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Variation on an Src-like Theme

Minireview

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The modularity of protein architecture and the diversity of protein domains hint at a vast combinatorial richness. But evolution appears to have been relatively conservative about selecting new combinations. When a particular grouping of domains within a polypeptide chain can perform a concerted function, that combination tends to reappear in multiple genomic contexts. In other words, once a molecular solution to a functional problem has emerged, it is reused rather than reinvented.

Consider the set of three domains found in Src family kinases—the Src-homology 3 or "SH3," the Src-homology 2 or "SH2," and the protein tyrosine kinase or "PTK" domains, respectively (Figure 1). Each domain can carry out its specific recognition or enzymatic function independently, but the ensemble creates coordinated regulatory properties as seen in the structures of the SH3-SH2-PTK fragments from Src and Hck (Figure 1; Sicheri et al., 1997; Xu et al., 1997; Williams et al., 1997). Members of at least two other protein kinase families—the Abl/Arg and Tec kinase types—have a precisely similar grouping of domains, and it has therefore been suspected that a Src-like switching mechanism might control them. Work reported in the present issue of *Cell* (Nagar et al., 2003; Hantschel et al., 2003) now confirms the correctness of that suspicion for c-Abl, by demonstrating that, like Src, it can adopt an "assembled" structure in which the SH3 and SH2 domains pack against the kinase domain and clamp it into an inactive state. Regulation by domain assembly also helps explain the surprising specificity of Abl inhibitors like STI571/imatinib and the distribution of imatinib resistance mutations (Azam et al., 2003 [also this issue of *Cell*]).

Protein serine/threonine and tyrosine kinases all contain a two-lobe enzymatic domain of conserved structure (Huse and Kuriyan, 2002). The catalytic site lies in a cleft between the smaller, amino-terminal lobe and the larger, carboxy-terminal one. The two domains can adopt a range of relative orientations, opening and closing the active-site cleft. Within each lobe is a polypeptide segment that has an "active" and an "inactive" conformation. In the small lobe, this segment is an α helix (the "C-helix") that in some kinases rotates and translates with respect to the rest of the lobe, making or breaking part of the catalytic site. In the large lobe, a loop (the "activation loop") reconfigures to make or break a different part of the catalytic region. In most kinases, including Src, the activation loop is stabilized in its active conformation by phosphorylation of one or more residues within it.

Kinases like Src and Abl have an elaborate built-in regulatory apparatus, made primarily from structural elements outside the kinase domain. In Src and its relatives, the control machinery has three critical components, which I will call the "switch," the "clamp," and the "latch." The switch is the kinase-domain activation loop and the coupling of its conformational state to a transition between active and inactive conformations of the whole domain (including a shift in the C-helix). The clamp is an assembly of the SH2 and SH3 domains (the "regulatory apparatus") on the back side of the kinase. The SH2 domain abuts the large lobe; the SH3 domain, the small lobe. The linker connecting SH2 and kinase is an adaptor element that runs through the ligand binding groove of the SH3 domain and interfaces it to the kinase. In Src-family kinases, the latch is a short, tyrosine-containing, C-terminal tail. When the tyrosine is phosphorylated, it binds the SH2 domain and fastens the clamp in place. In physico-chemical terms, it stabilizes the assembled state of the regulatory apparatus (Xu et al., 1999).

Alignment of sequences with those of Src-family members has left little doubt that Abl and Arg—and probably Tec-family kinases as well—would have Src-like switches and clamps. Indeed, mutagenesis experiments on Abl have confirmed various predictions of those similarities (for example, Barilá and Superti-Furga, 1998). But what about a latch? c-Abl lacks a simple, tyrosine-containing tail. Rather, it has a long set of other functional elements carboxy-terminal to the kinase. Its inactive form also lacks any phosphorylated tyrosines. Experiments published last year from Superti-Furga's laboratory (Pluk et al., 2002) suggested that the region *amino*-terminal to the SH3 domain might help stabilize the autoinhibited state of the enzyme. This amino-terminal "cap" bears an N-myristoyl group in one splice form of c-Abl (Abl 1b), but not in another (Abl 1a). A collaboration between the groups of Kuriyan and Superti-Furga has now come up with the unexpected answer that in Abl 1b it is the N-myristoyl group at the extreme end of the amino-terminal segment that keeps the SH3-SH2 clamp in place (Nagar et al., 2003; Hantschel, et al., 2003). The structural details of the latching mechanism involve the same corner of the large lobe of the kinase from which the carboxy-terminal tail emerges in Src, and the entire picture constitutes an elegant evolutionary variation on the familiar, Src-like theme.

How does the N-myristoyl group contribute to the assembled structure? The large lobe of the kinase presents a deep, hydophobic pocket for the fatty-acid chain, but the local structure depends on whether or not the myristoyl group is in place. In its absence, as seen in an earlier structure of the kinase domain alone (Schindler et al., 2000), rearrangement of two α helices that border the pocket destroys the docking surface for the SH2 domain. Thus, removal of the myristoyl group unlocks the clamp by destabilizing the SH2-kinase inter-

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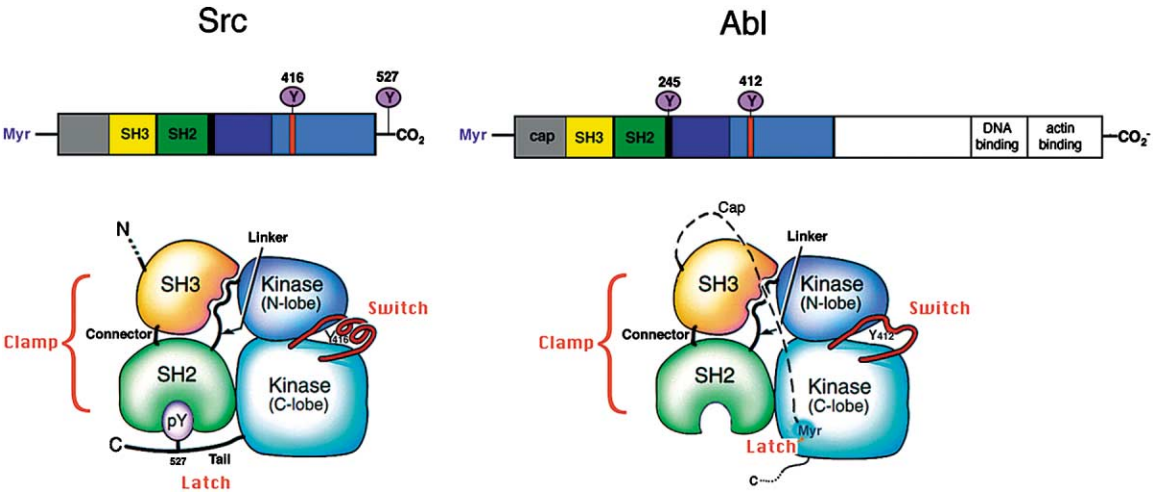

Figure 1. The Order of Domains in the Polypeptide Chains of Src and Abl, and Diagrams of Their Assembled, Autoinhibited States

In both cases, the SH3-SH2 clamp fixes the bilobed kinase domain in an inactive conformation. The domain color codes are SH3, yellow; SH2, green; kinase small lobe, dark blue; kinase large lobe, light blue. The activation loop in the large lobe is red. Connector, linker, and N- and C-terminal extensions are black. In Bcr/Abl, gene fusion has replaced the Abl cap by a long segment of Bcr.

action. The corresponding interactions in Abl 1a are not yet determined. The authors argue that hydrophobic amino-acid residues in the 1a cap might substitute for the fatty-acid side in the 1b cap, and the shape of the pocket appears to allow such a substitution. Other interactions, involving the SH3-proximal end of the cap, may also contribute: in one of the structure determinations described by Nagar et al. (2003), there is distinct but poorly ordered electron density attributable to this part of the polypeptide chain.

In what sense is the SH3-SH2 substructure a clamp? In solution, the two parts of the SH3-SH2 Src fragment are quite independent, and the short connector between them flexes freely (Fushman et al., 1999). But when organized into the asembled state of intact Src, the SH3-SH2 modules have strongly correlated dynamics, as demonstrated by some elegant and insightful simulations carried out by Young et al. (2001) and now replicated for Abl (Nagar et al., 2003). The computations show that the two submodules have correlated motions in the assembled structure. They predict that mutation of the SH3-SH2 connector might reduce the strength of the clamp; indeed such mutations do diminish autoinhibition, both in Src and in Abl (Young et al., 2001; Hantschel et al., 2003). Kuriyan and co-workers have called the clamp an "inducible snap lock", to capture the notion that the two flexibly joined parts snap together and rigidify the bilobed kinase domain.

Why does rigidification inhibit the kinase? The catalytic cleft must open and close, at least to some extent, to admit ATP and to release ADP. This capacity to flex, essential to the catalytic cycle, is effectively blocked by the snap-lock clamp assembly, which spans the two lobes and directly contacts the hinge between them. Perhaps equally important is that clamping enhances protection of Tyr 412. This is the position at which phosphorylation triggers rearrangement of the activation loop. Tyr 412 is sequestered in the inactive conformation of the kinase domain (Schindler et al., 2000; Nagar et al., 2003). By reinforcing this sequestration, the clamp blocks an event that would probably force disassembly of the autoinhibted state and effectively deregulate the enzyme (barring subsequent removal of the phosphate by a phosphatase). Src kinases in their autoinhibited conformations also bury their activation-loop tyrosines (Schindler et al., 1999; Xu et al., 1999).

The inactive conformations of Src and Abl, reinforced by essentially similar clamps, differ in subtle but important ways. In the "off" state of Src, the C-helix in the small lobe moves out of the active site, withdrawing a key glutamate residue. The comparable rearrangement does not occur in Abl. Moreover, the activation loops of the two kinases adopt dissimilar conformations in their respective autoinhibited states, and the differences induce different conformations even in conserved parts of the catalytic site. These observations help explain the remarkable specificity of inhibitors such as imatinib.

Imatinib is a 2-phenylaminopyrimidine derivative that occupies the ATP site of the kinase. It inhibits Abl, but not the very closely related Src. Structures of imatinib complexes of the Abl catalytic domain have shown that the drug requires an inactive kinase conformation for binding (Schindler et al., 2000; Nagar et al., 2002). Thus, the different responses of the Abl and Src catalytic domains to their autoinhibitory clamps underlie their divergent sensitivities to imatinib. Strong conservation of catalytic-cleft residues does not prevent longer-range structural features (e.g., in the activation loops) from imparting striking differential specifity. The inactive conformation of Abl is very similar to that of the insulin receptor (IR) tyrosine kinase. Imatinib does not inhibit IR, however, probably because of one or a few residues that differ from their Abl counterparts and block drug binding (Schindler et al., 2000). It does inhibit the platelet-derived-growth-factor-receptor (PDGFR) and the c-kit tyrosine kinases: presumably their inactive conformations resemble those of Abl and IR, without interfering amino-acid substitutions.

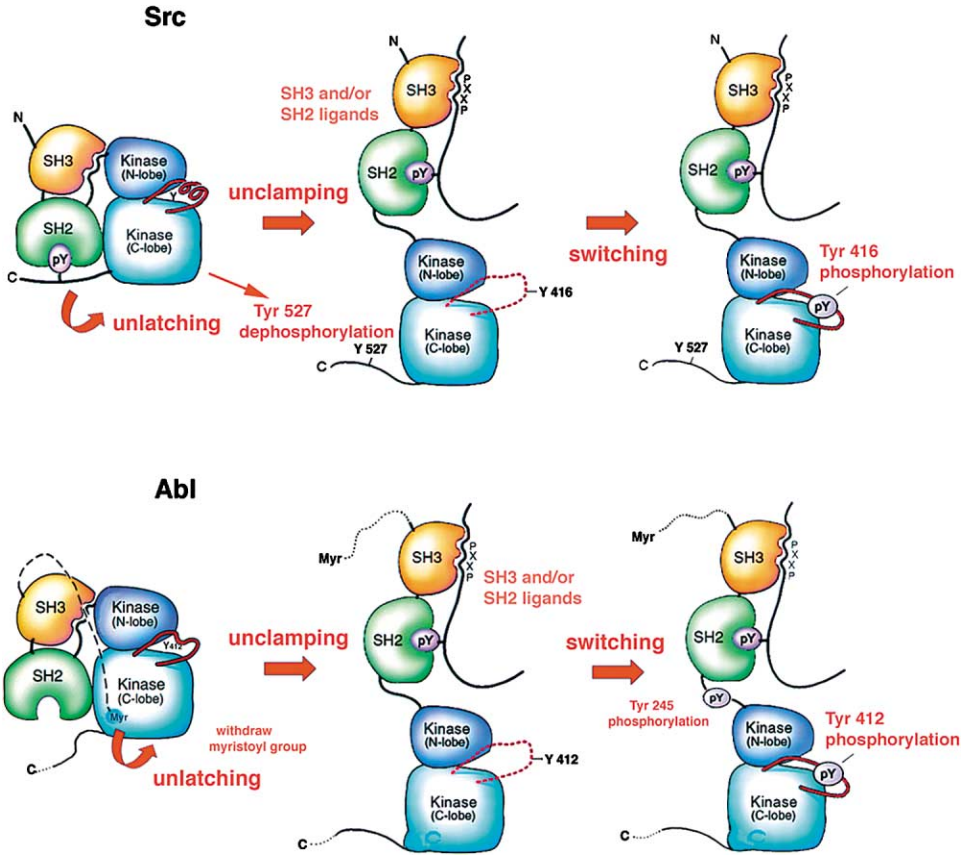

Figure 2. Modes of Activation for Src and Abl: Unlatching, Unclamping, and Switching

In Src-family members, the assembled state is unlatched by dissociation of the C-terminal tail from the SH2 domain and dephosphorylation of the exposed Tyr 527. In Abl, the assembled state is unlatched by release of the cap, anchored by a myristoyl group in Abl 1b and presumably by alternative hydrophobic interactions in Abl 1a. Competing SH2 and SH3 ligands can unclamp the assembled regulatory appartus of Src or Abl, and the kinase domain can then be switched into its active conformation by phosphorylation of a tyrosine in the activation loop. Linker phosphorylation further sets the switch in Abl.

The oncoprotein product of the Phildelphia chromosome in patients with chronic myeoloid leukemia (CML) is a deregulated version of Abl, in which fusion with the breakpoint-cluster-region product (Bcr) has deleted much of the N-terminal cap and hence the latch mechanism. Bcr fusion also leads to oligomerization, which probably enhances transphosphorylation of Tyr 412 and thus accelerates the switch. Patients treated with imatinib in the indolent chronic phase of CML experience complete remission; those treated in the more aggressive stages of the disease generally evolve drug-resistance. While most patient-derived resistance mutations are within the Bcr/Abl kinase domain, a cell-based screen for resistance mutations carried out by Daley and coworkers identifies point changes at a number of other sites (Azam et al., 2003). The results correlate extemely well with expectations from the structural analyses just described. Mutations conferring imatinib resistance appear not just at residues that contact imatinib directly or in parts of the kinase domain that might evidently influence its conformational preferences, but also in the cap, at the interfaces between the regulatory apparatus and the kinase domain, in the SH3-SH2 connector, and in the SH2-kinase linker. These mutations probably destabilize the assembled state of the enzyme. They suggest that the clamp can affect imatinib sensitivity by influencing the assembled-disassembled conformational equilibrium and by indirectly hindering phosphorylation of Tyr 412. Phosphorylation of the activation loop will render the enzyme insensitive to the drug. Note, however, that only if the kinase opens up occasionally will the drug bind at all. Thus, mutations that destabilize the assembled state may, paradoxically, enhance inhibition by imatinib in vitro, if the measurements are done under conditions that do not allow phosphorylation of Tyr 412 (Roumiantsev et al., 2002; Azam et al., 2003; Hantschel et al., 2003).

Kinases have become fashionable targets for the pharmaceutical industry, not the least because of the clinical success of imatinib in treating CML. The properties of the assembled state of Abl show why drug developers should look well beyond the catalytic sites of their enzymes. Imatinib's spectrum of targets (Abl, PDGFR, c-kit, but not Src) corresponds not just to conservation of active-site residues but also to conformational properties that extend across much of the kinase domain. The possibility of enhancing specificity or potency for Abl by simultaneously targeting the myristoyl pocket is

an obvious conclusion from the present discoveries. Moreover, proper analysis of imatinib resistance clearly requires understanding the assembled state of a multidomain protein. The complexities of potential patient resistance profiles implicit in the results of Azam et al. (2003) recall the history of understanding resistance of HIV to nucleoside-analog reverse-transcriptase (RT) inhibitors: a hard-won structure of a catalytic HIV-RT complex finally helped sort out apparent discrepancies between in vitro and in vivo observations (Huang et al., 1998; Boyer et al., 2001).

What sort of control device is the latch-clamp-switch of Abl and Src? Activating inputs are likely to come in several forms (Figure 2). *Unlatching* by withdrawl of the myristoyl group from its pocket is probably one form of activation, and a specific myristoyl binding protein could be part of the larger control circuitry. *Unclamping* by SH3 and SH2 ligands is a second kind of potential input. Although the phosphotyrosine pocket of Abl's SH2 domain is not occupied in the assembled structure, as it is in Src-family kinases, the ligand-binding site is partially occluded, and strong phophotyrosine-bearing peptides (e.g., docking sites on receptor tyrosine kinases) are likely to trigger disassembly (Hantschel et al., 2003). SH3 ligands will probably do the same. *Switching* the enzyme into its active state, by phosphorylation of Tyr 412, is a third type of input. This switch will be reversible only through the action of a phosphatase, but it will be require at least partial unclamping ("breathing open" of the kinase) to be set in the first place. A similarly hard-to-reverse, switch-like input is phosphorylation of Tyr 245, in the linker between SH2 and kinase domains (Brasher and Van Etten, 2000). Modification of this residue breaks the SH3-linker ineraction and prevents assembly into the autoinhibited structure.

The assembled state of c-Abl illustrates an important principle that modelers and redesigners of signal transduction networks will need to bear in mind. There is a limit to interchangeability. Recent work on the redesign of MAP kinase signaling complexes has shown that many aspects of the phosphorylation pipeline can be preserved by installing generic tethers on the kinases and creating a generic scaffold (Park et al., 2003). Had the SH3 and SH2 domains been part of Src or Abl only to recruit the kinase function to an appropriate target or scaffold, then considerably more leeway might have been possible in the way they could be linked to the catalytic domain and to each other. In practice, they form, along with the catalytic domain, an integrated structure with critical internal coherence. Unlike many man-made computers, which have been designed for programmability, the hardware of biological control circuits has coevolved with its program.

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