Chemistry & Biology
Preview

Ins and Outs of Kinase DFG Motifs

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http://dx.doi.org/10.1016/j.chembiol.2013.06.001

In this issue of *Chemistry & Biology*, Hari and colleagues show that two positions in kinase active sites, including the well-known "gatekeeper" residue, regulate "in" versus "out" conformations of the conserved "DFG" motif. These findings suggest yet another role for the gatekeeper residue.

The impact of the kinase conformational state on inhibitor potency and selectivity is an important but poorly understood problem in kinase inhibitor drug discovery. The breakthrough drug imatinib showed us several years ago that the plasticity of kinase structure can enable the development of selective kinase inhibitors despite the high sequence conservation within this large protein family. Imatinib is classified as a "type II" kinase inhibitor because it contacts both the ATP cofactor binding site and an adjacent "allosteric" site available only when the kinase assumes a catalytically inactive conformation where the "Asp-Phe-Gly (DFG)" motif at the N terminus of the activation loop is flipped "out" relative to its conformation in the active state ("in") (Figure 1B) (Nagar et al., 2002). In contrast, type I inhibitors including VX-680 (and dasatinib) bind at the ATP site but do not penetrate the allosteric pocket and therefore do not depend on specific kinase conformations for binding (Figure 1A). Type II inhibitors are generally more selective than type I inhibitors across the enormous human kinome (518 members) (Davis et al., 2011), but the reasons for this selectivity advantage are not well understood. Are the additional contacts made by type II inhibitors in the less well-conserved allosteric pocket critical for selectivity? Is it that many kinases do not adopt, or only poorly adopt, the "DFG-out" conformation required for type II inhibitor binding? Or is it some of both? The results presented by Hari et al. (2013) in this issue of *Chemistry & Biology* address this question and suggest that inherent differences in the ability of kinases to adopt the DFG-out conformation can indeed contribute to the selectivity of type II inhibitors.

Based upon the mutagenesis data presented, the authors propose that two residues influence the ability of kinases to adopt a DFG-out conformation (Figure 1). One of these is perhaps the best studied residue in kinases, known as the "gatekeeper", so named because kinases typically have bulky amino acids occupying this position, and mutation at this position to Gly or Ala can enable molecules access to a deeper hydrophobic pocket. Bishop et al. (2000) previously exploited this concept to generate mutant alleles of kinases that could be selectively inhibited by compounds that require access to the hydrophobic pocket to stably bind kinases and inhibit kinase activity. In a similar manner, Kevan Shokat's group demonstrated that direct substrates of these mutant alleles can be identified with the use of a bulky ATP analog, which can efficiently act as a cofactor for the modified kinase (Allen et al., 2007). The importance of this residue to pharmacology was first hinted at in a seminal study by John Kuriyan's group, which provided the first evidence that the prototypic small molecule tyrosine kinase inhibitor imatinib binds to ABL kinase in an inactive, DFG-out (type II) manner (Schindler et al., 2000). Moreover, Kuriyan speculated that the gatekeeper Thr in ABL was critical for the ability of imatinib to bind due to its contribution of an important stabilizing H-bond (Schindler et al., 2000). It was therefore scientifically gratifying when Charles Sawyers' group found that several patients with loss of response to imatinib had evolved bulky Ile substitutions at this residue, which not only destroyed the ability to establish an H-bond, but presumably further contributed to a high degree of imatinib resistance as a result of steric clash (Gorre et al., 2001). This mutation, known commonly as breakpoint cluster region-ABL/T315I, is highly resistant not only to imatinib, but also to three other approved second generation ABL inhibitors: dasatinib, bosutinib (type I inhibitors), and nilotinib (a type II inhibitor). Only recently has a clinically effective kinase inhibitor that retains activity against this mutant been identified and approved (ponatinib, a type II inhibitor). Importantly, among multiple kinases that have been effectively targeted clinically (KIT, PDGFRA, EGFR, EML4-ALK, and FLT3), gatekeeper mutations are commonly found to confer resistance. Notably, for EGFR, the gatekeeper T790M mutation has been demonstrated to confer resistance to EGFR inhibitors not for steric reasons, but due to an increased affinity for ATP (Yun et al., 2008). Interestingly, there is evidence that gatekeeper residue substitutions can have effects on kinase activation. Azam and colleagues demonstrated that select gatekeeper substitutions of ABL and SRC kinases can be activating, and proposed a mechanism whereby the hydrophobic spine in this region of the kinase is stabilized by these substitutions (Azam et al., 2008). Additionally, the identity of the gatekeeper residue can influence substrate specificity (Skaggs et al., 2006).

In the accompanying study by Hari et al. (2013), yet another activity has been ascribed to the gatekeeper residue: the ability (along with the residue referred to as xDFG immediately N-terminal to the conserved Asp-Phe-Gly [DFG] motif at the base of the activation loop) to influence the capacity of kinases to sample a DFG-out conformation. Should these predictions hold true across the entire

kinome, it can be safely assumed that perhaps the majority of kinases will be difficult to selectively target with type II inhibitors. Whereas some compelling data to support the importance of these residues in kinase conformation are presented in the manuscript, it appears that there will likely be other contextual influences as well. For example, the authors provide evidence that mutating Leu to Cys at xDFG prevents JNK3 from binding to their type II conformation indicator probe, thereby suggesting that Cys immediately N-terminal to DFG destabilizes the DFG-out conformation. However, FLT3 contains a Cys at this position and clearly has the ability to access a DFG-out conformation, as evidenced by the clinically active type II FLT3 inhibitors such as quizartinib, sorafenib, and ponatinib. Furthermore, examples of nonselective type II inhibitors have been reported, including AST-487 and EXEL-2880, which have low nM activity on many kinases, including p38-delta (Davis et al., 2011), which was shown by Hari et al. (2013) to be resistant to other type II inhibitors unless mutated at the gatekeeper position. It will therefore be important to assess the validity of the authors' predictions in the context of what is currently known about the ability of members of the human kinome to adopt a DFG-out conformation. Type I inhibitors, which are generally not sensitive to the DFG conformation, should also be tested against the reported mutant "type II-sensitive" and wild-type "type II-insensitive" kinases to further establish the identified residues as regulators of DFG conformation. Nevertheless, the study provides a welcome initial framework for thinking about intrinsic factors that govern kinase conformation, and the identified mutants will be valuable tools for future studies aimed at understanding how inhibitor type impacts potency and selectivity in vitro and in vivo.

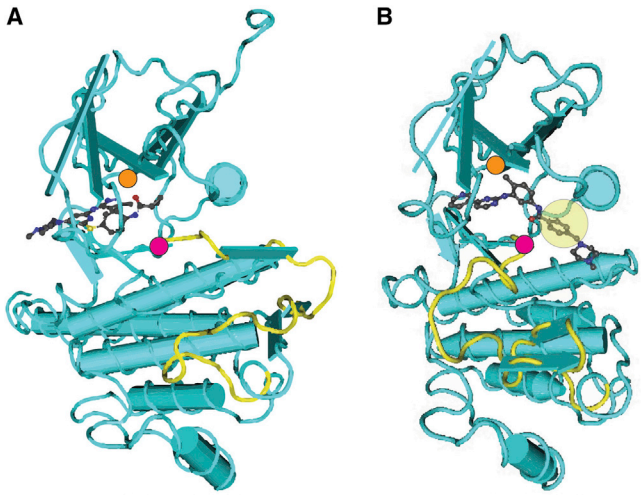

Figure 1. DFG-In and -Out Conformations of Inhibitor-Bound ABL

(A) Cocrystal structure of the type I inhibitor VX-680 (gray) bound to ABL (cyan) (Young et al., 2006). The activation loop (yellow) adopts the active DFG-in conformation. The gatekeeper and xDFG residues shown by Hari et al. (2013) to govern DFG conformation are indicated by orange and magenta circles, respectively.

(B) Cocrystal structure of the type II inhibitor imatinib (gray) bound to ABL (cyan) (Nagar et al., 2002). The activation loop adopts the inactive-DFG-out conformation exposing the allosteric pocket (transparent, yellow circle) occupied by imatinib. In (A), this pocket is occupied by the DFG motif, which adopts the "in" conformation.

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