Academic cross-fertilization by public screening yields a remarkable class of protein phosphatase methylesterase-1 inhibitors

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>As Published</td>
<td><a href="http://dx.doi.org/10.1073/pnas.1015248108">http://dx.doi.org/10.1073/pnas.1015248108</a></td>
</tr>
<tr>
<td>Publisher</td>
<td>National Academy of Sciences</td>
</tr>
<tr>
<td>Version</td>
<td>Final published version</td>
</tr>
<tr>
<td>Accessed</td>
<td>Mon Dec 03 07:16:52 EST 2018</td>
</tr>
<tr>
<td>Citable Link</td>
<td><a href="http://hdl.handle.net/1721.1/66989">http://hdl.handle.net/1721.1/66989</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>Article is made available in accordance with the publisher’s policy and may be subject to US copyright law. Please refer to the publisher’s site for terms of use.</td>
</tr>
<tr>
<td>Detailed Terms</td>
<td></td>
</tr>
</tbody>
</table>
Academic cross-fertilization by public screening yields a remarkable class of protein phosphatase methylesterase-1 inhibitors

Daniel A. Bachovchin*, Justin T. Mohr, Anna E. Speers, Chu Wang, Jacob M. Berlin, Timothy P. Spicer, Virneliz Fernandez-Vega, Peter Chase, Peter S. Hodder, Stephan C. Schürer, Daniel K. Nomura, Hugh Rosen*, Gregory C. Fu†, and Benjamin F. Cravatt*†

*The Skaggs Institute for Chemical Biology and Department of Chemical Physiology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037; †Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139; *Lead Identification Division, Molecular Screening Center, The Scripps Research Institute, 130 Scripps Way, Jupiter, FL 33458; and †The Scripps Research Institute Molecular Screening Center, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

Edited by Stuart L. Schreiber, Broad Institute, Cambridge, MA, and approved January 13, 2011 (received for review November 24, 2010)

National Institutes of Health (NIH)-sponsored screening centers provide academic researchers with a special opportunity to pursue small-molecule probes for protein targets that are outside the current interest of, or beyond the standard technologies employed by, the pharmaceutical industry. Here, we describe the outcome of an inhibitor screen for one such target, the enzyme protein phosphatase methylesterase-1 (PME-1), which regulates the methylesterification state of protein phosphatase 2A (PP2A) and is implicated in cancer and neurodegeneration. Inhibitors of PME-1 have not yet been described, which we attribute, at least in part, to a dearth of substrate assays compatible with high-throughput screening. We show that PME-1 is assayable by fluorescence polarization-activity-based protein profiling (fluopol-ABPP) and use this platform to screen the 300,000+ member NIH small-molecule library. This screen identified an unusual class of compounds, the aza-β-lactams (ABLs), as potent (IC50 values of approximately 10 nM) covalent PME-1 inhibitors. Interestingly, ABLs did not derive from a commercial vendor but rather an academic contribution to the public library. We show using competitive-ABPP that ABLs are exquisitely selective for PME-1 in living cells and mice, where enzyme inactivation leads to substantial reductions in de-methylated PP2A. In summary, we have combined advanced synthetic and chemoproteomic methods to discover a class of ABL inhibitors that can be used to selectively perturb PME-1 activity in diverse biological systems. More generally, these results illustrate how public screening centers can serve as hubs to create spontaneous collaborative opportunities between synthetic chemistry and chemical biology labs interested in creating first-in-class pharmacological probes for challenging protein targets.

Protein phosphorylation is a pervasive and dynamic posttranslational protein modification in eukaryotic cells. In mammals, more than 500 protein kinases catalyze the phosphorylation of serine, threonine, and tyrosine residues on proteins (1). A much more limited number of phosphatases are responsible for reversing these phosphorylation events (2). For instance, protein phosphatase 2A (PP2A) and PP1 are thought to be responsible together for >90% of the total serine/threonine phosphatase activity in mammalian cells (3). Specificity is imparted on PP2A activity by multiple mechanisms, including dynamic interactions between the catalytic subunit (C) and different protein-binding partners (B subunits), as well as a variety of posttranslational chemical modifications (2, 4). Within the latter category is an unusual methylesterification event found at the C terminus of the catalytic subunit of PP2A that is introduced and removed by a specific methyltransferase (leucine carboxymethyltransferase-1 or LCMT1) (5, 6) and methylesterase (protein phosphatase methylesterase-1 or PME-1) (7), respectively (Fig. 1A). PP2A carboxymethylation (hereafter referred to as “methylation”) has been proposed to regulate PP2A activity, at least in part, by modulating the binding interaction of the C subunit with various regulatory B subunits (8–10). A predicted outcome of these shifts in subunit association is the targeting of PP2A to different protein substrates in cells. PME-1 has also been hypothesized to stabilize inactive forms of nuclear PP2A (11), and recent structural studies have shed light on the physical interactions between PME-1 and the PP2A holoenzyme (12).

Notwithstanding the aforementioned models and findings, the actual functional consequences of perturbing PP2A methylation remain largely unexplored. In yeast, LCMT1 deletion caused severe growth defects under stress conditions, while PME-1 deletion did not result in an observable cellular phenotype (9). Disruption of the PME-1 gene in mice, on the other hand, caused early postnatal lethality (13), which has limited the experimental opportunities to explore methylation of PP2A in animals. Recent studies have found that RNA-interference knockdown of PME-1 in cancer cells leads to activation of PP2A and corresponding suppression of protumorigenic phosphorylation cascades (14), indicating that PME-1 could be an attractive drug target in oncology. Changes in PP2A methylation have also been implicated in Alzheimer’s disease, where this modification may stimulate PP2A’s ability to promote neural differentiation (15).

Despite the critical role that PME-1 plays in regulating PP2A activity and function, PME-1 inhibitors have not yet been described. This deficiency may be due to a lack of PME-1 activity assays that are compatible with high-throughput screening (HTS). Assessment of PME-1 activity typically involves either Western blotting with antibodies that recognize specific methylation states of PP2A (7, 13) or monitoring the release of 3H-methanol from radiolabeled-C subunits (16), but neither assay is easily adapted for HTS. PME-1 is, however, a serine hydrolase and therefore susceptible to labeling by active-site-directed fluorophosphonate (FP) probes (17). We have recently shown that FP probes can form the basis for a fluorescence polarization-activity-based protein profiling (fluopol-ABPP) assay suitable for HTS (18). Here, we apply fluopol-ABPP to screen the 300,000+ National Institutes of Health (NIH) compound library for PME-1 inhibitors. From this screen, we identified a set of aza-β-lactam (ABL) compounds that act as remarkably potent and selective pharmacological probes for challenging protein targets.


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

†To whom correspondence may be addressed. E-mail: cravatt@scripps.edu or gcff@mит.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1015248108/*IDSupplemental.
PME-1 inhibitors. We show that these ABLs covalently inactivate PME-1 with high specificity in living cells and animals, where disruption of this enzyme leads to substantial decreases in demethylated PP2A.

Results
PME-1 Inhibitor Screening by Fluopol-ABPP. Because PME-1 is a serine hydrolase that is known to interact with reporter-tagged FP probes (17, 19), we reasoned that this enzyme would be assayable by competitive ABPP methods. However, lower-throughput, gel-based competitive ABPP screens have not succeeded in identifying lead PME-1 inhibitors (20), indicating the need to survey larger compound libraries. We therefore asked whether PME-1 could be assayed using the recently introduced, HTS-compatible fluopol-ABPP platform (18). This technique, where compounds are tested for their ability to block the increase in fluopol signal generated by reaction of a fluorescent activity-based probe with a much larger protein target, has enabled inhibitor screening for a wide range of probe-reactive enzymes (http://pubchem.ncbi.nlm.nih.gov/). We confirmed that purified, recombinant wild-type PME-1, but not a mutant PME-1 in which the serine nucleophile was replaced with alanine (S156A), labels with a fluorophosphonate rhodamine (FP-Rh) (21) probe (Fig. 1B). This reaction generates a strong, time-dependent increase in fluopol signal that is not observed in the absence of enzyme or with the S156A mutant PME-1 enzyme (Fig. 1C). In collaboration with the Molecular Libraries Probe Production Centers Network (MLPCN), we screened 315,002 compounds for PME-1 inhibition using fluopol-ABPP (see Fig. 1D for a representative subset of the primary screening data). Following a confirmation screen on initial hits, we identified 1,068 compounds as potential PME-1 inhibitors. As an initial filter, we selected compounds for follow-up studies that had <5% hit rates in all other bioassays reported in the PubChem database, <30% inhibition in three fluopol-ABPP screens performed on other enzymes (http://pubchem.ncbi.nlm.nih.gov/), and >40% inhibition of PME-1 in the confirmation screen. This filter yielded approximately 300 candidate PME-1 inhibitors.

Discovery of aza-β-lactam (ABL) Inhibitors of PME-1. The approximately 300 hit compounds were next analyzed by gel-based competitive ABPP (18, 22) in soluble lysates from HEK 293T cells overexpressing PME-1. This convenient selectivity screen assessed in parallel the activity of lead compounds against approximately 25 gel-resolvable, FP-Rh-reactive serine hydrolases expressed in HEK 293T cells and rapidly eliminated false-positive and nonselective compounds. Among the compounds that selectively inhibited PME-1 (Fig. 1S) were four ABLs (ABL127, ABL103, ABL105, ABL107) that were similar in structure, all with a branched alky group at R1 and with R stereochemistry at this position (Fig. 2A). The MLPCN library contained 22 other ABLs, including the enantiomers of ABL127 (ent-ABL127), ABL103 (ent-ABL103), and ABL105 (ent-ABL105), that were all considerably less active toward PME-1 in the primary screen (Table S1). Intriguingly, the ABLs did not originate from a commercial compound collection but rather were submitted by the academic chemistry laboratory of our coauthor Gregory Fu, who generated these compounds as part of an investigation into the synthetic utility of chiral 4-pyrrolidinopyridine catalysts (23). We further noted that the ABLs are structurally unusual compared to the rest of the MLPCN library, lying a considerable distance in a chemical space plot from the typical structures that populate the compound collection (Fig. 2B).

The ABL hits were next titrated into the soluble proteome of MDA-MB-231 cells, a cell line that endogenously expresses PME-1, to assess their potency (Fig. 2C). The two compounds bearing cycloalkyl substituents at R1, ABL127 and ABL103, were extraordinarily potent inhibitors of PME-1, with IC50 values of 4.2 and 2.1 nM, respectively (Fig. 2C and Fig. S2). The two compounds with isopropyl substituents, ABL105 and ABL107, exhibited lower IC50 values (92 and 24 nM, respectively) but were still good inhibitors of PME-1. Interestingly, a strong preference for the R enantiomer of ABL127 was observed, as the S enantiomer of ABL127 (ent-ABL127) was at least two orders of magnitude less potent at inhibiting PME-1 (Fig. 2C and D). Indeed, some of the apparent activity of the S enantiomer may be due to the small amount of residual R enantiomer in the >99% ee sample.

Before proceeding further, we wanted to confirm that ABLs could inhibit the ability of PME-1 to demethylate PP2A. We therefore treated HEK 293T soluble lysates with ABL127 (500 nM, 30 min) or DMSO before adding purified recombinant PME-1 for an additional hour. In DMSO-treated lysates, we observed the expected time-dependent increase in demethylated PP2A and concomitant decrease in methylated PP2A, as determined by immunoblottting with antibodies that specifically recognize either form of the C terminus of PP2A (Fig. 2E). In lysates containing ABL127, however, we observed little or no change in the methylation state of PP2A (Fig. 2E), indicating that ABL127 blocks PME-1’s activity on its physiological substrate. As ABL127 and ABL103 are highly similar structures and emerged as virtually indistinguishable in these assays, we selected one of these compounds, ABL127, for in-depth characterization.

ABLs Covalently Inhibit PME-1. Based on scientific precedent showing that other serine hydrolases can react with and open β-lactam

Fig. 1. A fluopol-ABPP assay for PME-1. (A) Schematic representation of the reversible C-terminal methylation of PP2A introduced by LCM1 and removed by PME-1. (B) FP-Rh (75 nM) labels purified wild-type PME-1 (1 μM) but not the catalytically dead S156A PME-1 mutant (1 μM). Top panel: Fluorescent gel image shown in gray scale. (C) Time course for fluopol signal generated by reactions of wild-type PME-1 (1 μM) with FP-Rh (75 nM). No signal increase is observed in the absence of enzyme or with the S156A PME-1 mutant. The indicated 45-min time point (Z2 = 0.77), prior to reaction completion, was selected for HTS. Data are presented as mean values ± SD. (D) Scanning data for a representative 15,000 compound set of the NIH small-molecule library. Compounds that reduced the FP-Rh fluopol signal by >26.13% were designated as hits for PME-1 (red squares).

Bachovchin et al.
In both cell lines, we observed highly potent and selective inhibition (Fig. 4) of our competitive ABPP-MudPIT technology (20, 25) that targets of ABL127 at concentrations under 10 \( \mu \)M. We wanted to confirm the identity of the expected acylation/adduct was confirmed by liquid chromatography-mass spectrometry (LC-MS) analysis of purified, recombinant PME-1 treated with ABL127 (Fig. 3C).

**ABL127 Selectively Inhibits PME-1 in Cells.** We next investigated whether ABLs could inhibit PME-1 in living cells. We incubated MDA-MB-231 and HEK 293T cells with a concentration range of ABL127 for 1 h, harvested the soluble proteomes, and then reacted these lysates with the FP-Rh probe (Fig. 4A and Fig. S3). In both cell lines, we observed highly potent and selective inhibition of PME-1 with IC\(_{50}\) values of 11.1 nM and 6.4 nM, respectively (Fig. 4B and Fig. S3). Although these gel-based competitive ABPP studies did not reveal any additional serine hydrolase targets of ABL127 at concentrations under 10 \( \mu \)M, we wanted to verify this selectivity profile by higher-resolution LC-MS/MS methods. To accomplish this, we employed an advanced version of our competitive ABPP-MudPIT technology (20, 25) that utilizes stable-isotope labeling of amino acids in cell culture (SILAC) (26). SILAC involves differential labeling of proteins with stable isotopes to generate isotopically “light” and “heavy” samples, which, when pooled and analyzed by MS, yield accurate quantification by comparing intensities of light and heavy peptide peaks. SILAC has previously been used to identify enzymes targets of activity-based probes (27) and small-molecule-binding proteins in cell lysates (28). In our competitive ABPP-SILAC experiments, cells grown in light and heavy media were treated with DMSO or ABL127, respectively, for 1 h. Proteomes were then harvested, combined at a 1:1 total protein ratio, and treated with purified PME-1 (500 nM, 1 h) blocked PP2A demethylation as determined by Western blotting with antibodies that recognize specific methylation states of PP2A.

![Diagram](image1.png)

**Fig. 2.** Discovery of selective ABL inhibitors of PME-1. (A) Four ABLs (ABL127, ABL103, ABL105, and ABL107) identified as active in the MLPCN screen. (B) Chemistry space co-ordinates for compounds (colored red in high occupancy cells; colored blue in low occupancy cells) that were screened against PME-1. The 26 ABLs (shown as enlarged squares; all other compounds are shown as small circles) are located in a sparsely populated region of chemical space. This optimized 6-dimensional BCUTs (31) visualization of chemistry space for the compound library was generated using the standard 3D hydrogen suppressed descriptors with Diverse Solutions (Diverse Solutions, Tripops), with coverage illustrated by compression into an optimized two-dimensional BCUTs space as described in more detail previously (32). (C) Evaluation of compounds by gel-based competitive ABPP with FP-Rh (2 \( \mu \)M) in the soluble proteome (1 mg/mL protein) of MDA-MB-231 cells. (D) Complete PME-1 IC\(_{50}\) curves for ABL127 (4.2 nM; 95% confidence limits 2.3–7.5 nM) and ent-ABL127 (450 nM; 95% confidence limits 240–850 nM) in the soluble proteome of MDA-MB-231 cells (for IC\(_{50}\) curves of ABL103, ABL105, and ABL107, see Fig. S2). (E) Pretreatment of HEK 293T proteomes with ABL127 (500 nM, 30 min) before addition of purified PME-1 (500 nM, 1 h) blocked PP2A deamidation as determined by Western blotting with antibodies that recognize specific methylation states of PP2A.

**Fig. 3.** ABLs are covalent inhibitors of PME-1. (A) Proposed mechanism for covalent PME-1 inhibition by ABL127. (B) PME-1 (500 nM) was incubated with DMSO or ABL127 (5 \( \mu \)M), and each reaction was split into two fractions. One fraction was directly labeled with FP-Rh (left panels), and the other was subjected to gel-filtration to remove free inhibitor and then reacted with FP-Rh (right panels) to determine the reversibility of inhibition. (C) MS1 traces for the PME-1 active site peptide not adducted (top panel) or adducted (bottom panel) to ABL127. See SI Materials and Methods for more information on this experiment. Traces were obtained from tryptic digests of purified, recombinant PME-1 (10 \( \mu \)M) treated with ABL127 (50 \( \mu \)M, red trace) or DMSO (black trace).
in the levels of demethylated PP2A (35% and 80%, respectively; Fig. 4D and E). A trend toward increases in methylated PP2A was also observed in ABL127-treated HEK 293T cells, but this change did not reach statistical significance (p = 0.12) (Fig. 4D and E). No difference in methylated PP2A was observed in MDA-MB-231 cells treated with ABL127 (Fig. 4D and E). These outcomes might be expected if the vast majority of PP2A is constitutively methylated under standard cell culture conditions. To investigate this hypothesis, we stably overexpressed PME-1 in HEK 293T cells (Fig. 4F), which resulted in a dramatic increase in demethylated PP2A and a significant decrease in methylated PP2A relative to a control cell line stably expressing GFP (Fig. 4G and H). These ABL127-treated cells also showed a significant increase in methylated PP2A (Fig. 4G and H). Time course studies revealed that a single treatment of ABL127 resulted in sustained inactivation of PME-1 and reductions in demethylated PP2A for at least 24 h (Fig. 4I). These data, taken together, indicate that ABL127 selectively inactivates PME-1 in living cells, which in turn causes significant changes in the methylation state of PP2A.

A Clickable ABL Confirms Proteome-Wide Selectivity for PME-1. Our competitive ABPP results showed that ABL127 is highly selective for PME-1 among members of the serine hydrolase family, but they did not address the possibility that ABL127 might react with other proteins in the proteome. To investigate this possibility, we synthesized ABL112, an analog of ABL127 that contains alkyne groups to serve as latent affinity handles amenable to modification by reporter tags using the copper(I)-catalyzed azide–alkyne cycloaddition reaction (“click chemistry”) (30) (Fig. 5A). First, we confirmed that ABL112 retains inhibitory activity for PME-1 by gel-based competitive ABPP in MDA-MB-231 lysates, where it showed only a threefold reduction in potency (IC₅₀ = 13.8 nM; Fig. 5B) compared to the parent inhibitor ABL127 (Fig. S5). Next, we treated MDA-MB-231 cells with ABL127 (100 nM)
These results demonstrate that the remarkable selectivity displayed by ABL127 for PME-1 extends not only across the serine hydrolase family but also the greater mammalian proteome.

**ABL127 Inactivates PME-1 in Mice.** As mentioned earlier, PME-1 (−/−) mice are not viable (13), which has hindered experimental efforts to characterize this enzyme’s function (and the functional significance of PP2A methylation) in animals. Pharmacological inhibition of PME-1 would thus offer a potentially powerful means to study this enzyme in vivo. With this goal in mind, we asked whether ABL127 could inhibit PME-1 in mice. C57Bl/6 mice were treated with ABL127 (50 mg/kg, i.p., 2 h) or vehicle, sacrificed, and their brain proteomes assayed for PME-1 activity by competitive ABPP. Gel-based profiles indicated that brain PME-1 was inactivated by ABL127 (Fig. 6A), but overlapping serine hydrolase activities precluded a confident assessment of the extent of inactivation. For enhanced resolution of the activity state of PME-1 and other brain serine hydrolases, we performed competitive ABPP-MudPIT studies using FP-biotin. These LC-MS profiles confirmed complete inactivation of PME-1 (Fig. 6B) and no substantial reductions in any of the other approximately 40 brain serine hydrolases detected in this experiment. We also observed an approximately 35% reduction in the amount of demethylated PP2A in brain tissue from mice treated with ABL127 (Fig. 6 C and D). These results confirm that ABL127 can selectively inhibit PME-1 in mice and this inhibition alters the methylation state of brain PP2A.

**Discussion**

We report herein a class of ABL inhibitors that show remarkable selectivity for PME-1 and equipotent activity in both cell-free and living cell assays. The lead ABL, ABL127 (designated as NIH Probe ML174), is capable of inactivating PME-1 in both human cancer cells and mice, suggesting that it should serve as a versatile pharmacological probe for evaluating PME-1 function in a multitude of living systems. We found that PME-1 inhibition causes a significant reduction in demethylated PP2A and, in cells with high levels of PME-1 activity, also a concomitant increase in methylated PP2A. Future studies with ABL127 should facilitate a more detailed understanding of the role that methylation plays in regulating PP2A function. Will, for instance, alterations in methylation state impact the composition and/or stability of specific PP2A complexes? Structural studies have confirmed that...
PME-1 is a component of this complex, where its interactions with the catalytic subunit of PP2A appear to be mediated, at least in part, by the PME-1 active site (12). It is thus possible that inhibition of PME-1 will affect PP2A complexes not only through altering methylation but also through disrupting physical interactions between PME-1 and the catalytic subunit of PP2A. Determining how such changes in PP2A complexes affect substrate interactions and the broader phosphoproteome should represent an exciting area of research. On this note, recent studies point to an important role for PME-1 in negatively regulating the tumor-suppressive function of PP2A in cancer cells (14). PME-1 inhibitors may thus have utility as anticancer agents. Finally, we speculate that the net effect of PME-1 inhibition on PP2A methylation state will be dictated by the expression levels of not only PME-1 (see Fig. 3G) but also LCMT1. Imbalances in the relative expression of these two enzymes may thus mark specific cellular states where PME-1 inhibitors will produce their most dramatic pharmacological effects.

There were several keys to the success of our probe development effort. First, screening for inhibitors of PME-1 benefited from the fluopanal-ABPP technology, which circumvented the limited throughput of previously described substrate assays for this enzyme. Second, we were fortunate that the NIH compound library contained several members of the ABL class of small molecules. These chiral compounds, which represent an academic contribution to the NIH library, occupy an unusual portion of structural space that is poorly accessed by commercial compound collections. Although at the time of their original synthesis (23) it may not have been possible to predict whether these ABLs would show specific biological activity, their incorporation into the NIH library provided a forum for screening against many proteins and cellular targets, culminating in their identification as PP2A inhibitors. We then used advanced chemoproteomic assays to confirm the remarkable selectivity displayed by ABLs for PME-1 across (and beyond) the serine hydrolase superfamily.

That the mechanism for PME-1 inhibition involves acylation of the enzyme’s conserved serine nucleophile (Fig. 3) suggests that exploration of a more structurally diverse set of ABLs might uncover inhibitors for other serine hydrolases. In this way, the chemical information gained from a single high-throughput screen may be leveraged to initiate probe development programs for additional enzyme targets.

Projecting forward, this research provides an example of how public small-molecule screening centers can serve as a portal for spawning academic collaborations between chemical biology and synthetic chemistry labs. By continuing to develop versatile high-throughput screens and combining them with a small-molecule library of expanding structural diversity conferred by advanced synthetic methodologies, academic biologists and chemists are well-positioned to collaboratively deliver pharmacological probes for a wide range of proteins and pathways in cell biology.

Materials and Methods

PME-1 Protein Expression and Purification. Human recombinant PME-1 was expressed in BL21(DE3) Escherichia coli and purified at approximately 5 mg/L as detailed in SI Materials and Methods.

PME-1 Fluopanal-ABPP Assay. See SI Materials and Methods for details.

Competitive ABPP Assays in Proteomes. See SI Materials and Methods for details.

Synthesis of ABLs. See SI Materials and Methods for details.

ACKNOWLEDGMENTS. We thank Tianyang Ji, David Milliken, Kim Masuda, Benjamin Kidder, and Jaycin M. Murphy for technical assistance. This work was supported by National Institutes of Health grants CA132630 (B.F.C.), MH084512 (H.R.), GM57034 (G.C.F.), and GM086040 (postdoctoral fellowship to J.T.M.); the National Science Foundation (predoctoral fellowship to D.A.B.); the California Breast Cancer Research Program (predoctoral fellowship to D.A.B.); and The Skaggs Institute for Chemical Biology.


115:1455–1465.