Magic bullets for protein kinases

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A chemical-genetic method for the generation of target-specific protein kinase inhibitors has been developed recently. This strategy utilizes a functionally silent active-site mutation to sensitize a target kinase to inhibition by a small molecule that does not inhibit wild-type kinases. Tyrosine and serine/threonine kinases are equally amenable to the drug-sensitization approach, which has been used to generate selective inhibitors of mutant Src-family kinases, Abl-family kinases, cyclin-dependent kinases, mitogen-activated kinases, p21-activated kinases and Ca^{2+}/calmodulin-dependent kinases. The designed inhibitors are specific for the sensitized kinase in a cellular background where the wild-type kinase has been inactivated. By these means, kinase-sensitization has been used systematically to generate and analyze conditional alleles of several yeast protein kinases *in vivo*.

Protein kinases regulate a wide variety of eukaryotic signal-transduction pathways[1]. The large size of the protein kinase superfamily (>120 in *Saccharomyces cerevisiae*, ~900 in humans) has made it difficult to assign precise signaling roles to each individual kinase[2,3]. Cell-permeable inhibitors that are highly specific for individual protein kinases would be invaluable tools for helping to define kinase functions. Such inhibitors would provide several key advantages over the genetic approaches to enzyme inactivation. Inhibitor treatment allows the cell no time to compensate for the missing activity of the target kinase. In addition, small molecules can be administered to a cell in varying concentrations, allowing for the observation of the effects of partial as well as total inhibition of the target kinase. Finally, inhibition of kinase activity at the catalytic active site should not disrupt important cellular localization and protein–protein binding interactions that are generally mediated through regions of the protein that are spatially separated from the ATP-binding site.

This article summarizes recent progress in the design and use of cell-permeable compounds that specifically inhibit a drug-sensitized kinase target but do not inhibit wild-type protein kinases[4–9]. Such compounds are generated by chemically modifying a known kinase inhibitor to complement a rationally designed enlargement of the ATP-binding site of the target kinase. When the 'analog-sensitive' protein kinase is expressed in living cells that lack the activity of the wild-type kinase, the effects of highly specific inhibition can be assayed directly (Fig. 1). Inhibitor-sensitive alleles of a mitogen-activated protein kinase (MAPK; Fus3p), a cyclin-dependent kinase (CDK; Cdc28p) and a p21-activated kinase (PAK; Cla4p) have been used to elucidate the kinase-dependent roles of these enzymes in budding yeast[7,8].

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Structure-based design of allele-specific inhibitors

Convergent engineering of protein–small-molecule interfaces has emerged as a powerful method for the development of novel ligand–receptor pairs as tools for studying complex signaling systems (see Ref. 9 for a comprehensive review). The design strategy of the kinase-sensitization approach utilizes the engineering of a 'hole' in the ATP-binding site of the target kinase and a corresponding 'bump' on the small molecule inhibitor (Fig. 1). Site-directed mutagenesis of a large amino acid side chain to a small side chain (alanine or glycine) generates the unique hole in the kinase of interest. The necessary criteria for the sensitizing mutation are similar for any target kinase:

- the wild-type residue should be large enough such that site-directed mutagenesis to glycine or alanine would create a novel binding pocket;
- the corresponding residue should not be occupied by an alanine or glycine in other protein kinases;
- mutation of the residue should be functionally silent;
- ideally, the residue identified for drug-sensitization would be generalizable to other protein kinases. This eliminates the need to redesign the kinase–inhibitor interface for each target kinase.

Initial efforts to engineer unique inhibitor sensitivity were aimed at the oncogenic tyrosine kinase v-Src. This work identified a functionally conserved residue in the ATP-binding pocket of v-Src (Ile338) that could be mutated to glycine ('analog-sensitive v-Src #1', v-Src-as1) or alanine (v-Src-as2) without altering the biological function of the kinase[4,10,11]. Crystal structures of various ATP-bound protein kinases have revealed a conserved close-contact interaction between the residue corresponding to position 338 and ATP (Fig. 2)[12,13]. Analysis of protein kinase sequence alignments confirmed that, in all known eukaryotic protein kinases, residue 338 contains a bulky side chain (usually Thr, Ile, Leu, Met or Phe)[14]. Thus, a glycine or alanine mutation at the 338 position can create a novel pocket that is not present in any wild-type kinase.

The allele-specific kinase inhibitors also must satisfy specific criteria:

- the binding orientation of the parent compound must be known or readily predictable;
- rationally derivatized analogs of the parent molecule (whose substituents are directed towards the 338 position) must be synthetically accessible;
- the inhibitor analogs should rapidly cross cell membranes and, ideally, possess oral activity in mammals.

These criteria seriously limit the number of possible inhibitor scaffolds. Successful structure-based design is further complicated by the fact that only a limited number of inhibitor-bound kinase crystal structures have been solved to date[15–24]. From these considerations, two very different classes of kinase inhibitors, one based

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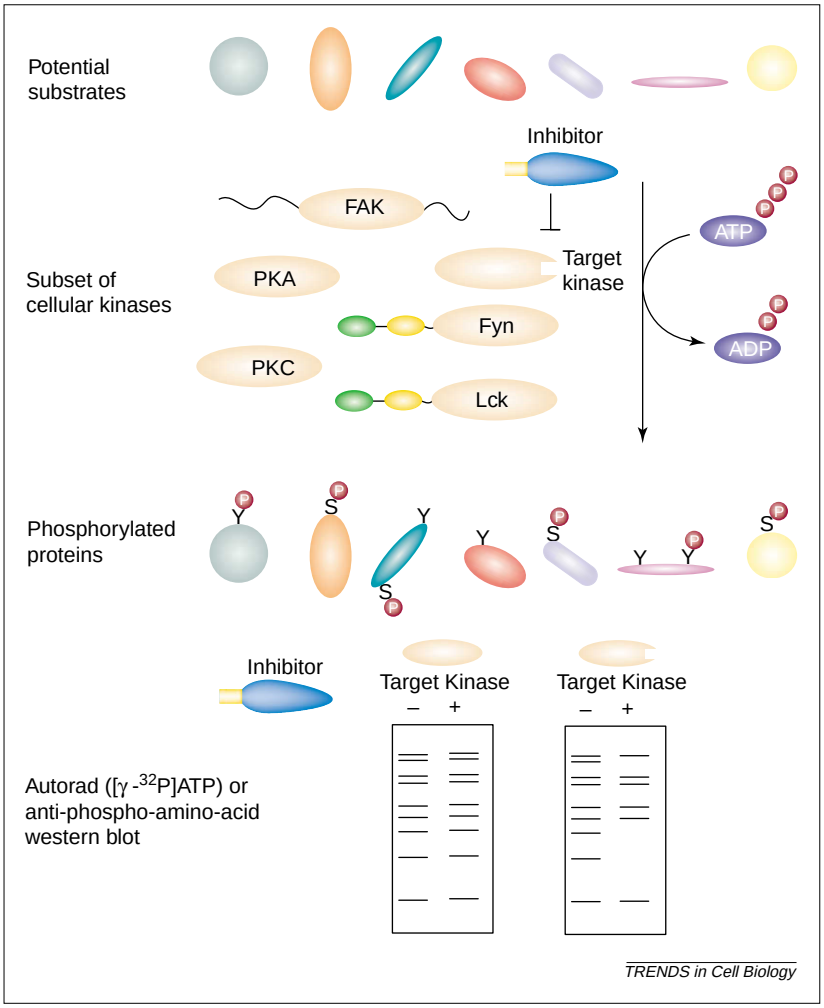

Fig. 1. Schematic diagram of the protein kinase inhibitor-sensitization strategy discussed in this review. A known inhibitor is modified (bump) to fit the active site of a rationally engineered target kinase (notch). Because the modified inhibitor does not inhibit wild-type protein kinases, the direct cellular effects of target-specific kinase inhibition can be readily assayed. Abbreviations: FAK, focal-adhesion kinase; PKA, protein kinase A; PKC, protein kinase C; S, serine residue; Y, tyrosine residue.

on the synthetic pyrazolo[3,4-*d*]pyrimidine ring system and one based on indolocarbazole natural products, have been investigated for their ability to act as scaffolds for the design of analog-sensitive kinase inhibitors.

PP1 (Fig. 3) is a synthetic pyrazolo[3,4-*d*]pyrimidine that received attention owing to its ability to disrupt signal transduction mediated through the T-cell receptor by inhibition of the Src-family kinases Lck and Fyn[25]. This compound is strongly (but not rigorously) Src-family kinase selective[26,27]. PP1 was predicted to bind in kinase-active sites, with the pyrazolo[3,4-*d*]pyrimidine ring system essentially taking the place of the adenine of ATP[4]. This prediction was confirmed by two different PP1/Src-family kinase crystal structures (Fig. 4a)[19,24]. Both the N^4-amine and the C(3)-phenyl ring of PP1 are in close contact with residue 338 (<4.0 Å away) in this orientation. Thus, two small panels of PP1 analogs [N^4-derivatized and C(3)-derivatized] have been synthesized and tested for their ability to selectively inhibit sensitized Src-family kinases[5,7]. It was found that both derivatization strategies yielded very selective inhibitors of sensitized Src kinases. However, the C(3)-derivatized PP1 analogs were generally much more potent inhibitors than the N^4-derivatives (Fig. 5a) presumably because substituents at N^4 disrupt the ATP-like hydrogen-bonding interactions of the exocyclic amine with the kinase backbone.

K252a (Fig. 3) is an indolocarbazole natural product that is a very potent and general inhibitor of protein kinases. It has been reported to be a potent ($IC_{50} \leq 30$ nM) inhibitor of protein kinase C, protein kinase A, cGMP-dependent protein kinase, myosin light-chain kinase, Trk-family tyrosine kinases, Src-family kinases, CDKs and Ca^{2+}/calmodulin-dependent kinases (CAMKs)[7,28,29]. The binding of K252a has been predicted from crystal structures of kinases bound to the structurally similar indolocarbazole (+)-staurosporine (Figs 3 and 4b)[22,23]. The two natural products are identical in their aglycone moiety, which binds in the back of the active site (in the region of 338). In this orientation, the residue corresponding to 338 is close to C(7) of K252a (3.7 Å). Thus, it was anticipated that developing unique indolocarbazole-based inhibitors of sensitized kinases would require manipulation of C(7) (Fig. 5b). This was achieved through total synthesis, using an approach that allows for the modular assembly of C(7)-modified K252a derivatives[30,31].

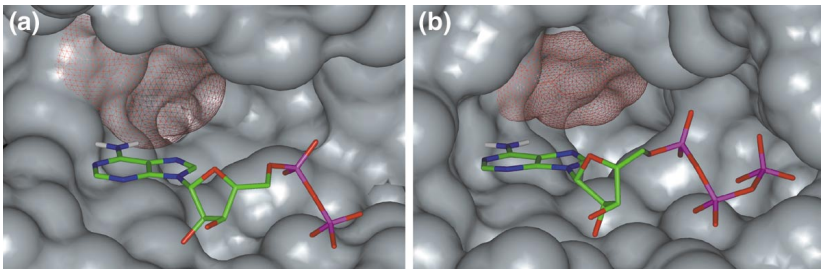

Fig. 2. Active sites of the tyrosine kinase c-Src (a) and the cyclin-dependent kinase CDK2 (b) with bound ADP and ATP, respectively. The surface of the target residue (Thr338 in c-Src, Phe80 in CDK2) is shown as a meshed surface in each case. Coordinates of both complexes are available from the Protein Data Bank [PDB ID numbers 2SRC (Ref. 50) and 1HCK (Ref. 51), respectively]. Several residues in front of the binding site are removed for clarity.

Breadth of the kinase-sensitization strategy

From the published pharmacology of PP1 and the indolocarbazoles, one would predict that PP1 derivatives could act as efficient inhibitors of sensitized Src-family kinases and that K252a analogs would be better suited to inhibit a wide range of sensitized protein kinases. To test this idea, both classes of inhibitors were screened against an array of kinases (wild-type and sensitized) from several divergent subfamilies (v-Src, c-Fyn, c-Abl, CDK2, CAMKIIα)[7].

The C(7)-2-methyl-propyl (MP; Fig. 3), *S*-benzyl, and 2-phenyl-ethyl (PE; Fig. 3) K252a analogs were all potent and selective inhibitors of the engineered Src-family kinases, v-Src-as1 and c-Fyn-as1. MP bound to these targets with exquisite selectivity (≥3000-fold compared with all wild-type kinases in the panel) and subnanomolar IC_{50} values (230 pM and 550 pM, respectively). PE is a potent ($IC_{50} = 4$ nM) and selective inhibitor for CAMK IIα-as1. The same molecule inhibits

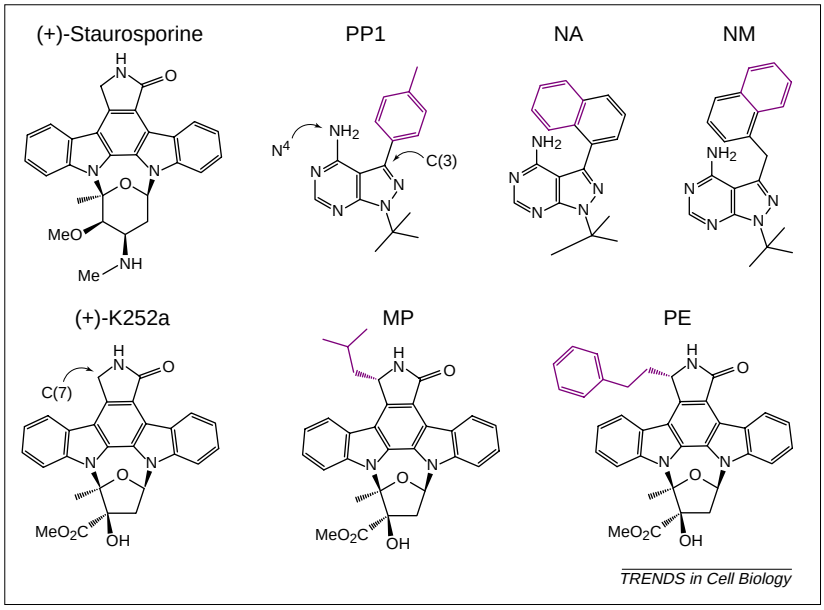

Fig. 3. Chemical structures of the protein kinase inhibitors discussed in this review: (+)-staurosporine, PP1, 1-naphthyl-PP1 (NA), 1-naphthylmethyl-PP1 (NM), (+)-K252a, *S*-C(7)-2-methyl-propyl-K252a (MP) and *S*-2-phenyl-ethyl K252a (PE).

CDK2-as1 ninefold less effectively (IC_{50} = 37 nM), with moderate selectivity. However, no selective inhibitor was identified for c-Abl-as2. c-Abl is the only wild-type protein kinase in the panel that is not effectively inhibited by the parent molecule K252a, suggesting that sensitivity to K252a is a predictive determinant for whether or not other kinases would be well suited for inhibition by K252a analogs.

Screening of PP1 derivatives against the same panel of kinases yielded interesting results[7]. As suspected, sensitized Src-family kinases can be selectively inhibited by PP1 analogs. In fact, the potency of the PP1 analog–*as* kinase pair can substantially exceed the potency of the PP1–wild-type kinase pair. For example, the IC_{50} of c-Fyn with PP1 is 50 nM, whereas c-Fyn-as1 is inhibited by 1-naphthylmethyl-PP1 (NM; Fig. 3) at 3.2 nM. Surprisingly, it was also found that derivatives of PP1 could effectively inhibit the non-Src-family sensitized kinases c-Abl-as2 {[1-naphthyl-PP1 (NA; Figs 3 and 5a); IC_{50} = 7.0 nM]}, CDK2-as1 (NM; IC_{50} = 5.0 nM) and CAMK IIα-as1 (NM; IC_{50} = 8.0 nM) with high target selectivity. The wild-type forms of these enzymes show only moderate-to-low PP1 sensitivity (IC_{50} = 0.30 μM, 22 μM and 17 mM, respectively) and possess low-to-moderate sequence identity to Src (51%, 26%, 27%, respectively). These facts, coupled with the observation that every tested kinase was readily sensitized, suggest that many other kinase subfamilies might be similarly susceptible to PP1 analog sensitization. This would greatly expand the utility of the kinase-sensitization strategy as it eliminates the need to identify a lead inhibitor (and synthesize analogs) for every targeted kinase subfamily.

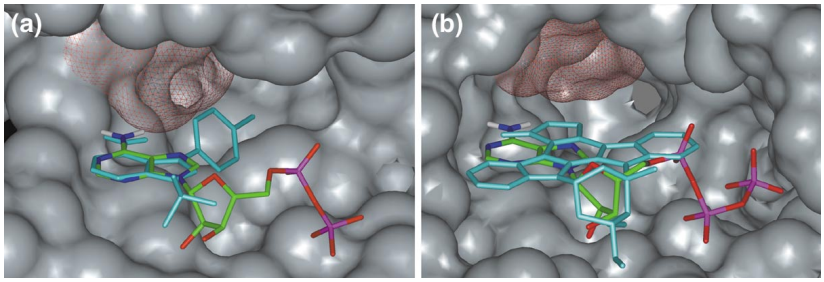

Fig. 4. Binding orientations of PP1 in the c-Src active site (a) and staurosporine in the CDK2 active site (b) overlaid with ADP and ATP. Structures of inhibitors are colored blue. Coordinates of the CDK2–staurosporine complex are available from the Protein Data Bank [ID number 1AQ1 (Ref. 23)]. The position of PP1 in the active site of c-Src was derived from its complex with another Src-family member, protein kinase Lck [PBD ID number 1QPE (Ref. 24)].

In vivo inhibition of Fus3p

Ideally, the kinase-sensitization strategy could be extended to identify analog-sensitive alleles of other families of protein kinases without individually purifying and assaying every enzyme *in vitro*. To test this idea, Bishop *et al.*[7] selected the *S. cerevisiae* MAP kinase Fus3p, which is required for induction of pheromone-inducible genes, cell-cycle arrest and cell fusion during mating[32]. In the presence of mating factor pheromones, Fus3p phosphorylates Far1p and Ste12p. These phosphorylation events are required for G1 cell-cycle arrest and transcription of genes required for mating[33,34].

Since the three-dimensional structure of Fus3p has not been solved, an analog-sensitive mutant of Fus3p (Fus3-as1; Q93G) was generated based solely on primary sequence alignments. This mutant complemented the deletion of wild-type *FUS3* as shown by mating assays and transcriptional analysis of pheromone-inducible transcripts[7]. It was also shown that NA (more potently) and NM specifically disrupted mating in cells carrying a plasmid that encodes Fus3-as1 but had no effect on the mating process in cells that express Fus3p. This result provided the first evidence that the ubiquitous MAP kinase family can be readily targeted through the kinase-sensitization strategy. More broadly, the *in vivo* target-specific inhibition of Fus3-as1 demonstrated that phenotype-based screens can be used for the identification of a selective kinase inhibitor, omitting the need for labor-intensive *in vitro* kinase assays.

Chemical-genetic analysis of Cdc28p

Bishop *et al.* also used kinase-sensitization to study the effects of target-specific inhibition of the Cdc28p CDK from *S. cerevisiae*[7]. The genome of budding yeast encodes five CDKs (Cdc28p, Pho85p, Kin28p, Ctk1p, Ssn3p)[2]. Cdc28p is the major CDK in budding yeast and is essential for advancement through multiple stages of the cell cycle[35,36]. As noted earlier, the mammalian CDK2-as1 is highly sensitive to selective inhibition by the NM inhibitor. Since Cdc28p has high identity to CDK2 (>60%), it was predicted that the similarly sensitized Cdc28p (F88G; Cdc28-as1) would accept the same compound. Indeed, it was shown that Cdc28-as1 is potently inhibited by NM, with very strong selectivity with respect to Cdc28p. This compound is also active and specific *in vivo* as it disrupts Cdc28-as1-mediated

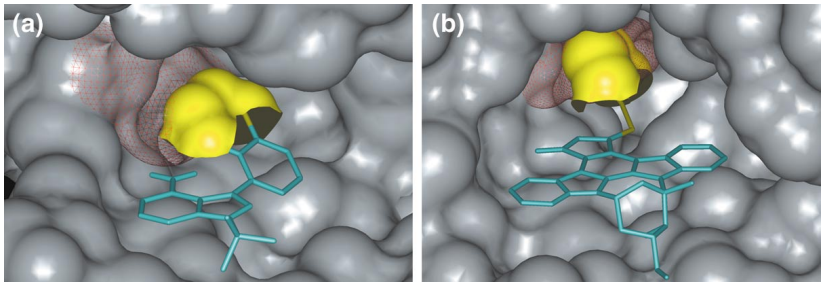

Fig. 5. Steric clash between the wild-type residue (meshed surface) and the 'bump' on the inhibitor (solid yellow surface) in the active sites of c-Src (a) and CDK2 (b). The portion of the inhibitor structure that corresponds to the parent molecule is colored blue. Positions of the derivatized inhibitors were modeled with molecular docking program AutoDock[52] using the PDB coordinates of the corresponding proteins. Structures of the inhibitors were built and refined with Spartan 5.1 (Wavefunction Inc., Irvine, CA 92612, USA).

cell growth at concentrations that have no effect on a strain that expresses Cdc28p[7].

The authors went on to characterize further the morphology of Cdc28-as1-expressing cells that were exposed to NM[7]. Synchronization of *cdc28-as1* cells in G1 phase, followed by release into low concentrations of NM (500 nM), caused the vast majority of these cells to arrest with large, hyperpolarized buds and a 2C DNA content. These cells have a single DNA mass correctly positioned at the bud neck but lack a mitotic spindle. At 5 μM NM, the same cell line initially fails to bud and eventually arrests with a 1C DNA content and hyperpolarized buds. This delayed budding was shown to be dependent on Pho85p activity (as opposed to residual Cdc28-as1 activity) as it did not occur in a strain that lacked the Pho85p-specific cyclins Pcl1p and Pcl2p.

These results suggest that the G2/M functions of Cdc28p are more sensitive to low inhibitor concentrations than are its G1 functions. This finding was substantiated by measuring the genome-wide transcriptional profile of NM-treated *cdc28-as1* cells using oligonucleotide arrays[7,37]. It was shown that both 30-min and 120-min treatments with 500 nM NM caused a strong and specific suppression of transcripts that normally peak at G2/M[38]. These transcripts include many established mitotic regulators including *CLB2*, *SWI5*, *CDC20* and *CDC5*. Prolonged (120 min) drug treatment also led to a less pronounced increase in G1-peaking transcripts, including those encoding the G1 cyclins Cln2p and Pcl1p.

This evidence that G2/M progression is most sensitive to Cdc28p inhibition is in agreement with the notion that CDK activity increases throughout the cell cycle, requiring maximum levels at mitosis[39]. However, these studies seem to contradict previous results with temperature-sensitive *cdc28* mutants, most of which arrest as unbudded cells in G1 phase[40]. It is not uncommon for a cellular protein kinase to function in ways that are not directly attributable to its kinase activity. This can be shown most clearly in cases where exclusive expression of a kinase-deficient form of a protein kinase gives a phenotype that is distinct from the knockout[41]. These differences are presumably due to the loss (in the knockout) of kinase-independent protein–protein interactions that help to form signaling complexes. Similar complications also exist in temperature-sensitive kinases, as the molecular details of protein inactivation are seldom known. A significant advantage of using analog-sensitive conditional alleles of protein kinases, therefore, is that any inhibition-induced phenotype can be attributed directly to the diminution of kinase activity in the target.

Chemical-genetic analysis of Cla4p

Weiss *et al.* have utilized the kinase-sensitization strategy to study the physiological functions of Cla4p, a PAK from budding yeast[8]. Cla4p (and the partially redundant PAK Ste20p) binds to and is activated by Cdc42p, a Rho-family GTPase that is required for the G1 cell polarization that precedes bud emergence during the *S. cerevisiae* cell cycle[42]. The presence of either Cla4p or Ste20p is necessary for cell viability[43]. Both *cla4Δ* and *ste20Δ* cell lines are impaired in their ability to nucleate F-actin assemblies but, surprisingly, do not show substantial defects in actin polarization[44]. It is important to note that the study of actin polarization-related processes through temperature-sensitive mutations is often complicated by heat-induced effects on the wild-type systems. For example, heat shock of wild-type yeast causes Rho-1-mediated depolarization of the actin cytoskeleton[45].

To elucidate the functional roles of Cla4p, Weiss *et al.* generated an analog-sensitive Cla4p (Cla4-as3) that contained two mutations in the ATP-binding site of the kinase domain (M659A, T701A)[8]. Plasmid-borne expression of Cla4-as3 rescued a *cla4Δ ste20Δ* strain, with a doubling time that was almost identical to rescue with Cla4p. Importantly, this Cla4-as3-dependent growth was specifically blocked by NM, which inhibited immunoprecipitated Cla4-as3 with an IC_{50} of 240 nM. *In vivo* inhibition of Cla4-as3 (in the absence of Ste20p) gave rise to a dramatic phenotypic response. Increasing concentrations of NM at 25°C led to an elongated cell morphology, with F-actin concentrated at the tips of the buds. It was also shown that these elongated cells possessed an altered distribution of the septins Cdc3p and Cdc11p.

Importantly, the authors were able to use the specific Cla4-as3 inhibitor to determine the timing of the role of Cla4p in cytokinesis[8]. Using a micromanipulator, individual *cla4-as3* cells were grouped based on whether they possessed a small bud or no bud. When the initially unbudded cells were treated with 30 μM NM, the overwhelming majority of them developed elongated buds. By contrast, initially budded cells grew normal daughter buds (for one generation only) when exposed to the inhibitor, suggesting that Cla4p kinase activity is only necessary for bud morphogenesis at or before bud emergence.

The elongated morphology of NM-treated *cla4-as3* cells was dependent on the presence of the *SWE1* gene product. It had previously been proposed that Swe1p controls a 'checkpoint' that arrests the budding yeast cell cycle in response to disruptions of normal septin structure, F-actin organization or cell polarity[46].

Weiss *et al.* therefore constructed a *cla4-as3 swe1Δ* strain to investigate the role of Swe1 in controlling the NM-induced morphology[8]. It was shown that exposure of *cla4-as3 swe1Δ* cells to NM led to a delay in bud emergence and an eventual increase in the population of multinucleated and multibudded cells. These results indicate that the Swe1p protein is necessary for the cell-cycle arrest observed in NM-treated *cla4-as3* cells. In addition, the combination of delayed bud emergence and multinucleation suggest the intriguing possibility that the *SWE1*-dependent checkpoint might regulate both negative (cell-cycle arrest) and positive (bud emergence repair mechanisms) responses to the disruption of normal bud morphogenetic and cytokinetic processes[8].

Limitations of the kinase-sensitization strategy

The development of kinase inhibitor-sensitization adds another tool to the array of techniques available to study the signaling functions of protein kinases. Not unlike traditional genetic and biochemical approaches, the sensitization strategy contains a number of limitations that will place some boundaries on its utility.

Clearly, the functional integrity of the mutant kinase and the true target-specificity of the inhibitor are crucial considerations. The currently used inhibitor-sensitizing mutation generally affects the ATP-binding ability of the kinase. Owing to the high (millimolar) quantities of cellular ATP, this has not typically been a major obstacle for *in vivo* studies. However, the catalytic activity of some protein kinases will undoubtedly be diminished by inhibitor sensitization (e.g. T315G c-Abl)[47]. Thus, the analysis of a novel inhibitor-sensitized target must include a careful comparison of the relevant phenotypes of cells expressing the wild-type and sensitized kinases in the absence of the inhibitor. Ideally, future work will lead to the identification of inhibitor-sensitizing mutations that do not appreciably affect the kinetic parameters of canonical protein kinases.

The usefulness of the kinase-sensitization is also limited by the specificity of the small-molecule inhibitors. One can never know absolutely that the inhibitor is not acting on any wild-type signaling molecule in a particular cell type. Thus, every sensitized kinase inhibition experiment must be performed side-by-side with the equivalent experiment in the wild-type system to ensure that any observed drug effect is specific to cells carrying the inhibitor-sensitive allele.

The requirement that *in vivo* studies be carried out in the absence of the wild-type kinase is also non-trivial. Analysis of the cellular inhibition of a kinase of interest requires (at the very least):

- generating a target kinase knockout cell line;
- generating a drug-sensitive mutant;
- finding a suitable expression system.

For protein kinases that are required for cell viability (or for whole-animal studies), this approach would need to be replaced by a 'knock-in' strategy in which the wild-type gene is directly replaced by the drug-sensitive allele. To date, these technical requirements have limited the application of kinase inhibitor sensitization to yeast. The technical hurdles of gene replacement in mammalian systems are somewhat more daunting – but not insurmountable[48]. The recent advent of the high-throughput method double-stranded RNA interference (dsRNAi) for inactivation of protein kinases in cultured cells should lessen the difficulty of obtaining a null background for a specific protein kinase[49]. Current efforts in several laboratories are aimed at using inhibitor-sensitization to help elucidate the cellular roles of a variety of mammalian kinases in cell culture and whole organisms.

Conclusions and future directions

The goal of using chemical genetics to study cell biology is to develop systems that allow for a combination of the advantages of small 'drug-like' chemicals and genetic manipulations. Cell-permeable drugs act on their targets quickly and reversibly but often have multiple cellular targets. Genetic mutations are highly specific but do not allow for rapid or precise control of protein function. The major limitation of the chemical-genetic approach is fairly clear – it is extremely difficult to find small molecules that act specifically on a cellular target of interest. The recent advances in the breadth of the kinase-sensitization approach indicate that this limitation might be rendered moot for one of the largest eukaryotic gene families – the protein kinases. A wide variety of protein kinases (the list is currently growing) can be rapidly targeted by making a site-directed mutation that is readily identifiable from primary sequence alignments. The work reviewed here demonstrates that analog-sensitive kinases are already being used to make significant contributions in understanding the phosphorylation events that control the budding yeast cell cycle.

One common theme has emerged from the chemical-genetic analyses of protein kinases reviewed here. Purely genetic means of perturbing kinases (i.e. knockout and temperature-sensitive alleles) can suggest roles for the kinase being studied that are distinct from those identified in chemical-genetic studies. It is not the case that one method is 'right' and one is 'wrong'; yet it is clear that the two approaches offer complementary information about the cellular function of a given kinase. The information from chemical inhibitors is particularly relevant to drug-discovery efforts because one would like to know how a specific inhibitor of a particular kinase would affect a given cell signaling pathway. Thus, 'target validation' of protein kinases for drug discovery is best done through chemical means because cell signaling pathways respond subtly, but significantly differently, to purely genetic perturbations and chemical perturbations.

Another area that we believe the method described here could be relevant to is in assay development for drug discovery. Currently, high-throughput assays for assessing kinase inhibitor specificity are carried out by overexpressing each kinase and developing a peptide phosphorylation assay. However, these assays do not reflect the special microenvironments in cells where kinases actually catalyze substrate

phosphorylation reactions. A potentially more biologically relevant screen for drugs would be based on whole-cell assays. Yet, since hundreds of different kinases are expressed in any given cell, measuring the 'desired' effect of inhibition of one kinase in the cell is impossible without the availability of a perfectly specific kinase inhibitor. Thus, we see the chemical-genetic approach being used extensively to develop 'blueprints' for specific inhibition of one kinase in a cell. Such phenotypic, genotypic or proteomic blueprints could serve as the basis of high-throughput cell-based assays for kinase-inhibitor discovery.

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