uncertain (by a factor of 3 or worse). Within this large margin the observed number of R CrB stars is consistent with the merger scenario as the only formation channel. Crucial model parameters will soon be known much better from a large survey of double white dwarfs with the Very Large Telescope of the European Southern Observatory (*9*).

The driving agent that leads to the merging of the white dwarfs is the emission of gravitational waves. Such waves have not yet been measured. First detections may be possible soon with sensitive ground-based laser-interferometric detectors, due to become operational by the end of the year (*10*). But the low-frequency gravitational waves emitted by double white dwarfs will be buried in the background noise. Their detection will have to await the operation of the space-born LISA observatory, planned for launch in the next decade.

In the far and dark future of the universe, when all stars have burnt their nuclear fuel and turned into cold, compact remnants, lots of double white dwarfs should exist. He white dwarfs should then be more numerous. Their merger will lead to the rebirth of He-burning stars (*11*) known as sdB stars (*12*), which are less luminous (about 30 times the solar luminosity) but live longer (100 million years) than R CrB stars. The dark universe will then be lit from time to time by R CrB and sdB stars for a while, until all of them have contracted to the white dwarf graveyard.

References and Notes

1. T. Marsh, *New Astron. Rev.* **44**, 119 (2000).
2. G. Nelemans, L. R. Yungelson, S. F. Portegies Zwart, F. Verbunt, *Astron. Astrophys.* **365**, 491 (2001).
3. L. D. Landau, E. Lifshitz, *The Classical Theory of Fields*, (Pergamon Press, Oxford, 2nd ed.,1962).
4. R. A. Saffer, J. Liebert, E. W. Olszewski, *Astrophys. J.* **334**, 947 (1988).
5. R. F. Webbink, *Astrophys. J.* **277**, 355 (1984).
6. H. Saio, C. S. Jeffery, *Mon. Not. R. Astron. Soc.* **333**, 121 (2002).
7. M. Asplund, B. Gustaffson, D. L. Lambert, N. K. Rao, *Astron. Astrophys.* **353**, 287 (2000).
8. C. S. Jeffery, R. L. C. Starling, P. W. Hill, D. Pollacco, *Mon. Not. R. Astron. Soc.* **321**, 111 (2001).
9. R. Napiwotzki *et al.*, *Astron. Astrophys.* **386**, 957 (2002).
10. B. F. Schutz, *Class. Quantum Grav.* **16**, A131 (1999).
11. H. Saio, C. S. Jeffery, *Mon. Not. R. Astron. Soc.* **313**, 671 (2000).
12. Unlike the R CrB stars, the white merger process is probably only a minor formation channel for the sdB stars observed in the present-day Galaxy.

PERSPECTIVES: SIGNAL TRANSDUCTION

MAP Kinase Signaling Specificity

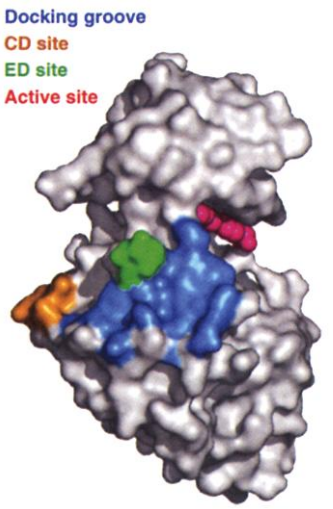
Claire R. Weston, David G. Lambright, Roger J. Davis

Where would cells be without mitogen activated protein kinases (MAPKs)? These molecules are components of signaling pathways that relay, amplify, and integrate signals from a variety of extracellular stimuli, thereby controlling the genomic and physiological response of a cell to its environment. In mammals, MAPKs guide cellular maturation and can induce inflammation and apoptosis. The MAPK family includes extracellular signal–regulated kinases (ERKs), which are activated by mitogens, and c-Jun NH_2-terminal kinases (JNKs) and p38 MAPKs that are primarily activated by cytokines and in response to cellular stress. With all of these related kinases in the same place, how does the cell keep the pathways distinct from one another? It turns out that many mechanisms exist to prevent cross talk between pathways, thereby ensuring that the cell responds correctly to each environmental challenge.

One way that MAPKs maintain specificity is to physically bind to other proteins in their information cascade through highly specific docking sites (*1*, *2*). Many MAPKs contain the common docking (CD) site and the Glu-Asp (ED) site; MAPK interacting proteins have D domains. The structural basis for these precise interactions remained elusive until Chang and colleagues resolved the crystal structure of p38 MAPK bound to the docking domains of a p38 substrate and a p38-activating enzyme (*3*).

In their report in *Molecular Cell*, Chang and colleagues used isolated D domains from transcription factor MEF2A (a p38 substrate), and from the p38-activating enzyme MKK3b. Both of these docking domains, like other D domains, contain basic residues and a hydrophobic ØA-X-ØB motif (where ØA and ØB are hydrophobic residues Leu, Ile, or Val). The D domain—first identified in c-Jun (which binds to JNK), MEF2 (which binds to p38), and Elk-1 (which binds to ERK and JNK) (*4*–*6*)—contains two to six residues that separate a hydrophobic ØA-X-ØB motif from a cluster of at least two basic residues (Lys, Arg). Both the basic and the hydrophobic residues of the D domain are vital to the recognition and binding of specific MAPK isoforms (*1*, *2*). Many different types of MAPK-interacting proteins, including activating enzymes (MAPKK), scaffold proteins, phosphatases, and substrates, have similar D domains, while some interacting proteins contain the related Phe-X-Phe-Pro (FXFP) MAPK binding domain (*1*, *2*).

Chang and colleagues uncovered many details in the crystal structure of p38 MAPK bound to the docking domains of MEF2A and MKK3b. For example, both domains bind to the same site, a groove present between α helices αd and αe and the reverse turn between β7 and β8 in the COOH-terminal region of the kinase (see the first figure). Residues Ile^{116} and Gln^{120} in this docking groove are essential for the recognition of the ØA-X-ØB motif and thus for the docking interaction. This docking groove is separate from the kinase active site and from the CD site previously implicated in MAPK docking interactions (*7*). Docking grooves similar to the one identified by Chang and colleagues appear in other MAPKs, so the groove is not unique to p38 (*3*). Nevertheless, many of the residues in p38 that contact the D domain differ between MAPK isoforms, which suggests that the structure of the docking groove in individual MAPK isoforms determines MAPK selectivity.



The binding site. Molecular surface representation of p38α MAPK, showing the docking groove (blue) that binds the D domains of the substrate MEF2A and the activator MKK3b. The CD site (orange) and ED site (green) that were identified in biochemical studies. The active site of p38 MAP kinase is bound to the inhibitor SB203580 (red).

In previous biochemical studies, researchers identified the CD site as a common docking site that mediates MAPK interactions with other proteins and is distinct from the catalytic active site of MAPK (*8*). Specifically, the acidic residues in the CD site were proposed to interact with the basic residues in the D domain of the binding partner. Chang and colleagues did not observe this interaction in the p38 MAPK crystal structures. The failure to confirm this proposed structure may reflect differences in the interactions of MAPK with various substrates. Alter-

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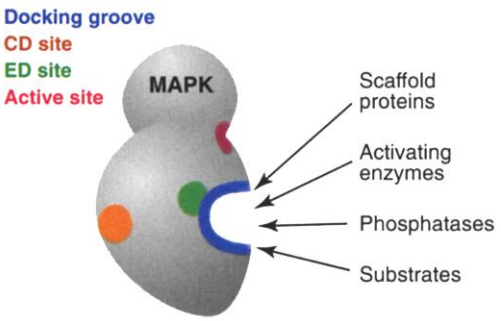


MAP kinase interactions with scaffold molecules, MAPKKs, phosphatases, and substrates.

natively, the CD site interaction may occur only with activated MAPK or full-length proteins; the structures reported by Chang and colleagues include nonactivated p38 MAPK and isolated D domains. A second MAPK site implicated in the interaction of MAPKs with MAPK-activated protein kinases (MAPKAPKs), the ED site (*7*), also did not participate in the interaction of p38 MAPK with MEF2A or MKK3b in the crystal structures (*3*). The ED site, however, is located close to the docking groove in the crystal structures (see the first figure). Thus, the ED site may aid the docking of some interacting proteins, although the mutational analysis used to identify the ED site may have indirectly affected the docking groove identified by crystallography.

Chang and colleagues unexpectedly observed that the binding of proteins to D domains induces conformational changes in p38 MAPK. The largest change occurs in the loop between αd and αe, which narrows the binding groove between these helices and the β7-β8 reverse turn. The binding and conformational changes that MEF2A and MKK3b induce differ and may contribute to p38 regulation. For example, alterations in the p38 activation loop caused by docking to MKK3b could aid p38 phosphorylation, and the conformational changes caused by docking MEF2A may activate p38.

Disease research may benefit from understanding the mechanisms that regulate MAPK signaling specificity. For example, lethal factor (LF), a protease that binds to and proteolytically cleaves the NH_2-terminal region of MAPKK, blocks MAPK function during anthrax infection (*9*, *10*). The proteolysis separates the NH_2-terminal D domain of MAPKK from the catalytic kinase domain that phosphorylates and activates MAPK (*9*). Loss of the D domain prevents recognition of the cognate MAPK by the cleaved MAPKK. This observation could lead to the use of small molecules (that is, drugs) to disrupt MAPK interactions and to block selectively individual MAPK pathways.

The structural insights of Chang and colleagues will provide a foundation for future studies of the molecular basis of MAPK signaling specificity. These studies will further our understanding of MAPK signaling networks in health and disease.

References

1. A. D. Sharrocks *et al.*, *Trends Biochem. Sci.* **25**, 448 (2000).
2. H. Enslen, R. J. Davis, *Biol. Cell* **93**, 5 (2001).
3. C. I. Chang *et al.*, *Mol. Cell* **9**, 1241 (2002).
4. R. J. Davis, *Cell* **103**, 239 (2000).
5. S. H. Yang *et al.*, *Mol. Cell. Biol.* **19**, 4028 (1999).
6. S. H. Yang *et al.*, *EMBO J.* **17**, 1740 (1998).
7. T. Tanoue *et al.*, *EMBO J.* **20**, 466 (2001).
8. T. Tanoue *et al.*, *Nature Cell Biol.* **2**, 110 (2000).
9. N. S. Duesbery *et al.*, *Science* **280**, 734 (1998).
10. A. D. Pannifer *et al.*, *Nature* **414**, 229 (2001).

PERSPECTIVES: MOLECULAR DYNAMICS

Biomolecules See the Light

David W. Pratt

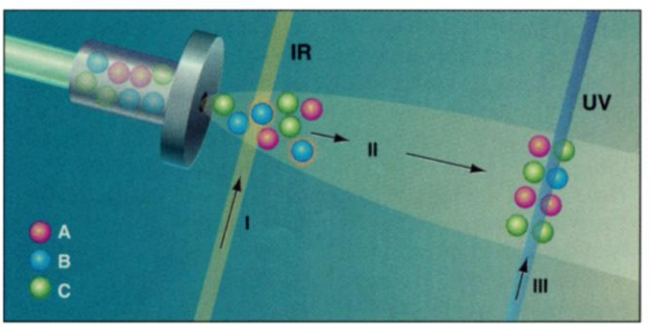
Molecular-level understanding of the complex dynamics of biological processes such as protein folding will greatly advance the treatment of human disease. Rapid progress toward this goal has been made in the past few years, owing to advances in experimental and theoretical techniques (*1*–*3*). For example, multidimensional nuclear magnetic resonance techniques have helped to determine the solution structures of large proteins and probe their dynamical behavior (*4*).

But existing approaches cannot fully elucidate the heterogeneity of biological systems, their intricate energy landscapes, and the possible role of solvent in the dynamics. On page 2369 of this issue, Dian *et al.* report the development of a new approach that overcomes some of these limitations (*5*). The method provides surprisingly detailed insights into the dynamics of a small biomolecule.

Using a supersonic jet, infrared (IR) and ultraviolet (UV) lasers, and ab initio quantum chemical calculations, the authors isolated a biomolecule in the gas phase, and identified and determined the relative populations of its energetically accessible "conformational substates" under collision-free conditions. They then manipulated these populations with tunable laser light, using collisions to relax the molecules back into their lowest energy conformations after they had visited less favorable regions of the energy landscape. Surprisingly, they found that the resulting population distributions depend upon the precise nature of the excitation, demonstrating for the first time that there are distinguishable pathways for conformational change.

The authors studied a methyl-capped dipeptide called NATMA (*N*-acetyl-tryptophan methylamide). The 36-atom molecule is small by biomolecule standards but nonetheless complex in its dynamics. The energy landscape of the molecule is calculated to have 164 minima connected by 714 transition states, with 65 minima lying within 40 kJ/mol of the global minimum (*6*). Different minima correspond to geometries with extended or partially folded peptide chains. Comparison of the relative abundances of the different conformations before and after IR excitation provides information about how a given structure evolves along a particular pathway.

The experiment of Dian *et al.* (*5*) is illustrated schematically in the figure. NATMA was heated to 150°C, entrained in helium, and passed through a ~1-mm orifice into a vacuum chamber, creating a supersonic expansion that cools each molecule into one of



Exploring the energy landscape with light. NATMA molecules expanded in a supersonic jet of helium are observed by UV spectroscopy (III) to exist in three different conformational substates (A, B, and C), corresponding to geometries with extended or partially folded side chains. Irradiating the ensemble with IR light (I) and relaxing it by collisions (II) changes the population ratio in a conformation-specific way, demonstrating that unique pathways govern the folding dynamics of this dipeptide.

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