Cancer

Escape from inhibition

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Mutations in the Abl protein cause some leukaemias. The determination of its structure and the identification of Abl variants resistant to an anti-leukaemia drug reveal regions of the protein needed for its regulation.

The enigmatic protein Abl has just given up another of its secrets. This enzyme is important because in mutated, activated form it causes some types of human leukaemia — so researchers have been keen to learn how it is regulated. That goal became still more precious when it was realized that the resistance of leukaemia to Gleevec (a drug that inhibits Abl) often results from further mutations in Abl itself. But until now the protein has stubbornly resisted attempts to understand it.

Writing in *Cell*, however, Hantschel and colleagues[1] and Nagar and co-workers[2] describe how they used a combination of mutational and crystallographic analyses to solve the puzzle. In a surprising twist, the regulation of Abl turns out to closely resemble that of a related enzyme, Src, despite key structural differences. Meanwhile, a complementary paper by Azam and colleagues[3] in the same issue describes the use of an *in vitro* assay to screen for Gleevec-resistant variants of Abl. This work reveals that Abl can escape Gleevec inhibition by mutating several amino acids now shown to be key to its regulation. Together these findings have implications for the design of the next generation of Abl inhibitors, and provide an unbiased assay with which drug resistance in Abl can be probed in the future.

Abl is a tyrosine kinase — an enzyme that transfers phosphate groups to tyrosine amino acids in proteins — and is expressed in all tissues of the body. One end of the protein (the amino-terminal half) resembles the related kinases from the Src family[4], with a region for binding proline-rich peptides (the SH3 domain), a domain that binds proteins containing phosphorylated tyrosine residues (the SH2 domain), and a catalytic portion (Fig. 1). But whereas Src-family kinases have, following the catalytic domain, a short 'tail' (which is crucial for their regulation), Abl has a long extension with domains that dictate its subcellular localization, and regions for protein and DNA binding[5]. Alternative processing of the Abl-encoding messenger RNA produces two proteins, called type 1a and type 1b, which differ in their extreme amino termini. Both forms of Abl are tightly regulated in cells, generally have very low kinase activity, and are distributed between the cell cytoplasm and nucleus.

So how is Abl so tightly controlled? One reason why researchers are interested in this question is because deregulation of Abl's kinase activity causes certain forms of blood cancer. More than 95% of chronic myelogenous leukaemias (CMLs), and some 10% of acute lymphocytic leukaemias, result from a chromosomal translocation that fuses part of the *bcr* gene from chromosome 22 with part of the *abl* gene on chromosome 9. The result is a fusion protein, Bcr–Abl[6], that is missing the extreme amino terminus of Abl (Fig. 1) and has high kinase activity, although it spends some time in the inactive state. The drug Gleevec (also known as STI-571 or imatinib), recently approved to treat CML, inhibits the catalytic activity of Bcr–Abl by binding to the inactive form of the kinase, thus stabilizing it[7,8]. It is a very successful treatment for patients in the early, chronic stage of the disease. But most patients in the later stage of the disease, although responding well at first, eventually

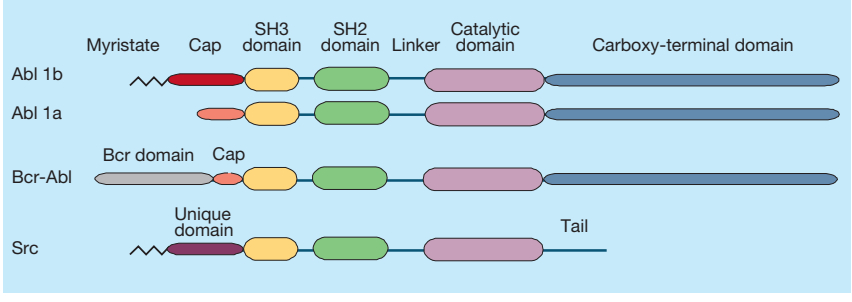

Figure 1 **The domain structure of the enzymes Abl and Src. The domains of Abl 1a and 1b (the two alternatively processed forms of Abl), Bcr–Abl and Src are shown. Note that the Bcr domain is not depicted in its entirety. The Abl carboxy-terminal domain contains sites that can be phosphorylated, sequences for import into and export from the nucleus, motifs with the sequence PxxP (where P is proline and x is any amino acid), a DNA-binding domain and a region that binds the cytoskeletal protein actin. These have been omitted for simplicity.**

100 YEARS AGO

The trouble of compiling pedigrees and their unmanageable size led me to devise a method of recording relationships in a form suitable to my own particular wants. As it promises to answer exceedingly well, and to be of more extending utility, I venture to publish it. The system of relationships between those who live or have lived in a long-established community is wide in extent, of indefinite depth, and interlaced in all directions. The problem is how to arrange its records so that when any individual is selected as a point of departure, it shall be easy to trace his relationship in every direction, whether ascending, descending, or collateral, so far as materials exist. The representation of such a system is wholly beyond the powers of a chart, but its object can be attained by breaking it up into what will be called "Family Groups," each of which slightly overlaps those with which it is immediately connected. A family group, in the sense used here, consists of (1) a parental couple, (2) all their sons and daughters, (3) the wives and husbands of them. Their names are supposed to be written on one page of a register, and the group, as a whole, to be defined by the No. of that page. The group is also… indexed under the joined surnames of the parental couple. **Francis Galton**
From *Nature* 23 April 1903.

50 YEARS AGO

New terms of grant were offered to nine research associations for the next five-year period… The new block grant for the British Hat and Allied Feltmakers' Research Association for 1952–54 will be £7,000 a year, conditional on £10,000 from the industry, with up to £5,000 on the £100 for £100 basis. The Association reports advances in the knowledge of mercuric carrotting of rabbit fur, which are leading to a better understanding of the process, and an investigation has been started on the effect of different types of dyeing machine on the quality of felt produced. For the two years 1952–53, the British Food Manufacturing Industries Research Association will receive a block grant of £9,000 a year, conditional on £20,000 from industry, with up to a further £6,000 on the customary basis… the Association is investigating the graining and rheological behaviour of chocolate, which are important in controlling the physical character of the chocolate and in preventing 'bloom'.
From *Nature* 25 April 1953.

relapse[9]. Some relapses are due to mutations in the catalytic domain of Bcr–Abl. So, understanding the effect of these mutations requires that we understand how Abl is regulated under normal circumstances.

The regulation of Src, an enzyme closely related to Abl, is well understood (Fig. 2a). In the inactive form, a phosphorylated tyrosine in the tail of the Src protein binds to the protein's own SH2 domain. This triggers the association of the SH3 domain with sequences in the 'linker' that lies between the SH2 domain and the catalytic domain, which in turn forces the catalytic domain to adopt a conformation incompatible with catalysis[10]. Another domain of Src, the 'unique domain', has no role in its regulation, but is required to attach the protein to membranes (with linkage of the small membrane lipid myristate to the first amino acid of the unique domain being particularly important).

Given the similarities between Abl and Src, why was the regulation of Abl so difficult to understand? The answer lies partly in the fact that Abl lacks a tyrosine-phosphorylated tail, and indeed is not detectably tyrosine-phosphorylated in cells. Furthermore, mutation of Abl's SH2 domain (so that it can no longer bind phosphorylated tyrosines) does not activate the enzyme. This, together with the observation that Abl's SH3 domain is required for regulation, led to models that invoked not 'self-regulation' (as for Src), but rather regulation by other proteins binding to the SH3 domain[5].

Prompted by two key observations, Hantschel *et al.*[1] and Nagar *et al.*[2] have now proposed a new model, in which regulation is in fact an intrinsic property of the amino-terminal half of Abl. The first observation was that sequences at the extreme amino-terminus of Abl 1b, called the cap, are critical[1,11]. In particular, mutation of the first amino acid (which prevents attachment of myristate) activates the enzyme both *in vitro* and *in vivo*, without affecting its subcellular localization. Second, incubation of Abl with high concentrations of tyrosine-phosphorylated peptides activates catalysis, suggesting a role for the SH2 domain[1].

Analysis of several crystal structures of Abl 1b, with and without the cap and/or catalytic inhibitors, reveals how the cap and SH2 domains contribute to the self-inhibition of Abl[2] (Fig. 2b). Remarkably, the trigger that generates the inhibited state is the binding of the myristate-attached cap to a deep pit at the base of the catalytic domain, causing a conformational change in this domain. This in turn forces the SH2 domain to dock onto the catalytic domain in a way that prevents SH2 interacting with proteins containing phosphorylated tyrosines. As in Src, the SH3 domain, rigidly linked to the SH2 domain, makes contact with linker sequences. Despite the overall similarity between the inhibited forms of Abl and Src, there are some key differences, particularly in the conformation of the catalytic domain, that help to explain why Gleevec inhibits Abl but not Src.

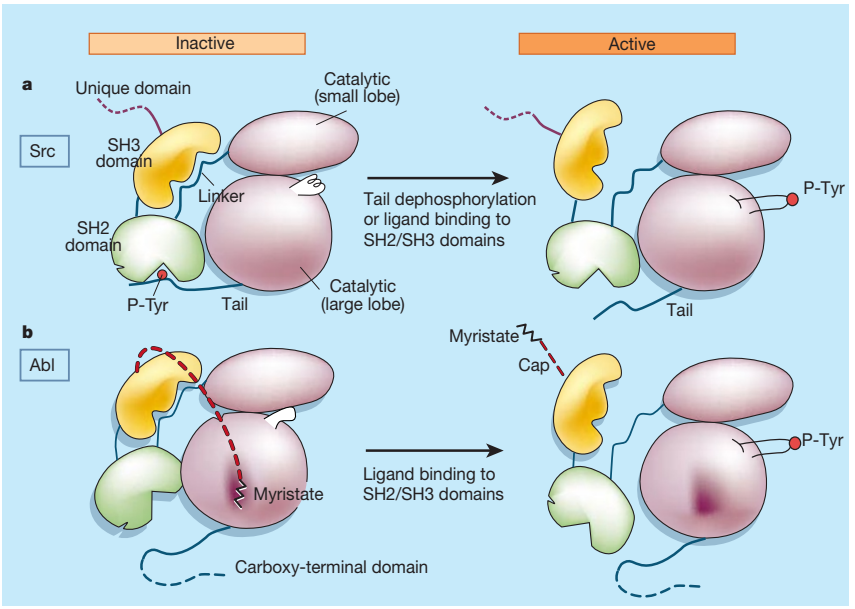

Figure 2 **The regulation of Src and Abl.** a, **The inactive form of Src is initiated by an intramolecular interaction between the SH2 domain and the tyrosine-phosphorylated tail (P-Tyr), and also involves the SH3 domain and linker.** b, **Abl is locked into an inactive state by binding of the myristate-attached cap to a pit in the large lobe of the catalytic domain. The SH2 domain binds the catalytic domain in a way that occludes the SH2 domain's phosphotyrosine-binding site. The substrate-binding face of the SH3 domain interacts with the linker. These locking mechanisms cause the catalytic domains of Abl and Src to adopt (different) inactive conformations. Activation of Abl occurs when high-affinity ligands for the SH3 and/or SH2 domains break the intramolecular contacts. Activation of Src can also occur in this way, and by dephosphorylation of the tail. Note that except for the myristate group, the cap sequences were not visible in the latest crystal structure of Abl[2] (hence the dotted lines). Neither the carboxy-terminal domain of Abl nor the unique domain of Src was present in constructs used for structural analysis.**

In searching for mutations in Bcr–Abl that confer such resistance, most studies have concentrated on the catalytic domain. Azam *et al.*[3] have conducted a more comprehensive survey, by testing randomly mutated Bcr–Abl proteins *in vitro* to identify Gleevec-resistant variants. Among the variants generated, the authors found most of the known catalytic-domain mutations that occur in patients, but also several others that affect the non-catalytic domains. Many of these mutations are in sites that the crystal structure now shows to be required in maintaining the inactive form of Abl —that is, in the cap, the SH3 and SH2 domains, and the linker. In these variants, the inhibited form of Abl is probably destabilized, so that the active enzyme, which cannot bind Gleevec, predominates.

In sum, we now understand that the cap, the SH3 domain and the SH2 domain are required both for regulation of Abl under normal circumstances, and for inhibition of Bcr–Abl by Gleevec. It is likely that, as Gleevec use expands, mutations outside the catalytic domain will also be detected in some drug-resistant patients. New Abl inhibitors that are being developed to treat Gleevec-resistant patients should in the future be screened to see if they work on the most common of these mutant forms. Furthermore, Bcr–Abl is not attached to myristate; future drug-discovery efforts might therefore fruitfully be geared to identifying compounds able to bind the myristate-attachment site and restore full regulation to the enzyme.

But Abl has not given up all of its secrets yet. Although the structural analysis[2] revealed the myristate group in the pit of the catalytic domain, it was not possible to see the relationship between the rest of the cap and Abl. Also, the alternatively processed form Abl 1a is fully regulated even though its cap lacks myristate; and Bcr–Abl only has a partial cap. How a non-myristylated cap contributes to Abl regulation will surely be the subject of intense study. ■

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