Horváth and Rábai also provide a dramatic dye-based visual demonstration that the miscibility of fluorous and nonfluorous phases can depend on temperature. Hence, reactions could be conducted under homogeneous conditions at elevated temperatures and then cooled to effect product separation. Other engineering advantages that could be associated with FBS chemistry are easily imagined. For example, a reaction involving a fluorinated catalyst might be conducted in a single organic phase, and a fluorous phase loop in the product stream could be used for catalyst recovery. Alternatively, in an environmental application, toxic wastes could be extracted from product streams by immobilized fluorous binding agents. It should also be kept in mind that interfacial reactions may be dominant in some FBS chemistry. As the field develops, there will be a particular need for data on this point and the effect of solvent and ponytail structure on phase properties, solubilities, and related phenomena.

The above FBS hydroformylation can also be analyzed in the context of other rhodium-catalyzed reactions involving phosphines designed to confer special phase properties. First, sulfonated aryl phosphines have been shown to similarly immobilize rhodium catalysts in the aqueous phases of organic-aqueous biphase systems. Commercial hydroformylation plants making use of this technology have been in operation since 1984 (*5*). However, rates are constrained by the limited solubilities of the reactants in the aqueous phase. Second, rhodium has also been ligated to phosphines containing poly(alkene)oxide chains, $(CHRCH_2O)_n$ (*6*). Such oligomeric units often give rise to water solubilities that are inversely dependent on temperature. Accordingly, the resulting hydrogenation catalyst shows an abrupt but reversible cessation of activity upon heating. This property, which has been correlated to a phase separation or precipitation of the catalyst, could have practical application as a means of controlling exotherms.

The protocol developed by Horváth and Rábai is remarkable in its conceptual elegance and insight. Its timeliness is enhanced by several parallel developments. For example, there have been significant recent advances in methodology for perfluorinating sizable, functionalized organic compounds that lack existing fluorine (*7*). There is also a rapidly growing body of data on the fundamental properties of metal complexes with perfluorinated ligands (*8*, *9*). Realistically, it does remain to be seen whether catalysts and reagents with fluorine-rich ponytails will simply be this season's fashion statement or a lasting addition to the chemist's haberdashery or trousseau. However, the strategy in this game is even easier than that in "pin the tail on the donkey," as any point of attachment can in principle produce a winner. Given the large number of industrial and academic research laboratories that will likely want to step up and play, it will be surprising if practical and widely adopted applications do not result.

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14-3-3: Modulators of Signaling Proteins?

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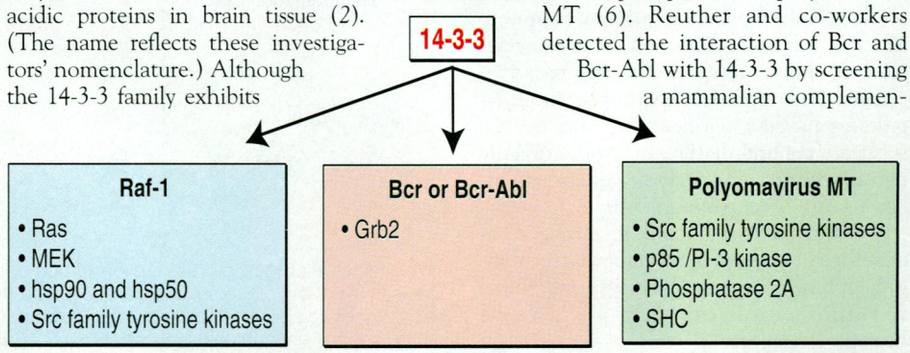
In spite of their unlikely name, the 14-3-3 proteins have been attracting attention recently. These molecules are highly conserved and are found in a broad range of organisms and tissues. At least seven mammalian isoforms of 14-3-3 have been identified, and multiple isoforms are present in most cells (*1*). 14-3-3 proteins were first identified by Moore and Perez as a series of very abundant 27- to 30-kilodalton (kD) acidic proteins in brain tissue (*2*). (The name reflects these investigators' nomenclature.) Although the 14-3-3 family exhibits a bewildering array of biological activities (*1*), many recent findings, particularly in fission yeast, point to the participation of these proteins in cell cycle control (*3*). In this issue (*4*, *5*) and in three previous reports (*6*–*8*) in *Science*, the 14-3-3 family acquires another feature of interest: Its members associate with the products of proto-oncogenes and oncogenes—in particular, Raf-1, Bcr-Abl, and the polyomavirus middle tumor antigen (MT)—suggesting that 14-3-3 proteins participate in cell transformation and mitogenic signaling pathways.



Associates of Raf-1, Bcr, Bcr-Abl, and polyomavirus MT. 14-3-3 is only one of many proteins that interact with these proto-oncogene and oncogene products.

The evidence that 14-3-3 interacts with proto-oncogene and oncogene products is compelling. 14-3-3 associates with Raf-1 in the yeast two-hybrid interaction system (*7*, *8*) and in binding assays in vitro (*4*) and is present in immunoprecipitates of Raf-1 expressed in insect cells and from mammalian cells (*4*, *8*). By protein sequencing analysis, Pallas and collaborators identified 14-3-3 in immunoprecipitates of polyomavirus MT (*6*). Reuther and co-workers detected the interaction of Bcr and Bcr-Abl with 14-3-3 by screening a mammalian complementary DNA expression library with a purified fragment of Bcr and showed that these proteins associated in vitro and coimmunoprecipitated from mammalian cells (*5*). These disparate techniques and approaches provide convincing evidence that this family of proteins indeed associates with proto-oncogene and oncogene products.

14-3-3 associates with Raf-1 at multiple sites, with the primary interaction sites located in the amino-terminal regulatory domain (*4*, *8*). The association of 14-3-3 with Raf-1, however, does not alter or interfere with the interaction of Raf-1 with Ras (*7*, *8*), which also occurs in this domain (*9*). For Bcr and Bcr-Abl, the 14-3-3 interaction site is located in the sequences encoded by

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the first exon of *BCR* (*5*). Cysteine- and serine-rich regions are common elements present in these regions of Raf-1, Bcr, and polyomavirus MT and may be determinants for 14-3-3 binding, although other regions of these proteins also contribute.

In contrast to the definitive association data, the functional effect of 14-3-3 association is still ambiguous. The most appealing suggestion is that 14-3-3 is the long-sought direct activator of the Raf-1 kinase. In mammalian cells, treatment with growth factors induces the association of Raf-1 with Ras (*10*); this interaction serves to translocate Raf-1 to the membrane, where it becomes activated (*11*). The exact mechanism by which Raf-1 is activated at the membrane is unclear. Could 14-3-3 be part of this mechanism?

Two recent reports suggest that it may be, by demonstrating that 14-3-3 modulates Raf-1 activity in yeast (*7*, *8*). Using a genetic screen, Irie and co-workers identified BMH1, the yeast homolog of 14-3-3, as a protein that when overexpressed enhanced the function of mammalian Raf-1 in budding yeast, and further showed that BMH1 was required for Raf-1 to be activated by Ras in this system (*7*). Freed and colleagues also found that overexpression of mammalian 14-3-3 proteins in yeast stimulated the biological activity of mammalian Raf-1 and observed that mammalian Raf-1 immunoprecipitated from yeast strains overexpressing 14-3-3 had three- to fourfold more enzymatic activity than Raf-1 from yeast strains lacking 14-3-3 expression (*8*). In addition, Fantl and colleagues will report next week (*12*) that expression of 14-3-3 in *Xenopus* oocytes induces meiotic maturation and that Raf immunoprecipitated from these oocytes had increased kinase activity. These data would suggest that 14-3-3 does alter Raf-1 function, but is 14-3-3 directly regulating Raf-1 enzymatic activity? Irie and co-workers approached this question by adding recombinant 14-3-3 in vitro to mammalian Raf-1 isolated from yeast. The 14-3-3 protein increased Raf-1 activity three- to fourfold, although this assay did not measure Raf-1 catalytic activity directly (*7*). When Fu and collaborators did measure catalytic activity directly, they found that one preparation of purified 14-3-3 enhanced the activity of baculovirus-expressed Raf-1 two- to threefold, while four other preparations had no stimulatory activity (*4*). Interpreting the significance of these findings is complicated by the observation that in vivo 14-3-3 always associates with Raf-1 regardless of the subcellular location or activation state of Raf-1 or whether Raf-1 is bound to Ras (*4*, *8*). It is unclear how exogenously added 14-3-3 could activate Raf-1 that is already associated with 14-3-3 and how a constitutive interaction could become a regulated activational event. Therefore, 14-3-3 binding alone cannot be sufficient to activate the Raf-1 kinase. Although 14-3-3 may be a cofactor involved in the stimulation of Raf-1 activity, it is difficult to conclude that 14-3-3 is indeed the direct activator of Raf-1.

An alternative function for 14-3-3 is suggested by the constitutive association of these highly abundant 14-3-3 proteins with oncogene and proto-oncogene products: 14-3-3 may play a structural role in stabilizing the activity or conformation of signaling proteins. This function would be similar to that proposed for the heat shock protein, hsp90, which also binds to Raf-1 (*13*). Binding of hsp90 to steroid and tyrosine kinase receptors is thought to stimulate or stabilize biologically active conformations of these receptors (*14*). A structural role for 14-3-3 could explain the enhancement of Raf-1 activity in yeast strains expressing 14-3-3 and in vitro. When Raf-1 is overexpressed in yeast where the amounts of 14-3-3 (BMH1) may be limiting, the overexpression of 14-3-3 (or the addition of 14-3-3 to yeast-expressed Raf-1 in vitro) may allow more Raf-1 molecules to become functionally competent. 14-3-3 may thus be required for the conformational stabilization of the kinase rather than for actual stimulation of Raf-1 activity. 14-3-3 may also regulate protein trafficking, since these proteins share a short stretch of sequence homology with the annexins. Members of the annexin family can act as receptors for activated protein kinase C and mediate the translocation of protein kinase C from the cytoplasm to the membrane (*15*).

Another role for 14-3-3 is suggested by the intrinsic adenosine diphosphate (ADP)–ribosylation cofactor activity of these proteins identified by Fu and co-workers (*16*). Because Ras and other small guanosine triphosphate–binding proteins (G proteins) can be ADP-ribosylated (*17*)—and Raf-1, Ras, and 14-3-3 may exist in a ternary complex—it is possible that Raf-1-bound 14-3-3 induces the ADP-ribosylation of Ras. However, Ras has not been shown to be ADP-ribosylated in vivo, and an effect of this modification on Ras activity has not been reported. 14-3-3 can also serve as a kinase substrate. Reuther and co-workers show that 14-3-3 is phosphorylated in vitro by Bcr and Bcr-Abl and that it is a substrate for these proteins in vivo (*5*). This work identifies the 14-3-3 protein Bap-1 as the first physiological substrate of Bcr. It is not known whether 14-3-3 proteins are substrates of other oncogene and proto-oncogene products or whether phosphorylation modifies the interactions of 14-3-3 with cellular proteins or alters any of its activities.

Other potential functions of 14-3-3 can be envisioned. Because 14-3-3 proteins can form dimers in vitro (*18*), they may form bridges that connect proto-oncogene and oncogene products with other signaling or cytoskeletal proteins. Therefore, the signaling proteins associating with Raf-1, Bcr-Abl, and polyomavirus MT might also interact with or be modified by 14-3-3.

One can only conclude from the available data that much more has yet to be learned about the binding of 14-3-3 with proto-oncogene and oncogene proteins and the function of these interactions. Of utmost importance are experiments identifying mutants of Raf-1, Bcr-Abl, and polyomavirus MT that cannot interact with 14-3-3. If 14-3-3 modulates function, mutational analysis should show a correlation between 14-3-3 association and the biological or biochemical activities of wild-type and mutant versions of these proteins. The fact that there are seven identified mammalian 14-3-3 family members (*1*, *5*), each possibly having a distinct specificity for (and ability to activate) proto-oncogene and oncogene products, will complicate this study, but the techniques are available to provide a clearer view of these interactions.

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