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JB Commentary

Phosphorylation in the activation loop as the finishing touch in c-Kit activation

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Receptor tyrosine kinases are a group of transmembrane proteins that transmit signals in response to stimulation by ligands including growth factors and cytokines. They share a common mechanism of activation through receptor dimerization or oligomerization, but subsequent routes to their full activation appear to be various. A recent paper published by DiNitto *et al.* (Function of activation loop tyrosine phosphorylation in the mechanism of c-Kit autoactivation and its implication in sunitinib resistance. *J. Biochem.* 2010;147:601–609) analysed a process of c-Kit autoactivation in detail. They revealed that phosphorylation in the activation loop, which is crucial for activation of many types of tyrosine kinases, is dispensable for c-Kit activation. However, the phosphorylation affects the sensitivity of c-Kit to kinase inhibitors, thus representing the finishing touch in c-Kit activation.

Keywords: tyrosine kinase/the A-loop/juxtamembrane domain; c-Kit.

Receptor tyrosine kinases play key functions in various intercellular communication processes that drive embryonic development as well as maintain homeostasis in adults. They have a low basal kinase activity in the resting state and are activated through *trans*-autophosphorylation of their intracellular kinase domain upon ligand-induced dimerization or oligomerization (*1*). In many textbooks of biochemistry, activation of tyrosine kinases is often explained referring the insulin receptor kinase, in which tyrosine phosphorylation in the activation loop (A-loop) is the crucial step that triggers kinase activation (*2*). Upon phosphorylation by a basal kinase activity, the A-loop, which occludes the catalytic site in the resting state, changes its conformation to allow access of substrates and ATP, leading to phosphorylation of substrates. Consistently, many tyrosine kinases lose their activity upon mutation of conserved tyrosine residue(s) in the A-loop (*3*). However, there is no rule without exceptions. One representative exception is epidermal growth factor receptor (EGFR), in which mutation in the conserved tyrosine located in the A-loop does not affect receptor activation and induction of cell proliferation upon ligand stimulation (*4*). EGFR is activated through formation of an asymmetric dimer (*5*). Another exception to this rule is c-Kit.

c-Kit is a receptor tyrosine kinase that is activated in response to stimulation by stem cell factor. Physiologically, it transmits signals required for development of various types of cells including primordial germ cells, haematopoietic progenitor cells, melanocytes and mast cells (*6*). Total loss of c-Kit activity is embryonic lethal. Abnormal signal transduction of c-Kit due to gain-of-function mutations is implicated in several malignant tumours, among which well studied is gastrointestinal stromal tumour (GIST) (*7*). GIST is the most common mesenchymal tumour in the human digestive tract, which appears to be originated from the interstitial cells of Cajar, pacemaker cells for gut peristalsis. More than 80% of GISTs have gain-of-function mutations in c-Kit, two-thirds of which were found in the juxtamembrane (JM) domain (*7*), suggesting an important role of the JM domain during c-Kit activation. Subsequently, c-Kit kinase activity was shown to be autoinhibited by the JM segment through intramolecular interaction in the resting state (*8–10*) (Fig. 1A). Phosphorylation or mutation in the JM segment releases it from the autoinhibitory position. Thus, phosphorylation in the JM domain is the initial event during c-Kit activation. Tyrosine residues in other regions are also phosphorylated. A phosphotyrosine in the 'kinase insert' domain (KID) (tyrosine 703) is known to serve as a docking site for Grb2 (*11*). In contrast, the specific role of phosphorylation of tyrosine 823 (Y823), located in the A-loop, has not been well understood (*9*).

In the recent report, DiNitto *et al.* (*12*) analysed a time course of tyrosine phosphorylation during *in vitro* autoactivation of c-Kit using mass spectrometry. They found that 14–22 phosphates are added during activation. They also monitored kinase activity of c-Kit and examined its correlation with the tyrosine phosphorylation profile (Fig. 1B). As predicted, the extent of tyrosine phosphorylation in the JM domain was well correlated with the kinase activity. Notably, Y823 in the A-loop was phosphorylated only after the kinase activity reached near its maximum (>90% activity), suggesting that Y823 phosphorylation is not required for c-Kit activation. Consistently, Y823F mutant of c-Kit had a specific activity comparable to that of wild-type c-Kit.

They next examined sensitivity of the Y823F mutant to therapeutic kinase inhibitors. Mutations that cause resistance to the inhibitors are found in the A-loop (D816H/V) (*13*). Two tyrosine kinase inhibitors have been approved for the treatment of GISTs: imatinib mesylate (marketed as Gleevec™) and sunitinib malate (marketed as Stent™, for advanced

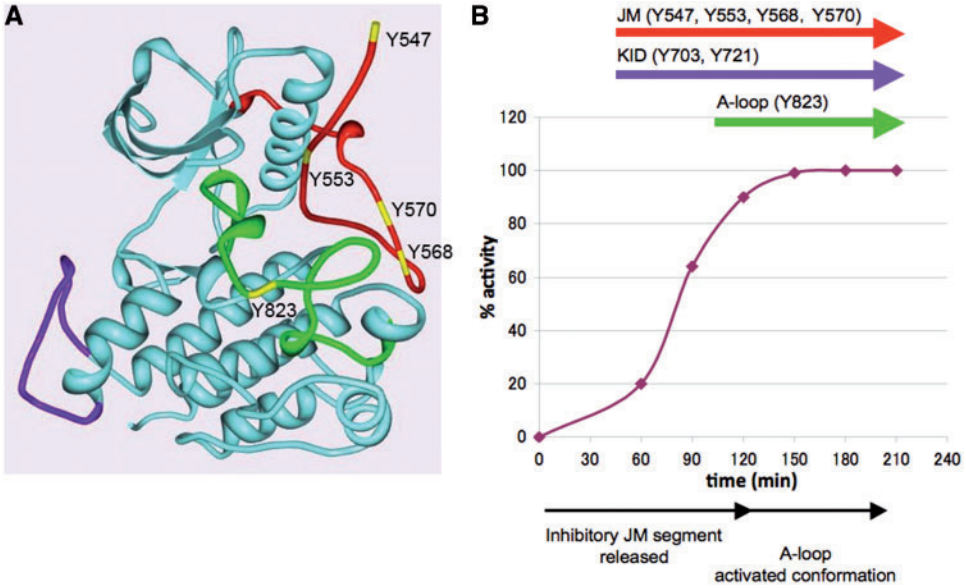

Fig. 1 Time course of the tyrosine phosphorylation profile of c-Kit during autoactivation. (A) Three-dimensional structure of the auto-inhibited form of c-Kit intracellular domain (Protein Data Bank code 1T45) (*11*). The JM segment (residues 547–581, shown in red) is in the auto-inhibitory position. The KID is shown in purple, the activation loop (A-loop) in green. Tyrosine residues phosphorylated upon activation in the JM domain (Y547, Y553, Y568, Y570) and the A-loop (Y823) are shown in yellow. Those in the KID are not visible because the KID 694–753 was deleted in the construct for crystallography. (B) Time course of autoactivation is shown in the graph. Tyrosine phosphorylation status is shown above the graph. The JM domain is released from its autoinhibitory position, followed by fixation of the A-loop in the fully activated conformation. Original data are derived from Ref. (*12*).

imatinib-resistant GISTs). Both drugs inhibit c-Kit activity through activation state-dependent interaction: they bind to c-Kit when the A-loop is in the unactivated conformation but not when it is in the activated conformation, although their modes of binding to c-Kit are distinct (*10*, *14*). These drugs were thus used as probes to monitor conformational equilibrium of the A-loop in c-Kit. The Y823F mutant remained to be sensitive to the drugs even after kinase activation, indicating that the A-loop in the Y823F mutant adopts the unactivated form to some extent.

The activation process of c-Kit thus consists of at least two steps. First, tyrosine residues in the JM segment are phosphorylated, which release it from the autoinhibitory position, leading to activation of the kinase. This process is not enough for the A-loop to exclusively adopt the fully activated conformation because the authors also found that c-Kit lacking the JM domain is still sensitive to imatinib and sunitinib (*12*). Secondly, Y823 in the A-loop is phosphorylated, which results in shift of the A-loop conformation towards the fully activated one. At present, the precise role of this second step in c-Kit activation remains to be elucidated because the apparent kinase activity is not significantly affected by states of the A-loop conformation. Phosphorylated Y823 may serve as a docking site for some downstream effector proteins. Alternatively, conformational transition of the A-loop may somehow affect downstream signalling events. These possibilities could be examined using cell-based assay systems, instead of the *in vitro* autoactivation system analysed in the present study. Intriguingly, a mutation of tyrosine 823 to aspartic acid (Y823D) has been reported as one of the secondary mutations in imatinib-resistant GISTs (*15–17*). However, the single Y823D mutation resulted in inactivation of the Kit receptor kinase (*12*). Therefore, the combinatorial effect of Kit mutations appears to be an interesting issue to be addressed in the future.

Currently, various chemical compounds have been used in the biochemical research field. Some of them are quite useful in determining contribution of specific signalling pathways to cell responses of interest, if appropriately used (*18*). Many researchers may not be interested in the mechanisms by which chemical compounds affect activity of target proteins. Molecular mechanisms underlying actions of imanitib and sunifenib were elucidated just because the resistance to these drugs is a critical problem in the treatment of GISTs. However, the present study provides us with a good example of effective usage of therapeutic drugs with defined mechanisms in basic science. Knowledge on inhibitory mechanisms of chemical compounds would be important not only in avoiding misunderstanding of experimental data, but also in expanding their applications in basic research.

Conflict of interest
None declared.

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