cys_2$–$His_2$ class comprise one of the largest families of eukaryotic DNA-binding motifs and recognize a diverse set of DNA sequences. These proteins have a relatively simple modular structure and key base contacts are typically made by a few residues from each finger. These features make the zinc finger motif an attractive system for designing novel DNA-binding proteins and for exploring fundamental principles of protein–DNA recognition. The authors report the X-ray crystal structures of zinc finger–DNA complexes involving three variants of Zif268, with multiple changes in the recognition helix of finger 1. To help elucidate the differential basis for site-specific recognition, the structures of four other




complexes containing various combinations of these peptides with alternative binding sites have also been determined. The protein–DNA contacts observed in these complexes reveal the basis for the specificity demonstrated by these Zif268 variants. Many of the contacts can be rationalized in terms of a recognition code, but the predictive value of such a code is limited. The structures illustrate how modest changes in the docking arrangement accommodate the new sidechain–base and sidechain–phosphate interactions. Such adaptations help explain the versatility of naturally occurring zinc-protein finger proteins and their use in design.
15 April 1998, Research Paper, *Structure*.

☐ **Three-dimensional reconstructions from cryoelectron microscopy images reveal an intimate complex between helicase DnaB and its loading partner DnaC.**
Carmen San Martin, Michael Radermacher, Bettina Wolpensinger Andreas Engel, Caroline S Miles, Nicholas E Dixon and José-María Carazo (1998). *Structure* **6**, 501–509.

DNA helicases play a fundamental role in all aspects of nucleic-acid metabolism and defects in these enzymes have been implicated in a number of inherited human disorders. DnaB is the major replicative DNA helicase in *Escherichia coli* and has been used as a model system for studying the structure and function of hexameric helicases. The native protein is a hexamer of identical subunits, which in solution forms a complex with six molecules of the loading protein DnaC. DnaB is delivered from this complex




onto the DNA template, with the subsequent release of DnaC. The authors report here the structures of the DnaB helicase hexamer and its complex with DnaC as determined using three-dimensional cryoelectron microscopy. The DnaB structure reveals that six DnaB monomers assemble as three asymmetric dimers to form a polar, ring-like hexamer. The hexamer has two faces, one displaying threefold and the other sixfold symmetry. The six DnaC protomers bind tightly to the sixfold face of the DnaB hexamer. The structures of the DnaB helicase and its complex with DnaC reveal some interesting structural features relevant to helicase function and to the assembly of the two-protein complex.
15 April 1998, Research Paper, *Structure*.