compensate for this unknown lignification regulator. An important approach to discovering such unknown factors will be to identify the direct targets of the dehiscence regulators, all of which seem to encode DNA binding transcription factors.

The other issue that needs to be tackled is how the initial pattern of this regulatory network is set up. Fortunately, we know quite a bit about the early steps of flower development, which begins with the decision of a plant to make individual flowers in response to environmental and intrinsic cues. The immediate result of floral induction is the transcriptional activation of a small set of integrator genes. These in turn positively regulate homeotic genes that control the identity of floral organs including the central carpels, which produce the fruit. Surprisingly, *FUL* activation occurs independently of carpel formation, while *SHP* expression does not. Similarly mystifying is the observation that several genes affecting other aspects of fruit patterning are also expressed independently of whether or not carpels are made (Heisler et al., 2001; Sessions et al., 1997).

Finally, as alluded to in the beginning, there is tremendous interspecific variation in the degree of fruit dehiscence. As the Yanofsky and Sundaresan laboratories have shown, it is quite easy to make *Arabidopsis* fruits indehiscent, both by knocking out or overexpressing various fruit regulators. They have not yet shown, however, how the system can be tuned to supercharge the dehiscence process and create explosive seedpods. Since *Arabidopsis* has close relatives such as hairy bitter-cress, *Cardamine hirsuta*, which can fling seeds a couple of feet, one is hopeful that this will not be too tough a nut to crack.

Jan U. Lohmann[1] and Detlef Weigel[1,2]
[1]Department of Molecular Biology
Max Planck Institute for Developmental Biology
D-72076 Tübingen
Germany
[2]Plant Biology Laboratory
The Salk Institute for Biological Studies
La Jolla, California 92037

Selected Reading

Ferrándiz, C., Liljegren, S.J., and Yanofsky, M.F. (2000). Science *289*, 436–438.

Heisler, M.G., Atkinson, A., Bylstra, Y.H., Walsh, R., and Smyth, D.R. (2001). Development *128*, 1089–1098.

Liljegren, S.J., Roeder, A.H.K., Kempin, S.A., Gremski, K., Østergaard, L., Guimil, S., Khammungkhune, D., and Yanofsky, M.F. (2004). Cell *116*, this issue, 843–853.

Rajani, S., and Sundaresan, V. (2001). Curr. Biol. *11*, 1914–1922.

Roeder, A.H., Ferrándiz, C., and Yanofsky, M.F. (2003). Curr. Biol. *13*, 1630–1635.

Sessions, A., Nemhauser, J., McColl, A., Roe, J., Feldmann, K., and Zambryski, P. (1997). Development *124*, 4481–4491.

Oncogenic Mutations in B-Raf: Some Losses Yield Gains

A study by Wan et al. in this issue of *Cell* demonstrates that the majority of oncogenic mutations in the B-Raf protein kinase result in increased catalytic activity, through disruption of the autoinhibited state of the kinase domain. Surprisingly, several mutations lead to impaired B-Raf kinase activity, yet these mutants are nevertheless capable of stimulating downstream signaling through transactivation of C-Raf.

The Raf-MEK-ERK protein kinase cascade is a highly conserved signaling pathway in eukaryotes, which links, for example, growth factor stimulation to cell proliferation. Raf is a protein serine/threonine kinase for which there are three mammalian isoforms: A-Raf, B-Raf, and C-Raf. Activation of the Raf proteins is mediated principally through binding of the small GTPase Ras in its GTP bound state. The importance of this signaling pathway in cell growth control is underscored by the finding that approximately 30% of all human cancers have activating *ras* mutations (Bos, 1989). Furthermore, somatic mutations in *B-raf* are found in a majority of human malignant melanomas (Davies et al., 2002). As might be expected for a protein kinase that sits atop an important growth control pathway, Raf proteins are subject to a complex regulatory scheme, including autoinhibition of the kinase domain by the N-terminal region, Ras binding and translocation to the plasma membrane, stimulatory and inhibitory serine/threonine and tyrosine phosphorylation, and possibly interaction with 14-3-3 proteins (Kolch, 2000).

Over 30 missense mutations in the *B-raf* gene have been identified in human cancers, most of which map to the kinase domain. Wan et al. (2004) characterized the catalytic activity of 22 of these mutants by measuring their ability to phosphorylate MEK in vitro. Seven of the B-Raf mutants exhibited highly elevated activity, above the level achieved by activating wild-type B-Raf with an oncogenic form of Ras. Eleven of the mutants phosphorylated MEK above the basal level (non-Ras-stimulated) for wild-type B-Raf, but below the level reached by Ras activation of wild-type B-Raf. Both the high- and intermediate-activity B-Raf mutants, when transfected into cells, stimulated hyperphosphorylation of ERK. Finally, and most curiously, four of the cancer-associated mutants were found to have impaired kinase activity versus wild-type B-Raf. Despite this, three of the four impaired-activity mutants, when transfected into cells, were more efficient at stimulating phosphorylation of endogenous ERK than wild-type B-Raf.

How to explain this seeming paradox? It had been shown previously, and again by Wan et al. (2004), that B-Raf and C-Raf are capable of heterooligomerization, and therefore one possibility is that the impaired-activity B-Raf mutants stimulate ERK phosphorylation through transactivation of C-Raf. This hypothesis was tested, and it could be demonstrated that the three kinase-impaired B-Raf mutants were considerably more effective than wild-type B-Raf in stimulating C-Raf activity. Moreover, phosphorylation of ERK in cells transfected

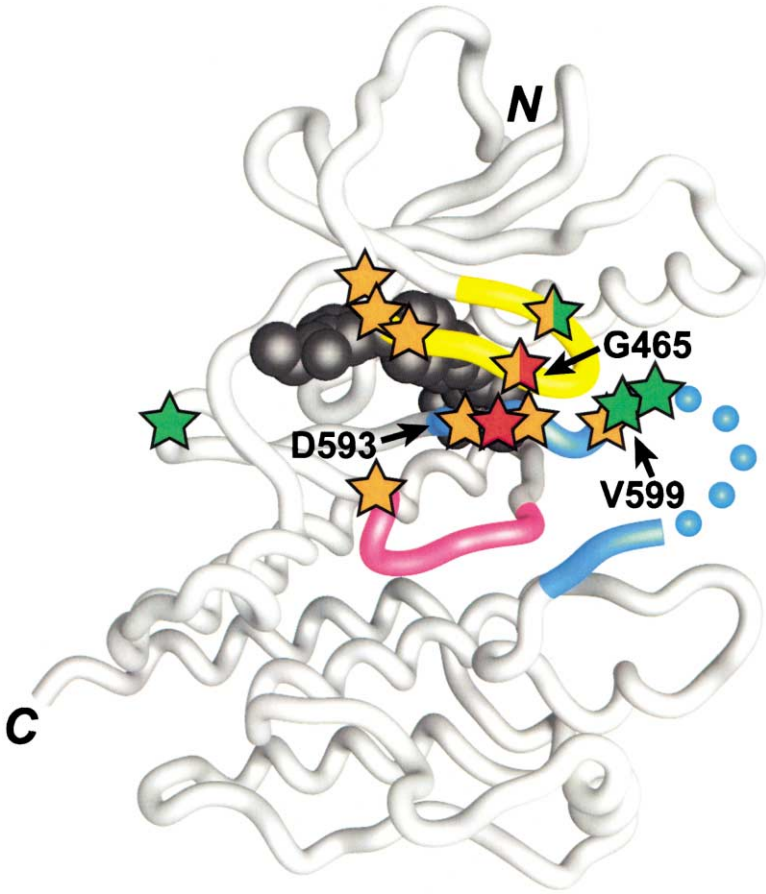

Figure 1. Oncogenic Mutations in the B-Raf Kinase Domain

The kinase domain of B-Raf is shown in backbone representation and colored gray, except for the nucleotide binding loop (yellow), activation segment (blue), and the catalytic loop (magenta). A portion of the activation segment, represented by blue spheres, is disordered in the crystal structure. The N- and C termini are denoted by *N* and *C*. The small-molecule inhibitor (BAY43-9006) cocrystallized with the B-Raf kinase domain is shown in full-sphere representation and colored black. Oncogenic mutations have been mapped onto the backbone representation as stars, color coded according to class. Mutations that strongly activate B-Raf kinase activity are colored green, those that result in intermediate activation are colored orange, and those that result in impaired B-Raf catalytic activity are colored red. In the nucleotide binding loop (yellow), mutations exist that, depending on the exact amino acid change, fall into more than one activation class. Such mutations are represented by bicolored stars. The positions of two sites of oncogenic mutation, Gly-465 (P loop), and Val-599 (activation segment), are indicated, as well as the position of Asp-593 of the DFG motif in the activation segment.

with the kinase-impaired B-Raf mutants was dependent on endogenous C-Raf activity. The high- and intermediate-activity B-Raf mutants also stimulated C-Raf activity, but C-Raf activity was not required for these B-Raf mutants to stimulate ERK phosphorylation in cells.

To gain insights into the molecular basis for oncogenicity of selected B-Raf mutants, Wan et al. (2004) determined crystal structures of the kinase domain of wild-type B-Raf and of a high-activity mutant, Val-599→Glu, which is the predominant B-Raf mutant in human malignant melanomas (Davies et al., 2002). To obtain crystals of suitable quality, it was necessary to cocrystallize the B-Raf kinase domains, wild-type and mutant, with a small-molecule inhibitor of Raf, BAY43-9006. The crystal structure of the B-Raf kinase domain displays the prototypical architecture of protein serine/threonine and tyrosine kinases, comprising an N-terminal and C-terminal lobe, between which lies the ATP binding cleft, which is occupied by the inhibitor in these crystal structures.

Like the majority of protein kinases, Raf kinases (wild-type) require phosphorylation (serine/threonine) in the activation segment for functional catalytic activity (Chong et al., 2001). Phosphorylation of the 15–20 residue activation segment in protein kinases stabilizes a configuration of this segment that is optimal for catalysis (Johnson et al., 1996). The activation segment begins with the protein kinase-conserved DFG sequence motif. The aspartic acid of this motif coordinates an active site Mg^{2+} ion during phosphoryl transfer. The wild-type (and mutant) protein was unphosphorylated in the activation segment, and thus the crystal structure should represent the inactive state of the B-Raf kinase.

A striking feature of the oncogenic mutations in the B-Raf kinase domain is their clustering to the nucleotide binding loop (P loop) and to the N-terminal portion of the activation segment (see Figure 1). The physical interaction between the P loop and the activation segment, as observed in the crystal structure, is the key to understanding the mechanism of oncogenesis for the high- and intermediate-activity B-Raf mutants. In particular, Val-599 in the activation segment (four residues beyond the DFG motif) makes hydrophobic contacts with Phe-467 in the P loop in the wild-type B-Raf kinase structure. With Val-599 stabilized in this position, the residues of the DFG motif are not properly aligned for phosphoryl transfer. Therefore, mutations that disrupt this kinase-inactive state and/or stabilize the active state will result in elevated kinase activity. Both effects are likely achieved through mutation of Val-599 to glutamic acid.

If Val-599 is important in stabilizing the inactive state of the B-Raf kinase, then one would expect that in the crystal structure of the Val-599→Glu mutant, the activation segment would adopt a different conformation, one more similar to the phosphorylated, active conformation. Curiously, though, the activation segment conformation in the mutant structure is nearly identical to that in the wild-type structure. Here, the cocrystallized inhibitor is probably the culprit for this obfuscation. BAY43-

9906, like the c-Abl inhibitor STI-571/Gleevec (Schindler et al., 2000), has higher affinity for (and thus stabilizes) the unphosphorylated, inactive conformation of the activation segment. Thus, in the Val-599→Glu B-Raf crystal structure, the inhibitor dictates the conformation of the activation segment more so than glutamic acid at residue 599.

For the impaired-activity class of B-Raf mutants, loss of kinase activity is relatively easy to rationalize. For example, two mutations in this class lead to substitutions of a protein kinase-invariant glycine in the P loop (Gly-465) with valine or glutamic acid. Any amino acid other than glycine at this position would adversely affect ATP binding. What is less easy to rationalize is how this class of mutants results in *trans*-activation of C-Raf. Wan et al. (2004) propose that the unifying feature for all three classes of oncogenic mutants is the resultant destabilization of the inactive conformation of the activation segment. For the impaired-activity mutants, one conceivable hypothesis is that the conformational change in the B-Raf activation segment, induced through mutation, is transmitted to a C-Raf molecule in the same complex, resulting in its activation. Although the detailed mechanisms are surely different, the ability of B-Raf to elicit a biological response through a conformational change rather than by phosphorylation is reminiscent of IRE1, a protein kinase involved in the unfolded protein response (Papa et al., 2003).

The study by Wan et al. (2004) provides biochemical insights into the mechanisms by which B-Raf, a protein kinase important in cell growth control, can be deregulated through mutation. From a human disease standpoint, the results of this study are sobering for two reasons. First, despite multiple regulatory mechanisms governing the activation state of B-Raf, a single point mutation is sufficient to trump these safeguards and lead to transformation. Second, and more insidious, mutations in this same protein kinase that cripple the enzyme can nevertheless result in a gain-of-function growth phenotype—losses yielding gains.

Stevan R. Hubbard
Skirball Institute of Biomolecular Medicine
and Department of Pharmacology
New York University School of Medicine
New York, New York 10016

Selected Reading

Bos, J.L. (1989). Cancer Res. *49*, 4682–4689.

Chong, H., Lee, J., and Guan, K.L. (2001). EMBO J. *20*, 3716–3727.

Davies, H., Bignell, G.R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M.J., Bottomley, W., et al. (2002). Nature *417*, 949–954.

Johnson, L.N., Noble, M.E.M., and Owen, D.J. (1996). Cell *85*, 149–158.

Kolch, W. (2000). Biochem. J. *351*, 289–305.

Malumbres, M., and Barbacid, M. (2003). Nat. Rev. Cancer *3*, 459–465.

Papa, F.R., Zhang, C., Shokat, K., and Walter, P. (2003). Science *302*, 1533–1537.

Schindler, T., Bornmann, W., Pellicena, P., Miller, W.T., Clarkson, B., and Kuriyan, J. (2000). Science *289*, 1938–1942.

Wan, P.T.C., Garnett, M.J., Roe, S.M., Lee, S., Niculescu-Duvaz, D., Good, V.M., Cancer Genome Project, Jones, C.M., Marshall, C.J., Springer, C.J., et al. (2004). Cell *116*, this issue, 855–867.

Obedient and Wayward Synaptic Behavior

Synapse formation is a complex process that culminates in the linking up and locking in of pre- and postsynaptic membranes. Shen et al. (2004 [this issue of *Cell*]) begin to dissect the molecular instructions that govern target selection of pre- and postsynaptic membrane interactions.

Synaptogenesis is the process by which nascent pre- and postsynaptic membranes link up and lock in to form an adhesive junction across which information in the form of chemical or electrical signals is transferred. It is the end product of an extraordinary process that has been conveniently partitioned into sequentially observed phenomena—axon guidance, gross target recognition, fine target recognition, and ultimately, synapse formation (Holt and Harris, 1998). These stages of synapse development correlate with observations in situ in well-studied invertebrate and vertebrate systems. However, the underlying controlling elements—the molecular basis for synaptic specificity and synaptic junction formation—are largely unknown. The development continuum that begins with neurite outgrowth and culminates with the formation of a specific synapse is the result of a continual interplay between the unfolding neural genetic program and local environmental influences on the outgrowing neurite (Benson et al., 2001).

Although we are still in the dark in terms of very basic questions about synaptogenesis, some recent experiments have uncovered certain instructive subtleties that are quite revealing about the nature of the controlling molecules that operate near the end of the line, that is, when the presynaptic fiber is in close approach to its target. Although there are now many examples of this, an interesting case in point is in the visual system of *Drosophila*, where the selective ablation of N-cadherin from photoreceptor neurons engenders visual defects due to disruptions in the photoreceptor cell connections; synapses form, but they become in a sense wayward (Lee et al., 2001). In this case, they miss the mark and synapse on the wrong cells. Thus, removal of a key molecule from the normal "synaptogenic program" releases presynaptic axons from their restrictive precommitments.

So, by denying neurites a single critical molecular cue needed to make the correct synaptic decisions, the neurites synapse aberrantly. In fact, it is becoming clear that certain local environmental cues that a neurite encounters in its search for the right target may not only encourage the correct target selection, but also serve to restrict or constrain neurites from inappropriate synapse formation.