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A Fission Yeast-based Platform for Phosphodiesterase Inhibitor HTSs and Analyses of Phosphodiesterase Activity

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Abstract

Fission yeast strains have been engineered so that their growth behavior reflects the activity of heterologous cyclic nucleotide phosphodiesterases (PDEs). These strains can be used in High Throughput Screens (HTSs) for PDE inhibitors that possess ‘drug-like’ characteristics, displaying activity in a growth stimulation assay over a 48-hr period. Through three generations of development, a collection of strains expressing 10 of the 11 mammalian PDE families that is appropriate for small molecule inhibitor screening has been generated in our laboratory. Strains unable to synthesize cyclic nucleotides allow characterization of PDE activity in that the enzyme’s potency is reflected in the amount of either cAMP or cGMP that must be added to the growth medium in order to stimulate cell growth. In the future, this system could be used to screen cDNA libraries for biological regulators of target PDEs and for the construction of strains that co-express PDEs and associated regulatory proteins to facilitate molecular and genetic studies of their functions and, in particular, to identify if different PDE-partner protein complexes show distinct patterns of inhibitor sensitivity.

1 Introduction

Mammals express eleven families of cyclic nucleotide phosphodiesterases (PDEs), encoded by 21 genes that generate over 100 distinct PDE isoforms by variations in transcriptional start sites and splicing. Each PDE family includes proteins encoded by as many as four genes (designated A through D), and are grouped, in part, according to substrate specificity: PDE4, PDE7 and PDE8 are cAMP-specific; PDE5, PDE6 and PDE 9 are cGMP-specific; and PDE1, PDE2, PDE3, PDE10, and PDE11 display dual-specificity. PDE families are also grouped according to conserved domains outside of the catalytic domain and their relative sensitivity to various chemical inhibitors and other molecules such as calcium/calmodulin or cGMP (Bender and Beavo 2006; Conti and Beavo 2007; Lerner and Epstein 2006; Soderling and Beavo 2000). In addition, tissue-specific expression and subcellular localization of PDEs permit members of this superfamily, though acting on only two substrates, to influence tissue-specific biological processes or even distinct processes in a single cell through compartmentalization of cAMP signaling (Houslay 2010). Thus, inhibition of a specific PDE sub-population in a cell may have a therapeutic benefit without altering other cAMP- or cGMP-controlled processes in the affected cell.

Most though not all approaches to PDE inhibitor development focus on compounds that act by binding to the catalytic site. This is true of medicinal chemistry approaches that produce and characterize analogs of nonselective PDE inhibitors, as well as rational drug design approaches that are guided by crystal structures of target PDEs (Card et al. 2005; Card et al. 2004). While these methods have led to the development of many selective PDE inhibitors, there remains a need to develop specific inhibitors for certain PDE families (PDE1, PDE6, PDE8, PDE11), as well as subtype-selective inhibitors for families encoded by multiple genes (PDE1, PDE3, PDE4, PDE6, PDE7, and PDE8).

This chapter describes a fission yeast-based platform for PDE inhibitor screens that can also be used to characterize PDEs in live cells. Differing from traditional inhibitor screens, this approach allows inexpensive screening of full-length enzymes expressed in a eukaryotic cell, following the yeast genetic philosophy of discovery: “first find something that does what you are looking for and then figure out the mechanism”. As such, this screening platform is open to the discovery of both active-site and allosteric inhibitors that are structurally unrelated to current PDE inhibitors. In addition, it can be used to characterize the PDEs under conditions that more closely resemble their natural cellular environment in contrast to *in vitro* enzyme assays that are frequently carried out on biochemically-stable fragments of PDEs expressed in and purified from *E. coli*. Finally, this system is amenable to conducting cDNA library screens for genes that encode biological regulators of a target PDE and genetic screens for mutations that affect PDEs or their regulators.

2 Use of Yeast to Study Mammalian Proteins

As unicellular eukaryotes, the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe* serve as important model organisms for the study of biological processes that are conserved in human cells. Both yeasts have been used to clone human genes by functional complementation of a defective gene in the host yeast strain. For example, the human Cdc2 cyclin-dependent kinase gene was first cloned by its ability to suppress the temperature-sensitive growth of a *S. pombe cdc2⁻* mutant strain (Lee and Nurse 1987). Yeast strains expressing human proteins are used to study the function of the human protein and to identify mutations that alter function, as in a study of the p53 protein expressed in *S. pombe* (Bischoff et al. 1992). Furthermore, yeast growth-based HTSs have been successfully deployed to identify inhibitors of various eukaryotic proteins, including sirtuin family NAD-dependent deacetylases (Grozinger et al. 2001), mammalian K⁺ channels (Zaks-Makhina et al. 2004), and mammalian p38 α MAP kinase (Friedmann et al. 2006).

Studies using *S. cerevisiae* first demonstrated the feasibility of cloning and studying mammalian PDE genes in a yeast system. *S. cerevisiae* expresses two PDE proteins, the Pde1 low-affinity enzyme and the Pde2 high-affinity enzyme (Nikawa et al. 1987b; Sass et al. 1986). Cells lacking Pde1 and Pde2 have high cAMP levels that confer heat-shock sensitivity to stationary phase cells. Library screens for mammalian genes that restore heat-shock resistance led to the identification of clones of rat PDE4B and human PDE7A (Colicelli et al. 1989; Colicelli et al. 1991; Michaeli et al. 1993). In addition, yeast strains that express mammalian PDE4B display a heat-shock survival phenotype that is sensitive to

treatment with the PDE4 specific inhibitor, rolipram (Engels et al. 1995; McHale et al. 1991; Pillai et al. 1993; Pillai et al. 1994; Torphy et al. 1992). The ability to monitor PDE4 activity via heat shock resistance and the sensitivity of yeast-expressed rat PDE4B to rolipram allowed for the isolation of rolipram-resistant forms of PDE4B (Pillai et al. 1993). The amino acids altered in these proteins were later shown to be part of the nucleotide-binding site in the active site. While this system facilitated the cloning and study of PDEs, it is not amenable to high throughput screening as abnormally high concentrations of rolipram are required for this assay. This may be due to the need to work with stationary phase cells that are relatively impenetrable to test compounds.

3 cAMP Signaling and *fbp1* Transcriptional Regulation in the Fission Yeast *S. pombe*

Both *S. pombe* and *S. cerevisiae* produce cAMP signals in response to glucose detection (D'Souza and Heitman 2001; Hoffman 2005a; 2005b; Lengeler et al. 2000; Thevelein et al. 2000; Thevelein and de Winde 1999). In these yeasts, the increase in cAMP levels is due to adenylyl cyclase activation, while feedback regulation to limit the cAMP signal is, in part, a function of PDE activity (Ma et al. 1999; Nikawa et al. 1987a; Wang et al. 2005).

Most of the components of the *S. pombe* cAMP pathway have been identified from mutant strains that are defective in glucose repression of transcription of the *fbp1* gene, which encodes the gluconeogenic enzyme fructose-1,6-bisphosphatase (Hoffman and Winston 1990; 1991). Key to these genetic selections is a reporter construct in which the *fbp1* promoter drives expression of the *ura4* OMP decarboxylase gene. The *ura4* gene is a selectable marker as its expression is required for uracil biosynthesis, which is needed for growth in medium lacking uracil. It is also a counterselectable marker, as the pyrimidine analog 5-fluoroorotic acid (5FOA) is toxic to cells that express it. Normally *S. pombe* represses *fbp1-ura4* transcription in glucose-rich medium, preventing growth in medium lacking uracil while allowing growth in 5FOA medium. Mutants defective in glucose/cAMP signaling, which is required to activate the cAMP-dependent protein kinase (PKA), constitutively transcribe the *fbp1-ura4* construct so as to confer growth on medium lacking uracil, as well as 5FOA-sensitivity (5FOA^S). These strains carry mutations that affect the Git3 G protein coupled receptor (Welton and Hoffman 2000), any subunit of the heterotrimeric G protein composed of Gpa2 (Nocero et al. 1994), Git5 (Landry et al. 2000), and Git11 (Landry and Hoffman 2001), the Git2/Cyr1 adenylyl cyclase (Hoffman and Winston 1991), the Git1 adenylyl cyclase-binding protein (Kao et al. 2006), Hsp90 (Alaamery and Hoffman 2008), the Git7 Hsp90 co-chaperone (Schadick et al. 2002), or the Pka1 PKA catalytic subunit (Jin et al. 1995) (Fig. 1).

Negative regulators of PKA were also identified using *fbp1-ura4* reporter-based screens. Mutations in the *cgs1* gene, which encodes the PKA regulatory subunit, were identified among suppressors of an adenylyl cyclase deletion (Stiefel et al. 2004), while mutations in the *cgs2* gene, which encodes the only *S. pombe* PDE, were identified among suppressors of an activation-defective form of adenylyl cyclase (Wang et al. 2005). In both selections, the *cgs1*[−] and *cgs2*[−] mutations restore 5FOA-resistant (5FOA^R) growth by repressing *fbp1-ura4* transcription. Thus this system for studying *fbp1* transcriptional regulation is able to identify

mutations that either reduce PKA activity to stimulate growth in medium lacking uracil or increase PKA activity to stimulate growth in 5FOA medium. The ability to use positive growth selections for mutations that either decrease or increase cAMP levels greatly enhances the robustness of these genetic selections. While this system was initially used to identify mutations that alter PKA activity, we have recently deployed it as a HTS to detect small molecule inhibitors of heterologously-expressed PDEs (Alaamery et al. 2010; Ivey et al. 2008). The screen capitalizes on the conversion of a PDE-expressing strain from 5FOA^S into 5FOA^R in the presence of a PDE inhibitor.

4 First Generation Screens

The cell growth based screen to detect PDE inhibitors was developed by exploiting mutations that reduce glucose detection and cAMP synthesis. Glucose is sensed by the Git3 GPCR, which activates the Gpa2 Gα subunit of the Gpa2-(Gα) Git5-(Gβ) Git11-(Gγ) heterotrimeric G protein. Gpa2-mediated activation of adenylyl cyclase produces a cAMP signal to activate PKA, which represses *fbp1* transcription by multiple mechanisms (Fig. 1). Deletion of any of the *git3*, *gpa2*, *git5*, or *git11* genes confers a 5FOA^S phenotype due to increased expression the *fbp1-ura4* reporter (Landry and Hoffman 2001; Landry et al. 2000; Nocero et al. 1994; Welton and Hoffman 2000). Assays of *fbp1-lacZ* expression in these strains reveal that the relative importance of these proteins in glucose repression is Gpa2 > Git3 > Git5 > Git11. Loss of Cgs2 PDE activity restores 5FOA^R growth and repression of *fbp1-lacZ* expression in all of these mutants, providing the conceptual basis for PDE inhibitor screens.

The key to developing a strain suitable for a PDE inhibitor HTS lies in the interaction between the heterologous PDE and the endogenous cAMP signaling pathway. The PDE must lower the intracellular cAMP level sufficiently to create a 5FOA^S growth phenotype that would be converted to 5FOA^R upon inhibition of the PDE. For highly active PDEs, little or no additional engineering is necessary to attain 5FOA sensitivity; for example, strains expressing either of two isoforms of the highly active human PDE4D enzyme (Conti and Beavo 2007; Houslay et al. 2007) display a leaky 5FOA^S in cells with an intact cAMP signaling pathway and require the loss of only the Git11 Gγ to produce a tight 5FOA^S phenotype. Strains expressing PDE4A or PDE4B enzymes encoded by two other genes of the PDE4 family, or PDE7A or PDE7B high-affinity cAMP-specific enzymes (Bender and Beavo 2006; Conti and Beavo 2007) require more severe disabling of cAMP signaling conferred by the loss of the Gpa2 Gα to produce a tight 5FOA^S phenotype. Loss of Gpa2 is not sufficient to confer 5FOA^S growth in strains expressing the cAMP-specific IBMX-insensitive PDE8A enzyme (Bender and Beavo 2006; Conti and Beavo 2007), suggesting very low activity of this enzyme when expressed in *S. pombe* (in these strains, the full-length mammalian genes are expressed from the yeast PDE gene promoter at its genomic locus). The 5FOA^S strains are suitable for HTSs for PDE inhibitors that elevate cAMP levels and thus repress *fbp1-ura4* transcription to allow 5FOA^R growth (Fig. 2). However, this approach is not amenable for screening strains expressing PDEs such as PDE8A whose activity is insufficient to confer 5FOA^S growth in these mutant backgrounds.

HTSs were optimized using a 384 well format. Assay development involves optimizing growth conditions prior to screening to prevent Ura4 reporter expression (thus PDE inhibitors maintain, rather than establish, repression of the reporter) and initial cell density. A 48-hr incubation period is required for actively growing strains to reach saturation, allowing the greatest contrast in optical density between vehicle-treated cultures ($OD_{600}=0.05-0.2$) and PDE inhibitor-treated or cAMP-treated cultures that reach a saturated cell density ($OD_{600}=1.2-1.3$). Optimized assays generally produce Z-factors of 0.7 to 0.9, indicative of highly robust screens.

HTSs using strains that express mouse PDE4A1 (Cherry et al. 2001), rat PDE4A5 (McPhee et al. 1995), mouse PDE4B3 (Cherry et al. 2001), human PDE7A1 (Michaeli et al. 1993), and *S. pombe* Cgs2 (DeVoti et al. 1991) were carried out at the Broad Institute's screening facility (Alaamery et al. 2010; Ivey et al. 2008). Of ~74,000 compounds screened at ~20 μ M in duplicate using a strain expressing PDE4A1, the PDE4 selective inhibitor rolipram (Wachtel 1982) ranked 34th and the PDE3/4 inhibitor zardaverine (Schudt et al. 1991) ranked 27th by Composite Z score. Ninety-one compounds produced average OD_{600} values of >1.2, which represent cultures grown to near saturation. The ability of these compounds to stimulate this level of growth suggests that they are permeable to the yeast cells, not toxic to yeast, and remain active for most or all of the 48-hr incubation period. Low toxicity serves as a proxy for selectivity, since compounds that bind to many proteins would likely inhibit some essential yeast proteins and prevent cell growth. The relative potencies of the compounds that confer saturated growth cannot be determined from these data alone.

To examine potency as judged by this assay, dose-response curves were carried out to measure the ability of compounds, such as those shown in Fig. 3, to stimulate cell growth at < 20 μ M (the concentration used in the initial screens). As seen in Fig. 4, rolipram increases cell growth of a strain expressing human PDE4B2 (Bolger et al. 1993) to $OD_{600} = 0.6$ (half that of a saturated culture) when present at < 2 μ M, while some structurally-diverse compounds identified in these screens, such as BC35 (identified in the PDE4 screens) and BC54 (identified in PDE4 and PDE7 screens), are effective at significantly lower concentrations. Therefore, this assay is very sensitive with regard to inhibitor detection. *In vitro* enzyme assays confirm the relative potencies of these PDE inhibitors.

5 Second Generation Screens

Second generation screening strains were developed to allow screening for inhibitors of enzymes such as PDE8A (Fisher et al. 1998a) for which the activity is insufficient to confer 5FOA^S growth even in strains lacking the Gpa2 G α . Unlike the budding yeast *S. cerevisiae*, *S. pombe* strains that lack either adenylyl cyclase or PKA are viable. Deletion of the *git2* adenylyl cyclase gene abolishes cAMP synthesis, so that PKA can be regulated by addition of cAMP to the growth medium. The expressed PDE activity reduces cell growth in response to exogenous cAMP (Fig. 2), allowing one to determine the optimal concentration of cAMP to add to the medium for inhibitor detection. For PDE8A-expressing cells, 40 μ M cAMP is optimal for achieving differential growth in 5FOA medium, as this level of cAMP stimulates growth to $OD_{600} > 1.0$ of a strain lacking PDE activity, while having little effect on a PDE8A-expressing strain (Fig. 5). These conditions were used to measure PDE8A

inhibition by 56 PDE4 or PDE7 inhibitors identified from the first generation screens and revealed that the PDE4 inhibitor BC69 (Fig. 3) is an effective inhibitor of PDE8A (Fig. 5). Further work with BC69 allowed the optimization of a PDE8A inhibitor HTS.

6 Third Generation Screens

The screens described above exploit the fact that the expressed PDE can hydrolyze endogenously-produced or exogenously-added cAMP to reduce PKA activity. As such, these screens detect inhibitors of cAMP hydrolysis. We have found that *S. pombe* PKA can also be activated by exogenously-added cGMP, promoting 5FOA^R growth of a strain that lacks both adenylyl cyclase and PDE activity (Fig. 6). Similar to the second-generation screens, cGMP stimulated growth is reduced upon expression of a PDE that hydrolyzes cGMP. This allows for screens to detect inhibitors of cGMP-specific PDEs, while inhibitors of dual specificity PDEs can be screened for using exogenous cAMP or cGMP (Fig. 2).

The ability to use exogenous cGMP for screening was validated using strains expressing cGMP-specific PDE5A (McAllister-Lucas et al. 1993) and PDE9A (Fisher et al. 1998b). As expected for a strain lacking both adenylyl cyclase and PDE activity, a higher concentration of cGMP is required to stimulate growth relative to cAMP. 40μM cGMP produces an OD₆₀₀ = 0.6 as compared to 12.5μM cAMP, while 100μM cGMP results in saturated growth as compared to 25μM cAMP. Expression of either PDE5A or PDE9A completely eliminates the response to cGMP at these, as well as significantly higher, concentrations. As described above for PDE8A, a screen of inhibitors obtained in either PDE4 or PDE7 HTSs led to the identification of compound BC76 (Fig. 3) as an effective PDE5A inhibitor. *In vitro* enzyme assays confirm that BC76 is a PDE5A inhibitor with an IC₅₀ of ~250nM (Sharron Francis, personal communication).

These three iterations of screening strains and methods have allowed us to construct strains that express mammalian PDEs from 10 of the 11 PDE families (with the exception of the PDE6 family of the visual system), all of which display sufficient activity to permit inhibitor screens based on 5FOA^R growth.

7 Profiling PDE Activity in Strains Lacking Adenylyl Cyclase

The expression of PDEs in strains lacking adenylyl cyclase provides a favorable context for observing the properties of PDEs in live cells (although to be sure, *S. pombe* lacks many of the PDE-interacting proteins found in mammalian cells). By comparing the cyclic nucleotide-mediated growth response of a strain that lacks both adenylyl cyclase and PDE activity to that of a PDE-expressing strain, one can assess the relative activity of the PDE against cAMP and cGMP (Fig. 6). We see, for example, that a strain expressing PDE4B requires a final concentration of ~4 mM cAMP in the medium to stimulate cell growth to OD₆₀₀ = 0.6, while the same strain grown in the presence of 40 μM rolipram (Fig. 6a) or a strain lacking PDE activity (Fig. 6b, c) requires < 50μM cAMP in the medium to reach this OD₆₀₀. In contrast, expression of PDE4B shifts the concentration of cGMP needed to stimulate growth by only ~60μM (Fig. 6a), although this may represent enough cGMP hydrolyzing activity by PDE4B to play a role in controlling cGMP levels in certain mammalian cells. A strain expressing the cGMP-specific PDE9A requires >3mM more

cGMP to stimulate growth to $OD_{600} = 0.6$ than does a strain lacking PDE activity, while these strains show identical responses to cAMP (Fig. 6b). A strain expressing the dual cAMP/cGMP hydrolyzing PDE1B enzyme (Repaske et al. 1992) requires 10 μ M more cAMP or 70 μ M more cGMP in the medium to stimulate growth to $OD_{600} = 0.6$ than does a strain lacking PDE activity (Fig. 6c). Low activity of mammalian calcium/calmodulin-activated PDE1B expressed in *S. pombe* could be due to the fact that *S. pombe* calmodulin is only 74% identical to mammalian calmodulin and may be unable to activate PDE1B.

8 Additional Uses of this System

The reciprocal growth behavior of strains expressing the *fbp1-ura4* reporter in medium containing 5FOA (in which high cAMP levels are required for growth) versus medium lacking uracil (in which low cAMP levels are required for growth), allows for screens of PDE activators as well as PDE inhibitors. While such compounds should be detectable by their ability to promote growth of strains with high cAMP levels in medium lacking uracil, two pilot screens failed to identify compounds that activated either PDE7A or PDE8A. This may be due to the fact that PDE activators will be much less common than PDE inhibitors, just as dominant gain-of-function mutations are less common than recessive loss-of-function mutations. The reciprocal growth behavior in medium lacking uracil versus 5FOA medium conferred by the *fbp1-ura4* reporter could also be used to facilitate growth enrichment and genetic screens for strains expressing mutant alleles of PDE genes that encode inhibitor-resistant enzymes. The ability of *S. pombe* to maintain autonomous plasmids allows for the screening of cDNA libraries, cloned in *S. pombe* expression vectors, for either PDE-activating or inhibiting proteins. Stably incorporating genes expressing such proteins into PDE-expressing strains would facilitate genetic studies of PDE regulator activity or small molecule screens similar to those described above. In summary, the introduction of PDEs (which could include those from non-mammalian sources) into *S. pombe* strains allows for a wide variety of classical and chemical genetic screens to enhance our understanding of these enzymes, and to advance the discovery of chemical tools and the development of therapeutic compounds.

9 Conclusions

Three generations of strain development has produced a *S. pombe*-based screening platform that can be used to detect inhibitors of a wide range of full-length PDEs expressed in a eukaryotic cell. The compounds detected in this manner are stable, cell permeable, and of low toxicity to *S. pombe*, characteristics that may make them attractive candidates for drug development. In addition to facilitating new inhibitor discovery and drug development, this system can be used to characterize PDEs and to study PDEs together with biological regulators in a heterologous system that is amenable to detecting subtle changes in PDE activity.

References

- Alaamery MA, Hoffman CS. Schizosaccharomyces pombe Hsp90/Git10 is required for glucose/cAMP signaling. Genetics. 2008; 178:1927–1936. [PubMed: 18430926]

- Alaamery MA, Wyman AR, Ivey FD, Allain C, Demirbas D, Wang L, Ceyhan O, Hoffman CS. New Classes of PDE7 Inhibitors Identified by a Fission Yeast-Based HTS. *J Biomol Screen*. 2010 in press.
- Bender AT, Beavo JA. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol Rev*. 2006; 58:488–520. [PubMed: 16968949]
- Bischoff JR, Casso D, Beach D. Human p53 inhibits growth in *Schizosaccharomyces pombe*. *Mol Cell Biol*. 1992; 12:1405–1411. [PubMed: 1549103]
- Bolger G, Michaeli T, Martins T, St John T, Steiner B, Rodgers L, Riggs M, Wigler M, Ferguson K. A family of human phosphodiesterases homologous to the dunce learning and memory gene product of *Drosophila melanogaster* are potential targets for antidepressant drugs. *Mol Cell Biol*. 1993; 13:6558–6571. [PubMed: 8413254]
- Card GL, Blasdel L, England BP, Zhang C, Suzuki Y, Gillette S, Fong D, Ibrahim PN, Artis DR, Bollag G, Milburn MV, Kim SH, Schlessinger J, Zhang KY. A family of phosphodiesterase inhibitors discovered by co-crystallography and scaffold-based drug design. *Nat Biotechnol*. 2005; 23:201–207. [PubMed: 15685167]
- Card GL, England BP, Suzuki Y, Fong D, Powell B, Lee B, Luu C, Tabrizizad M, Gillette S, Ibrahim PN, Artis DR, Bollag G, Milburn MV, Kim SH, Schlessinger J, Zhang KY. Structural basis for the activity of drugs that inhibit phosphodiesterases. *Structure*. 2004; 12:2233–2247. [PubMed: 15576036]
- Cherry JA, Thompson BE, Pho V. Diazepam and rolipram differentially inhibit cyclic AMP-specific phosphodiesterases PDE4A1 and PDE4B3 in the mouse. *Biochim Biophys Acta*. 2001; 1518:27–35. [PubMed: 11267656]
- Colicelli J, Birchmeier C, Michaeli T, O'Neill K, Riggs M, Wigler M. Isolation and characterization of a mammalian gene encoding a high-affinity cAMP phosphodiesterase. *Proc Natl Acad Sci U S A*. 1989; 86:3599–3603. [PubMed: 2542941]
- Colicelli J, Nicolette C, Birchmeier C, Rodgers L, Riggs M, Wigler M. Expression of three mammalian cDNAs that interfere with RAS function in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A*. 1991; 88:2913–2917. [PubMed: 1849280]
- Conti M, Beavo J. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. *Annu Rev Biochem*. 2007; 76:481–511. [PubMed: 17376027]
- D'Souza CA, Heitman J. Conserved cAMP signaling cascades regulate fungal development and virulence. *FEMS Microbiol Rev*. 2001; 25:349–364. [PubMed: 11348689]
- DeVoti J, Seydoux G, Beach D, McLeod M. Interaction between ran1⁺ protein kinase and cAMP dependent protein kinase as negative regulators of fission yeast meiosis. *Embo J*. 1991; 10:3759–3768. [PubMed: 1657594]
- Engels P, Sullivan M, Muller T, Lubbert H. Molecular cloning and functional expression in yeast of a human cAMP-specific phosphodiesterase subtype (PDE IV-C). *FEBS Lett*. 1995; 358:305–310. [PubMed: 7843419]
- Fisher DA, Smith JF, Pillar JS, St Denis SH, Cheng JB. Isolation and characterization of PDE8A, a novel human cAMP-specific phosphodiesterase. *Biochem Biophys Res Commun*. 1998a; 246:570–577. [PubMed: 9618252]
- Fisher DA, Smith JF, Pillar JS, St Denis SH, Cheng JB. Isolation and characterization of PDE9A, a novel human cGMP-specific phosphodiesterase. *J Biol Chem*. 1998b; 273:15559–15564. [PubMed: 9624146]
- Friedmann Y, Shriki A, Bennett ER, Golos S, Diskin R, Marbach I, Bengal E, Engelberg D. JX401, A p38alpha inhibitor containing a 4-benzylpiperidine motif, identified via a novel screening system in yeast. *Mol Pharmacol*. 2006; 70:1395–1405. [PubMed: 16847144]
- Grozinger CM, Chao ED, Blackwell HE, Moazed D, Schreiber SL. Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotypic screening. *J Biol Chem*. 2001; 276:38837–38843. [PubMed: 11483616]
- Hoffman CS. Except in every detail: Comparing and contrasting G protein signaling in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Eukaryotic Cell*. 2005a; 4:495–503. [PubMed: 15755912]

- Hoffman CS. Glucose sensing via the protein kinase A pathway in *Schizosaccharomyces pombe*. *Biochem Soc Trans*. 2005b; 33:257–260. [PubMed: 15667320]
- Hoffman CS, Winston F. Isolation and characterization of mutants constitutive for expression of the *fbp1* gene of *Schizosaccharomyces pombe*. *Genetics*. 1990; 124:807–816. [PubMed: 2157626]
- Hoffman CS, Winston F. Glucose repression of transcription of the *Schizosaccharomyces pombe fbp1* gene occurs by a cAMP signaling pathway. *Genes Dev*. 1991; 5:561–571. [PubMed: 1849107]
- Houslay MD. Underpinning compartmentalised cAMP signalling through targeted cAMP breakdown. *Trends Biochem Sci*. 2010; 35:91–100. [PubMed: 19864144]
- Houslay MD, Baillie GS, Maurice DH. cAMP-Specific phosphodiesterase-4 enzymes in the cardiovascular system: a molecular toolbox for generating compartmentalized cAMP signaling. *Circ Res*. 2007; 100:950–966. [PubMed: 17431197]
- Ivey FD, Wang L, Demirbas D, Allain C, Hoffman CS. Development of a fission yeast-based high-throughput screen to identify chemical regulators of cAMP phosphodiesterases. *J Biomol Screen*. 2008; 13:62–71. [PubMed: 18227226]
- Jin M, Fujita M, Culley BM, Apolinario E, Yamamoto M, Maundrell K, Hoffman CS. *sck1*, a high copy number suppressor of defects in the cAMP-dependent protein kinase pathway in fission yeast, encodes a protein homologous to the *Saccharomyces cerevisiae* SCH9 kinase. *Genetics*. 1995; 140:457–467. [PubMed: 7498728]
- Kao RS, Morreale E, Wang L, Ivey FD, Hoffman CS. *Schizosaccharomyces pombe* Git1 is a C2-domain protein required for glucose activation of adenylate cyclase. *Genetics*. 2006; 173:49–61. [PubMed: 16489217]
- Landry S, Hoffman CS. The *git5* G β and *git11* G γ Form an Atypical G $\beta\gamma$ Dimer Acting in the Fission Yeast Glucose/cAMP Pathway. *Genetics*. 2001; 157:1159–1168. [PubMed: 11238401]
- Landry S, Pettit MT, Apolinario E, Hoffman CS. The fission yeast *git5* gene encodes a G β subunit required for glucose-triggered adenylate cyclase activation. *Genetics*. 2000; 154:1463–1471. [PubMed: 10747045]
- Lee MG, Nurse P. Complementation used to clone a human homologue of the fission yeast cell cycle control gene *cdc2*. *Nature*. 1987; 327:31–35. [PubMed: 3553962]
- Lengeler KB, Davidson RC, D'Souza C, Harashima T, Shen WC, Wang P, Pan X, Waugh M, Heitman J. Signal transduction cascades regulating fungal development and virulence. *Microbiol Mol Biol Rev*. 2000; 64:746–785. [PubMed: 11104818]
- Lerner A, Epstein PM. Cyclic nucleotide phosphodiesterases as targets for treatment of haematological malignancies. *Biochem J*. 2006; 393:21–41. [PubMed: 16336197]
- Ma P, Wera S, Van Dijk P, Thevelein JM. The PDE1-encoded low-affinity phosphodiesterase in the yeast *Saccharomyces cerevisiae* has a specific function in controlling agonist-induced cAMP signaling. *Mol Biol Cell*. 1999; 10:91–104. [PubMed: 9880329]
- McAllister-Lucas LM, Sonnenburg WK, Kadleck A, Seger D, Trong HL, Colbran JL, Thomas MK, Walsh KA, Francis SH, Corbin JD, et al. The structure of a bovine lung cGMP-binding, cGMP-specific phosphodiesterase deduced from a cDNA clone. *J Biol Chem*. 1993; 268:22863–22873. [PubMed: 8226796]
- McHale MM, Cieslinski LB, Eng WK, Johnson RK, Torphy TJ, Livi GP. Expression of human recombinant cAMP phosphodiesterase isozyme IV reverses growth arrest phenotypes in phosphodiesterase-deficient yeast. *Mol Pharmacol*. 1991; 39:109–113. [PubMed: 1847489]
- McPhee I, Pooley L, Lobban M, Bolger G, Houslay MD. Identification, characterization and regional distribution in brain of RPDE-6 (RNPDE4A5), a novel splice variant of the PDE4A cyclic AMP phosphodiesterase family. *Biochem J*. 1995; 310(Pt 3):965–974. [PubMed: 7575434]
- Michaeli T, Bloom TJ, Martins T, Loughney K, Ferguson K, Riggs M, Rodgers L, Beavo JA, Wigler M. Isolation and characterization of a previously undetected human cAMP phosphodiesterase by complementation of cAMP phosphodiesterase-deficient *Saccharomyces cerevisiae*. *J Biol Chem*. 1993; 268:12925–12932. [PubMed: 8389765]
- Nikawa J, Cameron S, Toda T, Ferguson KM, Wigler M. Rigorous feedback control of cAMP levels in *Saccharomyces cerevisiae*. *Genes Dev*. 1987a; 1:931–937. [PubMed: 2828175]

- Nikawa J, Sass P, Wigler M. Cloning and characterization of the low-affinity cyclic AMP phosphodiesterase gene of *Saccharomyces cerevisiae*. *Mol Cell Biol*. 1987b; 7:3629–3636. [PubMed: 2824992]
- Nocero M, Isshiki T, Yamamoto M, Hoffman CS. Glucose repression of *fbp1* transcription of *Schizosaccharomyces pombe* is partially regulated by adenylate cyclase activation by a G protein α subunit encoded by *gpa2* (*git8*). *Genetics*. 1994; 138:39–45. [PubMed: 8001792]
- Pillai R, Kytle K, Reyes A, Colicelli J. Use of a yeast expression system for the isolation and analysis of drug-resistant mutants of a mammalian phosphodiesterase. *Proc Natl Acad Sci U S A*. 1993; 90:11970–11974. [PubMed: 7505450]
- Pillai R, Staub SF, Colicelli J. Mutational mapping of kinetic and pharmacological properties of a human cardiac cAMP phosphodiesterase. *J Biol Chem*. 1994; 269:30676–30681. [PubMed: 7982987]
- Repaske DR, Swinnen JV, Jin SL, Van Wyk JJ, Conti M. A polymerase chain reaction strategy to identify and clone cyclic nucleotide phosphodiesterase cDNAs. Molecular cloning of the cDNA encoding the 63-kDa calmodulin-dependent phosphodiesterase. *J Biol Chem*. 1992; 267:18683–18688. [PubMed: 1326532]
- Sass P, Field J, Nikawa J, Toda T, Wigler M. Cloning and characterization of the high-affinity cAMP phosphodiesterase of *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A*. 1986; 83:9303–9307. [PubMed: 3025832]
- Schadick K, Fourcade HM, Boumenot P, Seitz JJ, Morrell JL, Chang L, Gould KL, Partridge JF, Allshire RC, Kitagawa K, Hieter P, Hoffman CS. *Schizosaccharomyces pombe* Git7p, a member of the *Saccharomyces cerevisiae* Sgt1p family, is required for glucose and cyclic AMP signaling, cell wall integrity, and septation. *Eukaryot Cell*. 2002; 1:558–567. [PubMed: 12456004]
- Schudt C, Winder S, Eltze M, Kilian U, Beume R. Zardaverine: a cyclic AMP specific PDE III/IV inhibitor. *Agents Actions*. 1991; (Suppl 34):379–402. [PubMed: 1665311]
- Soderling SH, Beavo JA. Regulation of cAMP and cGMP signaling: new phosphodiesterases and new functions. *Curr Opin Cell Biol*. 2000; 12:174–179. [PubMed: 10712916]
- Stiefel J, Wang L, Kelly DA, Janoo RTK, Seitz J, Whitehall SK, Hoffman CS. Suppressors of an adenylate cyclase deletion in the fission yeast *Schizosaccharomyces pombe*. *Eukaryotic Cell*. 2004; 3:610–619. [PubMed: 15189983]
- Thevelein JM, Cauwenberg L, Colombo S, De Winde JH, Donation M, Dumortier F, Kraakman L, Lemaire K, Ma P, Nauwelaers D, Rolland F, Teunissen A, Van Dijck P, Versele M, Wera S, Winderickx J. Nutrient-induced signal transduction through the protein kinase A pathway and its role in the control of metabolism, stress resistance, and growth in yeast. *Enzyme Microb Technol*. 2000; 26:819–825. [PubMed: 10862891]
- Thevelein JM, de Winde JH. Novel sensing mechanisms and targets for the cAMP-protein kinase A pathway in the yeast *Saccharomyces cerevisiae*. *Mol Microbiol*. 1999; 33:904–918. [PubMed: 10476026]
- Torphy TJ, Stadel JM, Burman M, Cieslinski LB, McLaughlin MM, White JR, Livi GP. Coexpression of human cAMP-specific phosphodiesterase activity and high affinity rolipram binding in yeast. *J Biol Chem*. 1992; 267:1798–1804. [PubMed: 1309798]
- Wachtel H. Characteristic behavioural alterations in rats induced by rolipram and other selective adenosine cyclic 3'-5'-monophosphate phosphodiesterase inhibitors. *Psychopharmacology (Berl)*. 1982; 77:309–316. [PubMed: 6182575]
- Wang L, Griffiths K, Zhang YH, Ivey FD, Hoffman CS. *Schizosaccharomyces pombe* adenylate cyclase suppressor mutations suggest a role for cAMP phosphodiesterase regulation in feedback control of glucose/ cAMP signaling. *Genetics*. 2005; 171:1523–1533. [PubMed: 16143612]
- Welton RM, Hoffman CS. Glucose monitoring in fission yeast via the *gpa2* G α , the *git5* G β , the *git3* putative glucose receptor. *Genetics*. 2000; 156:513–521. [PubMed: 11014802]
- Zaks-Makhina E, Kim Y, Aizenman E, Levitan ES. Novel neuroprotective K⁺ channel inhibitor identified by high-throughput screening in yeast. *Mol Pharmacol*. 2004; 65:214–219. [PubMed: 14722253]

Fig. 1.
The *S. pombe* glucose/cAMP signaling pathway presented using the ProteinLounge Pathway Builder Tool.

Fig. 2.

First, second and third generation 5FOA-growth screens. a) 5FOA^S growth in a first generation screening strain is due to a defective glucose-sensing Git pathway. b) 5FOA^S growth in a second or third generation screening strain is due to PDE degradation of exogenously added cAMP or cGMP. c, d) PDE inhibition elevates intracellular cAMP or cGMP levels to repress *fbp1-ura4* transcription and confer 5FOA^R growth.

Fig. 3.
Structures of rolipram and four PDE inhibitors identified in HTSs using strains expressing PDE4 or PDE7 enzymes.

Fig. 4. Effect of rolipram, BC35, and BC54 (structures shown in Fig. 3) on 5FOA^R growth of a PDE4B2 expressing strain. OD₆₀₀ values were measured in the presence of various concentrations of compounds in 384 well microtiter dishes after 48-hr growth.

Fig. 5. 5FOA^R growth conferred by exogenous cAMP on strains lacking adenylyl cyclase. OD₆₀₀ values were determined for strains lacking adenylyl cyclase and either lacking PDE activity or expressing PDE8A. The X-axis indicates the concentration of cAMP (μM) added to the 5FOA medium. At 40μM cAMP (vertical dashed line), the OD₆₀₀ for the strain lacking PDE activity is five-fold higher than for the strain expressing PDE8A. Addition of 20μM BC69 (Fig. 3; identified in a PDE4 inhibitor screen) shifts the cAMP response curve of the PDE8A-expressing strain toward that of the strain lacking PDE activity.

Fig. 6.
cAMP/cGMP profiles for PDE4B, PDE9A and PDE1B. See text for details.