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Rheostat-ing Mitosis

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Ark1, the unique Aurora kinase in *Schizosaccharomyces pombe*, regulates multiple aspects of mitosis. In this issue of *Chemistry & Biology*, Kawashima and colleagues report the discovery and validation of a fungal Ark1 inhibitor, which they employ to evaluate the mitotic outputs of endogenous Ark1 signaling.

Mitosis is a prime example of a dynamic cellular transition orchestrated by multiple protein kinase activities. Untangling which kinases do what, and when and where they do it, represents a major challenge in the field. Unfortunately, we currently lack validated tools to distinguish between on/off (switch-like) and wide-range (rheostat-like) catalytic outputs of kinases, the latter potentially enabling diverse cellular outputs to be spatially and temporally programed using a single enzyme. This question is pertinent to cell-cycle regulated kinases such as Mps1, Aurora, and Polo-like kinases, which are reversibly activated during mitosis and integrate the sequential steps required to successfully execute cell division (Bayliss et al., 2012). One technique for studying kinase biology involves the exploitation of small molecule inhibitors, whose rapid and often reversible binding to kinases can be successfully harnessed to probe signaling in cells. However, to be really effective as biological tools, these compounds must inhibit a cellular kinase target in a specific and/or highly tractable fashion (Cohen, 2009). Unfortunately, most protein kinase inhibitors are fallible in this regard, due to high promiscuity toward multiple ATP-binding sites and the complex challenges associated with unequivocal "on-target" validation, which is a widespread problem in molecular pharmacology. However, because protein kinases contain prominent amino acid loci whose physiochemical properties create "selectivity filters", the discriminatory capacity of many kinases for diverse compounds targeting the ATP site can be rationalized in a rather general manner (Balzano et al., 2011; Bishop et al., 2000; Eyers et al., 1998; Huang et al., 2010). Four key positions bordering the hinge region of protein kinases dictate resistance or sensitivity to differing ligands, and we term these amino acids a "resistance tetrad" (Figure 1). Critically, catalytically silent mutation of these residues can create resistant (or sensitized) versions of kinases, permitting phenotypic effects observed with small molecules to be validated with high levels of certainty (Balzano et al., 2011; Eyers et al., 1999; Scutt et al., 2009; Sloane et al., 2010; Zunder et al., 2008).

The fission yeast *Schizosaccharomyces pombe* is a valuable laboratory model for studying mitosis. Unfortunately, it is resistant to many small molecules that traverse the cell membrane of vertebrate cells, potentially restricting its usefulness for mechanistic drug discovery. Two recent studies reported in *Chemistry & Biology* by the Nurse and Kapoor laboratories have addressed this major obstacle to progress in the field. In their initial paper, Kawashima et al. (2012) described the generation of a drug-sensitive fission yeast strain (MDR-sup) in which five multidrug resistance (MDR) genes were deleted, enhancing the ability of small molecules to accumulate and induce quantifiable biological effects. Hot on the heels of this advance, the same group (Kawashima et al., 2013; in this issue of *Chemistry & Biology*) reports the discovery of Arkin-1 (Figure 1A), an inhibitor of the endogenous *S. pombe* kinase Ark1, a key regulator of mitosis related to vertebrate Aurora kinases (Petersen et al., 2001). By combining a small molecule screen with chemical genetics in Ark1 wild-type and drug-resistant isogenic strains, evidence for rheostat-like behavior of Ark1 during mitosis was uncovered, which is consistent with the

gradients of substrate phosphorylation catalyzed by human Aurora B (Wang et al., 2011).

In order to reach these conclusions and target "endogenous" Ark1 (rather than an artificial Ark1 mutant), several challenges had to be overcome. Initially, an in vitro panel of small molecules that inhibit recombinant human and fungal Aurora kinases was assembled. Surprisingly, potent Aurora inhibitors such as VX-680 (Figure 1A) displayed little effect on cellular S10 phosphorylation of histone H3 (a physiological Ark1 phosphorylation site), even when tested in an MDR-sup strain. However, by using an ingenious "synthetic-lethal" chemical screen, the pyrrolopyrimidine small molecule Arkin-1 (Figure 1A) was discovered. Arkin-1 blocks Ark1 activity in vitro, induces mitotic phenotypes directly attributable to its inhibition (including effects on chromosome compaction, nucleolar separation, and defective kinetochore-microtuble correction), and is highly toxic toward the MDR1-sup strain. One question raised by these findings was if Arkin-1 induces these effects through the predicted "on-target" (i.e. Ark1-mediated) mechanism. To investigate this question, nine drug-resistant yeast clones were isolated from a chemically mutagenized strain exposed to 7.5 μM Arkin-1. Sequencing of Arkin-resistant clones confirms an Ark1-dependent inhibitory mechanism, because each expresses a G172 point mutation in Ark1, while exogenous expression of G172D Ark1 induces biochemical and phenotypic resistance to Arkin-1. No crystal structure of Ark1 is available to confirm the Arkin-1 binding mode, but G172 is equivalent to G160 in human Aurora B, a hinge-loop amino acid that is part of the Aurora kinase "resistance-tetrad" controlling affinity toward inhibitors like VX-680 (Figure 1B). These new data are also entirely consistent with previous work describing drug-resistant human Aurora A and B kinase alleles (Scutt et al., 2009). With the cellular target of Arkin-1 established, Kawashima et al. (2013) investigated the effects of exposing yeast strains to differing concentrations of Arkin-1 by evaluating the dose-dependence of phenotypes provoked by the compound. Interestingly, a low dose (2 μM) of Arkin-1 inhibits mitotic chromosome condensation, suggesting that this process might require high levels of Ark1 activity, whereas higher doses (5 μM) reveal additional defects in spindle checkpoint function, suggesting that lower Ark1 activity is needed to successfully correct microtubule/kinetochore interactions. The authors also provide evidence that Ark1 activity regulates an unknown chromosome compaction factor in addition to the condesin subunit Cnd2/Barren, because Arkin-1 still blocks chromosome arm separation in the presence of a constitutively active cnd2-3E mutant (Kawashima et al., 2013).

Taken together, these studies herald a new era for empirical analysis of kinase inhibitors in fission yeast, with the principles of this work transposable into other systems where inhibitors are employed to evaluate cell signaling. Indeed, it is possible that closer analysis of rheostat-like signaling capabilities among therapeutically relevant protein kinases will reveal the optimal levels of catalytic blockade that are essential for kinase inhibitors to induce a desired cellular phenotype or to provoke a defined "on-target" clinical response.

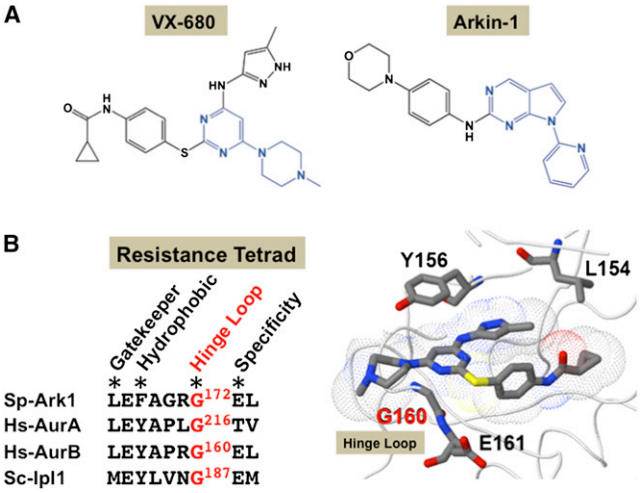

Figure 1. Arkin-1 Is a Target-Validated Fungal Aurora Kinase Inhibitor

(A) Chemical structures of VX-680 (Tozasertib) and Arkin-1, with prominent chemical groups shaded in blue. The piperazine group of VX-680 (blue) lies adjacent to G160 in the co-crystal structure with human Aurora B, and mutation to bulkier amino acids induces drug-resistance, presumably due to steric hindrance (Scutt et al., 2009). Based on this model, the equivalent G172 residue in Ark1 is likely to accommodate the N-7 pyridin-2-yl extension to the pyrrolopyrimidine ring of Arkin-1 (blue), and mutation to larger amino acids should induce phenotypic resistance in Aurora kinases to Arkin-1 and related compounds (Kawashima et al., 2013; Moriarty et al., 2006).

(B) Alignment of amino acids in the ATP-binding site of fission yeast (Sp), human (Hs) and baker's yeast (Sc) Aurora kinases. Four key amino acids make up the malleable resistance tetrad, corresponding to the four residues labeled in the human Aurora B and VX-680 cocrystal structure, which is derived from published coordinates (Protein Data Bank ID code 4AF3).

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