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Using yeast to screen for inhibitors of protein tyrosine phosphatase 1B

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Abstract

Inhibition of protein tyrosine phosphatase 1B (PTP1B) has been proposed as a novel therapy to treat type 2 diabetes and obesity. In order to identify novel PTP1B inhibitors, we have developed a robust screen in *Saccharomyces cerevisiae* where growth is dependent on PTP1B catalytic activity. This was based on the observation that overexpression of v-Src, a tyrosine kinase, in yeast leads to lethality through mitotic dysfunction and this lethality can be reversed by co-expression of PTP1B. The expression levels of v-Src and PTP1B were optimized to obtain a balance between robust growth and sensitivity to inhibitors. Screening was carried out in 96-well plates and growth of the liquid culture measured by absorbance at 600 nm. Initial characterization was performed using vanadate as well as some novel PTP1B inhibitors. Vanadate specifically inhibited PTP1B-dependent growth in a dose dependent manner with an EC_{50} of 0.92 ± 0.07 mM. This simple yeast growth interference assay has the potential for use as a high throughput screen for PTP1B inhibitors in sample collections or crude mixtures.

Keywords: Protein tyrosine phosphatase; PTP1B; v-Src; Biological assay; Enzyme inhibitors; *Saccharomyces cerevisiae*

Protein tyrosine phosphatase 1B (PTP1B) (EC 3.1.3.48) is a negative regulator of insulin action and has become an important diabetes and obesity drug target [1–3]. Results from PTP1B mouse gene knockouts [4,5] and antisense oligonucleotide treatment in diabetic rodent models [6–8] have validated PTP1B as having a role in the development of insulin resistance and obesity. The identification of potent and selective PTP1B inhibitors has become a major focus of many drug development programs. However, there are a number of issues in establishing both intrinsic as well as cell-based screens for PTP1B inhibitors. One of these is oxidation of the active site cysteine by oxidizing agents in sample collections that can lead to false positives. Another is the lack of a robust cell-based assay for evaluating the biological activity of PTP1B inhibitors. The typical functional readout for cellular PTP1B inhibition has been to measure IR phosphorylation [9,10]. Yet, the increase in IR phosphorylation due to PTP1B inhibition is usually variable, dependent on cell type and at most two to three-fold over controls, thus, making it unsuitable for screening. Furthermore, because many of the current potent PTP1B inhibitors contain a phosphonate or a phosphate mimetic they are highly charged and therefore cell permeability becomes an issue. Without a robust cellular functional readout and questionable cell permeability of the potent PTP1B inhibitors, it becomes difficult to reconcile the reason for the lack of activity of PTP1B inhibitors in cell-based assays. In order to circumvent some of these issues, a yeast-based PTP1B assay was developed. Yeast has been used as a model system for the screening of various pharmacological agents, it provides a protective

Abbreviations: PTP1B, protein tyrosine phosphatase 1B; IR, insulin receptor; ATCC, American type tissue collection; DiFMUP, difluoromethylumbelliferyl phosphate; BzN-EJJ-amide, *N*-benzoyl-L-glutamyl-[4-phosphono(difluoromethyl)]-L-phenylalanyl-[4-phosphono(difluoromethyl)]-L-phenylalanineamide

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cellular environment to prevent PTP1B oxidation and measuring yeast growth is both an easy and sensitive screening assay [11]. In addition, yeast cell permeability has been shown not to be an issue in concentrating compounds internally, but like mammalian cells, the rate limiting factor in internalization of compounds has been due to the function of efflux pumps [11].

v-Src, a tyrosine kinase from the Rous Sarcoma virus, is lethal when overexpressed in the yeast *Saccharomyces cerevisiae* [12]. The lethality of v-Src has been attributed to the hypertyrosine phosphorylation of multiple yeast proteins leading to mitotic dysfunction but the exact target responsible for the toxicity remains to be identified [13–17]. Yeast can be rescued from the v-Src lethality by co-expressing the catalytic domain of PTP1B [16]. This rescue is presumably due to the activity of PTP1B in dephosphorylating the various yeast proteins phosphorylated by v-Src. Using these initial observations, we have developed a yeast cell-based assay to screen for PTP1B inhibitors. In this report we describe the optimization and the characterization of this screen with both known as well as some novel PTP1B inhibitors.

1. Materials and methods

1.1. Yeast strain

YPH499: *Mat***a** ura 3-52 lys 2-801^{amber} ade 2-101^{ocher} trp 1-Δ63 his3-Δ200 leu2Δ1 (Stratagene).

1.2. Plasmids constructs

EcoRI and SalI were used to cut out the catalytic domain (amino acids 1-320) of human PTP1B previously cloned in a pFLAG vector [18]. The resulting fragment was gel purified and ligated to p416GAL1 (American type culture collection (ATCC) 87332) previously digested with EcoRI and SalI and de-phosphorylated. The plasmid p416GAL1-PTP1B was sequenced to confirm the correct construction. PTP1B mutants C215S (amino acids 1-320) and D181A (amino acids 1-298) were cloned into p416GAL1 in a similar manner. The plasmid p416GAL1 is a low-copy vector containing the URA3 marker for growth in uracil-deficient media.

v-Src was amplified by PCR using the pEcoRIB clone obtained from ATCC (41005) as a template. The forward primer used was: CG*TCTAGA*ATGGGGAGTAGCAAGAGCAAGCC, the reverse primer used was: *GTCGAC*CTACTCAGCGACCTCCAACACACAAGC and the primers also contained sequences for the introduction of XbaI and SalI restriction sites (italicized) into the PCR product. The amplified DNA was inserted into pCR2.1-TOPO (Invitrogen) to give the TA-v-Src plasmid. The fragment obtained from restriction digest of TA-v-Src with XbaI and SalI was gel purified (Qiagen) and ligated to p415GAL1 (ATCC 87326), p415GALL (ATCC 87338) or p415GALS (ATCC 87346) [19] previously digested with XbaI and SalI and de-phosphorylated. The resulting constructs were then sequenced. All three plasmids are low-copy vectors containing the LEU2 marker for growth in leucine-deficient media.

1.3. Growth curves

Yeast were transformed [20] with the various plasmids and the colonies formed 3 days after were transferred to leucine and uracil dropout media containing raffinose as the sole carbon source and grown overnight. Serial dilutions of the inhibitors were arrayed in 96-well plates containing leucine and uracil dropout media with 4% galactose. The plates were inoculated with the overnight yeast cultures at a final concentration of 10^6 cells per ml [21]. The wells were overlaid with mineral oil, incubated at 30 °C and read periodically at 600 nm for 4 days. The growth curves generated were used to obtain EC_{50} values for the various PTP1B inhibitors.

1.4. Western blots

Total yeast protein was extracted using Y-PER™ (Pierce) and quantified using Coomassie® Plus Reagent (Pierce). The extract was run (15 μg per lane) on 4–12% NuPage® BisTris gel (Invitrogen) in 3-(*N*-morpholino) propanesulfonic acid (MOPS) buffer and transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with 5% BSA in TTBS (0.1% Tween 20, 10 mM Tris–HCl pH 7.5, 100 mM NaCl), probed with an anti-phosphotyrosine antibody (4G10 from Upstate) in 1% BSA in TTBS, washed and developed with chemiluminescence (NEN Life Sciences). The blot was visualized using a CCD camera (Fujifilm).

1.5. Inhibitors and IC_{50} determinations

Inhibitors 1–3 and BzN-EJJ-amide [22] were synthesized at Merck Frosst Canada. Details of the synthesis of the novel PTP1B inhibitors used in this assay can be found in [23]. Inhibitor 1: 7-bromo-6-phosphono(difluoromethyl)-3-napthalenonitrile, inhibitor 2: 3-[*p*-(chloro)phenyl]-1-{*m*-bromo-*p*-[phosphono(difluoromethyl)]phenyl}-2-thiapropane, inhibitor 3: 2,3-biphenyl-1-{*m*-bromo-*p*-[phosphono(difluoromethyl)]phenyl}propan-3-one, BzN-EJJ-amide: *N*-benzoyl-L-glutamyl-[4-phosphono(difluoromethyl)]-L-phenylalanyl-[4-phosphono(difluoromethyl)]-L-phenylalanineamide.

Hydrolysis of 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) (Molecular Probes) by PTP1B was monitored by fluorescence (excitation 360 nm, emission 450 nm) continuously for 10 min using a SpectraMaxGemini from Molecular Devices. IC_{50} values were calculated

from non-linear regression fit of initial rates of hydrolysis obtained by reacting 0.14 nM of recombinant PTP1B (expressed and purified as in [24]) with 5.5 μM of DiFMUP (K_M of PTP1B) in assay buffer (2% glycerol, 0.01% triton, 5 mM *N*,*N*′-dimethyl-bis(mercaptoacetyl)hydrazine, 50 mM Bis–Tris, 2 mM EDTA pH 6.3) [24] in the presence of varying concentrations of inhibitor.

2. Results

2.1. Optimization of the rescue of yeast from v-Src lethality by PTP1B

It was previously shown that expression of v-Src in yeast results in the arrest of cell growth and this cell arrest can be rescued by the co-expression of PTP1B [16]. To reproduce these results and to establish the optimal balance between this lethality and the rescue mediated by PTP1B, the expression levels of v-Src in yeast were varied by using mutated GAL promoters [19]. In fact, v-Src expression driven by any of the GAL promoters, GAL1 (wild type) or the attenuated promoters GALL or GALS (missing upstream activation sequences) abolished yeast growth to the same extent (Fig. 1A). However, co-expression of PTP1B with v-Src resulted in the rescue of yeast growth only if v-Src expression was under the control of the GALL promoter (Fig. 1A). This suggests that the correct stoichiometry must be achieved between v-Src and PTP1B for rescue to occur. This was evident when the level of yeast protein tyrosine phosphorylation was examined by Western blotting (Fig. 1B). v-Src driven by either the GAL1 or GALS promoters resulted in a higher level of protein tyrosine phosphorylation than v-Src under the GALL promoter. Co-transformation with PTP1B effectively reduced the total tyrosine phosphorylation level for all the permutations of the GAL promoters driving v-Src yet could only rescue yeast from v-Src driven by the GALL promoter. Expression of PTP1B alone did not affect the yeast growth or the phosphorylation pattern compared to the wild type yeast.

Catalytically inactive PTP1B mutants were co-transformed with v-Src to verify that the effect of PTP1B on v-Src lethality was due to its enzymatic activity. Two mutants were assayed: C215S [25] where the active site cysteine was mutated to a serine and D181A [26] where the general acid/base involved in the phosphatase enzymatic reaction was replaced by an alanine. The results in Fig. 2A show that only wild type PTP1B can rescue yeast from v-Src lethality whereas the C215S or D181A mutants were ineffective. Furthermore, Western blots of total phosphotyrosine (Fig. 2B) revealed that only the catalytically active PTP1B and not the mutants attenuated the increase in tyrosine phosphorylation due to v-Src expression. These results indicate that the catalytic activity of PTP1B was required to rescue yeast

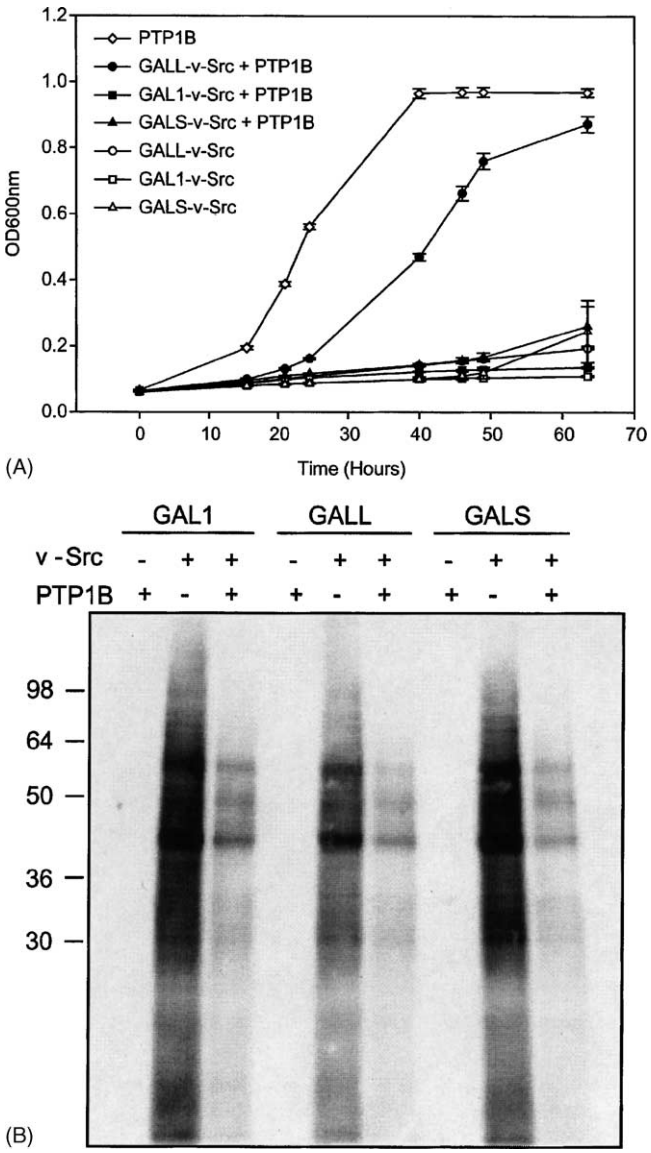

Fig. 1. The effect of PTP1B expression on the growth of yeast expressing v-Src under various GAL promoters. (A) Growth curves of yeast transformed with PTP1B (◇), GAL1-v-Src (□), GALS-v-Src (△) or GALL-v-Src (○) or co-transformed with GAL1-v-Src (■), GALS-v-Src (▲) or GALL-v-Src (●) and PTP1B. Error bars represent standard error (n = 3). (B) Western blots of total protein extracted from yeast transformed as in (A) induced in galactose for 63 h and probed with anti-phosphotyrosine antibody. GAL1, GALL and GALS refer to the promoter driving v-Src or the identity of the empty vector co-transformed with PTP1B.

from v-Src lethality and that co-transforming yeast with p415GALL-v-Src and p416GAL1-PTP1B yielded a yeast system that was strictly dependent on PTP1B activity for growth.

2.2. Screening compounds for PTP1B inhibition using a yeast-based system

Yeast were co-transformed with p415GALL-v-Src and p416GAL1-PTP1B and the colonies formed after 3 days of growth on double-dropout media were used to inoculate

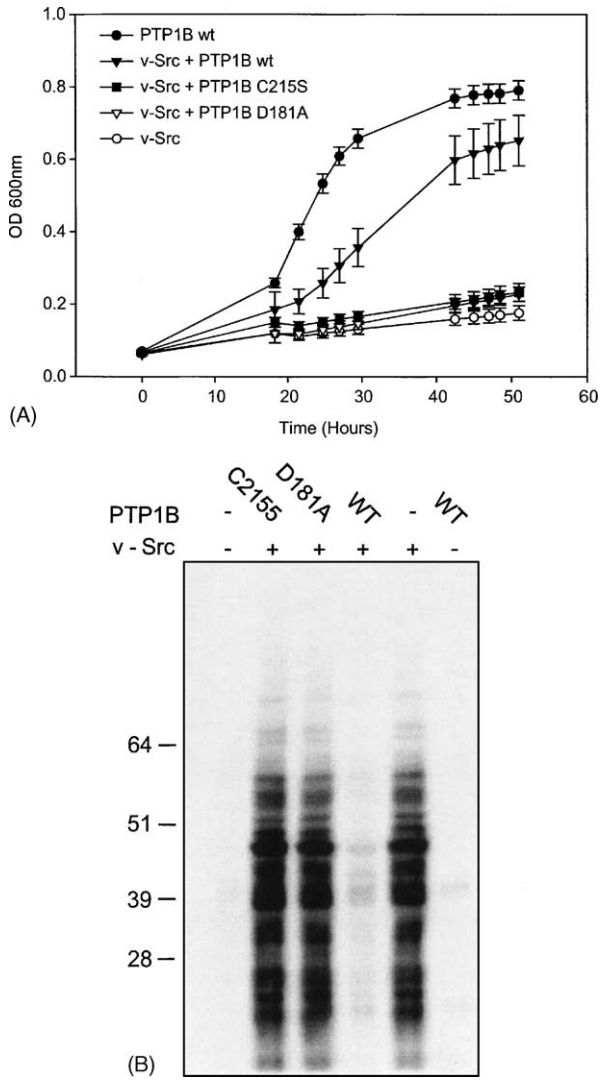

Fig. 2. The catalytic activity of PTP1B is required to rescue the yeast from v-Src lethality. (A) Growth curve of yeast transformed with PTP1B (●), v-Src (○) or co-transformed with v-Src and PTP1B (wild type (▼) or mutants (■or ▽)). Wild type is denoted wt, catalytically inactive PTP1B mutants are C215S and D181A. Error bars represent standard error (n = 3). (B) Western blot of total yeast protein extracted from yeast transformed as in (A) induced in galactose for 51 h and probed with anti-phosphotyrosine antibody.

liquid media for an overnight culture. The resulting PTP1B/v-Src screening culture was then diluted and aliquoted to a 96-well plate containing a serial dilution of known or novel inhibitors of PTP1B. The growth of the yeast was monitored by following the increase in absorbance at 600 nm over time. In addition, controls were included to identify compounds that were toxic to *S. cerevisiae* or compounds that could affect the lethality of v-Src. The controls were assayed as above but using yeast co-transformed with p415GALL and p416GAL1-PTP1B to check for toxicity or co-transformed with p415GALL-v-Src and p416GAL1 to check for an effect on v-Src lethality. Any compound that affected the growth of these controls would be rejected from the screen.

Initial characterization of the screen was performed with vanadate which is a general protein tyrosine phosphatase inhibitor [27]. Vanadate has an IC_{50} of $\sim$ 0.4 μM, when assayed using recombinant purified PTP1B in activity or binding assays [28]. The growth curves generated in the presence of vanadate are shown in Fig. 3A. Vanadate was not toxic to *S. cerevisiae* at the concentrations used since there was little effect on the growth of the PTP1B control yeast (Fig. 3A panel 1) nor did it affect the lethality of v-Src as shown by the absence of response for the v-Src control (Fig. 3A panel 3). However, with the addition of increasing concentrations of vanadate to the media, yeast dependent on PTP1B for growth displayed a dose-dependent decrease in growth (Fig. 3A panel 2).

We next tested various published tyrosine phosphatase inhibitors including the reversible active site inhibitors: BzN-EJJ-amide [22] and suramin [29] and the oxidizing agent phenylarsine oxide [30] (IC_{50} reported in [28] were 6 nM, 5 μM and 2 μM respectively). The potent peptide inhibitor, BzN-EJJ-amide, even up to 1 mM, did not inhibit yeast growth in the assay, nor was it toxic to yeast at this concentration (Fig. 3B). Suramin, appeared to have some minimal activity at 2 mM (Fig. 3C panel 2), but when assayed up to 12 mM, toxicity was observed at concentrations above 2 mM.[1] However, phenylarsine oxide, which inhibits PTP1B by oxidization, was toxic to yeast growth at concentrations as low as 10 μM (Fig. 3D).

In order to evaluate the assay with additional PTP1B inhibitors we tested a number of novel in-house synthesized PTP1B inhibitors in the assay (Fig. 4). These were non-peptidic PTP1B inhibitors but all contained the non-hydrolysable phosphotyrosyl mimetic 4′-phosphono(difluoromethyl)-phenylalanine (F_2PMP) [23]. Many of these compounds, although potent inhibitors like BzN-EJJ-amide, were also ineffective at inhibiting yeast growth in the assay. Nevertheless, a subgroup of these inhibitors did have significant effects on yeast growth. For example, PTP1B inhibitor 1 (IC_{50} 0.2 μM) inhibited yeast growth in a dose dependent manner and was not toxic to the cell even up to 2 mM concentration (Fig. 3E).

Using the data in Fig. 3, dose response curves were generated for vanadate, inhibitor 1 as well as for two other novel PTP1B inhibitors 2 and 3. Vanadate had an EC_{50} of 0.92 ± 0.07 mM for the inhibition of yeast growth whereas PTP1B inhibitor 1 had an EC_{50} of 0.17 ± 0.03 mM. The other two PTP1B inhibitors, 2 and 3, were about as effective as vanadate in inhibiting the growth with EC_{50}s of 1.2 ± 0.2 mM and 0.9 ± 0.1 mM respectively. These two inhibitors had essentially the same IC_{50} value, 0.3 μM, for the inhibition of the recombinant enzyme. It is of interest to note that vanadate and the three novel PTP1B inhibitors reported here all had similar IC_{50}s on purified PTP1B but inhibitor 1 was about five times more potent at inhibiting PTP1B-dependent yeast growth.

[1] Data not shown.

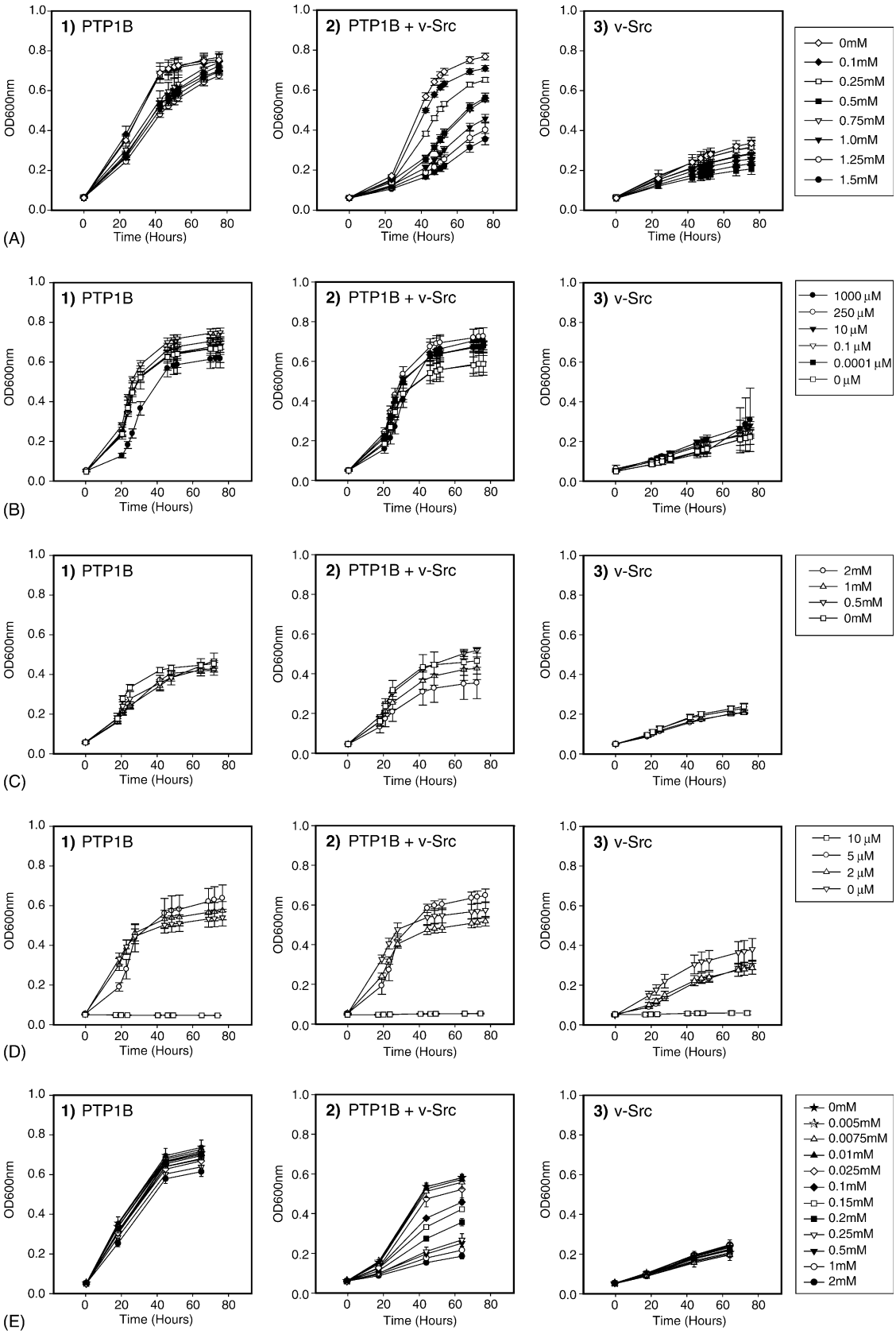

Fig. 3. Effect of PTP1B inhibitors on yeast growth. (A) Vanadate, (B) BzN-EJJ-amide, (C) Suramin, (D) Phenylarsine oxide and (E) Inhibitor 1. Panel 1. Toxicity control using yeast expressing PTP1B alone. Panel 2. Dose-dependent decrease in PTP1B-dependent growth by the inhibitor using yeast co-transformed with PTP1B and v-Src. Panel 3. Background control of the effect of the inhibitor on the lethality of v-Src using yeast expressing v-Src alone. The concentration of inhibitor used is shown boxed on the right. Error bars represents standard error (n = 3).

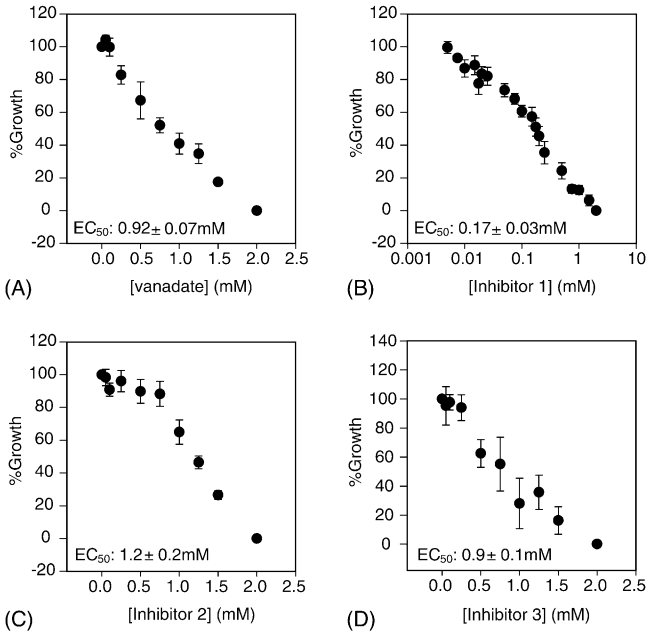

Fig. 4. Dose response profiles of selected PTP1B inhibitors. Yeast expressing PTP1B and v-Src were grown in the presence of increasing concentrations of the following PTP1B inhibitors: (A) vanadate, (B) inhibitor 1, (C) inhibitor 2 and (D) inhibitor 3. Growth curves were determined and the amount of growth at 68 h was used to generate the dose response curves. %Growth refers to growth at 68 h where 100% growth refers to growth in the absence of inhibitor. The data represent at least three independent experiments done in triplicate where error bars are standard error.

3. Discussion

A major issue with PTP1B drug screening programs is the lack of a robust cell-based functional assay. Typically, increased tyrosine phosphorylation of the IR or glucose uptake has been used as functional readouts for PTP1B inhibition. However, the effect on IR phosphorylation due to either PTP1B mRNA knockdown or inhibition, as measured by quantitation of Western blots, results in at most a two to three-fold increase in phosphorylation over unstimulated controls [9,10]. This small window and the variability introduced by quantitation of blots make it incompatible with high throughput screening. In addition, although glucose uptake can be a surrogate for IR activation, this event occurs much further downstream of IR activation and can be stimulated by several mechanisms thus results can be misleading since it is not a direct indicator of PTP1B inhibition. We wanted to develop a PTP1B cell-based assay where a biological function (growth) was completely dependent on PTP1B activity. Yeast is a model system to develop such an assay, since it provides the cellular environment (reducing) to maintain PTP1B in an active state, there is minimum compensation by yeast tyrosine phosphatases and growth interference is easily scoreable [8].

Based on the previous observation that co-expression of PTP1B can rescue yeast from v-Src lethality, we optimized the expression levels of PTP1B and v-Src such that inhibition of PTP1B activity results in growth interference. PTP1B catalytic activity was absolutely required for yeast growth since the catalytically inactive PTP1B mutants C215S and D181A failed to overcome the lethality of v-Src. There was a certain stoichiometry between PTP1B and v-Src expression that was required to obtain a robust growth phenotype and at the same time be sensitive to inhibitors. For example, it has been shown that expression from a high copy vector can result in inhibitor resistant phenotypes due to high levels of protein expression [31]. Previous reports [16,17] had utilized the GAL1 promoter to express PTP1B from high copy vectors. Therefore it was necessary to reduce the expression levels of PTP1B from the high copy vectors to a low-copy vector. However, diminishing the levels of PTP1B compromises its ability to rescue the yeast; thus v-Src was also expressed from a low-copy vector and expression was further decreased with the use of the attenuated GALL promoter. This combination yielded the greatest rescue while being sensitive to PTP1B inhibitors.

We had previously developed an insect cell assay to screen for PTP inhibitors using recombinant baculoviruses [32]. Although this assay was sufficient at evaluating compounds as PTP inhibitors it did not provide any indication of how efficient these compounds were at inhibiting cellular PTP1B biological activities since it monitored the conversion of the exogenously added substrate ρ-nitrophenylphosphate. In contrast, the PTP1B yeast cell-based assay is dependent on PTP1B catalytic activity for growth. For example, the oxidizing agent phenylarsine oxide, in the insect cell assay was effective at inhibiting PTP1B (IC_{50}16 μM), whereas in the yeast screen it was shown to be toxic to yeast growth and thus not a viable PTP1B inhibitor.

As mentioned in the introduction, an issue with many of the recently developed potent PTP1B inhibitors is that they are either highly charged or too large and because of this are not cell permeable and lack bioavailability [1]. For instance, the potent PTP1B peptide inhibitor BzN-EJJ-amide which has a M_r of 800 and contains five negative charges was shown to be inactive in this assay. This was also the fate of the other charged F_2PMP inhibitors with M_r ranging from 547 to 804. Likewise, the highly charged suramin with a M_r of 1429 was also minimally effective. The only compounds, besides vanadate, that had activity in the assay, contained two negative charges and had $M_r < 500$. These compounds as well as vanadate all have similar potency on the purified PTP1B enzyme but inhibitor 1 was significantly more potent at inhibiting yeast growth than the others. This is likely a reflection of the cell permeable properties of the compounds. It is of interest to note that the three active non-peptidic phosphonate inhibitors described

here all have significant bioavailability whereas the peptide inhibitor, BzN-EJJ-amide, is not bioavailable[2] [23].

Although potent PTP1B inhibitors have been described, cell permeability and bioavailability of these compounds still remains an issue [1]. In addition to providing a cellular environment for PTP1B, thereby protecting it from oxidation and inactivation, the use of this yeast system requires the compounds to have a certain degree of cell permeability. Because of these features and the simple scoreable phenotype, the PTP1B yeast screen can be easily established for high throughput screening of sample collections or complex mixtures (i.e. natural products) to identify lead cell permeable PTP1B inhibitors.

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References

[1] Taylor SD, Hill B. Recent advances in protein tyrosine phosphatase 1B inhibitors. Expert Opin Investig Drugs 2004;13:199–214.
[2] Zhang ZY, Lee SY. PTP1B inhibitors as potential therapeutics in the treatment of Type 2 diabetes and obesity. Expert Opin Investig Drugs 2003;12:223–33.
[3] Asante-Appiah E, Kennedy BP. Protein tyrosine phosphatases: the quest for negative regulators of insulin action. Am J Physiol Endocrinol Metab 2003;284:E663–70.
[4] Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, et al. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science 1999;283:1544–8.
[5] Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, et al. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol 2000;20:5479–89.
[6] Zinker BA, Rondinone CM, Trevillyan JM, Gum RJ, Clampit JE, Waring JF, et al. PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. Proc Natl Acad Sci USA 2002;99:11357–62.
[7] Rondinone CM, Trevillyan JM, Clampit J, Gum RJ, Berg C, Kroeger P, et al. Protein tyrosine phosphatase 1B reduction regulates adiposity and expression of genes involved in lipogenesis. Diabetes 2002;51:2405–11.
[8] Gum RJ, Gaede LL, Koterski SL, Heindel M, Clampit JE, Zinker BA, et al. Reduction of protein tyrosine phosphatase 1B increases insulin-dependent signaling in ob/ob mice. Diabetes 2003;52:21–8.
[9] Clampit JE, Meuth JL, Smith HT, Reilly RM, Jirousek MR, Trevillyan JM, et al. Reduction of protein-tyrosine phosphatase-1B increases insulin signaling in FAO hepatoma cells. Biochem Biophys Res Commun 2003;300:261–7.
[10] Xie L, Lee SY, Andersen JN, Waters S, Shen K, Guo XL, et al. Cellular effects of small molecule PTP1B inhibitors on insulin signaling. Biochemistry 2003;42:12792–804.
[11] Melese T, Hieter P. From genetics and genomics to drug discovery: yeast rises to the challenge. Trends Pharmacol Sci 2002;23:544–7.
[12] Brugge JS, Jarosik G, Andersen J, Queral-Lustig A, Fedor-Chaiken M, Broach JR. Expression of Rous sarcoma virus transforming protein pp60v-src in Saccharomyces cerevisiae cells. Mol Cell Biol 1987;7:2180–7.
[13] Kornbluth S, Jove R, Hanafusa H. Characterization of avian and viral p60src proteins expressed in yeast. Proc Natl Acad Sci USA 1987;84:4455–9.
[14] Boschelli F. Expression of p60v-src in Saccharomyces cerevisiae results in elevation of p34CDC28 kinase activity and release of the dependence of DNA replication on mitosis. Mol Cell Biol 1993;13:5112–21.
[15] Boschelli F. Expression of p60v-src in Saccharomyces cerevisiae results in elevation of p34CDC28 kinase activity and release of the dependence of DNA replication on mitosis. Mol Cell Biol 1994;14:6409.
[16] Florio M, Wilson LK, Trager JB, Thorner J, Martin GS. Aberrant protein phosphorylation at tyrosine is responsible for the growth-inhibitory action of pp60v-src expressed in the yeast Saccharomyces cerevisiae. Mol Biol Cell 1994;5:283–96.
[17] Trager JB, Martin GS. The role of the Src homology-2 domain in the lethal effect of Src expression in the yeast Saccharomyces cerevisiae. Int J Biochem Cell Biol 1997;29:635–48.
[18] Huyer G, Liu S, Kelly J, Moffat J, Payette P, Kennedy B, et al. Mechanism of inhibition of protein-tyrosine phosphatases by vanadate and pervanadate. J Biol Chem 1997;272:843–51.
[19] Mumberg D, Muller R, Funk M. Regulatable promoters of Saccharomyces cerevisiae: comparison of transcriptional activity and their use for heterologous expression. Nucleic Acids Res 1994;22:5767–8.
[20] Agatep R, Kirkpatrick RD, Parchaliuk DL, Woods RA, Gietz RD. Transformation of Saccharomyces cerevisiae by the lithium acetate/single-stranded carrier DNA/polyethylene glycol (LiAc/ss-DNA/PEG) protocol. Technical Tips Online; 1998.
[21] Hammonds TR, Maxwell A, Jenkins JR. Use of a rapid throughput in vivo screen to investigate inhibitors of eukaryotic topoisomerase II enzymes. Antimicrob Agents Chemother 1998;42:889–94.
[22] Desmarais S, Friesen RW, Zamboni R, Ramachandran C. [Difluro-(phosphono)methyl]phenylalanine-containing peptide inhibitors of protein tyrosine phosphatases. Biochem J 1999;337(Pt 2):219–23.
[23] Dufresne C, Roy P, Wang Z, Asante-Appiah E, Cromlish W, Boie Y, et al. The development of potent non-peptidic PTP-1B inhibitors. Bioorg Med Chem Lett 2004;14:1039–42.
[24] Asante-Appiah E, Ball K, Bateman K, Skorey K, Friesen R, Desponts C, et al. The YRD motif is a major determinant of substrate and inhibitor specificity in T-cell protein-tyrosine phosphatase. J Biol Chem 2001;276:26036–43.
[25] Guan KL, Dixon JE. Protein tyrosine phosphatase activity of an essential virulence determinant in Yersinia. Science 1990;249:553–6.
[26] Flint AJ, Tiganis T, Barford D, Tonks NK. Development of ''substrate-trapping'' mutants to identify physiological substrates of protein tyrosine phosphatases. Proc Natl Acad Sci USA 1997;94:1680–5.

[2] Kennedy et al. unpublished results.

[27] Swarup G, Cohen S, Garbers DL. Inhibition of membrane phosphotyrosyl-protein phosphatase activity by vanadate. Biochem Biophys Res Commun 1982;107:1104–9.
[28] Skorey KI, Kennedy BP, Friesen RW, Ramachandran C. Development of a robust scintillation proximity assay for protein tyrosine phosphatase 1B using the catalytically inactive (C215S) mutant. Anal Biochem 2001;291:269–78.
[29] Zhang YL, Keng YF, Zhao Y, Wu L, Zhang ZY. Suramin is an active site-directed, reversible, and tight-binding inhibitor of protein-tyrosine phosphatases. J Biol Chem 1998;273:12281–7.
[30] Garcia-Morales P, Minami Y, Luong E, Klausner RD, Samelson LE. Tyrosine phosphorylation in T cells is regulated by phosphatase activity: studies with phenylarsine oxide. Proc Natl Acad Sci USA 1990;87:9255–9.
[31] Sibley CH, Brophy VH, Cheesman S, Hamilton KL, Hankins EG, Wooden JM, et al. Yeast as a model system to study drugs effective against apicomplexan proteins. Methods 1997;13190–207.
[32] Cromlish WA, Payette P, Kennedy BP. Development and validation of an intact cell assay for protein tyrosine phosphatases using recombinant baculoviruses. Biochem Pharmacol 1999;58:1539–46.