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# Recognizing and exploiting differences between RNAi and small-molecule inhibitors

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## Abstract

The biology of RNA interference has greatly facilitated analysis of loss-of-function phenotypes, but correlating these phenotypes with small-molecule inhibition profiles is not always straightforward. We examine the rationale of comparing RNA interference to pharmacological intervention in chemical biology.

RNA-mediated silencing has rapidly arisen as a critical tool in the arsenal of the molecular biologist. This strategy facilitates the generation of loss-of-function mutations in individual cells or in whole organisms and enables investigators to interrogate the impact of these mutations. The rapid proliferation of small interfering RNA (siRNA) experimentation has provided a complementary view to traditional pharmacological approaches (which allow perturbation of a target through small-molecule inhibition) and to genetic knockouts (which generate loss-of-function events more often in whole organisms than in individual cells) (Fig. 1). These three modalities are typically seen as capable of providing answers to the same question: what is the phenotype when protein X is inactivated? Because genetic knockouts are often difficult to apply to cell culture and typically require a significant investment of resources and time (and are often compounded by compensatory changes in development and by lethality—both of which complicate interpretation), we limit this Commentary to the study of RNA interference (RNAi) and pharmacological manipulation in cells.

In many cases the answers arrived at by RNAi and pharmacology are aligned. The purpose of this Commentary is (i) to highlight situations in which RNAi and small-molecule approaches diverge in reading out complementary biology, (ii) to provide specific examples where the absence of a protein shows a different phenotype than inhibition of a protein that is physically intact and (iii) to highlight the importance of recognizing these differences. The motivation behind seeking an answer to the question “Is RNAi of target X likely to induce the same phenotype as a small-molecule inhibitor of target X?” is two-fold. First, in a target discovery mode, RNAi has proven powerful for identifying unexpected pathway components in many normal and disease processes. How likely is it that one could produce a small molecule to match the RNAi-induced phenotype? The second question is in some sense the reverse: on discovery of a new small-molecule entity, its true specificity for the stated target is often debatable. Thus it is often commented that the investigator should

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"validate" the small-molecule phenotype by checking to see whether RNAi against the same target provides a consistent phenotype. By highlighting two examples of kinase signaling (Aurora kinases and phosphatidylinositol-3-OH kinases) in which such readouts are inconsistent, we argue that the phenotypes need not necessarily be consistent and that a genuine difference between the two can be biologically informative and therapeutically important.

## Basic mechanisms of RNAi and kinase inhibition

RNAi typically involves generation of an siRNA or a small hairpin RNA (shRNA) that directs cleavage and degradation of complementary mRNA target molecules (reviewed in ref. [1]). siRNA duplexes are typically introduced into cells for short-term degradation of target molecules (days), whereas shRNA molecules can be delivered through expression vectors, allowing long-term and regulated delivery in single cells and whole organisms. Knockdown is typically observed 24–48 h after transfection and can be even more rapid using siRNA (in comparison with shRNA). Because protein half-lives can vary, interrogation of mRNA levels provides the most reliable measure of efficacy for RNAi. Proteins that are abundant and short lived (c-myc for example) are quite difficult to target and require a robust siRNA or shRNA for efficient knockdown.

Whereas siRNA and shRNA molecules can be obtained quickly and reasonably affordably, the generation of small-molecule inhibitors for specific proteins requires a more substantial investment. The pharmacological approach to obtaining a potent small-molecule inhibitor typically involves (i) screening a library of compounds to identify lead scaffolds, (ii) performing subsequent medicinal chemistry to identify regions of the small molecule for which substitutions lead to alterations in sensitivity or specificity and (iii) deriving additional derivatives to optimize the efficacy of the small molecule.

In general, pharmacological approaches have been quite successful for identifying potent inhibitors of classes of proteins that have a well-defined substrate and/or cosubstrate, such as kinases, proteases, nuclear hormone receptors, G protein–coupled receptors and ion channels. These approaches have been more challenging for the identification of agents that disrupt other aspects of protein function. In particular, there is a critical need for more effective small-molecule inhibitors of transcription factors, a major class of molecules that interact with other proteins and with DNA. Targeting protein-protein and protein-DNA interactions in a highly efficient manner has proven challenging, although many exciting new developments are emerging in this important area[2]. Our expertise is in the area of kinases, and kinases will thus be the focus of this Commentary. As kinases represent one of the largest and most highly conserved classes of drug targets in biology, the lessons learned from design and validation of specific small-molecule inhibitors of kinases should be applicable to other classes of small-molecule targets.

## Specificity and controlling for off-target effects

Issues of specificity complicate both siRNA and small-molecule methods. Off-target effects for small molecules may affect proteins of similar conformation. For example, kinase inhibitors that block ATP binding are more likely to have off-target effects among kinases than among other classes of proteins. In contrast, off-target effects for siRNA are much more difficult to classify, as short stretches of sequence homology may exist among RNA molecules encoding structurally distinct classes of proteins, or even in noncoding regions of DNA. Typically, detailed control experiments are required to distinguish on-target from off-target effects. For siRNA molecules, such experiments include use of siRNA at the lowest possible concentration, design of two different siRNA molecules that affect the same target, demonstration of knockdown at the mRNA and protein level, demonstration of a functional

readout, and (for maximal stringency) manipulation of the target gene to a form that is no longer affected by the siRNA (Box 1).

**Box 1**

## Designing effective experiments using small molecules and RNAi

### Guidelines for design of small-molecule inhibitor experiments

### Small-molecule design

There are many guides to avoiding particular functional groups in pharmacological agents because they are known to undergo biotransformation reactions resulting in nonspecific covalent modification of many targets. These and other features of frequent hitters are subjects of other excellent reviews[24].

### Demonstration of protein inhibition

At first approximation there should be a rational correlation between *in vitro* biochemically measured affinity and effective cellular concentration.

### Use at the lowest concentration

Avoid the use of compounds that are effective in cells only at concentrations >10 μM, as these are likely to target many proteins at this high concentration.

### Confirming causality

There are three stages to confirming the phenotypic effect of a molecule as being due to the target of interest.

- First, it is best to observe the effects of more than one molecular structure. With small-molecule agents, it is usually the case that multiple molecules of a similar structure (same scaffold but different substituents) are found to have a range of potencies against the target of interest. These analogs should have the same rank order potency on cells that they have in the biochemical assay.
- Second, it is important to use a negative control in each experiment. It is usually the case that a very closely related analog (for example, enantiomer, diastereomer, regioisomer, ± methyl) is inactive in the biochemical assay—this is an excellent negative control for helping to prove the target dependence of the phenotype—that is, the phenotype should not be induced by the structurally related negative control compound.
- Third, and most valuable but often not available, is an independent positive control, or a structurally unrelated (that is, from a chemically distinct chemical series) inhibitor that shows the same biochemical potency and still shows the same phenotype on cells. This is an important control because compounds of different structure that share one target would be predicted to have different off-targets, and this can help to confirm target dependence even when perfectly selective tools are not available.

### Rescue experiments

A powerful way to rule out off-target effects is to rescue the small molecule–induced phenotype by ectopic expression of a drug-resistant transgene. Though identification of drug-resistant mutants is extremely difficult, they provide ideal opportunities for delineating on- and off-target effects.

### Dose-dependent effect

There should be a dose-dependent effect on the cellular phenotype.

**Guidelines for design of siRNA experiments**

### Oligo design

In the first instance, the siRNA sequence should be carefully designed to ensure that it targets the specific protein isoform in question; database searches should be performed to select siRNA sequences that have minimal similarity to other mRNAs.

### Demonstration of protein knockdown

It is highly desirable to demonstrate that the siRNAs do indeed repress the level of the protein under investigation—for example, by immunoblotting. Some proteins are very stable, so exclusively monitoring mRNA levels—for example, by RT-PCR—may be misleading.

### Use at the lowest concentration

Having established that a given siRNA represses the protein of interest, siRNA titrations should be performed to identify the lowest possible concentration required for efficient knockdown, thereby reducing nonspecific effects.

### Confirming causality

There are similarly three stages to confirming the phenotypic effect of siRNA as being due to the target of interest.

- First, it is important to use pools of individually validated oligos. Pooling multiple siRNAs that efficiently repress the protein of interest allows the concentration of each to be reduced, thereby reducing nonspecific and off-target effects while maintaining efficient repression of the target. Note however that although purchasing ready-made pools is a cost-effective way to begin investigating a new target, it is important that each individual siRNA sequence be independently validated.
- Second, appropriate negative controls must be used. As a bare minimum, mock transfected cells should be analyzed in parallel to account for the effects of the transfection reagent. But this alone is not sufficient; a mismatched or scrambled siRNA should also be used as a negative control. Note however that siRNAs that do not target an endogenous mRNA may not engage the RNAi machinery. Therefore, a more suitable negative control would be an siRNA that engages the RNAi machinery but results in the repression of a protein not involved in the biological process under investigation.
- Third, independent oligos will provide strong support for the origin of the effect. Off-target effects seem to be sequence specific, not target specific[25]; therefore, demonstration that two or more independent siRNA sequences repress the target and yield the same phenotype is highly advantageous.

### Rescue experiments

A powerful way to rule out nonspecific and off-target effects is to rescue the RNAi-induced phenotype by ectopic expression of an RNAi-resistant transgene. Though such approaches can be technically challenging, once established they then facilitate structure-function experiments. In the case of enzymes, the expression of catalytically inactive mutants should phenocopy small-molecule-mediated inhibition.

### Appreciate “run down”

Because of the time delay between siRNA transfection and protein repression, and because of the run down that accompanies this delay (Fig. 1), it is important to consider whether or not the phenotype observed is an indirect consequence of an earlier defect.

For example, a multipolar mitosis may be the result of a cytokinesis defect in the previous cell cycle rather than centrosome amplification in the current cycle[26].

For small molecules, appropriate controls include measurement of concentration required to inhibit the target both *in vitro* and in cells, measurement of concentration required to inhibit closely related targets *in vitro* and possibly in cells, use of small molecules at the lowest possible concentration (ensuring that the doses in cells are correlated with the biochemically measured potency after taking into account competition with endogenous substrates, as in the case of ATP for kinases), demonstration of a functional readout and, where possible, demonstration of alignment of functional readouts using a structurally dissimilar inhibitor that blocks the same target[3] (Box 1). In either case, using the lowest possible concentration of the applied molecule helps to ensure specificity of the compound (suggested cutoff is 10 μM on cells), particularly in cases in which the selectivity of the compound is not well established. For example, for kinase inhibitors (for which the challenge of generating a selective inhibitor is notoriously difficult), the appropriate concentration can be determined by screening much of the kinome *in vitro* using a candidate inhibitor to identify alternate targets and to determine the difference in the concentration of inhibitor needed to block the intended target as compared with off-targets.

## Phenotypes do not always align

It has become customary for results of small-molecule inhibition studies to be verified using RNAi, with the expectation that the siRNA experiment becomes a control for off-target effects of the small molecule. In most cases, phenotypes do typically align. However, when such experiments are not congruent, the lack of alignment may reflect a true difference in biology between RNAi and pharmacological approaches (discussed below). But because reviewers may view this lack of congruence as a lack of specificity for the small molecule, specific and detailed examples of incongruence are often down-played, and they are typically relegated to supplementary data. As a result, identifying well-documented cases that illustrate a lack of alignment between RNAi and small-molecule inhibitor experiments is not trivial, and our ability to tabulate compelling examples for such lack of alignment is understandably limited.

A biological basis for this divergence between results observed using siRNA and those observed using pharmacological methods is typically attributed to protein-protein interactions. A protein treated with a small-molecule inhibitor may still act as a scaffold for protein-protein interactions that would be disrupted by siRNA treatment. In other words, if an enzyme's physical scaffold is itself important for biological function, then the siRNA treatment will inhibit both functions, whereas the small molecule will only inhibit one. To illustrate this point, here we consider two case studies: one revolving around the Aurora kinases, the other focusing on members of the phosphatidylinositol-3-OH kinase (PI(3)K) family.

## The Aurora B kinases

Recent studies on the Aurora kinases provide a useful case study illustrating the lack of congruence between RNAi and small-molecule inhibition. The Aurora kinases have been extensively reviewed[4,5]; therefore here we provide only a brief overview. The Aurora kinases are mitotic regulators conserved from yeast to man, and mammals express three Auroras: A, B and C. Aurora A, the "polar" kinase, localizes to the centrosome and spindle poles and is required for bipolar spindle assembly during mitosis. Aurora B, the "equatorial" kinase, is a chromosome passenger protein that first localizes to the centromeres and kinetochores during early mitotic stages but then traverses to the spindle midzone after

anaphase. In addition to phosphorylating histone H3 on Ser10, mitotic activation of Aurora B is required for regulating kinetochore-microtubule interactions, the spindle checkpoint and cytokinesis. Aurora C, another chromosome passenger protein, is expressed exclusively in the male germline.

Largely because Aurora A is overexpressed in many cancers, a number of Aurora inhibitors have been developed, several of which are being tested in the clinic for anticancer activity[6,7]. The best characterized Aurora inhibitors described thus far include ZM447439, hesperadin, VX-680 and MLN-8054, and all are useful tools for probing the roles of cellular Aurora kinase activity. Indeed, the first reports describing small-molecule Aurora inhibitors uncovered a unique aspect of Aurora B function in terms of spindle checkpoint signaling: inhibitor-treated cells could not mount a robust checkpoint response after paclitaxel-mediated microtubule stabilization[8,9]. By contrast, the checkpoint was activated when microtubule polymerization was prevented with nocodazole. This differential checkpoint response is consistent with the notion first developed in budding yeast[10] that Aurora B activity is required to trigger the spindle checkpoint only in response to a loss of tension, not in response to a loss of attachment. In the presence of paclitaxel, kinetochores attach microtubules, but dampened microtubule dynamics prevent kinetochores from pulling on the attached microtubule fibers, so tension does not accumulate. By contrast, with nocodazole, the lack of microtubules prevents both attachment and accumulation of tension.

RNAi-mediated inhibition of Aurora B also compromises the spindle checkpoint, but here the lack of congruence arises; Aurora B RNAi prevents checkpoint activation in nocodazole-treated cells. Though this could reflect an off-target RNAi effect, this is unlikely. Injection of anti-Aurora B antibodies or massive overexpression of a catalytically inactive Aurora B mutant yields phenotypes similar to those observed after RNAi, which leads to the hypothesis that the lack of phenotypic congruence reflects different modes of inhibition (Fig. 2a)[6,8]. This hypothesis draws on the fact that Aurora B, along with survivin, the inner centromere protein (INCENP) and borealin, is part of a chromosome passenger complex (CPC)[11]. RNAi-mediated depletion of Aurora B not only delocalizes remaining passenger components from the centromere but also destabilizes them, which in turn inhibits the structural contribution that the CPC has at the centromere, thus leading to pleiotropic effects. By contrast, the CPC is intact and correctly localized after small-molecule-mediated inhibition of Aurora B (ref. [8]). Indeed, it is the ability of small molecules to target enzymatic activity without affecting stoichiometry that makes them powerful tools for probing the physiological functions of enzymes.

Support for this hypothesis comes from experiments expressing Aurora B kinase mutants at near-physiological levels. Under these conditions the paclitaxel-nocodazole differential manifests[12]—that is, in this case, small-molecule-mediated inhibition is phenocopied by mild overexpression of catalytic mutants but not by RNAi-mediated repression. Whereas small-molecule inhibition and the moderate overexpression of catalytic mutants simply reduces Aurora B kinase activity in the cell, RNAi disrupts the CPC, leading to a more global kinetochore defect. Thus, because small-molecule inhibitors of Aurora B (in contradistinction to siRNA approaches) still allow binding of the CPC to centromeres, distinct differences in the biology are observed when using these two approaches.

Another anomaly arises when inhibiting Aurora B with these two approaches. Small-molecule inhibition potently suppresses Ser10 phosphorylation[6] (Fig. 2b). By contrast, RNAi-mediated inhibition often does not, although it does compromise other Aurora B functions. Is this due to an off-target effect of the inhibitors? This is unlikely; all the evidence suggests that Aurora B is the primary kinase responsible for Ser10 phosphorylation, and importantly, overexpression of Aurora B dominant negatives potently

inhibits histone H3 phosphorylation[12]. A more likely explanation is that residual levels of Aurora B are sufficient for H3 phosphorylation but not chromosome alignment. Note that evidence supporting the lack of a potent effect on H3 phosphorylation after Aurora B RNAi is largely anecdotal—possibly because when investigators do not get the expected result, they do not report it. One aim of this Commentary is to provide an intellectual framework to encourage the disclosure of noncongruence between RNAi and small-molecule approaches.

The issue of residual protein levels following RNAi is further highlighted by studies on the Aurora A kinase. In several model systems, such as flies, frogs and worms, inhibition of Aurora A prevents centrosome separation in mitosis, thereby yielding a monopolar spindle phenotype[13]. Though small-molecule-mediated Aurora A inhibition also results in monopolar spindles[12,14], bipolar spindles often form following Aurora A RNAi, although chromosome alignment appears defective (see ref. [15], for example). The small-molecule phenotype is indeed due to inhibition of Aurora A (as opposed to an off-target effect), as expression of a drug-resistant Aurora A mutant reverts the monopolar phenotype[12]. Furthermore, the monopolar phenotype also arises when Aurora A is repressed by RNAi along with simultaneous expression of an RNAi-resistant kinase mutant[12]. Thus, in this case it seems that RNAi alone (despite extensive repression) does leave residual kinase that, when concentrated at the spindle pole, is sufficient to promote reasonably efficient spindle assembly.

These observations derived from probing Aurora A function also highlight methodologies that can be used to resolve the differences that arise after small-molecule- and RNAi-based approaches. The expression of drug-resistant mutants is a powerful way to delineate on- and off-target effects, thereby validating small-molecule specificity in cells. In addition, the expression of RNAi-resistant kinase mutants following repression of the target should yield phenotypes consistent with small-molecule effects (Box 1). However, it is important to note that although investigators should be encouraged to consider these approaches, reviewers should also appreciate that the identification of drug-resistant mutants and the establishment of RNAi complementation assays is not always trivial.

From this case study, a key thematic issue arises: the biology of siRNA does not have to parallel the biology of small-molecule inhibition. The biological readouts using both siRNA and small-molecule approaches can be independently correct, and they are unlikely to be due to off-target effects when the appropriate control RNAi, control compounds and broad biochemical screening are brought to bear on assessing likely off-target effects. Both approaches have independent value toward approaching a biological problem, and there is no reason to assume that they should always be aligned.

## Divergent readouts with lipid kinases

Protein-protein interactions also serve an important role in sequestering or stabilizing protein partners. An example of this scenario stems from work using siRNA and pharmacologic approaches to study lipid kinase function. The class IA PI(3)K family of lipid kinases consists of three catalytic kinases: p110α, p110β and p110δ. Class IA PI(3)Ks have critical roles in many areas of cell biology[16]. Much of our current knowledge of these enzymes derives from experiments using pan-selective inhibitors of all PI(3)Ks. Although pan-selective inhibitors such as LY294002 and wortmannin have been critical to our current understanding of these enzymes, these compounds have proven to be poorly suited for use as clinical agents. A number of academic and pharmaceutical laboratories have therefore initiated efforts to identify inhibitors that are selective within the PI(3)K family. We have synthesized a large number of potent isoform-selective inhibitors of class I PI(3)Ks, and we have screened these in a variety of human cancers[17,18]. In particular, we have identified

three structurally different agents that have low-nanomolar activity against p110β. In experiments analyzing numerous cancer cell lines, none of these inhibitors showed activity in functional assays of cell cycle progression[17,18]. Yet we and others have identified potent antiproliferative activities in comparable experiments using siRNA against p110β (refs. [17,19,20]).

Thus, as in the case of Aurora kinase, siRNA and chemical genetic approaches provided clear insights into the biology of a complex system, but results using these approaches were not aligned. How can we explain this mysterious lack of congruence? A key issue contributing to these differences again stems from the fact that p110α, p110β and p110δ are catalytic proteins that exist within cells as heterodimers. Free p110 is rapidly degraded and exists stably only in association with p85 or its splice variants, which direct the interaction between PI(3)K and a large family of growth factor receptors. Two regulatory proteins exist (p85α and p85β) that as monomers act to negatively regulate PI(3)K activity. In addition to various possible heterodimers between p110α, p110β, p110δ and p85 and its splice variants, p110β protein can also heterodimerize with the βγ subunits of heterotrimeric G proteins[16]. Because p110β exists as a heterodimer with various regulatory subunits, we analyzed small-molecule and siRNA treatment for impact on levels of p85. Small-molecule inhibitors of p110β did not affect p85, likely because these inhibitors simply affect the activity of p110 without affecting the ability of p110 to bind to its regulatory partners. In contrast, siRNA against p110β led to knockdown of this kinase, which resulted in a considerable alteration in detectable levels of p85 (ref. [17]). The fact that siRNA against p110β led to alteration in levels of p85 means that siRNA against p110β is likely to affect the activities of other molecules that can heterodimerize with p85, thus resulting in altered biological function. Therefore in this instance, small-molecule inhibitors of p110β signaling likely assess the impact of blocking p110β specifically, whereas siRNA against p110β may block proliferation by modifying the activity of p110α or of another class I p110 molecule (Fig. 3). Genetic knockout experiments in the PI(3)K field have led to similar compensatory or unanticipated results—through which knockout of p110 or p85 molecules leads to dominant negative effects from altering the ratio of p110 to p85—and in this manner are aligned with the siRNA experiments[21–23].

The failure of concordance between siRNA and inhibitor studies, as depicted in both of these examples, occurs infrequently but has important implications in biology. Scientists worried about off-target effects of small-molecule inhibitors typically use siRNA validation. It is increasingly clear that siRNA and small-molecule approaches need not be aligned, and that this failure of concordance is not necessarily attributable to off-target effects of either approach. The need for siRNA validation must be considered within the context of careful experiments that use appropriate dosages of small-molecule inhibitors, and that demonstrate a consistent biological readout using multiple small-molecule inhibitors with distinct scaffolds, each of which blocks a common target (Box 1).

The lack of alignment between RNAi and inhibitor studies also presents an opportunity to develop fundamentally new drugs using RNAi. Although high-throughput pharmacological screens using small-molecule inhibitors are well established, similar screens using siRNA libraries are in a state of rapid evolution. With respect to the application of these approaches to treat people, both academic laboratories and companies developing RNAi-based therapeutics have the potential to treat disease in ways that are not possible using molecules that target the active sites in proteins. The examples given above exemplify these critical differences. Whereas small-molecule inhibitors of Aurora B block proliferation of cancer cells, small-molecule inhibitors of p110β do not. RNAi against Aurora B is less active than analogous small-molecule inhibitors. In contrast, RNAi against p110β a proliferative arrest in cancer and, in this regard, is more robust than pharmacological inhibition of p110β.

RNAi-based therapy may have fundamental differences in comparison with pharmacological approaches. The ability to recognize and exploit these opportunities will be critical in (i) reassessing the importance of cancer targets known to be dispensable using inhibitors and (ii) delivering RNAi therapeutics in new ways and with different results than analogous approaches using small molecules.

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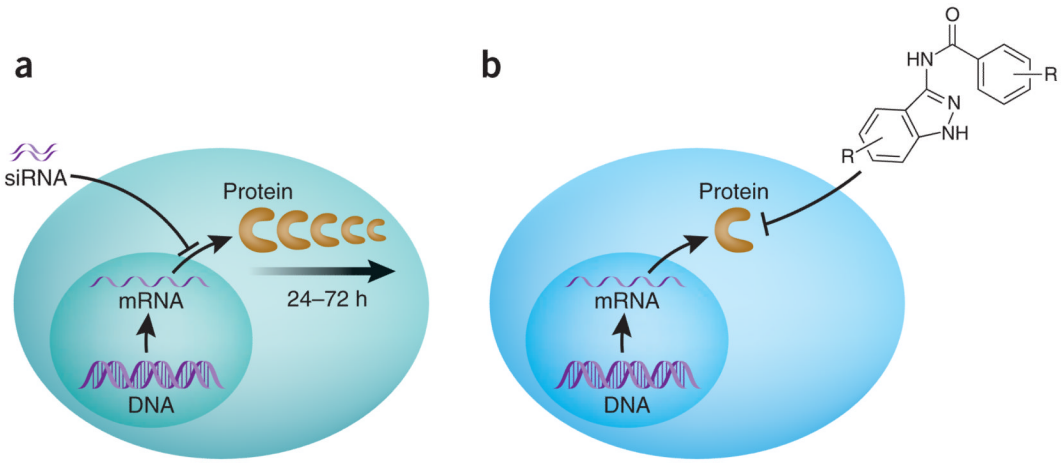


**Figure 1.**
Modes of action for inhibition of protein activity. (**a**) Inhibition of protein expression by siRNA. (**b**) Inhibition of protein activity by small molecules.

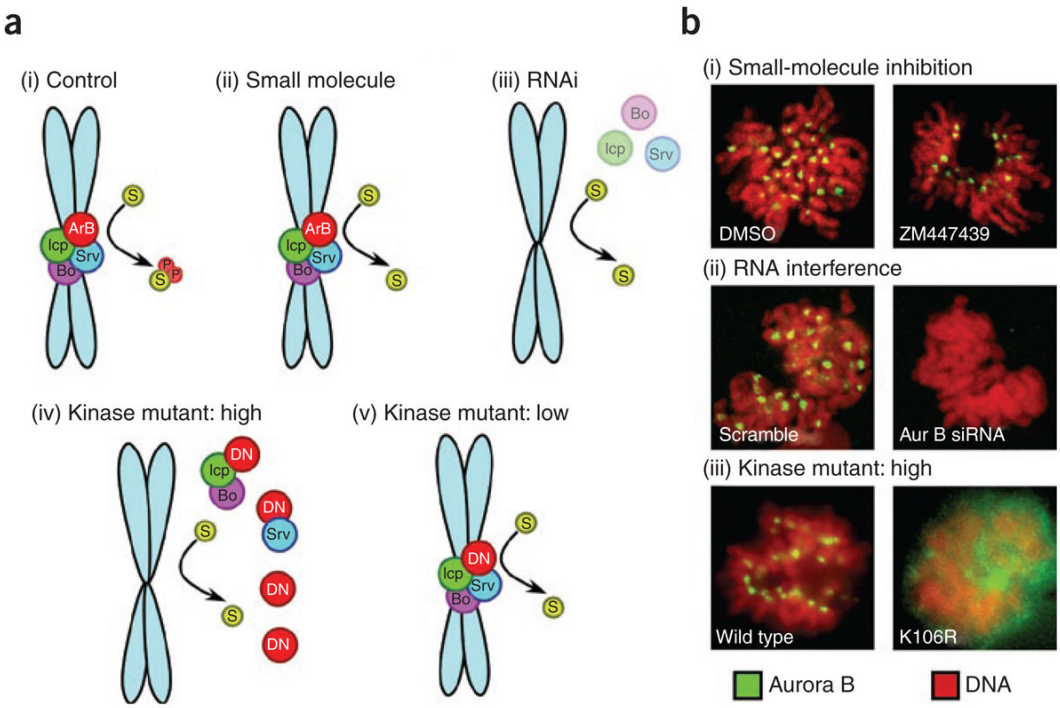


**Figure 2.**

Alternative modes of Aurora B blockade. (**a**) Schematic showing that in unperturbed cells (i), the CPC comprising Aurora B, INCENP, survivin and borealin localizes to centromeres and phosphorylates substrates. Exposure to small-molecule inhibitors (ii) prevents substrate phosphorylation but leaves the CPC intact. Aurora B RNAi (iii) or high overexpression of dominant negative (DN) mutants (iv) not only prevents substrate phosphorylation but also mislocalizes and destabilizes remaining CPC components. Low overexpression of dominant negative mutants (v) leaves the CPC intact but potently inhibits substrate phosphorylation. (**b**) Immunofluorescence images of human cells exposed to a small-molecule Aurora B inhibitor (i) or Aurora B siRNAs (ii), or transfected with a dominant negative mutant (iii), and then stained to detect Aurora B (green) and DNA (red). Small-molecule-mediated inhibition allows correct localization of Aurora B, whereas RNAi greatly reduces Aurora B levels and the kinase mutant mislocalizes the CPC. Reproduced from ref. [8]. Copyright 2003 Rockefeller University Press.

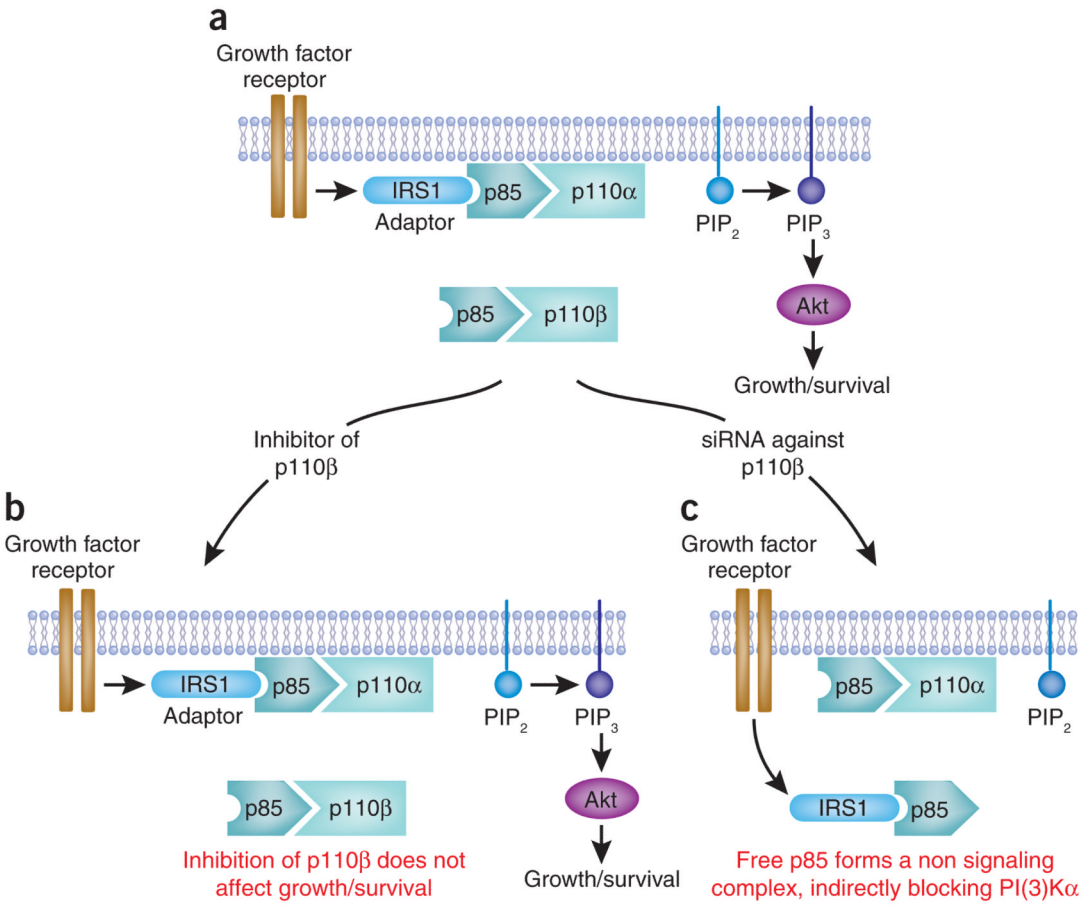


**Figure 3.**

Differential effects of siRNA and small-molecule inhibitors against p110β. (**a**) Activation of growth factor receptor signals through an adaptor protein such as IRS1 to bring PI(3)K to the membrane and to relieve basal inhibition of p110α by p85. Catalytically active p110α then phosphorylates phosphoinositide-4,5-bisphosphate ($PIP_2$), generating the lipid second messenger phosphoinositide-3,4,5-trisphosphate ($PIP_3$). $PIP_3$ in turn recruits Akt to the membrane, where it is phosphorylated and regulates a broad range of substrates that promote growth, proliferation and survival. (**b**) The role of p110β in responding to growth factor receptors and in activating growth and survival is less central than that of p110α. Small-molecule inhibitors of p110β do not affect signaling through p110α and therefore do not appreciably affect growth or survival. (**c**) In contrast, siRNA against p110β leads to growth arrest, possibly by freeing up monomeric p85, which can sequester IRS1 in a nonsignaling cytosolic protein complex, thereby abrogating signaling through p110α.