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Protein tyrosine and serine–threonine phosphatases in the sea urchin, *Strongylocentrotus purpuratus*: Identification and potential functions

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Abstract

Protein phosphatases, in coordination with protein kinases, play crucial roles in regulation of signaling pathways. To identify protein tyrosine phosphatases (PTPs) and serine–threonine (ser–thr) phosphatases in the *Strongylocentrotus purpuratus* genome, 179 annotated sequences were studied (122 PTPs, 57 ser–thr phosphatases). Sequence analysis identified 91 phosphatases (33 conventional PTPs, 31 dual specificity phosphatases, 1 Class III Cysteine-based PTP, 1 Asp-based PTP, and 25 ser–thr phosphatases). Using catalytic sites, levels of conservation and constraint in amino acid sequence were examined. Nine of 25 receptor PTPs (RPTPs) corresponded to human, nematode, or fly homologues. Domain structure revealed that sea urchin-specific RPTPs including two, PTPRLec and PTPRscav, may act in immune defense. Embryonic transcription of each phosphatase was recorded from a high-density oligonucleotide tiling microarray experiment. Most RPTPs are expressed at very low levels, whereas nonreceptor PTPs (NRPTPs) are generally expressed at moderate levels. High expression was detected in MAP kinase phosphatases (MKPs) and numerous ser–thr phosphatases. For several expressed NRPTPs, MKPs, and ser–thr phosphatases, morpholino antisense-mediated knockdowns were performed and phenotypes obtained. Finally, to assess roles of annotated phosphatases in endomesoderm formation, a literature review of phosphatase functions in model organisms was superimposed on sea urchin developmental pathways to predict areas of functional activity.

Keywords

Phosphatase; PTP; DSP; MKP; Serine-threonine phosphatase; PPP; PPM; Genome; *Strongylocentrotus*; Urchin

Introduction

Although often overlooked, the protein tyrosine and serine–threonine (ser–thr) phosphatases play critical roles in regulation of numerous cellular activities. They control cell receptors, signaling cascades, cytoplasmic regulatory proteins, transcription factors, posttranslational

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processes, and, in some instances, even act as switches between signaling pathways, activating one while inhibiting another. Most phosphatases regulate these processes by dephosphorylating specific substrate proteins (enzymes, regulatory proteins, receptors, transcription factors, etc.), generally counteracting the effects of kinases, proteins that phosphorylate substrates. Often, dephosphorylation inactivates the substrate, although, in other cases, the substrate is activated. Some phosphatases perform functions in addition to dephosphorylation. For example, there are phosphatases, such as DUSP11 (SHP2), that also act as adaptor proteins, linking components in a signal transduction pathway (Gomperts et al., 2003). Other phosphatases have completely lost the ability to dephosphorylate proteins and, instead, perform novel functions. In humans, about half of the myotubularin related protein tyrosine phosphatases (MTMRs) are catalytically inactive. Instead of dephosphorylating proteins, they are thought to modulate activities of catalytically active MTMRs by dimerizing with them (Laporte et al., 2003).

Protein phosphorylation and dephosphorylation generally occurs at tyrosine, serine, or threonine residues. Proteins that dephosphorylate tyrosine residues are called protein tyrosine phosphatases (PTPs) whereas those that act on serine or threonine sites are called serine–threonine (ser–thr) phosphatases.

This activity is dependent on the type of catalytic domain present. PTPs are categorized together because they all contain an invariant motif, CxxxxxR (CX_5R), which forms the active site of their catalytic domains. These are monomeric proteins and well-known members include the receptor protein tyrosine phosphatases (RPTPs), nonreceptor protein tyrosine phosphatases (NRPTPs), and dual specificity phosphatases (DSPs). DSPs differ notably from other PTPs in that they dephosphorylate both serine/threonine and tyrosine sites.

There are three families of ser–thr phosphatases: the PPPs (*P*hos*p*hoprotein *p*hosphatases), the PPMs (*P*hosphoprotein *p*hosphatases activated by *m*agnesium), and the FCPs (T*F*IIF stimulated *C*TD *p*hosphatases). PPPs are categorized together, in part, because each has a single conserved PP2A catalytic domain (PP2Ac domain). These polymeric proteins are activated when a single catalytic subunit interacts with one or more regulatory subunits. Identity of the regulatory subunit influences when or where the catalytic subunit acts. By utilizing different regulatory partners, one catalytic subunit can perform numerous cellular activities.

The other ser–thr phosphatases are monomeric proteins. PPMs are a fairly large family that contain a PP2C catalytic domain (PP2Cc) and depend on the presence of magnesium or manganese for catalytic activity. The FCPs are a minor group. They contain the signature motif of phosphotransferases and phosphohydrolases, DXDX(T/V), but are otherwise highly divergent from the PPPs and PPMs.

Since most protein phosphatases were discovered within the last 20 years, much remains to be learned about roles they play and how they are regulated. Initially, identifying phosphatase functions was quite difficult. Many early experiments relied on chemical inhibitors such as okadaic acid (a ser–thr inhibitor), calyculin (a ser–thr inhibitor), and sodium orthovanadate (a PTP inhibitor). These inhibitor experiments gave investigators a general idea about which types of phosphatases were acting, but not which specific phosphatase or pathway was affected. Other attempts to block single phosphatases were thwarted by the fact that more than one phosphatase acted on the substrate. In these instances, it was necessary to block several phosphatases to see an effect. Also, if catalytic activity was controlled by regulatory subunits, knocking out the catalytic subunit affected several cell processes simultaneously, again making it difficult to identify the substrate or

cell signaling pathway. As genomic data become available and, as more is learned about phosphatase functions in different model systems, it has become much easier to study specific roles of phosphatases in developmental processes.

This paper describes protein tyrosine and ser–thr phosphatases that were identified *in silico* using the *Strongylocentrotus purpuratus* genome. Expression of these proteins in the sea urchin embryo is also evaluated using high density oligonucleotide tiling microarrays and EST databases. Finally, several of the identified phosphatases were blocked in developing sea urchins using morpholino antisense oligonucleotide injection and their phenotypes are described.

One process that has been thoroughly investigated by the sea urchin community is endomesoderm specification. Although much is now known about this process, little has been reported about roles of phosphatases in regulation of sea urchin endomesodermal pathways. An additional goal of this paper is to identify potential functions of *S. purpuratus* protein phosphatases in endomesoderm formation. This should be a valuable resource to those wishing to study phosphatase function in development.

Materials and methods

Searching for phosphatase domains in the sea urchin genome

To find putative phosphatases in the sea urchin genome, sequences of known chordate phosphatases were obtained from NCBI Entrez Protein site (http://www.ncbi.nlm.nih.gov/) and blasted against Baylor's Human Genome Sequencing Center *Strongylocentrotus purpuratus* BLAST site (http://www.hgsc.bcm.tmc.edu/blast/blast.cgi?organism=Spurpuratus). The Baylor site retrieved matching sequences from the genome of a single urchin (compiled by the Baylor gene assembly team). Information concerning assembly of the genome appears in Weinstock and The Sea Urchin Genome Sequencing Consortium (2006). If retrieved sequence *E* values were 10^{-30} or less, identity of the urchin sequence was confirmed by reciprocal comparison against the non-redundant NCBI BLASTP site (http://www.ncbi.nlm.nih.gov/BLAST/). Throughout this paper, this process will be referred to as "back blasting." Putative urchin phosphatase sequences were then archived in the Baylor College of Medicine Sea Urchin Genome Project site (http://annotation.hgsc.bcm.tmc.edu/Urchin). To identify the seven genes reported in Wessel et al. (1995), their deduced amino acid sequences were blasted against Baylor's *Strongylocentrotus purpuratus* BLAST site.

The domain detection sites Pfam (http://www.sanger.ac.uk/Sortware/Pfam/), NCBI (http://www.ncbi.nlm.nih.gov/BLAST/), and SMART (http://smart.embl-heidelberg.de/smart/set_mode.cgi?NORMAL=1) were used to learn about structure of putative phosphatases. To find missing portions of sequences: A) Tiling data were examined to determine whether the gene was expressed in areas of the scaffold missing in the annotated sequence, B) Genboree (http://www.genboree.org/java-bin/login.jsp) was used to find alternate sequence predictions for the gene based on contig data from sites such as the NIDCR *S. purpuratus* Genome Search page (http://urchin.nidcr.nih.gov/blast/index.html) or NCBI, C) Sequences were checked for overlapping identical regions, D) PTPR D1 and D2 trees were compared to identify the second catalytic domain, and E) back blasting results not used in phylogenetic analyses were reexamined. In all cases, orientation of genes in scaffolds was considered and data were checked to ascertain that both parts of the gene were on the same DNA strand (positive or negative). Transmembrane domains were detected at TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) and the Mouse Genome Informatics site (http://www.informatics.jex.org/) was useful for determining alternate phosphatase names.

Evaluating protein expression

Tiling array data generated for the *S. purpuratus* genome project (Samanta et al., 2006) were examined. In each case, the raw data were evaluated and peak heights were measured for each predicted exon. Quantitative values were calculated for each gene by averaging all exon peaks when there were 5 peaks or less. When 6 or more peaks were present, low and high values were eliminated and remaining peaks were averaged. Averages were graded as no expression (<3), very low expression (3–4.99), low expression (5–9.99), moderate expression (10–29.99), or high expression (>30).

EST data were used to find expressed sequences undetected by the tiling array. If EST data for an annotated gene appeared in Geneboree, the gene was considered expressed even if tiling data were inconclusive. Also, annotated sequences were blasted against all sea urchin ESTs at the NCBI BLAST Sea Urchin Sequences site (http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=7668). Annotated sequences that matched an EST sequence were back blasted to the Baylor *S. purpuratus* Blast site. Sequences with *E* values of zero were accepted as expressed.

Finding human and/or invertebrate homologues

Representative phosphatase sequences for homologue analysis were collected from several sources. Vertebrate, *Drosophila melanogaster*, and *Cae-norhabditis elegans* RPTPs and NRPTPs were downloaded from the Novo Nordisk Science protein tyrosine phosphatase database (http://ptp.cshl.edu or http://science.novonordisk.com/ptp) while sequences for other phosphatases were found in GenBank (http://www.ncbi.nih.gov/entrez/query.fcgi?db=Protein), UniProt (http://www.pir.uniprot.org/), or Ensembl (http://www.ensembl.org/index.html). Potential cnidarian (sea anemone) homologues were obtained by domain searches of the *Nemtostella vectensis* genome at http://www.stellabase.org. (Accession numbers for non-sea urchin phosphatases used in these analyses appear in Suppl. 9). Sequences were aligned using ClustalX. Conserved regions were selected in McClade, and classification of the phosphatases by phylogenetic analysis was performed in PAUP. Rooted or unrooted neighbor-joining trees were generated, followed by bootstrap analysis of the data. Two thousand repetitions were used for bootstrap analysis.

For the RPTP analysis, most sequences were partial. Therefore phylogenetic comparison was limited to PTPc domain 1 (D1) or PTPc domain 2 (D2). Since PTPcD1 and D2 sequences segregate in neighbor joining trees, it was easy to distinguish *S. purpuratus* D1 sequences from D2 sequences by running them with known *Hs*PTPc D1 and *Hs*PTPc D2 sequences (not shown). Using this information, separate PTPc D1 and D2 trees were produced. The RPTP D2 neighbor-joining tree was used to confirm phylogenetic relationships observed in the D1 tree. Similarity of sea urchin RPTP D1 domains to those of other species was evaluated by neighbor-joining, maximum likelihood, maximum parsimony (not shown), and Bayesian analysis.

When duplications appeared in a tree, pairwise comparison of the genes was performed (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi) to ensure that they were not identical. Genboree was also used to detect null sequences (artifacts of genome sequencing), to compare locations of overlapping sequences in different scaffolds (to determine whether two sequences were complementary ends of the same protein), and to compare exon/intron boundaries (and neighboring sequences) when searching for haplotype pairs.

Determining potential functions of identified sea urchin phosphatases

To identify potential functions of annotated *S. purpuratus* phosphatases in endomesoderm formation, a list of sea urchin endomesodermal signaling pathways (and components that comprise these pathways) was generated. In addition, a list of genes known to affect sea urchin endomesoderm formation was obtained from the Endomesoderm Network site (http://sugp.caltech.edu/endomes/). Using Pubmed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) and Endnote, activities of phosphatases in endomesodermal signaling pathways were collected and tabulated.

Design and microinjection of anti-phosphatase MASOs

A list of phosphatase sequences from mouse was compiled and used to perform BLAST searches of *S. purpuratus* expressed sequence tags (ESTs) to identify phosphatases expressed in the embryo. ESTs encoding N-terminal protein sequences (based on similarity to the N-termini of mouse phosphatases) were then used to obtain morpholino antisense oligonucleotides (MASOs) from Genetools, LLC (Corvallis, OR). In all cases except one, the MASO sequences were targeted to the translation initiation site of respective mRNAs; the one exception is DUSP1/2/4/5, for which the GLEAN3 model was used to design a splice-blocking MASO that targets the first exon-intron junction (Table 1). The anti-phosphatase MASOs as well as a standard non-specific control MASO (Genetools) were initially injected by standard methods into fertilized eggs, at three different concentrations (injection solutions containing 200, 400, or 800 μM MASO and 120 mM KCl). Injected embryos developed at 15 °C, with periodic observation and notation of their phenotypes. Additional microinjections of several MASOs were subsequently carried out at the most effective dose for each MASO, and digital light micrographs were obtained on a Zeiss Axiovert 20 microscope equipped with an Axiocam MRm. Images were processed in Photoshop.

Results and discussion

Most human PTPs and ser–thr phosphatases have S. purpuratus homologues

The protein tyrosine phosphatases—There are four PTP superfamily classes: Class I Cysteine-based PTPs, Class II Cysteine-based PTPs, Class III Cysteine-based PTPs, and Asp-based PTPs (Alonso et al., 2004b). These proteins are categorized as PTPs because they all contain an invariant motif, CxxxxxR (CX_5R), which forms the active site of the catalytic domain.

Almost all PTPs are Class I Cysteine-Based PTPs, a group that can be subdivided into two categories: conventional PTPs (which dephosphorylate at tyrosine residues) and dual specificity protein phosphatases or VH1-like phosphatases (which primarily dephosphorylate at tyrosine and/or nearby serine/threonine residues).

Class II and III Cysteine-based PTPs are classified separately from Class I Cysteine-based PTPs because they evolved from distinct lineages. Class II Cysteine-based PTPs are closely related to bacterial arsenate reductases while Class III Cysteine-based PTPs are derived from bacterial rhodanese-like enzymes. Both of these classes are small. Humans, for example, have only one Class II Cysteine-based PTP and 3 Class III Cysteine-based PTPs.

Asp-based PTPs utilize a distinct catalytic mechanism from other PTPs. Class I, II, and III Cysteine-based PTPs, utilize cysteine as the nucleophile in the catalytic domain and form thiol–phosphate intermediates during substrate dephosphorylation (Alonso et al., 2004b). Asp-based PTPs differ in that their catalytic reactions are metal-dependent and rely on a

nucleophilic aspartic acid to form a phospho-aspartate intermediate (Rebay et al., 2005). Like Class II and III Cysteine-based PTPs, Asp-based PTPs only have a few members.

Class I cysteine-based PTPs

Conventional PTPs: The conventional PTPs include two groups: receptor protein tyrosine phosphatases (RPTPs), which are membrane-associated proteins (with the exception of a few splice variants), and nonreceptor protein tyrosine phosphatases (NRPTPs), which are cytoplasmic. Most data about these phosphatases come from studies of human proteins.

Structurally, RPTPs contain an intracellular and extra-cellular region. Intracellular portions of the protein tend to be highly conserved due to the fact that the PTP catalytic (PTPc) domain is intracellular. All RPTPs have either one or two PTPc domains. In cases where two PTPc domains are present, the domain closest to the cell membrane, PTPc D1 (D1), dephosphorylates substrate proteins while the distal domain, PTPc D2 (D2), is thought to aid in aligning the RPTP to substrate proteins. Of these, D1 is more highly conserved and is retained in RPTPs that have only one PTPc domain.

Extracellular portions of RPTPs vary quite a bit. In humans, there are at least 23 different RPTPs and most of this diversity is due to variation in extracellular domains. Functions of these domains are less well understood. In some cases, they may influence which extracellular signals the RPTP responds to. In other cases, they allow the protein to perform functions independent of substrate dephosphorylation. Extracellular domains found in human RPTPs include fibronectin 3 repeats (FN3), immunoglobulin (Ig) sequences, glycosylation sites, and MAM domains (Fig. 1).

RPTPs have been implicated in numerous processes including the regulation of density dependent growth (PTPRJ and possibly PTPRB and PTPRO; Schaapveld et al., 1997), regulation of adherens junction complexes (PTPRF, PTPRK, PTPRM; Wadham et al., 2003), regulation of axon guidance in motor neurons (DLAR, DPTP10D, and DPTP99A; Desai et al., 1997), and neuronal differentiation (PTPRA; Hertog et al., 1999).

Phylogenetic studies have clearly shown that NRPTPs share a common ancestor with the RPTPs. These phosphatases only have one PTPc domain (similar to PTPc D1) and, like the RPTPs, they often have additional domains. These other domains can aid in cellular localization of the NRPTP (Ex: PEST domains target activity to the cell membrane/cytoskeletal interface and SEC14 domains target activity to the perinuclear region), regulation of substrate access (Ex: SH2 domains), or other protein–protein interactions. Domain types that have been found in human NRPTPs include PEST, FERM, SH2, SH3, BRO1, KIND, PDZ, B41, and SEC14 domains (Andersen et al., 2001; Bhaduri and Sowdhamini, 2003; Alonso et al., 2004b; Gandhi et al., 2005). Some of the known functions of NRPTPs include regulation of Ha–Ras dependent cell growth (PTPN23; Cao et al., 1998), coupling of cell protrusion and retraction during cell migration (PTPN12; Sastry et al., 2006), blocking apoptosis (PTPN13; Foehr et al., 2005; Ivanov et al., 2006), promoting vesicle fusion (PTPN9; Huynh et al., 2004), inhibiting signal transduction by Src family kinases (PTPN18; Wang et al., 2001), and promoting cell migration while reducing cell–cell adhesion by dephosphorylating β-catenin (PTPN14 and PTPN1; Wadham et al., 2003).

Based on domain structure, protein function, and homologue analysis of conventional PTPs, Andersen et al. (2001, 2005) concluded that there are 10 types of RPTPs (R1/6, R2A, R2B, R3, R4, R5, R6, R7, R8, and R9) and 9 types of NRPTPs (NT1–NT9). With the exception of Type R9 RPTPs (found in *Drosophila*), these are all present in humans (See representatives of the RPTP categories in Fig. 4.) (For reviews see Brady-Kalnay and Tonks, 1995; Schaapveld et al., 1997; Neel and Tonks, 1997; Barford et al., 1998; Stoker and Dutta, 1998;

Hertog et al., 1999; Andersen et al., 2001; Bhaduri and Sowdhamini, 2003; Alonso et al., 2004b; Gandhi et al., 2005). Although this classification was initially based on structural similarities, phylogenetic analysis using PTPc D1 sequences has shown that extracellular domain structure may be a poor predictor of relatedness (Andersen et al., 2001, 2005). In fact, two of the RPTP clades, R7 and R8, are probably more closely related to the NRPTPs than to the RPTPs.

One difficulty in studying conventional PTPs is the nomenclature. When these were first being discovered and characterized, the same protein was often given different names. In this paper, PTP nomenclature is simplified as in Alonso et al. (2004b). RPTPs are identified using letters of the alphabet (Ex: PTPRA, PTPRS) and NRPTPs are indicated using numbers (Ex: PTPN1, PTPN11). For alternate names see Tables 1–3.

To identify homologues of human conventional PTPs, human sequences were back blasted against the *S. purpuratus* genome. Using this technique, 49 putative *Sp*RPTP and 12 putative *Sp*NRPTP sequences were detected (Table 1, Suppl. 10). Seventeen other sequences were also weakly similar to conventional PTPs (most were partial sequences).

Because many RPTP sequences are long and because extracellular regions are poorly conserved, this group was difficult to analyze. Many of the sequences crossed scaffolds and back blasting to find extracellular sequences was frequently useless. Because of these complications, full-length *S. purpuratus* sequences were not used to identify human RPTP and NRPTP homologues. Instead, PTPc domain analysis was performed (the standard procedure to identify conventional PTPs).

Since all RPTPs contain a D1 domain, more detailed analysis was performed on these data. Homologues to the *S. purpuratus* RPTP D1 sequences were detected using three methods: neighbor-joining (Fig. 1), maximum likelihood (Suppl. 1), and Bayesian analysis (Suppl. 2). Results showed that only 9 of 25 *S. purpuratus* D1 sequences grouped with RPTP subgroups and that only 3 of the 8 RPTP subgroups have sea urchin homologues strongly supported by bootstrap analysis (R2A, R5, and R7). Type R3-like and R9-like *S. purpuratus* RPTPs were also found, but these clades (when observed) were weakly supported. In contrast, the other 16 sea urchin sequences appear to be orphans, defining at least 2–3 new clades (these results are supported by high bootstrap values and by neighbor-joining, maximum likelihood, and Bayesian analysis). Intriguingly, one novel group of *Sp*PTPRs always forms a single clade (*Sp*PTPRLec1–6 and *Sp*PTPRFn1–2), suggesting that it arose by lineage specific expansion (the production of paralogues by duplication of an orthologue after speciation; for more about this, see Beane et al., 2006). Further, none of these sequences are identical or located on the same scaffold, indicating that they are likely to be real and distinct genes. Based on Bayesian and maximum likelihood analyses these genes and the other orphans may be distantly related to *Drosophila melanogaster* CG7180PA (a PTP with no clear human orthologue) and *Nematostella vectensis* 39268×. Based on tiling microarrays and EST data, 72% of the *Sp*RPTPs are expressed during development (Table 1). With the exception of *Sp*PTPR G/Z2, PTPR D/F/S and PTPRorph2 (present at moderate levels), expression of these genes is either undetectable, very low, or low (see Table 1).

The RPTP D2 neighbor-joining tree (Suppl. 3) corroborates findings in the D1 trees. Again, sea urchin homologues to the R2A and R5 RPTPs were detected, as was the *Sp*PTPRLec/Fn clade and the *Sp*PTPRY1/2 clade. Partial sequences containing the missing D2 domains of *Sp*PTPRW1 and *Sp*PTPRFn2 may have also been identified. This is supported by three observations: A) The detected D2 sequence is on the same strand as the known D1 sequence, B) these genes occupy complementary locations in each scaffold, and C) the D1 and D2 genes occupy the same clade in both trees.

As with most of the phosphatases studied, fewer NRPTPs were found in the sea urchin than in humans. While humans have 15 NRPTPs (Alonso et al., 2004b), only 8 are present in *S. purpuratus* (Fig. 1B). Since Type R8 RPTPs (PTPRN and PTPRN2) are thought to be more closely related to NRPTPs than to RPTPs, they were included in the *S. purpuratus* NRPTP tree. Eight of 10 NRPTP types were represented in the *S. purpuratus* genome. Only the Type NT8 (PTPN20) NRPTP and the Type R8 RPTP were missing. No novel NRPTPs were detected. All *S. purpuratus* NRPTPs were expressed at moderate levels except PTPN3/4 (expressed at low levels) (see Table 1).

Dual specificity phosphatases: The dual specificity protein phosphatases (DSPs) include 8 subfamilies: MAP kinase phosphatases (MKPs), atypical DSPs, Slingshots, PRLs, CDC14s, PTENs, tensins, and myotubularins. In *S. purpuratus*, 32 DSPs were found, far fewer than the 61 found in humans. Of these, at least one member of each major subfamily was present: 5 MKPs, 12 atypical DSPs, 2 Slingshots, 1 PRL, 3 CDC14s, 4 PTENs, and 5 myotubularins (Fig. 2, Table 2).

The MKPs are one of the most studied DSP groups. By dephosphorylating MAP kinases (MAPKs) at dual sites, both the threonine and tyrosine residues in MAPK motif -pTXpY-, MKPs halt MAPK activity. In vertebrates, there are three MAPKs: Erk, JNK/SAPK, and p38. While some MKPs only interact with one MAPK (for example, DUSP6/MKP-3 blocks Erk activity), others can dephosphorylate two or all three MAPKs (for example, DUSP4/MKP-2 blocks both Erk and JNK) (Ducruet et al., 2005). With the exception of DUSP10 (MKP-5), DUSP8 (VH5), and DUSP16 (MKP-7), MKPs contain two domains, a DUSP catalytic domain and an N-terminal MAPK binding domain (MKB domain). Other MKPs possess a third domain. The function of this domain is unknown in DUSP10/MKP-5, but, in DUSP8/VH5 and DUSP16/MKP-7, the third domain (a PEST domain) is thought to facilitate ubiquitination and proteolysis when phosphorylated (Farooq and Zhou, 2004). Five homologues of human DSPs were found in the sea urchin: *Sp*DUSP 1/2/4/5 (nuclear DSPs that act on all MAPK types), *Sp*DUSP 6/7/9 (cytosolic DSPs that act on Erks), *Sp*DUSP 8/16 (cytosolic DSPs that dephosphorylate Jnk and p38), *Sp*DUSP10 (also cytosolic, dephosphorylating Jnk and p38) and *Sp*MK-STYX (MKP functions from Alonso et al., 2004a). With the exception of SpMK-STYX (present at low levels), all were expressed at moderate to high levels during development.

Much less is known about atypical DSPs. These tend to be smaller proteins (less than 250 amino acids) that lack the MKB domain (Alonso et al., 2004a) and three dephosphorylate MAPKs (DUSP3, DUSP22, and DUSP14/MKP-6) (Farooq and Zhou, 2004). *S. purpuratus* homologues of human atypical DSPs identified include *Sp*DUSP11, *Sp*DUSP12, *Sp*DUSP14/18/21, *Sp*DUSP15/22a and b, *Sp*DUSP23, *Sp*RNGTT, *Sp*STYX, and three copies of *Sp*DUSP3: *Sp*DUSP3A, *Sp*DUSP3B, and *Sp*DUSP3C (Fig. 2). Two of these, DUSP3B and 3C, were formed by tandem duplication. Also, the identification of *Sp*DUSP15/22b is suspicious. This is definitely a DSP, but there appears to be a genome assembly error. The DUSP15/22b sequence is incomplete and lies between exons of *Sp*JAK. Based on the tiling microarrays and EST data, only 5 atypical DSPs are expressed in developing urchins: *Sp*DUSP11, *Sp*DUSP12, *Sp*DUSP14/18/21, *Sp*MK-STYX/DUSP24, and *Sp*RNGTT (Table 2). None are expressed above low levels.

Other DSP subfamilies also have DUSP domains, but are more distantly related to the MKPs and atypical DSPs. These include the Slingshot, PRL, Cdc14, PTEN, and myotubularin subfamilies. Members of the Slingshot (SSH) subfamily (SSH1, SSH2, and SSH3 in humans) regulate the actin cytoskeleton (Niwa et al., 2002; Baum, 2002; Huang et al., 2006). By dephosphorylating cofilin, an actin-binding protein that severs F actin, SSHs indirectly activate disassembly at the slow-growing ends of actin filaments. In the sea

urchin, two homologues of human SSH were found, *Sp*SSH1/2/3A, and *Sp*SSH1/2/3B (Fig. 2). During development, *Sp*SSH1/2/3A and B are expressed at very low and low levels respectively (Table 2).

Another subfamily, the PRLs (*p*hosphatase for *r*egenerating *l*iver), is involved in regulation of growth, cell proliferation, cell migration, and differentiation of specific tissues (such as digestive epithelial cells). These phosphatases are among the smallest known (usually 140–180 aa in length) and are fairly simple in structure, with only one catalytic domain and no regulatory or docking domains (Kozlov et al., 2004). Localization and activity of these phosphatases may be regulated through a prenylation site at the C terminus. Humans have 3 PRLs: PTP4A1, 2, and 3. Only one, expressed at high levels, was found in *S. purpuratus*, *Sp*PTP4A1/2/3 (Fig. 2, Table 2).

Members of the Cdc14 subfamily (including Cdc14A, Cdc14B, CDKN3, and PTP9Q22) interact with cyclin dependent kinases (CDKs/cyclins), inactivating them by removing phosphates (Torres-Rosell et al., 2005; Hannon et al., 1994). Effects of this differ depending on the cyclins involved. For example, Cdc14 promotes exit from mitosis when one group of cyclins associates with it, but when bound to a different cyclin, Cdc14 regulates mitotic entry (Trautmann and McCollum, 2002). Cdc14s have also been linked to nucleolar rDNA segregation during anaphase and to rDNA condensation. Three *S. purpuratus* homologues of human Cdc14s were found: *Sp*Cdc14A, *Sp*CDKN3, and *Sp*PTP9Q22. No Cdc14B homologue was detected (Fig. 2). During development, *Sp*CDKN3 and PTP9Q22 are expressed at low levels whereas *Sp*Cdc14A is expressed at high levels (Table 2).

Because strong sequence similarity exists between members of the PTEN subfamily (PTEN, PTPE, PTIP) and other PTPs, and because members of this subfamily have DUSP domains, it was originally thought that PTENs dephosphorylated proteins at serine/threonine or tyrosine sites. With the exception of one study (Raftopoulou et al., 2004), all work in this area has shown that the primary substrates of PTENs are D3-phosphorylated inositol phospholipids (Wishart and Dixon, 2002). Raftopoulou et al. recently suggested that, in some cases, PTENs also dephosphorylate focal adhesion kinase (FAK), a protein. Three members of this subfamily were found in *S. purpuratus: Sp*PTENa, *Sp*PTENb, *Sp*TPTE/TPIP (Fig. 2). Although BLAST searches classify *Sp*PTENb as a homologue of TPIP, neighbor-joining analysis does not support this, but categorizes it as a PTEN homologue. All subfamily members except *Sp*TPTE/TPIP were expressed during embryonic development (Table 2).

Another group of proteins that shares sequence homology with the PTENs (Haynie and Ponting, 1996; Lo, 2004) is the tensins (Tensin1, Tensin3, Tensin4, and Tenc1). These proteins have previously been classified with the PTPs (Alonso et al., 2004a,b) although, with the exception of Tenc1 (Hafizi et al., 2005), they lack phosphatase activity. Instead, Tensin1, Tensin3, and Tensin4 are generally categorized as phosphoproteins or focal adhesion associated proteins (Chen et al., 2002; Lo, 2004; Cui et al., 2004). Structurally, the tensins have domains that bind integrin β (PTB domain), actin (ABD domains), and tyrosine-phosphorylated proteins (SH2 domain) (Lo, 2004). They affect cell motility, cell shape, and cell proliferation (Chen et al., 2002; Lo, 2004; Cui et al., 2004; Hafizi et al., 2005), link receptor tyrosine kinase signaling to control of cell adhesion (Cui et al., 2004; Lo, 2004), and regulate Akt/PKB signaling (Hafizi et al., 2005). Low levels of *Sp*Tensin 1/3 were expressed in *S. purpuratus*.

PTENs are not the only PTPs that dephosphorylate lipids. Members of the myotubularin subfamily, myotubularin (MTM1) and the myotubularin-related proteins (MTMRs), are also lipid phosphatases. Humans have 14 myotubularins: MTM1 and MTMR 1 to 13.

Phylogenetic analysis has shown that metazoans have six types of myotubularins (Wishart et al., 2001). Three clades (*Hs*MTMR5/13, *Hs*MTMR9, and *Hs*MTMR10/11/12) contain catalytically inactive forms that lack the ability to dephosphorylate while the other three clades (*Hs*MTM1/MTMR1/2, *Hs*MTMR3/4, and *Hs*MTMR6/7/8) actively dephosphorylate phosphatidylinositol 3-monophosphate (PtdIns3P) and phosphatidylinositol 3,5-biphosphate (PtdIns3, 5P2). Lipid intermediates produced by dephosphorylation act in vacuolar transport and membrane turnover (Laporte et al., 2003). Because the catalytically active and inactive forms interact, it is thought that inactive forms may regulate active myotubularins by heterodimerizing with them (Laporte et al., 2003). Five of the six myotubularin types are present in *S. purpuratus* (Suppl. 4A). Three catalytically active forms were found: *Sp*MTMR1/2, *Sp*MTMR3/4, and *Sp*MTMR6/7/8. But only two non-catalytic myotubularins were annotated: *Sp*MTMR9 and *Sp*MTMR5/13. Homologues of *Hs*MTM1, *Hs*MTMR10, *Hs*MTMR11, or *Hs*MTMR12 were not observed. With the exception of *Sp*MTMR6/7/8 (not expressed), *S. purpuratus* MTMRs were expressed at levels ranging from very low to moderate during development (Table 2).

Class II and III cysteine-based PTPs—Only one Class II Cysteine-based PTP is found in humans, ACP (aka the low molecular weight PTPs) (Alonso et al., 2004b). This is a 180 kDa protein that can be alternatively spliced to form 4 different mRNA isoforms. The catalytic domain contains a typical PTP motif, but other portions of the phosphatase have limited similarity to the PTPs. This protein regulates growth factor induced mitotic growth by deactivating tyrosine kinase receptors that interact with growth factors (i.e. insulin, ephrin, and PDGF receptors) (Raugei et al., 2002). It also influences cytoskeletal activities by dephosphorylating p190RhoGAP, a regulator of Rho. No *Hs*ACP homologue was found in the *S. purpuratus* genome.

Class III Cysteine-based PTPs are critical in cell cycle regulation where they dephosphorylate cyclin dependent kinases (Cdk/cyclin). Humans produce three Class III Cysteine-based PTPs: Cdc25A, Cdc25B, and Cdc25C. Two of these, Cdc25B and Cdc25C, regulate progression of cells from G2 to M phase, and Cdc25A, in addition to modulating the G2 to M transition, also regulates the G1 to S transition and contributes to S phase and G2 phase progression (Kristjánsdóttir and Rudolph, 2004; Ducruet et al., 2005). Cdc25 phosphatases are also targets of cell checkpoint proteins. If cells are exposed to ultraviolet light, ionizing irradiation, replication inhibition, or other components that damage DNA, cell checkpoint proteins (such as Chk1-, Chk2-, and p38) phosphorylate Cdc25, resulting in cytoplasmic sequestration or ubiquitin/proteosome-mediated destruction of the phosphatase. Since Cdc25 is necessary for progression of cell cycle, phosphorylation by checkpoint proteins causes mitotic arrest, preventing replication of damaged DNA. These are short proteins (423–566 aas in humans) with a regulatory domain at the N-terminus and a catalytic domain closer to the C-terminus. In *S. purpuratus*, only one Cdc25 is found, *Sp*Cdc25A/B/C (Suppl. 4B). This protein is expressed at low levels during development (Table 2).

Asp-based PTPs: Eyes absent (EYA) is the only member of the Asp-based PTP Class and is notable because, in addition to dephosphorylating proteins, it acts in coordination with *Sine oculis* (SO) as a transcriptional coactivator (Rebay et al., 2005). It has been implicated in several developmental processes including formation of the eyes, kidneys, ears, and muscle. Recent *in vitro* data suggest that substrates dephosphorylated by EYA include the CTD domain of RNA polymerase II and EYA itself. In humans, there are 4 forms of EYA: *Hs*EYA1, *Hs*EYA2, *Hs*EYA3, and *Hs*EYA4. In *S. purpuratus*, only one form was found, *Sp*EYA1/2/4 (Suppl. 4C). This gene is expressed at moderate levels in developing embryos (Table 2).

The serine–threonine phosphatases—As mentioned in the introduction, many ser–thr phosphatases are multimeric proteins, with a catalytic subunit whose activity and localization are controlled by regulatory subunits. It was not within the scope of this study to identify both catalytic and regulatory subunits; instead efforts were focused on identification of the catalytic subunits. In *S. purpuratus*, 57 sequences were initially identified that blasted to ser–thr phosphatase sequences (catalytic and regulatory). Of these, 25 contained sequences of ser–thr phosphatase catalytic domains complete enough to be used in homologue analysis (2 others were discarded due to redundancy) (Table 3, Suppl. 10). Eighty-eight percent of the annotated sequences containing a catalytic domain were expressed (Table 3).

PPP phosphatases: The PPP family consists of five subfamilies: PP1 (*P*rotein *p*hosphatase 1), PP2A/PP4/PP6, PP5, PP7, and PP2B. Studies of PPP evolutionary relationships found that all 5 subfamilies are present in both humans (deuterostomes) and *Drosophila* (ecdysozoan protostomes) and that most, with the exception of PP7, are conserved throughout the Eukaryota. Subfamilies are classified in a manner consistent with the findings of Cohen (2004). These investigators reported that the PP1 and PP2A/PP4/PP6 subfamilies share a common ancestor. They also found that PP5 and PP7 phosphatases might be related to the PP2B phosphatases, sharing a distant common ancestor. Two members of the PP2B/PP5/PP7 clade, PP2B and PP7, are only activated in the presence of calcium while other members of the PPP family do not depend on ions for activation. All members of the PPP family contain a catalytic domain that is approximately 280 amino acids in length called the PP2A domain. Representatives of each PPP subfamily are found in the sea urchin (Fig. 3A and B, Table 3).

The PP1 subfamily: PP1 phosphatases (also known as the PPP1 subfamily) are among the most highly conserved eukaryotic proteins. In fact, an isoform present in *Giardia lamblia* is 72% identical to human PP1 (Ceulemans et al., 2002)! Humans have 3 catalytic subunits: PP1 alpha, PP1 beta, and PP1 gamma. These catalytic subunits cannot function independently, but must heterodimerize with variable regulatory proteins (over 50 in humans) that influence when and where they act (Ceulemans and Bollen, 2004). PP1 activity is also regulated by kinases. By phosphorylating threonine residues at the C-terminus of the catalytic subunit, kinases can reduce PP1 activity.

PP1 phosphatases function in various cell activities. They dephosphorylate transcription factors so they can be reused, process mRNA, downregulate ion pumps and channels, maintain G1 and G2 phases, allow exit from mitosis, control gene transcription by mRNA Polymerase II, and induce relaxation of actomyosin fibers (Ceulemans and Bollen, 2004). Three PP1 homologues were identified in *S. purpuratus: Sp*PP1 alpha/beta/gamma, *Sp*PP1 beta, and *Sp*PP1-like (Fig. 3A). Two of these, *Sp*PP1-like and *Sp*PP1 beta, are expressed at moderate levels in developing *S. purpuratus* and *Sp*PP1 alpha/beta/gamma is expressed at very low levels (Table 3). *Sp*PP1-like groups close to the PP1 clade, but does not cluster with any specific metazoan PP1 group (*Drosophila*, *Nematostella*, and human sequences were tested, not shown). It may be a highly divergent form or perhaps a pseudogene.

PP2A/PP4/PP6 subfamily: Like PP1, members of the PP2A/PP4/PP6 subfamily also interact with regulatory domains. In humans, catalytic subunits of two PP2A phosphatases have been identified, PP2A alpha and PP2A beta. Normally, the catalytic subunit (C subunit) associates with a second 65 kDa subunit (the A subunit or P65) to form the core enzyme. A second subunit then associates with the core enzyme. This subunit, the B subunit, can vary quite a bit. There are 4 families of B subunits: B (PR55), B′ (PR61), B″ (PR 72, PR130, PR59, and PR48), and B‴ (PR93, PR110). The PP2A (or PPP2) phosphatases are one of the most commonly utilized phosphatases in the cell. These phosphatases regulate

canonical and non-canonical Wnt signaling, regulate G_2/M transition, initiate and terminate translation, respond to DNA damage, and act in apoptosis. Kinases dephosphorylated by PP2A phosphatases include Erks, calmodulin-dependent kinases, PKA, PKB, PKC, and Cdks (Janssens and Goris, 2001). In *S. purpuratus*, one PP2A protein (*Sp*PP2A alpha/beta) was identified (Fig. 3A), and, as expected, it is expressed at high levels during development (Table 3).

The other two members of this subfamily, PP4 and PP6, are also highly conserved and are found in both deuterostomes and ecdysozoan protostomes. Like the PP2A phosphatases, PP4 and PP6 each bind a regulatory subunit to form a core protein. The core protein then interacts with a third variable subunit that influences phosphatase activity (Cohen et al., 2005). PP4 is involved in numerous cell activities including chromatin regulation, centrosome maturation, spliceosomal assembly, and regulation of signaling pathways (NF-kB and target of rapamycin) (Cohen et al., 2005). Less is known about PP6. Studies of yeast homologues have shown that this phosphatase is important for the G1/S transition, initiation of translation, and cell shape regulation (Bastians and Ponstingl, 1996; Cohen, 2004). *S. purpuratus* has one PP4 catalytic subunit (*Sp*PP4c) and one PP6 catalytic subunit (*Sp*PP6c) (Fig. 3A). PP4c is expressed at high levels and PP6c is present at low levels during sea urchin development (Table 3).

The PP5 and PP7 subfamilies: Like all members of the PPP family, PP5 and PP7 phosphatases both have a catalytic domain similar to PP2A. In PP7/PPEF (*P*rotein *p*hosphatase 7/*P*rotein *p*hosphatase with *EF* hand domains) phosphatases, however, this catalytic domain contains inserts that are absent in PP2A phosphatases (Luan, 2003). These inserts vary in length and there is evidence that they may be autoinhibitory regions (Andreeva and Kutuzov, 1999). It is also known that, like PP2B phosphatases, PPEF activity is calcium dependent. Protein activity is controlled in two ways, by calcium binding to C-terminal EF hand sequences and by interaction of calmodulin with the N-terminal domain (Kutuzov et al., 2002). PP7 phosphatases are important for vision. In mammals, PP7 expression is primarily limited to the retina and the brain (Cohen, 2004), and, if this gene is disrupted (either in mammals or *Drosophila*), photoreceptor function is impaired (Andreeva and Kutuzov, 1999; Ramulu et al., 2001). Although PP7 performs a different roles in mammals, *Drosophila* Rdg5, a PP7 homologue, is known to be necessary in dephosphorylation of rhodopsin.

PP5 probably originated from the same ancestral protein as PP7/PPEF, but it differs from PP7/PPEF in both structure and function. PP5 activity is not calcium dependent, but depends on interactions with regulatory proteins or lipids. The regulatory domain of the catalytic subunit is approximately 200 aa long, lies near the N-terminal, and contains 3 tetratricopeptide (TTP) tandem repeats. By interacting with regulatory molecules, PP5 influences atrial natriuretic peptide signaling, ion channel activity, steroid signaling, and RNA polymerase I and II function (Andreeva and Kutuzov, 1999).

In *S. purpuratus*, homologues were identified for both *Hs*PP5 and *Hs*PP7 (Fig. 3A). *Sp*PP7 is expressed at moderate levels during development and *Sp*PP5 is expressed at low levels (Table 3).

The PP2B Subfamily: The PP2B phosphatases (also known as PPP3 phosphatases) are activated in response to intracellular increases in calcium. When calcium levels rise in the cell, calcium binds both of the regulatory subunits (calmodulin and calcineurin B) needed to activate the catalytic subunit (calcineurin A, also known as PP2B alpha or PPP3A) (Gomperts et al., 2003; Luan, 2003). In humans, there are 3 catalytic subunits, calcineurin A alpha, calcineurin A beta, and calcineurin A gamma. This phosphatase acts in numerous

calcium dependent processes and regulates nuclear factor of activated T-cells (NF-AT) mediated transcription. It plays important roles in apoptosis, cardiac physiology, embryonic development, and regulation of metabolic processes (Groenendyk et al., 2004). A single homologue of *Hs*Calcineurin A, *Sp*Calcineurin A alpha/beta/gamma, is present in *S. purpuratus* (Fig. 3A). A second *Hs*Calcineurin A homologue may also be present. SPU_018404 contains a PP2A domain and back blasts to calcineurin A, however the sequence was too short to analyze using neighbor-joining. Both SPU_018404 and *Sp*Calcineurin A alpha/beta/gamma are expressed at moderate levels in developing embryos (Table 3).

PPM phosphatases: The PPM phosphatases only contain one subfamily, the PP2C subfamily. Unlike the PPP phosphatases, these ser–thr phosphatases are a structurally diverse group of monomeric proteins that are activated in response to magnesium or manganese. Although, like the PPP phosphatases, these proteins act on serine–threonine residues, they probably evolved independently of other ser–thr phosphatases (Schweighofer et al., 2004). A few of the cell activities regulated by members of this subfamily include spliceosome assembly during pre-mRNA splicing (Ppm1g; Murray et al., 1999), cell cycle (Ppm1a and b; Cheng et al., 2000), glycolysis to tricarboxylic acid cycle transition (PDP; Maj et al., 2006), p38 and JNK signaling (Ppm1a; Takekawa et al., 1998), and TAK1 signaling (Ppm1b; Hanada et al., 2001). Eleven homologues of human PP2Cs were identified: *Sp*Ppm1a/b, *Sp*Ppm1d, *Sp*Ppm1e/f, *Sp*Ppm1g, *Sp*Ppm1h/j/m, *Sp*Ppm1k, *Sp*Ppm1l, *Sp*ILKAP (a and b), *Sp*ILKAPb, *Sp*TAB1, *Sp*TA-PP2C, and *Sp*PDP1/2. No human homologues were found for the PP2C phosphatases SPU_026428 (*Sp*Ppm1h-like), SPU_014625 (*Sp*Ppm1g-likeA), or SPU_004300 (*Sp*Ppm1g-likeB) (Fig. 3B). All of these proteins are expressed during development except *Sp*Ppm1h, *Sp*Ppm1k and *Sp*ILKAP (Table 3).

FCPs: The FCP (T*F*IIF associated *C*TD *p*hosphatase) family is a small group of phosphatases that are highly divergent from other ser–thr phosphatases except that they have the signature motif of phosphotransferases and phosphohydrolases, DXDX (T/V) (Kamenski et al., 2004). Also, they typically contain 2 conserved regions, the N-terminal FCP1 homology region, which includes the DXDX(T/V) sequence, and the C-terminal BRCT (breast cancer protein-related) domain which binds the CTD domain of RNA polymerase II. By dephosphorylating RNAPII at serine residues, FCP1/CTDP regulates mRNA transcription. One FCP, *Sp*CTDP1, was found in *S. purpuratus*. Based on EST and tiling data, this gene is expressed at low levels during sea urchin development (Table 3).

Overview of the phosphatases found in S. purpuratus

A total of ninety-one phosphatases were identified in this study. Sixty-six are PTPs and 25 are ser–thr phosphatases. Based on tiling and EST data, it is estimated that 79% of the PTPs and 88% of the ser–thr phosphatases are expressed during *S. purpuratus* development. Highest levels of expression were observed in the MKPs, PRLs, Cdc14s, PTENs, PPPs, and PPMs. Genes expressed at moderate levels include the RPTPs (a few), NRPTPs, MKPs, myotubularins (one), EYA, and most of the PPPs and PPMs.

Why are some phosphatases highly expressed during development while others are only weakly expressed? The phosphatases transcribed at moderate to high levels regulate MAPK pathways, cell motility, cell cycle control, growth, transcription, organelle assembly, apoptosis, spliceosomal assembly, PI3K-PBP/ATK signaling, and ion channel activities. (For a detailed summary of the activities of phosphatases expressed at moderate to high levels, see Supplemental Table 8.) Many of these are multimeric proteins. By associating with different regulatory subunits they perform a wide array of cellular functions (Examples:

PP1, PP2A, and PP4). Since they are frequently utilized, it may be advantageous for the cell to express high levels of these proteins. Some of the highly expressed monomeric forms act in commonly utilized signaling cascades or signal transduction pathways (for example MKPs in MAPK signaling cascades or PTPN6/11 in PTK/Ras signal transduction). Others perform critical functions associated with morphogenesis such as regulating the actin cytoskeleton, cell division, and gene transcription.

Many phosphatases, such as the RPTPs and the myotubularins, are weakly expressed or not present at all. Why are they present at such low levels? In some cases, these proteins may perform specialized developmental tasks that are limited to a small group of cells or only occur for a short period of time. Alternately, these proteins may be exceptionally efficient in their roles or they may be more important in the adult than the embryo. Those that are never expressed may be pseudogenes.

It was common to find that phosphatases in the human genome were absent in *S. purpuratus*. There may be a number of reasons for this. First, gene duplication was common in chordate evolution. Since any common ancestor shared between *S. purpuratus* and humans would be basal to the phylum Chordata, it is not surprising that a single *S. purpuratus* homologue is often found for multiple vertebrate genes (Example: *Sp*MTMR6 is homologous to *Hs*MTMRs 6, 7, and 8).

Other genes missing in the *S. purpuratus* genome may be present, but were not identified in this study. Although every effort was made to be thorough and comprehensive, we have been conservative in the identification of genes. If two sequences were highly similar (95–100% identical) and possibly overlapped, they were identified as a single sequence. Also, several sequences had to be omitted because they were too incomplete for analysis, improperly assembled in the genome, improperly identified by software, or had other problems associated with this sort of *in silico* analysis.

Homologues may also be missing because, in some cases, two genes with similar functions could have evolved in the urchin. In this situation, loss of the gene homologous to that in humans would not have been detrimental. Other genes may be absent in the sea urchin because they were unnecessary. Potential candidates include PTPRA, PTPRE, PTPRC, PTPN20, DUSP13, CDC14B, or MTM1. Even though these genes are expressed in members of both the protostomes and other deuterostomes, they are absent in *S. purpuratus* (or they just were not detected).

Sea urchin specific RPTPs were found using domain analysis

Most of the *S. purpuratus* RPTPs identified in this study have no obvious homologues. One way to better characterize these novel proteins is by domain analysis. As mentioned before, extracellular domains commonly found in RPTPs include the fibronectin type III (FN3), immunoglobulin-like (Ig), carbonic anhydrase-like (CA), and Meprin/A5/μ (MAM) domains as well as RDGS adhesion recognition motifs and heavily glycosylated regions (Andersen et al., 2005). Domain prediction programs detected six extracellular domain types in *S. purpuratus*: FN3, Ig, EGF, MAM, Lectin C (CLECT), and scavenger receptor cysteine-rich (SRCR) domains. Sequences corresponding to MBOAT, RVT, and xanthine uracil permease (Xan_ur_permease) were also predicted, but these are probably from neighboring genes or, in the case of RVT, an error in contig assembly (Raw data presented in Suppl 5 and 6). As in other invertebrates, extracellular domain structure did not necessarily correlate to similarity in catalytic domain structure (probably because evolution of extracellular regions is less constrained than that of PTPc domains).

Although *S. purpuratus* RPTPs were seldom identical to their homologues, they do share some structural similarities (Fig. 4). Like the Type R2A human homologues, *Sp*PTPR D/F/S has two PTPc domains, a series of FN3 repeats and Ig domains (although only 2, not 3 were found). Also, *Sp*PTP69D-like (a Type 9 RPTP) is structurally similar to *Dm*PTP69D. Both have Ig and FN3 domains as well as two catalytic domains. They differ in that *Sp*PTP69D only has one Ig and Fn3 domain while *Dm*PTP69D has two of each. The RPTP most similar to its homologue is *Sp*PTPR G/Z. This Type R5 RPTP is nearly identical to PTP99A, a *Drosophila* R5 RPTP.

One gene, *Sp*PTPR D/F/S, was previously identified in *S. purpuratus.* Using PCR primers designed to conserved portions of the PTPc domain, Wessel et al. (1995) identified seven *S. purpuratus* RPTPs and deduced partial amino acid sequences (83–85 aa in length) for these (6 of these sequences correspond to those in this study). *Sp*PTPR D/F/S appears to be *Sp*PTPSp108. Using Rnase protection assays, they also found that this gene is expressed at moderately high levels in ovary, egg, blastula, and gastrula stages and that expression drops slightly at the pluteus stage. Thus, *Sp*PTPR D/F/S may be a good candidate for developmental studies, where, like *Hs*PTPRF it may help regulate junctional complexes.

Domain structure of another identified homologue, *Sp*PTPRR (a Type R7 RPTP), remains a mystery. The *Sp*PTPRR sequence is incomplete, containing only the PTPc domain. Since this sequence is at the end of the scaffold and no extracellular sequence has been found, the suggested *Sp*PTPRR structure (Fig. 4) was based solely on known conformations of other PTPRR genes.

In the phylogenetic analysis, four *S. purpuratus* RPTP Type R3-like genes were identified: *Sp*PTPRH-like 1, *Sp*PTPRH-like2, *Sp*PTPRscav, and *Sp*PTPRO-like (Fig. 4, Suppl. 5 and 6). These probably correspond to four of the RPTPs identified by Wessel et al. (1995): PTPSp11 (PTPRH-like1), PTPSp135 (PTPRH-like2), PTPSp111 (PTPRscav), and PTPSp107 (PTPRO-like). Two of these proteins, PTPRH-like1 and PTPRH-like2, are structurally similar to human Type R3 PTPRs, with only one PTPc domain and a series of extracellular FN3 repeats. Structure of PTPRO-like was unclear because the sequence was incomplete. Wessel et al. determined that PTPRH-like2 is expressed at higher levels than PTPRH-like1 and that, in both, highest expression occurs during gastrulation (20× that in the egg or blastula). Based on this, PTPRH-like1 and 2 may act during gastrulation. Highest levels of PTPRO-like mRNA were observed in the ovary and egg, and expression decreased to half this level in the blastula, gastrula, and pluteus stages.

The other *S. purpuratus* Type R3-like gene is PTPRscav (*Sp*PTPSp111 in Wessel et al., 1995). Two *Sp*PTPRscav sequences are present in the neighbor-joining tree, but these are actually overlapping pieces of the same protein. The fully assembled sequence contains 3 extracellular SRCR repeats and 2 intracellular PTPc domains (most human Type 3 RPTPs have only one) (Fig. 4). Wessel et al. (1995) reported that this gene is expressed throughout development, and that highest mRNA levels are present in the egg (1.5×–2× more than in subsequent developmental stages).

To determine whether potential homologues exist for *Sp*PTPRscav, SMART was queried for proteins containing both PTPc and SRCR domains. No matches were found, suggesting that PTPRscav may be novel. Interestingly, SRCR domains are conserved throughout the animal kingdom, both in soluble and membrane-bound proteins (Sarrias et al., 2004). In mammalian myeloid cells, SRCR domain-containing proteins are thought to facilitate immune responses and, in sea urchins, SRCR domain-containing proteins found in coelomocytes (SpSRCR1 and SpSRCR5) are also presumed to function in the immune response (Pancer et al., 1999).

Other sea urchin SRCR domain-containing proteins, present in sperm, bind an egg jelly peptide (Dangott et al., 1989).

At least 4 unique RPTP groups were found in *S. purpuratus*: Unique group 1, Unique group 2, Unique group 3, and PTPRiz (Fig. 4, Suppl. 5 and 6). Unique group 1, the PTPRLec clade, contains 8 members (SpPTPRLec1–6 and SpPTPRFn1–2). Four of these proteins (*Sp*PTPRLec1, 3, 4 and 5) have 1–2 CLECT domains, 1 FN3 domain (weakly conserved in PTPRLec3 and 4), and 2 PTPc domains (Fig. 4, Supp. 5 and 6). Two proteins in this clade differ in structure (*Sp*PTPRFn1 and *Sp*PTPRFn2), containing 2 FN3 repeats and 2 PTPc domains. Sequences of the remaining members may be incomplete.

To identify potential homologues of the PTPRLec proteins, SMART was queried for proteins that contain both PTPc and CLECT domains. Only one protein was found, *Gallus gallus* PTPRQ isoform 1 precursor (UPI0000448F75, glomerular mesangial cell receptor PTP). This protein, whose function is unknown, contains 1 inactive PTPc domain, 3 VWD domains, 1 CLECT domain, and 23 FN3 repeats. Although it may be distantly related to PTPRLec phosphatases, it bears little resemblance. CLECT domains are found throughout the Metazoa (and in some non-metazoan groups). They generally bind carbohydrate moieties and are typically found in proteins involved in humoral/immune response, cell adhesion, or regulation of endocytosis (Zelensky and Gready, 2005).

The other two sea urchin specific groups, unique groups 2 (*Sp*PTPRY1, *Sp*PTPRY2) and 3 (*Sp*PTPRW1 and *Sp*PTPRW2) are predicted to contain PTPc D1 and D2 domains and FN3 domains. Unique group 2 has at least 1 FN3 domain and Unique group 3 has 4 FN3 domains, 3 spaced close together near the PTPc D1 domain and 1 that is closer to the carboxy terminal. Wessel et al. (1995) identified *Sp*PTPRW1 as *Sp*PTPSp12. This gene was not detectable in the Rnase protection assays, the tiling microarrays, or the EST datasets. If it is expressed, it is probably present at very low levels.

Finally, there is a fourth unique group with only one representative, *Sp*PTPRiz (Fig. 4, Suppl. 5 and 6). This RPTP has a single EGF binding domain at the carboxy-terminal followed by 13 FN3 repeats and 2 PTPc domains.

Further research will be necessary to determine functions of these novel phosphatases, however the domain types observed (FN3, Ig, Clec, EGF, MAM, SRCR) suggest that several of these RPTPs could be involved in cell–cell interactions, cell adhesion, or physiological receptor activity. For more about known and hypothesized roles of RPTP extracellular domains, see Brady-Kalnay and Tonks (1995); Bixby (2001).

Blocking expression of phosphatases interferes with endomesoderm formation

Previous studies of phosphatases in the sea urchin have mainly focused on their roles in fertilization (Kumano et al., 2001; Whalley et al., 1991; Tash et al., 1988; Brokaw, 1987; Murofushi et al., 1986), regulation of the egg cytoskeleton (Tosuji et al., 2000; Asano and Mabuchi, 2001; Henson et al., 2003), and cell cycle control (Hiriyanna et al., 1995; Johnston et al., 1994; Patel and Whitaker, 1991; Arion and Meijer, 1989; Marc et al., 2004; Philipova et al., 2005a,[b]; Tosuji et al., 1992, 2003; Gliksman et al., 1992; Wright and Schatten, 1995). Only a few types of phosphatases have been examined, primarily PP1, PP2A, DUSP1/2, or calcineurin, although, as mentioned above, Wessel et al. (1995) cloned and examined expression of several urchin-specific RPTPs. One reason so little is known is that most studies relied on chemical inhibitors that affect specific phosphatase groups. For example, sodium orthovanadate and other vanadate inhibitors were used to block PTP activity whereas okadaic acid or calyculin were used to block type1 and type 2A ser–thr phosphatases.

Inhibitor experiments must be cautiously evaluated. Sometimes these inhibitors affect proteins other than those of interest. For example, when okadaic acid and calyculin were first used, investigators thought that these would allow one to distinguish PP1 from PP2A. Okadaic acid is ~100 times more efficient at blocking PP2A activity than PP1 activity, but calyculin blocks both equally. When calyculin inhibited a reaction, but okadaic acid did not, investigators originally concluded that PP1 activity was responsible. Alternately, when calyculin and okadaic acid both blocked a reaction, they concluded that PP2A was acting. As new PPP phosphatases, also sensitive to okadaic acid and calyculin, were discovered, it became clear that the “PP2A” effects might instead be PP4, PP5, and/or PP6 effects. The same thing has happened with sodium orthovanadate. It blocks PTPs, but can also inhibit alkaline phosphatases, acid phosphatases, and aryl sulfatases.

With a few exceptions (Cervello et al., 1999; Wessel et al., 1995), studies of phosphatase activity in the sea urchin have not looked at developmental events following first cleavage. These events are of particular interest to those studying sea urchin development. In recent years, a primary objective of the sea urchin research community has been to understand interactions between genes regulating endomesoderm specification. To better understand these relationships, the sea urchin gene regulatory network was developed (http://sugp.caltech.edu/endomes/). Although much has been learned about signal transduction pathways and transcription factors acting in endomesoderm formation, very little is known about the phosphatases and kinases that regulate these pathways.

To assess roles of the identified urchin phosphatases in endomesoderm formation, a literature review of phosphatase functions in model organisms was superimposed on sea urchin signal transduction pathways (Table 5). PTP and ser–thr phosphatases regulate or are influenced by a number of endomesoderm network genes including *Ets*, *Alx1*, *Gcm*, *Snail*, *Brachyury*, and *Hox11/13b*. Signaling pathways necessary for endomesoderm formation that are regulated by phosphatases include the canonical Wnt, non-canonical Wnt, Delta/Notch, EGF, FGF, PDGF, and VEGF pathways. Table 5 gives examples of phosphatases that may influence endomesoderm formation in the urchin. Based on this compilation, the following sea urchin phosphatases are hypothesized to affect endomesoderm formation: PP1 alpha/beta/gamma, PP1 beta, PP1-like, PP2A alpha/beta, PP4c, PP2B (calcineurin), PTPR D/F/S, PTPRR, PTPN 1/2, PTPN 13, PTPN 14/21, DUSP 6/7/9, PTENa, PTENb, and/or CDC25 A/B/C. Undoubtedly, there are many additional phosphatases critical for endomesoderm specification. For example, phosphatases that influence cell motility (such as PTP4A1) are surely critical for ingression of primary mesenchyme cells and invagination of the archenteron, and those that regulate gene transcription (such as EYA or Ctdp) are likely to be utilized in the production of proteins needed for endomesoderm cell specification and differentiation.

What functional data are available concerning the roles of phosphatases in later embryonic development? In one of the only studies looking at roles of phosphatases after first cleavage, Cervello et al. (1999) used chemical inhibitors to block PTPs and ser–thr phosphatases in *Paracentrotus lividus*. They used sodium orthovanadate to block PTPs, and okadaic acid to block ser–thr phosphatases. In these experiments, embryos exposed to 0.5–1 mM sodium orthovanadate between the hatching blastula and early gastrula stages formed smaller spicules or completely failed to undergo skeletogenesis. They expressed lower levels of type I fibrillar collagen. If, instead, embryos were exposed to 20–40 nM okadaic acid, development proceeded as it normally would, leading the authors to conclude that PTPs regulate micromere differentiation and skeletogenesis in the sea urchin, but that ser–thr phosphatases are not involved.

As we mentioned earlier, much more is known about the effects of okadaic acid now than was known 7 years ago. This inhibitor blocks PP1, PP2A, PP4, PP5, and PP6, but not PP2B, PP7, or PPMs. Also, it is ~100× less efficient at blocking PP1 than the type 2A ser–thr phosphatases. Thus, it is entirely possible that ser–thr phosphatases unaffected by okadaic acid could act in skeletogenesis.

The recent availability of *S. purpuratus* EST and genomic data have made it much easier to clone individual genes and look at the effects of overexpressing phosphatases or selectively blocking activity by injecting MASOs (a much more specific means of blocking phosphatase activity). To screen for phosphatases that have specific developmental functions in the embryo, a panel of morpholino antisense oligonucleotides (MASOs) targeted to fourteen different developmentally expressed phosphatases was developed (Suppl 7). The MASOs were each injected at three different concentrations into zygotes, and the morphology of the injected embryos was scored at 24, 48, and 96 h post-fertilization (when control embryos were mesenchyme blastula, late gastrula, and pluteus stages, respectively). Five of the fourteen MASOs (PP4c, PP6c, Ppm1a, Ppm1g, and Ptpn9) did not produce consistently obvious abnormalities at any of the doses used. Two MASOs (PP1 alpha and DUSP10) caused cleavage-stage arrest and/or lethality at all doses (data not shown). The remaining seven (PP1 beta, PP2A alpha/beta, PPEF2, Ppm1d, PTP4A 1/2/3, PTPN 1/2, and DUSP 1/2/4/5) are necessary for skeletogenesis, either for patterning or for formation of spicules (Table 4 and Fig. 5). Based on this it is likely that ser–thr phosphatases as well as PTPs affect spiculogenesis. Several phosphatases may also influence formation of the gut. In five cases, PP2A alpha/beta, PPEF2, Ppm1d, PTP4A 1/2/3, and DUSP 1/2/4/5, formation of the endoderm was abnormal or absent. While further controls are necessary both to determine the efficacy of each MASO in depleting its target phosphatase and whether the observed morphological defects are specifically caused by depletion of that phosphatase, results of this preliminary study refute the conclusions of Cervello et al. (1999) that ser–thr phosphatases are unimportant in skeletogenesis and suggest that these phosphatases may indeed play critical roles in endomesoderm formation, warranting further investigation.

One interesting result of the MASO study was that PP2A alpha/beta, a phosphatase blocked by okadaic acid, lacked skeletogenic or gastrulation defects in the Cervello study, but exhibited these defects in response to MASO injection. A possible explanation for this is that the okadaic acid concentration may have been too low to see a noticeable effect. Many phosphatases overlap in function with other phosphatases. This redundancy is an important means of protecting the embryo during development. If one phosphatase is lost, development proceeds as it should because “backup” phosphatases allow the animal to regulate. In this case, PP2A alpha/beta may dephosphorylate the same protein as a second phosphatase. If levels of PP2A alpha/beta are decreased, the “backup” phosphatase may dephosphorylate well enough for development to proceed “normally.” If PP2A alpha/beta expression is completely abolished, however, the “backup” phosphatase may not be able to compensate and development is grossly disrupted. These sorts of functional redundancies are commonly encountered in phosphatase studies (Table 5).

It is also notable that the phenotypes observed for PP2A alpha/beta, PP1 alpha, PP1 beta, and some of the other multifunctional phosphatases are probably not the result of blocking a single cellular process, but are caused by blocking several. Very careful studies blocking these multifunctional phosphatases in limited regions of the embryo or for limited durations combined with knowledge of their functions in other animals will be useful to learn more about specific functions of these phosphatases in endomesoderm formation.

Conclusions

Numerous PTP and ser–thr phosphatases were detected in the *S. purpuratus* genome. In general, most phosphatases have been conserved although, as expected, a single phosphatase in *S. purpuratus* is often homologous to several phosphatases in chordates. One group, the RPTPs, is an exception. Only a few sea urchin RPTPs were homologous to chordate RPTPs and several sea urchin-specific clades were identified. At least one of these (the PTPRLec clade, Unique group 1) probably arose by lineage specific expansion.

Learning more about roles of phosphatases and kinases (see Bradham et al., 2006) during sea urchin development will be critical to understanding how endomesoderm formation and other developmental processes are regulated. Also, much remains to be learned about general roles of phosphatases. Little information is available for the atypical DSPs, and many of the RPTPs. Among the ser–thr phosphatases, detailed studies of PP5, PP6, PP7, and the PPMs would be useful.

Availability of the sea urchin genome will greatly facilitate investigation of phosphatases and proteins in echinoderm development. With genomic data it will be much easier to clone individual phosphatases, predict groups that may overlap in function, and block phosphatase activity *in vivo*. The *S. purpuratus* genome also provides an invaluable resource to those studying in deuterostome or metazoan evolution.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.ydbio.2006.08.050.

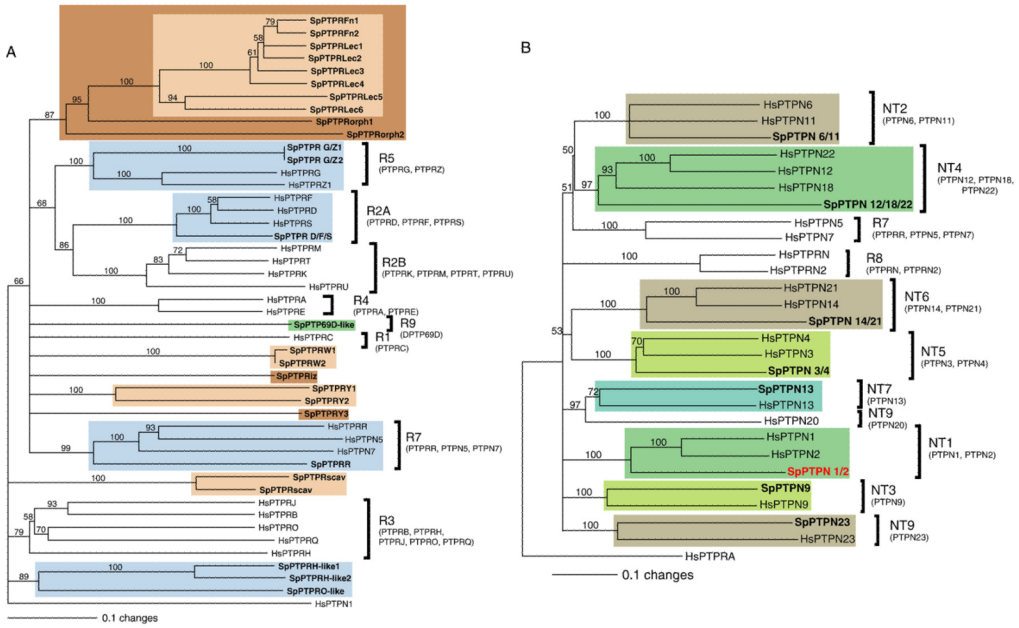

Fig. 1.

A) Phylogenetic relationships of *S. purpuratus* RPTPs to human forms. In this neighbor-joining tree (generated using sequences that align with residues 226–503 of *Hs*PTPRA; outgroup=HsPTPN1), the light blue clades contain *S. purpuratus* homologues of human RPTPs or human-like RPTPs, the green clade contains a *S. purpuratus* RPTP homologous to invertebrate RPTPs, and the tan or brown clades are orphan *S. purpuratus* RPTPs. RPTP subtypes are indicated at right edge. In all trees, *Sp*PTPRG/Z1 and *Sp*PTPRG/Z2 are in the R5 RPTP clade, and *Sp*PTPR D/F/S clusters with the R2A RPTPs. *Sp*PTPRH-like1, *Sp*PTPRH-like2, *Sp*PTPRO-like, and *Sp*PTPRscav group near the R3 subgroup, but are only weakly similar to this subgroup (see Suppl. 1 and 2). Three back blast to PTPRO or PTPRH, but, if they are R3 PTPRs, they are probably divergent. In all 3 trees, *Sp*PTPRR is a member of the R7 clade (PTPRR, PTPN5, and PTPN7). One other phosphatase, *Sp*PTP69D-like, forms a clade with the invertebrate specific Type R9. This is weakly supported and probably divergent. A tree generated using RPTP D2 sequences appears in Suppl. 3. B) Phylogenetic relationships of *S. purpuratus* NRPTPs to human forms. In this neighbor-joining tree (generated using sequences that align with residues 31–279 of *Hs*PTPN1), highlighted clades contain *S. purpuratus* homologues of human NRPTPs. NRPTP and RPTP subtypes are indicated at right edge. In both trees bootstrap values are shown at the dendogram node (maximum=100) and the horizontal distance indicates percent divergence (0.1 scale=10 substitution events per 100 amino acids). MASOs were produced and tested for *Sp*PTPN 1/2, shown in red. Sp=*Strongylocentrotus purpuratus*, Hs=*Homo sapiens*.

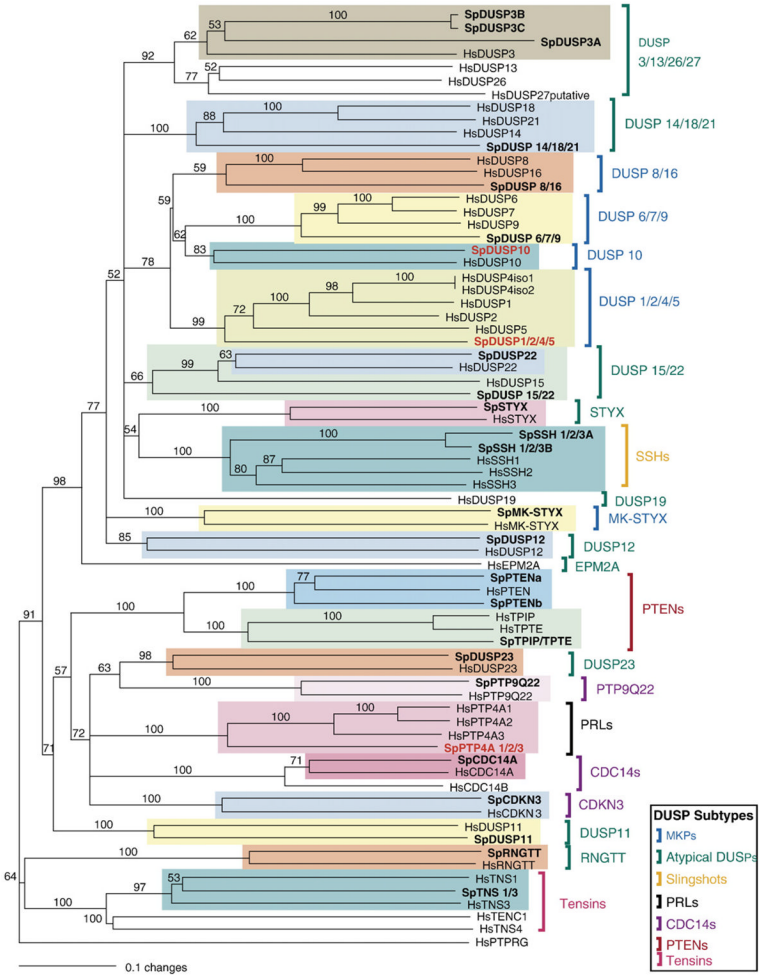

Fig. 2.

Phylogenetic relationships of *S. purpuratus* DSPs to human forms. In this neighbor-joining tree (generated using sequences that align with residues 306–531 of *Hs*DUSP1; outgroup=*Hs*PTPRG), highlighted clades contain *S. purpuratus* homologues of human DSPs. Bootstrap values are shown at the dendogram node (maximum=100) and the horizontal distance indicates percent divergence (0.1 scale=10 substitution events per 100 amino acids). MASOs were produced and tested for sequences shown in red. For relationships of myotubularins, CDC25s, or EYAs, see Suppl. 4A–C. Sp=*Strongylocentrotus purpuratus*, Hs=*Homo sapiens*.

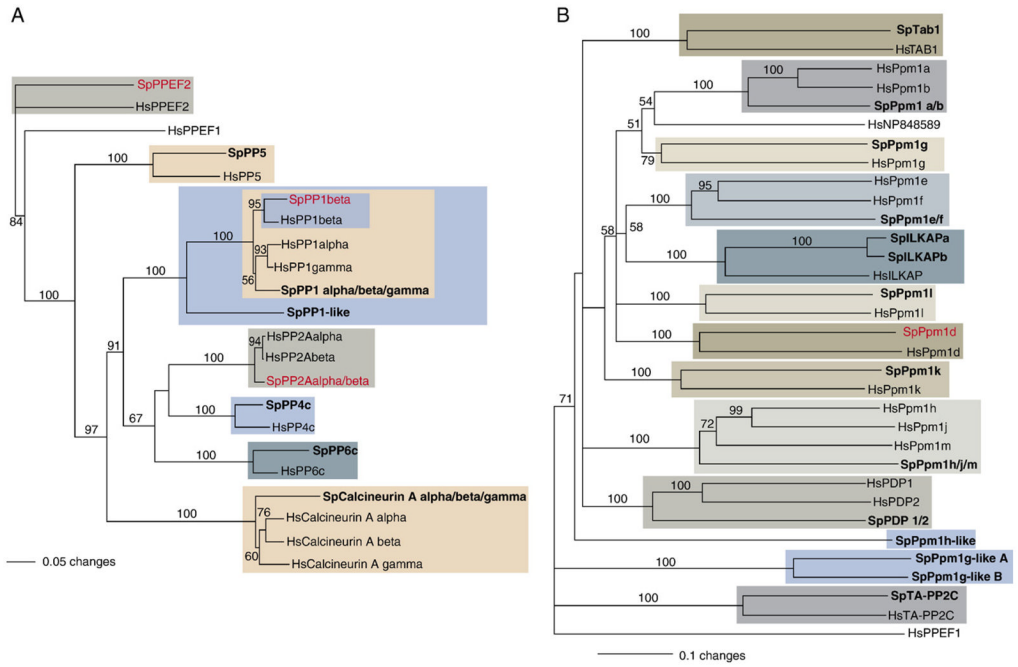

Fig. 3.

(A) Phylogenetic relationships of *S. purpuratus* PPPs to human forms (unrooted neighbor-joining tree generated using sequences that align with residues 59–322 of *Hs*PP1alpha). *S. purpuratus* homologues to human PPPs were found for all subfamilies. Clades containing these homologues are highlighted. (B) Phylogenetic relationships of *S. purpuratus* PPMs to human forms (rooted neighbor-joining tree generated using sequences that align with residues 60–343 of *Hs*Ppm1d; outgroup=*Hs*PPEF1). Sequences highlighted in blue are novel PPMs. Other highlighted sequences are *S. purpuratus* proteins with human homologues. In both trees, bootstrap values are shown at the dendogram node (maximum=100) and the horizontal distance indicates percent divergence (0.1 scale=10 substitution events per 100 amino acids). MASOs were produced and tested for sequences shown in red. Sp=*Strongylocentrotus purpuratus*, Hs=*Homo sapiens*.

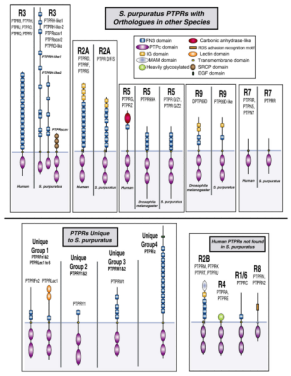

Fig. 4.

Hypothesized domain structures of *S. purpuratus* RPTP homologues compared to known human and *Drosophila* RPTPs. Predictions were based on: (A) results of Pfam, NCBI, and SMART domain searches, (B) known domain structures of human, *Drosophila*, and *C. elegans* RPTPs, (C) comparison of partial *S. purpuratus* sequences from the same clade, and (D) use of the modified *S. purpuratus* sequences described in methods. PTPRH-like2 contains 5 to 11 FN3 repeats, while PTPH-like 1 has 10–28 FN3 repeats (5 and 10 domains are shown based on the assumption that *e*-values less than 10^{-3} are insignificant). The only portion of *Sp*PTPRR that was found was the PTPc domain. Structure of this protein was postulated based on PTPRR proteins in other species. Transmembrane domains were identified in 15 of 25 *S. purpuratus* RPTPs (all except *Sp*PTPR D/F/S, G/Z1, H-like2, Lec3, Lec6, orph1, R, W1, W2, and Y2). Several sequences lacking transmembrane domains were partial. Since software sometimes misses sequences, transmembrane domains have been drawn here in known or expected positions. Human and *Drosophila* structures were redrawn from figures on the Novo Nordisk Science protein tyrosine phosphatase database (http://ptp.cshl.edu or http://science.novonordisk.com/ptp). RPTP domain structure raw data are presented in Suppl. 5 and 6.

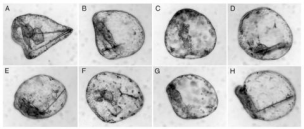

Fig. 5.

Examples of 4- to 5-day larval phenotypes obtained by injecting zygotes with the following morpholino antisense oligonucleotide (MASOs): (A) non-specific control MASO (normal pluteus); (B) anti-PP1 beta; (C) anti-PP2A alpha/beta; (D) anti-Ppm1d; (E) anti-PPEF2; (F) anti-PTP4A 1/2/3; (G) anti-PTPN 1/2; and (H) anti-DUSP 1/2/4/5. All embryos are oriented more or less the same, with the oral side toward the left (except in panel C, which is radialized), and the anal side down.

Table 1

A survey of the Class I Cysteine-based conventional PTPs in the *S. purpuratus* genome and their predicted expression based on tiling and EST data

Category	PTP gene	Alternative names	Urchin gene	Annotated ID	Tiling data	EST data
PTPs						
A. Class I Cys-Based PTPs						
A. 1. Conventional PTPs						
A. 1.1. Receptor PTPs						
R1/6	PTPRC	CD45, LCA	None			
R2A	PTPRD	RPTP-delta	PTPR D/F/S	SPU_020050	Moderate (11.6)	No
	PTPRF	LAR				
	PTPRS	RPTP-sigma, PTPNU3, CRYPalpha				
R2B	PTPRK	RPTP-kappa	None			
	PTPRM	RPTP-mu				
	PTPRT	RPTP-rho				
	PTPRU	PTP-psi, PTP-lambda, PTP-omicron				
R3	PTPRB	PTP-beta	PTPRH-like1	SPU_016937	Low (6.6)	No
	PTPRH	SAP 1, PTPBEM2	PTPRH-like2	SPU_028717	Very Low (4.8)	No
	PTPRJ	CD148; DEP1; RPTP-eta; PTPBYP	PTPRscav	SPU_004193/005860	Very Low (4.5)	No
	PTPRO	PTP-phi, PTP-BK, PTPBEM1, PTPoc, PTPcryp2	PTPRO-like	SPU_001461	Very Low (3.8)	Yes
	PTPRQ	PTPS31, PTPGMC				
	PTPRV	OST-PTP, Esp, OST				
R4	PTPRA	RPTP-alpha	None			
	PTPRE	RPTP-epsilon				
R5	PTPRG	RPTP-gamma	PTPR G/Z1	SPU_011335	No	No
	PTPRZ	RPTP-zeta	PTPR G/Z2	SPU_021599	Moderate (12.4)	Yes
	DmPTP99A					
R7	PTPRR	PC12-PTP1, PCPTP, PCPTP1, PTPBR7, PTP-SL	PTPRR	SPU_008670	Very Low (4.4)	No
	PTPN5	STEP, STEP61				
	PTPN7	HePTP, LCPTP, BPTP-4, C920001D21Rik				
R8	PTPRN	IA-2, Islet cell antigen 512	None			
	PTPRN2	IA-2β, Phogrin, PTPRP, RPTP-pi, PTPNP, acPTPIA2beta				
R9	DmPTP69D-like		PTP69D-likc	SPU_027101	Low (5.7)	No

Category	PTP gene	Alternative names	Urchin gene	Annotated ID	Tiling data	EST data
Urchin Specific Group 1			PTPRFn1	SPU_016143/016144	Very Low (4.3)	Yes
			PTPRFn2	SPU_027014/003581*	No	Yes
			PTPRLec1	SPU_019852	No	No
			PTPRLec2	SPU_022839	Very Low (3.4)	Yes
			PTPRLec3	SPU_020604	Very Low (3.9)	Yes
			PTPRLec4	SPU_016053	No	No
			PTPRLec5	SPU_023162	No	No
			PTPRLec6	SPU_024820	No	No
Urchin Specific Group 2			PTPRY1	SPU_015923	Very low (4)	No
			PTPRY2	SPU_020542	Low (6.12)	No
Urchin Specific Group 3			PTPRW1	SPU_027290/025766*	No	No
			PTPRW2	SPU_024074	No	No
Miscellaneous Urchin RPTPs			PTPRiz	SPU_023115	Very Low (3.3)	Yes
			PTPRorph1	SPU_016411	Low (6.5)	Yes
			PTPRorph2	SPU_008466	Moderate (12.1)	No
			PTPRY3	SPU_028575	Very Low (3.2)	Yes
A. 1.2. Non-receptor PTPs						
NT1	PTPN1	PTPIB	PTPN1/2	SPU_020281	Moderate (23)	Yes
	PTPN2	MPTP, PTP-S, TCPTP				
NT2	PTPN6	HCP, PTP1C, SHP1, SH-PTP1	PTPN6/11	SPU_013810	Moderate (14.6)	Yes
	PTPN11	PTP1D, PTP2C, SH-PTP3, SHP2, SH-PTP2, Syp				
NT3	PTPN9	PTP-MEG2	PTPN9	SPU_019984/000776	Moderate (21)	Yes
NT4	PTPN12	PTPG1, PTP-P19, PTP-PEST, RKPTP	PTPN 12/18/22	SPU_019920	Moderate (16)	No
	PTPN18	BDP, PTP20, PTP-HSCF, PTPK1				
	PTPN22	LYP, LyPTP, PEP				
NT5	PTPN3	PTPH1	PTPN3/4	SPU_005885	Low (7)	No
	PTPN4	PTP-MEG1, TEP, PTPtep				
NT6	PTPN14	PEZ, PTP36, PTPD2	PTPN 14/21	SPU_028592	Moderate (15.9)	Yes
	PTPN21	PTP2E, PTPD1, PTP-RL10				
NT7	PTPN13	FAP-1, PTP1E, PTP-BAS, PTP-BL, PTPL 1, RIP, PTPBA14	PTPN13	SPU_022501	Moderate (15)	No
NT8	PTPN20	TypPTP; PTPTyp	None			

Category	PTP gene	Alternative names	Urchin gene	Annotated ID	Tiling data	EST data
NT9	PTPN23	DKFZP564F0923, HD-PTP, HDPTP, KIAA 1471, PTP-TD14	PTPN23	SPU_007282	Moderate (10.8)	No

Human PTPs and a few *Drosophila* PTPs (indicated by Dm) are shown under “PTP Gene” and *S. purpuratus* homologues or novel genes appear under “Urchin Gene”. Expression data are presented as well. Yellow highlighted genes are novel. Blue areas indicated genes that are homologous or very similar to human genes. MASOs shown in red block development. *Note*. Comparison of exon/intron boundaries suggests that none of the duplicate forms are haplotype pairs.

* Second domain is hypothesized based on A) clade formed in D2 tree, B) found on the same DNA strand, C) orientation of sequences at ends of scaffolds.

Table 2

A survey of the Class I Cysteine-based DSPs, Class II Cysteine-based PTPs, Class III Cysteine-based PTPs, and Asp-based PTPs in the *S. purpuratus* genome and their predicted expression based on tiling and EST data

Category	PTP gene	Alternative names	Urchin gene	Annotated ID	Tiling data	EST data
PTPs						
A. Class I Cys-Based PTPs						
A. 2. DSPs or VH1-like						
A.2. 1.MKPs						
	DUSP1	3CH134, CL100/HVH1, Erp, MKP-1, PTPN10	DUSP 1/2/4/5	SPU_021143	Moderate (21.5)	Yes
	DUSP2	PAC-1				
	DUSP4	hVH2/TYP, MKP-2				
	DUSP5	hVH3/B23				
	DUSP6	MKP-3/rVH6, PYST1	DUSP 6/7/9	SPU_028124	High (37.7)	Yes
	DUSP7	B59; MKP-X, PYST2				
	DUSP9	MKP-4, PYST3				
	DUSP8	HB5, hVH5, M3/6	DUSP 8/16	SPU_003348	Moderate (17.3)	Yes
	DUSP16	MKP-7, MKP-M				
	DUSP10	MKP-5	DUSP10	SPU_006513	High (49.7)	Yes
	MK-STYX		MK-STYX/DUSP24	SPU_000663	Low (5.4)	No
A.2. 2. Atypical DSPs						
	DUSP3	VHR; T-DSP11	DUSP3A	SPU_016669	No	No
			DUSP3B (Tandem Dupl)	SPU_016406	No	No
			DUSP3C (Tandem Dupl)	SPU_016409	No	No
	DUSP11	PIR1	DUSP11	SPU_001665	No	Yes
	DUSP12	G KAP; HYVH1; LMV-DSP4; YVH1	DUSP12	SPU_003149	No	Yes
	DUSP13A	BEDP	None			
	DUSP13B	TMDP; TS-DSP6				
	DUSP26	VHP; similar to RIKEN cDNA 0710001B24				
	DUSP27	DUPD1; FMDSP; similar to cyclophilin				
	DUSP14	MKP6; MKP-L	DUSP 14/18/21	SPU_000652	Very Low (3.1)	Yes
	DUSP18	DUSP20; LMW-DSP20				
	DUSP21	BJ-HCC-26; LMW-DSP21; tumor antigen				

Category	PTP gene	Alternative names	Urchin gene	Annotated ID	Tiling data	EST data
	DUSP15	Q9H1R2; VHY	DUSP 15/22A	SPU_013773	No	No
	DUSP22	JKAP; JSPI; LMW-DSP2: MKPX; TS-DSP2; VHX	DUSP 15/22B	SPU_022023	Low (8.1)	No
	DUSP19	DUSP17; LDP-2; SKRP1; TS-DSP1	None			
	DUSP23	MOSP; similar to RIKEN cDNA 2810004N20	DUSP23	SPU_004533	No	No
	DUSP24	MGC1136	MK-STYX/DUSP24	SPU_000663/018623	Low (5.4)	No
	DUSP25*	FLJ20442; LMW-DSP3; VHZ				
	EPM2A	Laforin	None			
	RNGTT	mRNA capping enzyme	RNGTT	SPU_008892	No	Yes
	STYX		STYX	SPU_011259	No	No
A. 2. 3. Slingshots						
	SSH1	Slingshot 1	SSH 1/2/3A	SPU_002366	Very Low (3.1)	Yes
	SSH2	Slingshot 2	SSH 1/2/3B	SPU_003158	Low (8.2)	Yes
	SSH3	Slingshot 3	None			
A. 2. 4. PRLs						
	PTP4A1	PRL-1	PTP4A 1/2/3	SPU_008895	High (31.4)	Yes
	PTP4A2	OV-1; PRL-2				
	PTP4A3	PRL-3				
A. 2. 5. CDC14s						
	CDC14A		CDC14A	SPU_015765	High (63.4)	Yes
	CDC14B					
	CDKN3	KAP	CDKN3	SPU_003479	Low (5.5)	Yes
	PTP9Q22		PTP9Q22	SPU_012073	Low (7.0)	No
A. 2. 6. PTENs						
	PTEN	MMAC1; TEP1	PTENa	SPU_009522	High (33.4)	Yes
			PTENb	SPU_023882/003142	Low (7)	No
	TPIP	TPIPα, TPTE and PTEN homologous	TPTE/TPIP	SPU_002928	No	No
	TPTE	PTEN-like; PTEN2				
A. 2. 7. Tensins						
	TNS1	Tensin 1	TNS 1/3	SPU_005602	Low (7.4)	Yes
	TNS3	Tensin 3				
	TNS4	Tensin 4				

Category	PTP gene	Alternative names	Urchin gene	Annotated ID	Tiling data	EST data
	TENCI	Tensin like C1 domain containing phoshatase, Tensin 2				
A. 2. 8. Myotubularins						
	MTM1	Myotubularin	None			
	MTMR1		MTMR 1/2	SPU_008287	Very Low (3.3)	No
	MTMR2					
	MTMR3	FYVE-DSPI	MTMR 3/4	SPU_024752	Moderate (11)	Yes
	MTMR4	FYVE-DSP2				
	MTMR5	SBF1	MTMR 5/13	SPU_005099	Low (7.7)	No
	MTMR13	CMT4B2; SBF2				
	MTMR6		MTMR 6/7/8	SPU_022669	No	Yes
	MTMR7					
	MTMR8					
	MTMR9	LIP-STYX	MTMR9	SPU_021537/018586	Low (5.5)	No
	MTMR10		None			
	MTMR11	CRAα/β				
	MTMR12	3-PAP				
	MTMR14*	hEDTP; hJumpy; FLJ22075	None			
	MTMR15*	KIAA1018	None			
B. Class 11 Cys-Based PTPs						
	ACP1	BHPTP; LMPTP; low Mr PTP; LMWPTP	None			
C. Class III Cys-Based PTPs						
	CDC25A		CDC25 A/B/C	SPU_019568	Low (5.4)	Yes
	CDC25B					
	CDC25C					
D. Asp-Based PTPs						
	EYA1		EYA 1/2/4	SPU_013869	Moderate (14.4)	No
	EYA2					
	EYA4					

Human PTPs are shown under “PTP Gene” and *S. purpuratus* homologues or novel genes appear under “Urchin Gene”. MASOs were produced for genes shown in red. No novel genes were detected. Morpholino blocks development. Note: Comparison of exon/intron boundaries in duplicate forms suggests that none of these are haplotype pairs.

*Sequence not available so not in orthologue analysis.

Table 3

A survey of the serine–threonine phosphatases in the *S. purpuratus* genome and predicted expression based on tiling and EST data

Category	Gene	Alternative names	Urchin gene	Annotated ID	Tiling data	EST data
PPPs						
A. PP1 Subfamily						
A. 1. PP1	PP1c alpha	Ser/thr protein phosphatase I, catalytic subunit alpha;PPP1-A;PP-1a	PP1 alpha/beta/gamma	SPU_000642/011110	Very Low (3.4)	No
	PP1c beta	Ser/thr protein phosphatase I, catalytic subunit beta;PPP1-B;PP-1b	PP1 beta	SPU_001788	Moderate (15.5)	Yes
	PP1c gamma	Ser/thr protein phosphatase I, catalytic subunit gamma				
Urchin Specific PP1			PP1-like	SPU_008700	Moderate (20.5)	Yes
Morpholino PP1			PP1 alpha-Partial seq*	SPU_006956	Moderate (23)	Yes
B. PP2 Subfamily						
B.1. PP2A	PP2A alpha	Ser/thr protein phosphatase 2A, catalytic subunit alpha	PP2A alpha/beta	SPU_025182	High (68.6)	Yes
	PP2A beta	Ser/thr protein phosphatase 2A, catalytic subunit beta				
B.2. PP4	PP4c	Serine/threonine protein phosphatase 4 catalytic subunit; Pp4; PP-X	PP4c	SPU_027953	High (100)	Yes
B.3. PP6	PP6c	Serine/threonine protein phosphatase 6, catalytic subunit; Ppp6c	PP6c	SPU_011983	Low (7.2)	Yes
C. PP5 Subfamily						
C.1. PP5 (PPP5)	PP5c	Serine/threonine protein phosphatase 5, catalytic subunit	PP5c	SPU_028312	Low (6.7)	Yes
D. PP7 Subfamily						
D.1. PP7	PPEF-1	Ser/thr protein phosphatase with EF-hand motifs 1, PP7, PPP7	None			
	PPEF-2	Ser/thr protein phosphatase with EF-hand motifs 2	PPEF-2	SPU_008844/011860/028868	Moderate (10.8)	No
E. PP3 Subfamily						
E.1. PP3 (PP2B)(PPP3)	CalcineurinA α	PPP3A alpha, PP2B A alpha	Calcineurin A alpha/beta/gamma	SPU_018404/016657	Moderate (22.2)	Yes
	CalcineurinA β	PPP3A beta, PP2B A beta				
	CalcineurinA γ	PPP3A gamma, PP2B A gamma				
PPMs						
A. PP2C Subfamily						

Category	Gene	Alternative names	Urchin gene	Annotated ID	Tiling data	EST data
	Ppm1a	Protein phosphatase 1A, PP1A, PP2C alpha	Ppm 1a/b	SPU_009810	Moderate (15)	Yes
	Ppm1b	Protein phosphatase 1B, PP1B, PP2C beta				
	Ppm1d	Protein phosphatase 1D, PP1D	Ppm1d	SPU_020266	High (68.8)	Yes
	Ppm1e	Protein phosphatase 1E, PP1E	Ppm1e	SPU_006378	Very Low (3.9)	Yes
	Ppm1f	Protein phosphatase 1F, PP1F				
	Ppm1g	Protein phosphatase 1G, PP1G	Ppm1g	SPU_011351	Moderate (15)	Yes
	Ppm1k	Protein phosphatase 1K, PP1K	Ppm1k	SPU_011705/017161	No	No
	Ppm1l	Protein phosphatase 1L, PP1L	Ppm1l	SPU_006132	Low (7.6)	Yes
	ILKAP	Integrin-linked kinase-associated protein phosphatase 2C	ILKAPa	SPU_011070	No	No
			ILKAPb	SPU_011294	No	No
	Ppm1h	Protein phosphatase 1H	Ppm1h/j/m	SPU_007363	No	No
	Ppm1j	Protein phosphatase 1J, PP1J				
	Ppm1m	Protein phosphatase 1M, PP1M				
	PDP1	Pyruvate dehydrogenase phosphatase 1	PDP1/2	SPU_025951	Moderate (21.5)	Yes
	PDP2	Pyruvate dehydrogenase phosphatase 2				
	TA-PP2C	T-cell activation protein phosphatase 2C	TA-PP2C	SPU_017346	Moderate (28.1)	No
	Tab1	Map3k7ip1;MAP kinase kinase kinase 7 interacting protein 1	Tab 1	SPU_005254	Very Low (4.0)	Yes
	Novel Group 1		Ppm1g-like A	SPU_014625	Moderate (22.4)	No
			Ppm1g-like B	SPU_004300	Moderate (17.3)	Yes
	Novel Protein 2		Ppm1h-like	SPU_026428	Very Low (3.9)	No
FCPs						
A. FCP						
	Ctdp1	Carboxy Terminal Domain, RNA Pol II, polypeptide A, phosphatase	Ctdp1*	SPU_021493	Low (5.7)	Yes

Human PTPs are shown under “PTP Gene” and *S. purpuratus* homologues or novel genes appear under “Urchin Gene”. Yellow highlighted genes are novel. Blue areas indicated genes that are homologous or very similar to human genes. MASOs produced for genes shown in red block development. *Note*. Comparison of the exon/intron boundaries of Ppm1g-like A and B suggests that these are not haplotype pairs.

*Sequence unconfirmed by phylogenetic analysis.

Table 4

Larval phenotypes obtained with anti-phosphatase MASOs

MASO target	Morphological defects
PP1 alpha (SPU_006956)	Late cleavage/early blastula stage arrest at all doses; cell division defects?
PP1 beta (SPU_001788)	Skeletal patterning defects (short skeletal rods, sometimes crossing or parallel; oral ectoderm defect?) at medium and high doses
PP2A alpha/beta (SPU_025182)	Gastrulation and skeletogenic defects; poorly differentiated/radialized ectoderm at medium and high doses
PPEF2 (SPU_028868)	Endoderm and oral ectoderm defects at low and medium dose; some loss of skeletons at high dose
Ppm1d (SPU_020266)	Nothing obvious at low and medium doses; global differentiation defects at high dose, with small guts and short spicules, rounded ectoderm
PTP4A 1/2/3 (SPU_008895)	Skeletal defects and small gut at medium dose; blastula stage arrest at high dose
PTPN 1/2 (SPU_020281)	Failure of skeletogenesis at low dose (ranging from no spicules, to short spicules or in some cases a single spicule); more general differentiation defects at medium and high doses
DUSP 1/2/4/5 (SPU_021143)	Endoderm and oral ectoderm malformed or diminished at medium dose
DUSP10 (SPU_006513)	Cleavage stage arrest/death at all doses

Table 5

Examples of phosphatases acting in endomeosderm formation

Pathway component	Phosphatase	Organism	Function?	Urchin phospatase	PMID # (Ref)
Canonical Wnt pathway	PP2A	Mammalian cells	Regulation of ß-catenin levels	SPU_028046	9920888
	PP2A	Mammalian cells	Regulation of ß-catenin levels	SPU_028046	10330403
	PP2A	*Xenopus,Danio*	Regulation of ß-catenin levels	SPU_028046	11007767
	PP2A	*Drosophila*	Regulation of ß-catenin levels	SPU_028046, 000178	14966281
	PP2A:B56 subunit	*Xenopus,* Mammal	Regulation of ß-catenin levels	SPU_028046, 000178	10092233
	PP2A:B56 subunit	*Xenopus*	Regulation of ß-catenin levels	SPU_028046, 000178	14522869
	PP2A:B/PR55 subunit	*Drosophila*	Regulation of ß-catenin levels	SPU_028046	14973271
	PP2A	Mammalian cells	Positive regulator of Wnt signaling interacting with ß-catenin & Dvl3.	SPU_009810*	10644691
Planar Cell Polarity or Ca/Wnt Pathway	FRIED (similar to PTPN13)	*Xenopus*	FRIED binds C-terminal PDZ binding motif of Fz8.	SPU_022501* (PTPN13)	16086323
	PP2A:Widerborst (PP2A B56 epsilon)	*Danio*	Convergent extension & asymmetric localization of Dsh & Fz.	SPU_028046, 000178	12091318
	PP2A:PR72	*Xenopus*	Increasing PR72 decreased Dsh levels.	SPU_028046, 004954*	15687260
	PP2A:PR72	Mammalian cells	Switch from canonical Wnt to PCP pathway.	SPU_028046, 004954*	15687260
	Puckered	*Drosophila*	Negative regulator of JNK activity.	SPU_006513* (DUSP10)	9472024
	Ppm1a (PP2C alpha)	Mammalian cells	Blocks Jnk MAPK cascade	SPU_009810*	9707433
	PTEN	Mammalian cells	Stimulate lipid phosphatase activity of PTEN	SPU_009522, 023882	9707433
	FRIED (similar to PTPN13)	*Xenopus*	Hypothesis: regulation of the actin cytoskeleton through Rac?	SPU_022501* (PTPN13)	16086323
	Ppm1a (PP2C alpha)	Yeast, Mammal	Blocks Jnk MAPK cascade	SPU_009810*	9707433
	PTEN	Mammalian cells	Stimulate lipid phosphatase activity of PTEN	SPU_009522, 023882	9707433
	FRIED (similar to PTPN13)	*Xenopus*	Hypothesis: regulation of the actin cytoskeleton through Rac?	SPU_022501* (PTPN13)	16086323
	Calcineurin	Mammalian cells	Calcineurin necessary for translocation of NFAT to the nucleus.	SPU_018404, 016657	8631904
	Calcineurin	Mammalian cells	Calcineurin necessary for translocation of NFAT to the nucleus.	SPU_018404, 016657	8799126
	Calcineurin	Mammalian cells	Calcineurin necessary for translocation of NFAT to the nucleus.	SPU_018404, 016657	7657645
	Calcineurin	*Xenopus*	Calcineurin necessary for translocation of NFAT to the nucleus.	SPU_018404, 016657	12015605
Notch pathway	String (similar to CDC25)	*Drosophila*	Notch activity represses this phosphatase.	SPU_019568	11731454
	LIP-1 (similar to DUSP6/MKP-3)	*C. elegans*	Inactivates MKP-1, blocking primary cell fate specification.	SPU_028124	11161219
	PP2A	Mammalian cells	Dephosphorylates furin. Transports between Golgi & early endosomes.	SPU_028046	9744873
	PTEN	Mammalian cells	Sites for CBF on PTEN. Notch increases PTEN activity.	SPU_003142	16096376
Hedgehog pathway	PP2A	Mouse	Expression of COUP-TFII mediated by a ser-thr phosphatase	SPU_028046	9395397
	CDC25B	Mouse/chick	CDC25B is transcriptionally upregulated by Shh/Gli.	SPU_019568	16564519
	PTPRD (RPTP delta)	Mammalian cells	MIM and PTPRD remodel the actin cytoskeleton.	SPU_020050	15684034
	Mts (PP2A):Widerborst (PP2A B56 eps)	*Drosophila* cells	Dephosphorylates PKA. May also act on CI.	SPU_028046, 000178	16311596
EGF Pathway	PTPRF	Mammalian cells	Reduces EGFR phosphorylation.	SPU_020050	1645724
	PTPRS	Mammalian cells	Reduces EGFR phosphorylation.	SPU_020050	10435588
	PTPN1 (PTP-1B)	Mammalian cells	Attenuates EGF signaling.	SPU_020281	11872838, 7963660
	PTPN11 (SHP-2)	Mammalian cells	Lateral transduction of EGF signal	SPU_003810	8197172,Gomperts,03
	PTEN	Review	Dephosphorylates PIP3 to form PIP2.	SPU_003142	Gomperts,2003
	PTPRR (PTP-SL)	Mammalian cells	Regulates activation of ERK.	SPU_002840	9857190
	PTPN5 (PTP-STEP)	Mammalian cells	Regulates activation of ERK.	SPU_008670	9857190
	PP2A	Mammalian cells	Inactivates ERK2 by dephosphorylating it.	SPU_028046	8631373
	PP4	Mammalian cells	PP4 required to stabilize JNK-1 activity.	SPU_027953	16238095
FGF Pathway	PTPN11 (SHP-2)	Review	Binds PDGFR to Ras & allows MAPK signaling.	SPU_013810	Gomperts,2003
	DUSP6 (Psyt1)	Chick	Inducible antagonist in FGF pathway. Regulates MAPK.	SPU_028124, 009718	12814546
PDGF Pathway	PTPN11 (SHP-2)	Review	Binds PDGFR to Ras & allows MAPK signaling.	SPU_013810	Gomperts,2003
	PTPN1 (PTP-1B)	Mammalian cells	Attenuates EGF signaling.	SPU_020281	11872838
VEGF Pathway	PTEN	Mammalian cells	VEGF levels decrease in cells transfected with PTEN.	SPU_009522	14748662
	PTPN6 (SHP-1)	Review	Suppresses cellular activity	SPU_013810	16084691
	PTPN11 (SHP-2)	Review	Increases cellular activity (signalling).	SPU_013810	16084691
NF-kappaB	PP4	Review	Regulates this	SPU_027953	15913612
Target of Rapamycin (TOR)	PP4	Review	Regulates this	SPU_027953	15913612
Urchin Endomesodermal Genes					
Ets1	Calcineurin	Mammalian cells	Activates Ets by dephosphorylating it.	SPU_018404, 016657	15123671
Ets1	Unidentified phosphatase	Urchin	Threonine phosphorylation of Ets acts in PMC specification.	Unknown	14973284
Alx1	Unidentified phosphatase	Urchin	Alx1 has PSTP motif, a potential MAPK phosphorylation site.	Unknown	14973284
Gcm	Numerous protein phosphatases	*Drosophila*	Gcm regulates expression of numerous phosphatases.	Not Available	12091301
Snail	String (CDC25)	*Drosophila*	Snail regulates expression of String.	SPU_019568	11731456
Brachyury	Unidentified (AF106702=PTPN3/4)	*Ciona*	Found a PTP that is downstream of Brachyury.	SPU_005885*	10898962
Hox11/13b	PP2A catalytic subunit.	*Xenopus,* Mammal	Interaction observed between Hox11 and PP2Ac.	SPU_028046	9009195, 15897879
Hox11/13b	PP1 alpha/beta/gamma catalytic subunit.	*Xenopus,* Mammal	Interaction observed between Hox11 and PP1c.	SPU_008700, 001788	9009195, 15897879

Based on the predictions of oligonucleotide tiling arrays, the genes in blue are highly expressed, those in peach are expressed at moderate levels, and those in yellow are present at low or very low levels. Genes that are not highlighted are predicted to be absent in developing sea urchins (or present at extremely low levels). MASOs were produced for genes shown in red. *Potentially similar sea urchin sequence.