understanding of our planet's deeper layers. ■

Sébastien Merkel is at the Institute for Solid State Physics, University of Tokyo, Kashiwanoha 5-1-5, Kashiwa, Chiba, 277-8581 Japan.
e-mail: smerkel@issp.u-tokyo.ac.jp

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Cancer

Enzymes play molecular tag

Deborah K. Morrison

The B-RAF protein is often mutated in human cancers, contributing to their development. Although most known mutations stimulate its catalytic activity, others, surprisingly, impair it — yet still cause cancer.

Like most other cellular events, cell proliferation is tightly regulated by signals from the surrounding environment. These cues are relayed from the cell surface to the nucleus by defined signal-transduction cascades. The core components of one such pathway are the RAS, RAF, MEK and ERK proteins. If this pathway is constantly switched on, it can cause cells to proliferate wantonly, resulting in cancer.

Researchers have known for some time that there are cancer-promoting mutations in the RAS protein that keep it permanently 'on'. Recently, large-scale genomic screens have also detected mutations in one member of the RAF family of proteins — B-RAF — in 65% of malignant melanomas[1] and many colorectal[2], ovarian[3] and papillary thyroid[4,5] cancers. How these mutations alter B-RAF's function is the topic of an elegant study, published in *Cell*, by Wan and colleagues[6]. The paper also provides structural information that should help to guide the search for more effective inhibitors of this protein family.

The RAF enzymes are central intermediates in this fundamental signalling cascade, transmitting signals from RAS to the downstream enzymes MEK and ERK (Fig. 1a, overleaf). In mammalian cells there are three members of the RAF family, A-RAF, B-RAF and C-RAF. Working out how these proteins are regulated has been a daunting task, largely because of the complexity of the process. There seem to be several mechanisms, including self-inhibition (involving a regulatory domain located at one end, the amino terminus, of the proteins), interactions with binding partners such as RAS and the 14-3-3 protein, and the phosphorylation of (covalent linkage of phosphate groups to) inhibitory and activating sites on the proteins[7,8].

The activation of RAF is typically

Developmental genetics

Bittersweet evolution

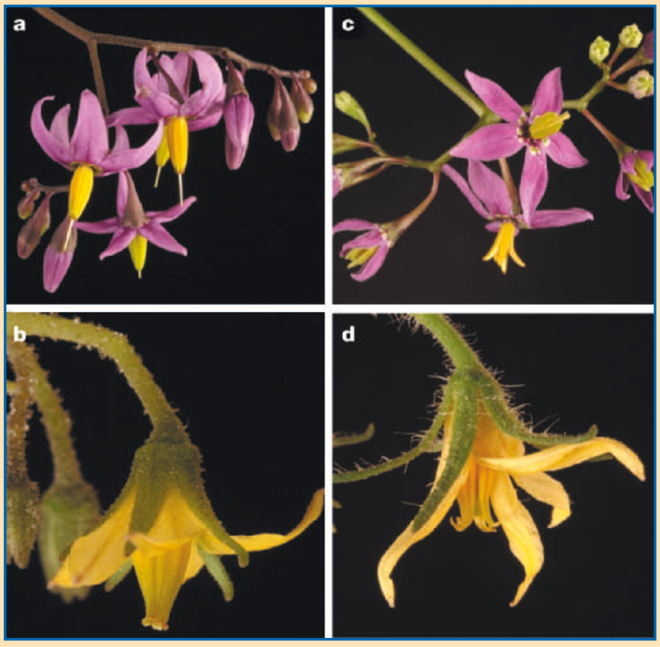
Structures that occur in closely related organisms and that look the same are usually considered to be homologous — their similarity is taken to arise from their common ancestry. Common sense suggests that the more complex such structures are, the less likely they are to have evolved independently and the more valuable they should be for studying systematics. But what if 'obviously' identical organs have arisen through two mutually exclusive developmental routes?

Beverley Glover and colleagues have revealed one such case (*Gene* **331,** 1–7; 2004). It occurs in the floral organs of the genus *Solanum* from the nightshade family. In one group of these species, the anthers — the flower's pollen-producing organs — are arranged as a cone, which functions like a 'pepperpot' (see the yellow, cone-like structures in a and b, right). In the pepperpot of bittersweet (*S. dulcamara*), the anther surfaces are held together by a glue-like secretion (a). In another species from the same group, tomato (*S. lycopersicum*), they are instead linked by interlocking hairs, or trichomes, along the edges of the anthers (b).

Glover *et al.* find that tomato trichomes are clearly required for pepperpot formation. In one form, the *dialytic* mutant, which lacks them, the pepperpot fails to develop (d). In bittersweet, however, trichomes surprisingly prevent pepperpot formation. Glover *et al.* show this using transgenic plants in which expression of a gene from snapdragon leads to the development of hairs on bittersweet anthers. The hairs push the glue-bearing surfaces apart, preventing pepperpot formation (c).

This result makes it unlikely that the tomato-type pepperpot originated from the bittersweet type, or vice versa, because the development of anther hairs in bittersweet-type cones would probably have caused the cone to fall apart, whereas the addition of glue to tomato-type cones already supported by trichomes would probably have carried no selective advantage. So the most plausible conclusion is that pepperpots originated twice independently in the lineages that led to tomato and bittersweet.

Molecular systematic analysis confirms that tomato and bittersweet are closely related, and the traditional view would be that their pepperpot cones are obviously homologous. But genetic tinkering and mutant analysis show that they probably are not — that they are convergent, having taken different routes to the same end. Life's potential to invent complex structures more than once may worry systematists, who depend on reliable characters to reconstruct relationships between organisms. But it will please anyone who admires nature's innovative power. **Günter Theißen**



A. DAVIS, JOHN INNES CENTRE

Günter Theißen is at Friedrich Schiller University, D-07743 Jena, Germany.
e-mail: guenter.theissen@uni-jena.de

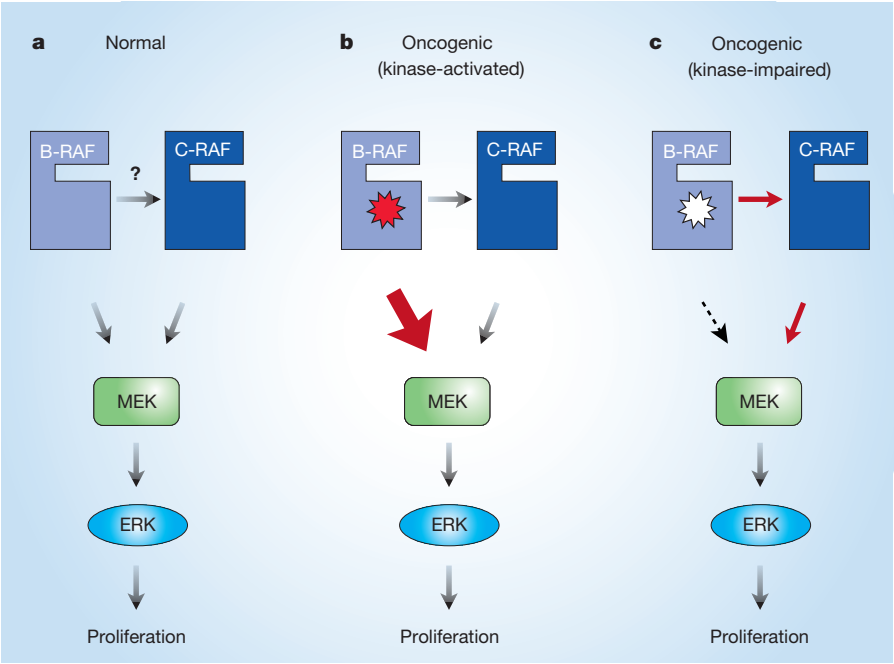


Figure 1 **Cells, signalling cascades and cancer.** a, **In one of the best-characterized signalling pathways that promotes cell proliferation, signals from outside the cell are passed via the RAS protein (not shown) to activate B-RAF and C-RAF, both of which activate MEK, which in turn switches on ERK.** b, **Most cancer-promoting (oncogenic) mutations in B-RAF stimulate its catalytic activity, resulting in MEK and ERK activation without the earlier events.** c, **Other oncogenic mutations impair B-RAF's kinase activity but somehow allow it to activate C-RAF, which stimulates MEK and ERK in its place. Although kinase-activated B-RAF mutants can also activate C-RAF, this is not required for later events. Whether B-RAF activates C-RAF under normal conditions is unknown.**

initiated by an interaction with RAS, which leads to RAF relocating from the cellular cytoplasm to the plasma membrane. This interaction also promotes conformational changes in RAF that remove self-inhibition and enable phosphorylation of its activating sites. In the mammalian C-RAF and A-RAF proteins, these sites are located in two regions of the catalytic ('kinase') domain — namely the negatively charged regulatory region (the N-region) and the activation segment[9,10].

In contrast, the N-region of B-RAF is constantly phosphorylated, so phosphorylation of the activation segment alone is enough to turn the enzyme on. Because this continuous phosphorylation of the N-region also disrupts self-inhibition, B-RAF is the member of this family that teeters closest to the brink of activation. This may explain why it is the only RAF protein so far that has been found to be activated by mutation in human cancers.

Of the more than 30 known tumour-associated B-RAF mutations, around 90% alter amino acids in the kinase domain, raising the question of whether these mutations promote cancer simply by increasing B-RAF's catalytic activity (Fig. 1b). To find out, Wan and colleagues[6] characterized the enzymatic properties of 22 such mutants. They found that most can be explained in this manner, including the highly prevalent V599E mutant (in which the amino acid valine, V, at position 599 in the protein is changed to glutamic acid, E).

Surprisingly, however, three mutants showed impaired, not enhanced, catalytic activity *in vitro* — but still stimulated ERK *in vivo*. How could these mutants switch ERK on? Remarkably, it seems that they do so by, in effect, playing tag, activating another RAF-family member, C-RAF (Fig. 1c). As has been reported previously[11], Wan and co-workers detected an association between B-RAF and C-RAF, which occurs whether B-RAF is normal or mutated. But C-RAF activity is increased only in cells that express the 'kinase-activated' or 'kinase-impaired' B-RAF mutants — and not in cells expressing normal B-RAF. Moreover, although both classes of mutant activate C-RAF, only the kinase-impaired proteins specifically require C-RAF in order to activate ERK (Fig. 1b, c), a finding confirmed in cell lines derived from human tumours with B-RAF mutations.

To gain further insight into the effects of these mutations, Wan *et al.* determined the structure of the normal and a cancer-associated B-RAF kinase domain — an impressive feat that researchers have struggled to accomplish for nearly a decade. As in other protein kinases, the B-RAF catalytic domain adopts a bi-lobal structure. Between the amino- and carboxy-terminal lobes is a cleft that binds the energy-providing molecule ATP.

Also consistent with other kinases, phosphorylation of the activation segment of the kinase domain is required for optimal catalysis — as noted above — and to stabilize the active shape of the protein. The crystal structure of the normal B-RAF kinase domain represents the inactive conformation. In this structure, the activation segment interacts with amino acids in a glycine-rich stretch — known as the P-loop — in the amino-terminal lobe. Strikingly, 70% of cancer-associated B-RAF mutations occur in either the P-loop or the activation segment, apparently destabilizing this inactive conformation. Some mutations, such as V599E, may inflict a double whammy by not only destabilizing the inactive structure but also mimicking the effect of phosphorylating the activation segment.

Interestingly, the mutations that impair kinase activity also localize to these regions, but instead they alter amino acids that are important in catalysis. Wan *et al.* propose that, like the kinase-activating mutations, these kinase-impairing changes destabilize the inactive structure, producing the active conformation. Crucially, however, the enzyme is still kinase-impaired, because amino acids involved in catalysis are mutated. Destabilization of the inactive structure could account for the cancer-promoting properties of this mutant class, but how it allows the impaired enzyme to activate the related C-RAF is unclear. Perhaps, by adopting an active structure, these mutants induce activating conformational changes in associated C-RAF proteins. Alternatively, they might recruit molecules that activate C-RAF, or titrate out negative regulators that inhibit it.

In this era of targeted therapeutics, these studies[6] should provide crucial structural information to guide the development of more effective inhibitors of this enzyme family. Given that the promotion of tumour development by RAF proteins is a family affair — involving both B-RAF and C-RAF, which share highly related catalytic domains and downstream substrates — inhibitors that effectively block the activity of both proteins could hold great promise as broad-spectrum treatments for cancer. ■

Deborah K. Morrison is in the Laboratory of Protein Dynamics and Signaling, Center for Cancer Research, National Cancer Institute, Frederick, Maryland 21702-1201, USA.
e-mail: dmorrison@ncifcrf.gov

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