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# Analysis of cytoskeletal and motility proteins in the sea urchin genome assembly

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**Abstract**

The sea urchin embryo is a classical model system for studying the role of the cytoskeleton in such events as fertilization, mitosis, cleavage, cell migration and gastrulation. We have conducted an analysis of gene models derived from the *Strongylocentrotus purpuratus* genome assembly and have gathered strong evidence for the existence of multiple gene families encoding cytoskeletal proteins and their regulators in sea urchin. While many cytoskeletal genes have been cloned from sea urchin with sequences already existing in public databases, genome analysis reveals a significantly higher degree of diversity within certain gene families. Furthermore, genes are described corresponding to homologs of cytoskeletal proteins not previously documented in sea urchins. To illustrate the varying degree of sequence diversity that exists within cytoskeletal gene families, we conducted an analysis of genes encoding actins, specific actin-binding proteins, myosins, tubulins, kinesins, dyneins, specific microtubule-associated proteins, and intermediate filaments. We conducted ontological analysis of select genes to better understand the relatedness of urchin cytoskeletal genes to those of other deuterostomes. We analyzed developmental expression (EST) data to confirm the existence of select gene models and to understand their differential expression during various stages of early development.


*Keywords:* Sea urchin; Cytoskeleton; Actin; Myosin; Intermediate filament; Tubulin; Microtubule; Microfilament; MAP; Dynein; Kinesin; Motility; Genome

## Introduction

The cytoskeleton is involved in all aspects of cell migration, mitosis and cytokinesis, organelle movements, ciliary and flagellar movements, and other cell shape changes. In developing sea urchin embryos, the cytoskeleton has long been studied by developmental and cell biologists, biochemists and biophysicists. In this early work, researchers used cells of sea urchins to make important and path breaking observations on aspects of cell division (Harvey, 1956; Hiramoto, 1968; Salmon, 1975). Sea urchins have been excellent models for the study of motility of sperm (Brokaw and Gibbons, 1973; Yokota et al., 1987), the proteins responsible for actin-based movements (Begg and Rebhun, 1979; Bryan et al., 1993; Edds, 1980; Fishkind et al., 1987; Kane, 1986; Mabuchi, 1986; Mabuchi and Spudich, 1980; Schroeder, 1968; Tilney and Gibbins, 1969; Tilney et al., 1973), and the proteins of the microtubule-based mitotic apparatus (Cohen and Rebhun, 1970; Kane, 1967; Scholey et al., 1985). In fact, the first dynein cloned and sequenced came from sea urchins (Gibbons et al., 1991; Ogawa, 1991). Moreover, the sea urchin zygote has served as one of the best model systems for the biophysical and biochemical investigation of cytokinesis and mitosis (Mabuchi, 1986; Rappaport, 1996; Salmon, 1975; Sluder, 1979). For example, antibody microinjection has been

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used to analyze the functions of myosin, kinesin-related, and dynein motor proteins in mitosis and cytokinesis in the early sea urchin embryo (Mabuchi and Okuno, 1977; Wright et al., 1993; Strickland et al., 2005). Cells from sea urchins remain just as valuable today as models for the study of mitosis, cytokinesis, ciliary assembly and beat, and cell shape transformation.

Because cells of sea urchins serve as such exquisite models for study of the cytoskeleton, a number of key cytoskeletal proteins were identified first in these cells using biochemical methods and were subsequently cloned and sequenced. The rich diversity of cloned (some partially cloned) and sequenced sea urchin cytoskeletal proteins ranges from actin- and microtubule-based motors (including myosins I, II, V, and X, kinesin-1, -2, -5, and 14-B, and dyneins), actin and associated actin-binding proteins (such as fascin, scruin, spectrin, actin-binding protein, moesin, villin, and dystrophin) and tubulin and various microtubule-binding proteins (such as MAPs). Such a wide variety of cloned cytoskeletal genes provides a rich established database that allows for significant assistance in annotating the sea urchin genome.

In addition, due to the fact that cytoskeletal proteins are so conserved evolutionarily, structurally and functionally, there is ample opportunity for detailed comparative analysis of the genome. Because the cytoskeleton is involved in fundamental cellular functions such as mitosis, cytokinesis, membrane translocations, and cellular motility, the genes encoding many cytoskeletal proteins are amenable to critical evolutionary analysis.

## Materials and methods

Lists of known major cytoskeletal proteins were generated for major classes of functional proteins. Using the common names for cytoskeletal and motility proteins, the category and number of proteins annotated in the sea urchin assembly were: actin (5), actin-binding proteins (36), myosins (12 classes, 29 proteins), intermediate filament proteins (5), dyneins (15 heavy chains, 10 light chains, 4 light intermediate chains, 4 intermediate chains), kinesins (14 families, 45 motors, 2 associated proteins), tubulin (15), microtubule-binding proteins (32), and regulatory proteins (16). Because of the abundance of cytoskeletal and motility proteins, we have focused this analysis on only the commonly known proteins and those previously known to be present in the sea urchin.

### *Determination of homologs from gene predictions*

In order to derive and annotate *Strongylocentrotus purpuratus* cytoskeletal and motility genes, lists of proteins of interest (called 'Indexing Gene Products') were compiled from a combination of literature and database sources. Amino acid sequences corresponding to homologs of these proteins were collected from the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/), EST libraries (Poustka et al., 2003; available at A. Poustka's website, www.molgen.mpg.de/~ag_seaurchin/; and Zhu et al., 2001), the Sea Urchin Genome Project website at the California Institute of Technology (BAC-end sequence: http://sugp.caltech.edu/), and the *Chlamydomonas* Flagellar Proteome Project web page (Pazour et al., 2005: http://labs.umassmed.edu/chlamyfp/index.php). These lists were then used to identify candidate orthologs by interrogating the list of predicted gene products (or 'GLEANs') derived from processing the April 2005 release (build 1.1) of the whole-genome shotgun sequence assembly of the *S. purpuratus* genome using the GLEAN3 algorithm (Sea Urchin Genome Sequencing Consortium, *Science*, in press). In brief, the list of predicted genes was queried with the individual protein sequences from the Indexing Gene Products List using the *S. purpuratus* Genome Search website at the National Institute of Dental and Craniofacial Research Developmental Mechanisms Unit (http://urchin.nidcr.nih.gov/blast/index.html) or the Sea Urchin Project website at the Human Genome Sequencing Center at Baylor College of Medicine (http://www.hgsc.bcm.tmc.edu/projects/seaurchin/) using the TBLASTN algorithm (Altschul et al., 1990). In most cases, TBLASTN returned a table of results consisting of one or more GLEAN gene models that represented 'strong hits' with a length and sequence consistent with the protein query. However, in the cases of myosin, kinesin, and dynein heavy chains, multiple sequence alignments (ClustalW, http://www.ebi.ac.uk/clustalw/) often showed that the strongest hits corresponded to no more than 25–60% of the full-length of the query sequence. In these cases, multiple GLEAN gene models identified by TBLASTN were used to manually extend the sequence of the predicted gene. Whenever possible, gene models from the same scaffold were used to construct full-length gene models and the GLEAN gene identity assigned to the most N-terminal sequence was used to annotate the complete concatenated sequence.

Strategies to fully annotate each of the superfamilies in this study (actins, myosins, tubulins, kinesins, and dyneins) varied somewhat. For myosins, in addition to genes identified by BLAST score alone, a list of GLEANs grouped by SMART and PFAM conserved domains was generated by Hynes and Whittaker which was subsequently made available to our group (http://luria.mit.edu/urchin/) (see this issue). We derived sequences encoding predicted myosin motor domains from this list to corroborate results derived from BLAST searches described above and for conducting phylogenetic analysis described below. For kinesins, a database of 147 candidate kinesin genes was generated by keyword search of NCBI GNOMON predictions and by kinesin motor domain PFAM search of GLEAN3 predictions. Amino acid sequences of all 147 candidates were assembled into a phylogenetic tree alone or with example homologs from human, mouse, *Danio*, *Drosophila*, and *Caenorhabditis elegans*, to narrow down the 147 kinesin-related proteins to a non-redundant set. Kinesin sequences were deemed redundant if they shared 100% identity with another candidate for 95 amino acids or more. GLEAN3 predictions were selected over redundant NCBI predictions if the GLEAN3 prediction was greater than half the length of a partially identical NCBI prediction. For any two GLEAN3 predictions deemed redundant, the sequence with the most complete motor domain was retained. In cases where phylogenetic trees did not unambiguously assign a gene prediction to a kinesin family, genes encoding putative kinesin motor domains were assigned to the most similar kinesin family as determined by BLAST score. For the dyneins, the chain of exons corresponding to each of the 15 heavy chain genes was obtained by mapping the full-length peptide sequence of the corresponding Indexing Gene Product onto the March 2005 scaffolds of the *S. purpuratus* genome by using the BLAT server (Kent, 2002) at the University of California at Santa Cruz Genome Bioinformatics world wide web site (http://genome.ucsc.edu). Approximately 10% of the dynein exons were either too divergent or too short to be obtained in this way and these were obtained by using one of the above Blast servers to perform *in silico* PCR between the neighboring, previously identified exons on each side. The identity of each sea urchin gene model obtained in this way was confirmed by using the BLAT server to map its translated peptide sequence back onto the human genome and verifying that its chromosomal location corresponded to that of the correct indexing gene. Some regions of the dynein genes are highly conserved and particular care was necessary to avoid crossing between genes in these regions. Because of the exceptionally large size of the dynein heavy chain genes, with each gene having 12–14 kb of coding sequence that spanned ~70 kb of genome, the exon chains for the different genes involved from 2 to 5 scaffolds each.

### *Phylogenetic analysis to infer gene homologies*

We used a phylogenetic approach to verify the predicted *Strongylocentrotus* amino acid sequences for the actin, CLIP170, dynein, fascin, gelsolin, kinesin, lamin B, myosin, tubulin, and profilin gene and gene families. For each gene and gene family, known or predicted homologs were recovered from GenBank using a number of strategies. For CLIP170, fascin, gelsolin, lamin B, and profilin, sequences for the following taxa were downloaded from GenBank when genomic data were available: human (*Homo sapiens*), mouse (*Mus musculus*), chicken (*Gallus gallus*), frog (*Xenopus laevis/gilli*), zebrafish (*Danio rario*), nematode (*C. elegans*), fruit fly (*Drosophila melanogaster*), tunicate (*Ciona intestinalis*), and sea anemone (*Nematostella vectensis*). When appropriate, yeast (*Saccharomyces cerevisiae*) sequences were included as an outgroup. Actin sequences were derived from Carlini et al. (2000) and from GenBank.

Myosin sequences were from Hodge and Cope (2000). Kinesin sequences were obtained from Miki et al. (2005). Dynein gene sequences were mostly obtained from GenBank. Additional dynein gene models for Chlamydomonas (Pazour et al., 2006) were obtained from the Chlamydomonas Genome version 3 produced by the US Department of Energy Joint Genome Institute (http://www.jgi.doe.gov/) and are provided for use in this publication only. Additional dynein gene models for Tetrahymena (Asai and Wilkes, 2004) were obtained from the Tetrahymena Genome Database (http://www.ciliate.org) and are based upon sequence determined by the Institute for Genome Research. Within each gene family, efforts were made to include representatives of all subfamily and family members to assure homology of the urchin sequences.

A multiple sequence alignment was performed using the program MAFFT version 5.743 (Katoh et al., 2005) for the CLIP170, fascin, gelsolin, lamin B, and profilin sequences. The program MUSCLE (Edgar, 2004) was used to align the sequences for actin, kinesin, myosin, and tubulin gene families due to the size of the datasets. Full-length sequences of dynein heavy chains were aligned with the program ClustalX (Thompson et al., 1997). In all cases, the resulting multiple sequence alignments were visually inspected and refined by eye using the multiple sequence editor in MacVector (www.accelrys.com/products/macvector/). All phylogenetic analyses were performed using the program package PHYLIP 3.65 (Felsenstein, 2005). The phylogenetic relationships among the aligned sequences within each gene/gene family were estimated by the Neighbor-Joining method (Saitou and Nei, 1987) with the pairwise divergence among sequences estimated using the JTT (Jones et al., 1992) method. Confidence levels for the branching patterns for actin, dynein, kinesin, myosin, and tubulin were assessed by nonparametric bootstrapping (Felsenstein, 1985). Predicted urchin sequences that deviated significantly from other family members for each gene or gene family were re-assessed as to the validity of the results.

### *Expression analysis of kinesins*

Expression profiles of select kinesins were downloaded as EST counts from NCBI's transcript-based EST libraries available for *S. purpuratus*: UniGene Build #9 described at http://www.ncbi.nlm.nih.gov/UniGene/UGOrg.cgi?TAXID=7668. Expression profiles suggested by analysis of EST counts were generated automatically from egg, cleavage, blastula, gastrula, and larva libraries (dbEST Library IDs. 13749, 13750, 13752, 13751, and 13748, respectively, submitted by A.J. Poustka), supplemented with additional blastula data (dbEST Library ID. 16781, submitted by J. Coffman), and primary mesenchyme cell data (dbEST Library ID. 17157, submitted by C.A. Ettensohn). UniGene IDs for kinesins or kinesin-associated proteins in *S. purpuratus* were identified by keyword (http://www.ncbi.nlm.nih.gov/UniGene/UGOrg.cgi?TAXID=7668), and expression profiles were generated for UniGene entries that matched a previously cloned *S. purpuratus* CDS or consistently matched the same kinesin family in all organisms. These UniGene entries included UniGene Spu.93: KRP180 (SPU_021317), UniGene Spu.964: Kinesin-C (SPU_009400), UniGene Spu.13111: KIF1B subfamily, UniGene Spu.11982: KIF1A subfamily, UniGene Spu.15: KRP85 (SPU_018378), and UniGene Spu.198: SpKAP115 (SPU_010954).

### *Analysis of relationship among major taxa based on cytoskeletal and motor proteins*

Based on the NJ trees derived for each gene/gene family, putative homologs were determined for CLIP170, fascin, gelsolin, lamin B, myosin II, and profilin. The homologous sequences for the nine taxa listed above plus *S. purpuratus* were concatenated into a single file and the phylogenetic relationship among the ten taxa determined using maximum likelihood methods as implemented in the program PHYML (Guindon and Gascuel, 2003). The JTT+I+G model was used estimating the proportion of invariant sites (*I*) and the gamma distribution parameter (*G*) from the data. Nonparametric bootstrapping (100 replications) was used to infer levels of confidence for the resulting branching patterns. Branch support was further assessed by Bayesian analysis using MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). Two heated Markov chains were run for 100,000 generations sampling every 100 generations using the JTT+G+I model. A burn-in time of 1000 generations was used to assure stationarity, and the remaining tree samples were used to generate a 50% majority rule consensus tree and to calculate the posterior probability of each clade.

## Results

A complete presentation of our results, showing each individual homolog identified from the GLEAN3 predictions can be found in Tables 1, 2 and 3. Overall, representatives of all major classes and families of cytoskeletal and motility proteins have been identified in the *S. purpuratus* genome. Selected representatives of major families are presented below.

### *Actin*

Actin is the most abundant protein in most cells and both muscle and non-muscle actins have been well studied in numerous systems. Because the high sequence conservation of actin across species, which is likely due to the conserved nature of the abundant protein–protein interactions that actin is involved in, searches for actin in the assembly are straightforward.

There are 36 different scaffolds containing genes predicted to have significant homology with actin (BLAST score>1e-7). Phylogenetic analysis of these sequences together with a broad range of actin sequences derived from diverse species shows single predicted genes corresponding to previously characterized *S. purpuratus* cytoskeletal actins CyI (SPU_009481 groups with equal similarity to CyIa and CyIb), CyIIa, CyIIb, CyIIIb and the skeletal M actin gene Table 1. All other putative actin-encoding sequences were ruled out as outliers as they did not group into clades containing defined actin orthologs (data not shown).

### *Myosins*

Myosins are actin-based motor proteins with a highly conserved ATPase and actin-binding globular head domain and a tail domain, allowing for tail–protein and tail–membrane interactions. Thorough analysis of the large myosin family of proteins, based on sequence analysis of the motor domain, reveals thirty-one sequences corresponding to twelve different classes of myosin (Fig. 1a and Table 1). The myosin family has been well studied in sea urchins. Initially, egg myosin II was characterized biochemically (Mabuchi, 1973; Kane, 1983), followed by the unconventional myosins I and VI (D'Andrea, et al. 1994). Other myosins that have been partially sequenced include amoeboid-like myosin I, V, VI, VII, IX (Sirotkin et al., 2000). Of these, only myosins V and VI have been completely sequenced. Predictions of the highly conserved and myosin class-specific motor domain sequence (MYSc) have allowed the accurate prediction of homologs corresponding to myosins I, II, V, VI, IX. Homologs pertaining to myosin classes III, IV, VII, X, XII, XV, XVI, XVII were not previously described in sea urchins and have been partially derived from whole-genome sequences. To date, homologs corresponding to myosins VIII, XI, XII, XIII, and XIV have not been identified.

### *Actin-binding proteins*

The ability of cells to change shape is largely due to the abundant variety of actin-binding proteins that function to

Table 1
Cytoskeletal and motor proteins in the *S. purpuratus* genome

| Gene name | Synonyms | SPU gene ID | Accession number of best hit | BLAST score |
|---|---|---|---|---|
| *Actin* | | | | |
| Sp-cytoskeletal-actin-I | CyI | SPU_009481 | 1703128 | 0.0 |
| Sp-cytoskeletal-actin-IIa | CyIIa | SPU_009482 | 47551037 | 0.0 |
| Sp-cytoskeletal-actin-IIb | CyIIb | SPU_009483 | 47550921 | 0.0 |
| Sp-cytoskeletal-actin-IIIb | CyIIIb | SPU_009433 | 47551035 | 0.0 |
| Sp-muscle-actin | muscle actin | SPU_006797 | 1703137 | 0.0 |
| *Actin-related* | | | | |
| Sp-Arp1 | Arp1/centractin | SPU_001366 | 18606465 | 0.0 |
| Sp-Arp3 | Arp3 | SPU_010631 | 6681670 | 0.0 |
| Sp-Arp6 | Arp6 | SPU_007479 | 72044622 | 1E-125 |
| Sp-Arp8 | Arp8 | SPU_027580 | 39812115 | 8E-102 |
| *Myosins* | | | | |
| Sp-myosin-I-subclass-2 | | SPU_007023 | * | * |
| Sp-myosin-I-subclass-4 | | SPU_019273 | * | * |
| Sp-myosin-I-subclass-4 | | SPU_014397 | * | * |
| Sp-myosin-II-smooth-and-non-muscle | | SPU_024055 | * | * |
| Sp-myosin-II-smooth-and-non-muscle | | SPU_006850 | * | * |
| Sp-myosin-II-smooth-and-non-muscle | | SPU_010054 | * | * |
| Sp-myosin-II-smooth-and-non-muscle-light-chain | | SPU_001613 | 67083885 | 2E-39 |
| Sp-myosin-III | | SPU_009340 | 35959282 | 4E-97 |
| Sp-myosin-IV | | SPU_005048 | * | * |
| Sp-myosin-IV | | SPU_007083 | * | * |
| Sp-myosin-V | | SPU_003901 | * | * |
| Sp-myosin-V | | SPU_001160 | * | * |
| Sp-myosin-V | | SPU_003900 | * | * |
| Sp-myosin-VI | | SPU_002723 | 8099610 | 0.0 |
| Sp-myosin-VI | | SPU_002723 | * | * |
| Sp-myosin-VI | | SPU_026648 | * | * |
| Sp-myosin-VII | | SPU_025990 | 9944237 | 2E-72 |
| Sp-myosin-VII | | SPU_013161 | 9944237 | 0.0 |
| Sp-myosin-VII | | SPU_022040 | 9944237 | 0.0 |
| Sp-myosin-IX | | SPU_020406 | * | * |
| Sp-myosin-X | | SPU_007248 | 50428778 | 0.0 |
| Sp-myosin-X | | SPU_023549 | * | * |
| Sp-myosin-X | | SPU_003746 | * | * |
| Sp-myosin-X | | SPU_001633 | * | * |
| Sp-myosin-XV | | SPU_022456 | 22547229 | 4E-177 |
| Sp-myosin-XVI | | SPU_009340 | * | * |
| Sp-myosin-XVI | | SPU_027367 | * | * |
| Sp-myosin-XVII | | SPU_013384 | * | * |
| Sp-myosin-XVII | | SPU_026002 | * | * |
| *Actin-binding/actin dynamics* | | | | |
| Sp-filamin | ABP278/filamin B | SPU_017634 | 32129530 | 0.0 |
| Sp-ABP620 | ABP620 | SPU_003260 | 5821434 | 0.0 |
| Sp-alpha-actinin | Alpha-actinin | SPU_020920 | 1070611 | 0.0 |
| Sp-adducin/drebrin | Adducin/Drebrin | SPU_026521 | 28382 | 2E-92 |
| Sp-calponin-1 | Calponin | SPU_015595 | 23491586 | 2E-33 |
| Sp-capz | CapZ | SPU_024912 | 38322686 | 9E-91 |
| Sp-cofilin-1 | Cofilin 1 | SPU_006172 | 2440185 | 6E-07 |
| Sp-Contactin-2 | Contactin 2 | SPU_021328 | 6981632 | 2E-88 |
| Sp-coronin | Coronin | SPU_000974 | 11463876 | 0.0 |
| Sp-drebrin | Drebrin | SPU_026521 | 20454881 | 2E-07 |
| Sp-dystrophin-1 | Dystrophin | SPU_021580 | 13377398 | 0.0 |
| Sp-dystrophin-2 | Dystrophin | SPU_021581 | 13377398 | 0.0 |
| Sp-EWAM | EWAM | SPU_027390 | 551452 | 2E-106 |
| Sp-fascin | Fascin | SPU_010943 | 47551048 | 0.0 |
| Sp-gelsolin | Gelsolin | SPU_000296 | 28916693 | 1E-20 |
| Sp-moesin | Moesin | SPU_018705 | 719272 | 0.0 |
| Sp-paxillin | Paxillin | SPU_008331 | 43415525 | 2E-125 |

Table 1 (*continued*)

| Gene name | Synonyms | SPU gene ID | Accession number of best hit | BLAST score |
|---|---|---|---|---|
| *Actin-binding/actin dynamics* | | | | |
| Sp-profilin | Profilin | SPU_020197 | 400849 | 0.0 |
| Sp-radixin | Radixin | SPU_007679 | 40804379 | 7E-35 |
| Sp-scruin | Scruin | SPU_019550 | 633238 | 1E-22 |
| Sp-alpha-spectrin | Spectrin, alpha | SPU_020836 | 158489 | 6E-174 |
| Sp-beta-spectrin | Spectrin, beta | SPU_007720 | 56206997 | 5E-61 |
| Sp-supervillin | Supervillin/Archvillin | SPU_007557 | 55957976 | 7E-149 |
| Sp-talin-1 | Talin | SPU_000241 | 45383127 | 0.0 |
| Sp-tropomodulin | Tropomodulin | SPU_027778 | 13928838 | 1E-50 |
| Sp-tropomyosin | TropoSp-myosin- | SPU_000128 | 38569885 | 5E-106 |
| Sp-troponin-C | Troponin C | SPU_016371 | 72012357 | 1E-79 |
| Sp-troponin-I | Troponin I | SPU_013183 | 18157232 | 6E-18 |
| Sp-troponin-T | Troponin T | SPU_006532 | 48255883 | 2E-06 |
| Sp-villin | Villin | SPU_000296 | 49257782 | 9E-128 |
| Sp-vinculin | Vinculin | SPU_028781 | 309553 | 8E-12 |
| Sp-Wiskott-Aldrich-syndrome-protein | WASP | SPU_003194 | 4633273 | 1E-17 |
| *Cytoskeletal regulators/signaling* | | | | |
| Sp-LIM-kinase | LIM Kinase | SPU_022207 | 1657756 | 1E-93 |
| Sp-Calponin | Calponin/Myophillin | SPU_015595 | 23491586 | 4E-33 |
| Sp-Cdc42 | Cdc42 | SPU_019494 | 62858789 | 1E-98 |
| Sp-citron | Citron | SPU_011918 | 56405460 | 0.0 |
| Sp-enabled | ENA (Enabled) | SPU_010763 | 58865872 | 6E-09 |
| Sp-formin-2 | Formin 2 | SPU_020333 | 55961780 | 1E-36 |
| Sp-formin-2-DAD-domain | DAD domain | SPU_020879 | 3834629 | 78 [a] |
| Sp-WISH | N-WASP binding protein | SPU_020040 | 49188650 | 1E-98 |
| Sp-Mob1 | Mob1 | SPU_024840 | 39645694 | 2E-106 |
| Sp-PAK-(p21) | PAK (p21) | SPU_003534 | 29791476 | 7E-31 |
| Sp-PAR3-1 | PAR3 | SPU_014037 | 45387877 | 2E-85 |
| Sp-PAR3-2 | PAR3 | SPU_005813 | 46405829 | 3E-45 |
| Sp-paxillin-1 | Paxillin | SPU_008331 | 42415525 | 5E-125 |
| Sp-profilin | profilin | SPU_020197 | 47551153 | 4E-42 |
| Sp-RhoA | Rho1/RhoA | SPU_011032 | 72038732 | 2E-94 |
| Sp-ROCK-1 | ROCK | SPU_026779 | 34784414 | 6E-106 |
| *Tubulins* | | | | |
| Sp-alpha-tubulin-1 | | SPU_021668 | NP_006000 | 0.0 |
| Sp-alpha-tubulin-2 | | SPU_021669 | NP_006000 | 0.0 |
| Sp-alpha-tubulin-3 | | SPU_016746 | NP_006000 | 0.0 |
| Sp-alpha-tubulin-4 | | SPU_028221 | NP_006000 | 0.0 |
| Sp-alpha-tubulin-5 | | SPU_019990 | NP_006000 | 0.0 |
| Sp-alpha-tubulin-6 | | SPU_024615 | NP_006000 | 0.0 |
| Sp-alpha-tubulin-7 | | SPU_012679 | NP_006000 | 0.0 |
| Sp-alpha-tubulin-8 | | SPU_007984 | NP_006000 | 0.0 |
| Sp-alpha-tubulin-9 | | SPU_006756 | NP_006000 | 0.0 |
| Sp-beta-tubulin-1 | | SPU_002788 | NP_006079 | 0.0 |
| Sp-beta-tubulin-2 | | SPU_001045 | NP_006079 | 0.0 |
| Sp-beta-tubulin-3 | | SPU_000062 | NP_006079 | 0.0 |
| Sp-beta-tubulin-4 | | SPU_013273 | NP_006079 | 0.0 |
| Sp-beta-tubulin-5 | | SPU_003894 | NP_006079 | 1e-92 |
| Sp-beta-tubulin-6 | | SPU_007425 | NP_006079 | 0.0 |
| Sp-gamma-tubulin | | SPU_020943 | NP_001061.2 | 0.0 |
| Sp-delta-tubulin | | SPU_004266 | AAF09584.1 | 1e-108 |
| Sp-epsilon-tubulin | | SPU002663 & SPU_013393 | NP_001016136.1 | 1e-172 |
| *Microtubule-associated proteins* | | | | |
| Sp-EMAP | 77 kDa MAP, 77 kDa microtubule-associated Protein | SPU_006911 & SPU_005744 | Q26613 | 0.0 |
| Sp-EMAP-like-1 | 77 kDa MAP-like, 77 kDa microtubule-associated protein-like | SPU_011819 | Q26613 | 1E-138 |
| Sp-EMAP-like-2 | 77 kDa MAP-like, 77 kDa microtubule-associated protein-like | SPU_026643 | Q26613 | 1E-95 |
| Sp-EMAP-like-3 | 77 kDa MAP-like, 77 kDa microtubule-associated protein-like | SPU_001991 | Q26613 | 2E-94 |
| Sp-EMAP-like-4 | 77 kDa MAP-like, 77 kDa microtubule-associated protein-like | SPU_020821 | Q26613 | 1E-76 |

*(continued on next page)*

Table 1 (*continued*)

| Gene name | Synonyms | SPU gene ID | Accession number of best hit | BLAST score |
|---|---|---|---|---|
| *Microtubule-associated proteins* | | | | |
| Sp-EMAP-like-5 | 77 kDa MAP-like, 77 kDa microtubule-associated protein-like | SPU_024323 | Q26613 | 8E-73 |
| Sp-Tektin-1-1 | | SPU_008777 | NP_444515.1 | 4E-90 |
| Sp-Tektin-1-2 | | SPU_019591 | NP_444515.1 | 4E-90 |
| Sp-Tektin-1-3 | | SPU_013841 | NP_444515.1 | 1E-109 |
| Sp-Tektin-1-4 | | SPU_006453 | NP_444515.1 | 1E-109 |
| Sp-Tektin-2 | | SPU_020728 | NP_055281.2 | 1E-101 |
| Sp-Tektin-3 | | SPU_023618 | NP_114104.1 | 1E-141 |
| Sp-HS-MAP | MAP2-like, MAP4-like,MAP215-like, Tau-like | SPU_013254 | NP_002366.2 | 2E-32 |
| Sp-MAP1B-like | MAP1B-like, MAP1A-like | SPU_004628 | NP_005900.1 | 3E-81 |
| Sp-MAP1A/1B_LC3-like-1 | Microtubule-associated proteins 1A/1B light chain 3B precursor, MAP1A/MAP1B LC3, MAP1A/1B light chain 3 | SPU_009444 | NP_115903.1 | 3E-44 |
| Sp-MAP1A/1B_LC3-like-2 | Microtubule-associated proteins 1A/1B light chain 3B precursor, MAP1A/MAP1B LC3, MAP1A/1B light chain 3 | SPU_008954 | NP_115903.1 | 1E-36 |
| Sp-RIB43A | flagellar protofilament ribbon protein | SPU_027579 | NP_001027762.1 | 1E-122 |
| Sp-TPX2-like-1 | | SPU_008221 | NP_036244.2 | 2E-22 |
| Sp-TPX2-like-2 | | SPU_022897 | NP_036244.2 | 2E-22 |
| Sp-WD-Repeat-domain-56 | | SPU_012598 | XP_545256.2 | 5E-74 |
| Sp-Adenomatous Polyposis Coli | APC | SPU_026745 | 6978509 | 5E-175 |
| Sp-bicaudal-D | Bicaudal | SPU_017959 | 51093830 | 2E-130 |
| Sp-diaphanous-1/3 | Diaphanous 1/3 | SPU_012710 | 55959274 | 3E-35 |
| Sp-diaphanous-1 | Diaphanous 1 | SPU_020879 | 62088544 | 7E-80 |
| Sp-EB1 | EB1 | SPU_027631 | 73980297 | 1E-25 |
| Sp-gephyrin | Gephyrin | SPU_017877 | 16605466 | 1E-112 |
| Sp-katanin-p60-subunit-A | Katanin | SPU_001000 | 3098603 | 0.0 |
| Sp-MAST/orbit/CLASP | MAST/orbit/CLASP | SPU_000096 | 66271020 | 3E-75 |
| Sp-CLIP170 | CLIP170/Restin | SPU_001154 | 23821025 | 4E-61 |
| Sp-dynactin-1 | Dynactin1/p150-glued | SPU_013935 | 33872165 | 0.0 |
| Sp-dynactin-2/dynactin-subunit-5 | Dynactin 2/Dynactin subunit 5 | SPU_028205 | 50926127/14789952 | 1E-74/1E-84 |
| Sp-dynamitin-p50 | Dynamitin | SPU_018500 | 28274444 | 1E-63 |
| *Intermediate filaments* | | | | |
| Sp-keratin-complex-1-acidic | Acidic keratin | SPU_011620 | 72105012 | 0.0 |
| Sp-enabled | ENA (enabled) | SPU_007845 | 6753754 | 1E-33 |
| Sp-Keratin-associated-protein | Keratin associated | SPU_019996 | 77157625 | 1E-33 |
| Sp-nestin | Nestin | SPU_024601 | 72108788 | 1E-155 |
| Sp-Lamin-B | Lamin B | SPU_004509 | 11386011 | 2E-66 |

Cytoskeletal, cytoskeletal-interacting, and motor proteins in the sea urchin genome assembly. "Gene Name" is the name we assigned to the gene in the annotation database. "Synonyms" include all names used to identify this gene or its homologs in any organism. "SPU Gene ID" is the unique and permanent identifier for this gene and it is based on identifiers originally assigned by the GLEAN3 gene identification process. Because the dynein heavy chain genes involve exons located on up to 5 genomic scaffolds per gene, they are in the annotation database as "novel genes" separate from the GLEAN system. "Accession Number of best hit" indicates an NCBI entry that will be most informative to a reader interested in studying this protein further. "BLAST score" is the BLAST score of the final GLEAN sequence deposited in the annotation database, vs. the sequence of the database entry corresponding to the Accession Number cited in the previous column. Gene models (i.e., SPUs) marked with an asterisk (*) were predicted to encode conserved Sp-myosin-motor domains (MYSc) by SMART/PFAM prior to bootstrap analysis, and BLAST scores were not derived independently for these sequences.

[a] Smith-Waterman score.

regulate actin assembly into filaments, form actin networks, meshworks and bundles, stabilize filaments, and control disassembly of filaments and three-dimensional assemblies. Previously cloned actin-binding genes and several novel homologs of actin-binding genes were identified from the sea urchin genome assembly (Table 1). The Arp2/3 complex regulates actin branching networks and promotes actin polymerization (Kelleher et al., 1995) and biochemical analysis has shown that this complex exists in sea urchins (Terasaki et al., 1997). A homolog of Arp3 has been predicted from the urchin genome. Subunits 2, 3 and 4 of the seven-subunit Arp2/3 complex have also been identified. Homologs of tropomyosin and tropomodulin, that bind actin on pointed ends and inhibit actin depolymerization, gelsolin, an actin severing protein, α-actinin, that crosslinks and bundles actin at higher concentrations and WASP, that stimulates the actin-nucleating activity of the Arp2/3 complex, were also found. A homolog of ENA/VASP that associates with barbed ends and inhibits filament capping by CapZ (Krause et al., 2003) was predicted. SpCoel1, an ortholog of profilin, a protein that regulates actin polymerization by sequestering actin monomers, was previously identified in sea urchin coelomocytes (Smith et al., 1992) and this gene has been identified in the genome.

Table 2
Kinesins and kinesin-associated proteins in the *S. purpuratus* genome

| Kinesin family | Gene name | Synonyms or closest homologs | SPU gene ID | Accession number of best hit | BLAST score |
|---|---|---|---|---|---|
| *Motor polypeptides* | | | | | |
| Kinesin-1 | Sp-KHC | Conventional kinesin heavy chain, kinesin family member 5B [Hs] | SPU_021656 | CAA40175 | 0.0 |
| Kinesin-2 | Sp-KRP85 | Kinesin II, 85 kDa [Sp]; kinesin family member 3A [Hs] | SPU_018378 | NP_999777 | 0.0 |
| Kinesin-2 | Sp-KRP95 | Kinesin-related protein 95 kDa [Sp]; kinesin family member 3B [Hs]; kinesin-like protein FLA10 (KHP1 protein) [Cr] | SPU_026280 | NP_999817 | 0.0 |
| Kinesin-2 | Sp-Osm-3 | Kinesin family member 17 [Hs] | SPU_009443 | NP_065867 | 5e-52 |
| Kinesin-3 | Sp_KIF13A2 | Kinesin-13A2 [Hs] | SPU_006602 | CAC20443 | 0.0 |
| Kinesin-3 | Sp-KIF1B-like1 | Kinesin family member 1B [Hs], kinesin family member 1Bbeta isoform II [Hs] | SPU_023560 | BAE02544 | 8e-106 |
| Kinesin-3 | Sp-KIF16-like | SNX23_HUMAN, kinesin-like motor protein C20orf23 [Hs], Sorting nexin 23 | SPU_026237 | CAI43180 | 0.0 |
| Kinesin-3 | Sp-KLP6-like1 | Kinesin family member 1B [Hs] | SPU_027784 | CAI95220 | 2e-128 |
| Kinesin-3 | Sp-KLP6-like2 | Kinesin family member 1B isoform alpha [Hs] | SPU_018764 | NP_904325 | 8e-109 |
| Kinesin-3 | Sp-KLP6-like3 | "axonal transport of synaptic vesicles" [Hs] | SPU_020634 | NP_004312 | 3e-118 |
| Kinesin-4 | Sp-KIF4 | KIF4, chromokinesin [Hs] | SPU_008110 | NP_036442 | 0.0 |
| Kinesin-4 | Sp-KIF21A | Kinesin family member 21A [Hs] | SPU_002954 | NP_060111 | 8e-123 |
| Kinesin-4 | Sp-KIF27 | Kinesin-related protein KIF27 [Mm] | SPU_012877 | NP_780423 | 0.0 |
| Kinesin-5 | KRP170 | BimC, Eg5, kinesin family member 15 [Hs] | SPU_020414 | AAG18583 | 0.0 |
| Kinesin-6 | KRP110 | KRP110 [Sp] | SPU_019165 | AAG18582 | 0.0 |
| Kinesin-6 | Sp-KIF20A | Kinesin family member 20A [Hs] | SPU_010570 | NP_005724 | 2e-91 |
| Kinesin-7 | Sp-CENPE | CENPE_HUMAN, Centromeric Protein E, CENPE-E protein, KIF10 | SPU_023126 | NP_001804 | 2e-101 |
| Kinesin-7 | Sp-KIF2-like | Kinesin heavy chain member 2 [Hs] | SPU_015437 | NP_004511 | 6e-168 |
| Kinesin-8 | Sp-KIF18 | Kinesin family member 18A [Hs] | SPU_022160 | NP_112494 | 3e-120 |
| Kinesin-8 | Sp-KIF19 | Kinesin family member 19 [Hs] | SPU_016655 | AAI10990 | 1e-111 |
| Kinesin-8 | Kinesin-8-like | Kinesin family member 19 [Hs] | SPU_025767 | AAI10990 | 2e-30 |
| Kinesin-9 | Sp-KIF6 | Kinesin family member 6 [Hs] | SPU_007505 | NP_659464 | 0.0 |
| Kinesin-9 | Sp-KIF9 | Kinesin family 9 isoform 3 [Hs] | SPU_010081 | NP_878906 | 1e-133 |
| Kinesin-9 | Sp-KIF6-like | Kinesin family member 6 [Hs] | SPU_003717 | NP_659464 | 5e-72 |
| Kinesin-10 | Sp-KIF22 | Kinesin family member 22 [Hs] | SPU_022296 | AAH28155 | 7e-115 |
| Kinesin-11 | Sp-KIF26 | KIF26B protein [Hs] | SPU_018533 | AAH35896 | 8e-147 |
| Kinesin-12 | KRP180 | Kinesin family member 15 [Hs] | SPU_021317 | NP_999656 | 0.0 |
| Kinesin-12 | Sp-KIF12 | Kinesin-like protein at 54D CG15844-PA [Dm], kinesin family member 12 [Hs] | SPU_022840 | NP_524883 | 3e-100 |
| Kinesin-13 | Sp-KIF24 | Kinesin family member 2C [Hs] | SPU_016067 | CAI12999 | 3e-84 |
| Kinesin-14 | Sp-kinesin-C | Kinesin-C [Sp] | SPU_009400 | AAF04841 | 0.0 |
| Kinesin-14 | Sp-KIFC1 | Kinesin family member C1 [Hs] | SPU_017289 | AAH73878 | 2e-45 |
| Kinesin-14 | Sp-KIFC3-like1 | Kinesin family member C3 [Hs] | SPU_013729 | AAH01211 | 7e-77 |
| Kinesin-14 | Sp-KIFC3-like2 | Kinesin family member C3 [Hs] | SPU_010770 | AAH01211 | 1e-75 |
| Orphan | Sp-KIF25-like | Kinesin family member 25 isoform 1 [Hs] | SPU_012452 | NP_085118 | 1e-49 |
| Orphan | Sp-KIF27-like | Kinesin family member 13A [Hs] | SPU_011353 | NP_071396 | 3e-61 |
| *Kinesin-associated proteins* | | | | | |
| Kinesin-2 associated | Sp-KAP | Kinesin II-accessory 115 kDa polypeptide [Sp]; KAP, kinesin II-associated protein [Cr]; kinesin-associated protein 3 [Hs] | SPU_010954 | NP_999823 | 0.0 |
| Kinesin-1 associated | Sp-KLC | Kinesin light chain [Sp] | SPU_018898 | Q05090 | 0.0 |

Kinesins in the sea urchin genome assembly. "Kinesin family" is the family, as per Lawrence et al. (2004), to which the motor domain of this gene is most closely similar. Other column headings are as in Table 1. Genus species name abbreviations are as described in Legend to Table 1.

Homologs for Arp1/centractin, that polymerizes to form a complex with p150-*glued* and vesicle-specific spectrins (Holleran and Holzbaur 1998), Arp6 and Arp8, shown to be involved in heterochromatin organization in yeast (Shen et al., 2003), were also identified. Partial homologs to diaphanous Diaphanous/mDia/formin were determined. The mouse formin gene (mDia/Fmn) is located over approximately 400 kb and is encoded by at least 24 different exons (Wang et al., 1997). It is likely that the presence of multiple exons and/or large intronic regions has complicated efforts to identify a full-length formin

Table 3
Dyneins in the *S. purpuratus* genome

| *Strongylocentrotus purpuratus* gene | | | | | Homologous genes | |
|---|---|---|---|---|---|---|
| Gene name | Common name | Synonyms (organism) | Predicted polypeptide length (%identity to Indexing Gene) | SPU gene ID | Indexing gene ID (Accn. number) | Other homologs used (Accn. number) |
| Sp-DYNC1H1 | Cytoplasmic dynein 1 heavy chain homolog | DYN1 (sc)<br>DYH1A (tg)<br>DHC1A (cr)<br>DHC64C (dm) | 4652 (74%) | SPU_030236 | hsDYNC1H1 (Q14204) | tgDYNC1H1 (CAA79935) |
| Sp-DYNC2H1 | Cytoplasmic dynein 2 heavy chain homolog | DHC1B (cr)<br>IFT dynein (cr)<br>Beethoven (dm)<br>che-3 (ce) | 4312 (95%) | SPU_030235 | tgDYNC2H1 (AAA63583.2) | hsDYNC2H1 (NP_001367) |
| Sp-DNAH1 | Axonemal dynein heavy chain 1 homolog | DYH6 (tg) | 4154 (64%) | SPU_030223 | hsDNAH1 (NP_056327.3) | tgDNAH1 (AAA63589) |
| Sp-DNAH2 | Axonemal dynein heavy chain 2 homolog | DYH5C (tg)<br>IDA1-beta (cr) | 4458 (65%) | SPU_030224 | hsDNAH2 (NP_065928.2) | tgDNAH2 (AAA63590) |
| Sp-DNAH3 | Axonemal dynein heavy chain 3 homolog | DYH7B (tg) | 4038 (64%) | SPU_030225 | hsDNAH3 (NP_060009.1) | tgDNAH3 (AAA63593) |
| Sp-DNAH4 | Axonemal dynein heavy chain 4 homolog | DYH7D (tg) | 4208 (56%) | SPU_030222 | hsDNAH3 (NP_060009.1) | tgDNAH4 (AAA63595) |
| Sp-DNAH5 | Axonemal dynein heavy chain 5 homolog | DYH3B (tg)<br>ODA-gamma (cr) | 4532 (62%) | SPU_030226 | hsDNAH5 (NP_001360.1) | tgDNAH5 (AAA63584) |
| Sp-DNAH6 | Axonemal dynein heavy chain 6 homolog | DYH5A (tg) | 4160 (64%) | SPU_030227 | cfDNAH6 (XP_532984.2) | tgDNAH6 (AAA63591) |
| Sp-DNAH7 | Axonemal dynein heavy chain 7 homolog | DYH7A (tg) | 3982 (68%) | SPU_030228 | hsDNAH7 (NP_061720.1) | tgDNAH7 (AAA63592) |
| Sp-DNAH8 | Axonemal dynein heavy chain 8 homolog | DYH3C (tg) | 4592 (66%) | SPU_030229 | hsDNAH8 (CAI42433.1) | tgDNAH8 (AAA63586) |
| Sp-DNAH9 | Axonemal dynein heavy chain 9 homolog | DHC-beta (tg)<br>DYH2 (tg)<br>OAD-beta (cr) | 4466 (95%) | SPU_030230 | tgDNAH9 (beta) (CAA42170) | hsDNAH9 (Q9NYC9)<br>hsDNAH11 (CAC60121.1)<br>cfDNAH17 (XM_533129.1) |
| Sp-DNAH10 | Axonemal dynein heavy chain 10 homolog | DYH4 (tg)<br>IDA1-alpha (cr) | 4592 (68%) | SPU_030231 | cfDNAH10 (XP_543369.2) | tgDNAH10 (AAA63587) |
| Sp-DNAH12 | Axonemal dynein heavy chain 12 homolog | DYH7C (tg) | 3935 (66%) | SPU_030232 | cfDNAH12 (XP_541831.2) | tgDNAH12 (AAA63594) |
| Sp-DNAH14 | Axonemal dynein heavy chain 14 homolog | DYH5B (tg) | 4337 (41%) | SPU_030233 | cfDNAH14 (XM_537236) | tgDNAH14 (AAA63588) |
| Sp-DNAH15 | Axonemal dynein heavy chain 15 homolog | DYH3A (tg) | 4647 (72%) | SPU_030234 | hsDNAH8 (CAI42433.1) | tgDNAH15 (AAA63586) |
| Sp-DYNC1I1 | Cytoplasmic dynein 1 intermediate chain 1 | | 645 (51%) | SPU_022687 | hsDYNC1I1 (NP_004402.1) | |
| Sp-DYNC1LI1 | Cytoplasmic dynein 1 light intermediate chain 1 | | 523 (45%) | SPU_015909 | hsDYNC1LI1 (NP_057225.1) | |
| Sp-DYNC2LI1 | Cytoplasmic dynein 2 light intermediate chain 1 | | 351 (48%) | SPU_018582 | hsDYNC2LI1 (NP_057092.2) | |
| Sp-DYNC2LI2 | Cytoplasmic dynein 2 light intermediate chain 2 | | 201 (48%) | SPU_008154 | hsDYNC2LI2 (NP_056337) | |
| Sp-DYNLL1 | Cytoplasmic dynein light chain, LC8-type 1 | | 89 (89%) | SPU_004009 | hsDYNLL1 (AAI00290.1) | |
| Sp-DYNLRB1 | Dynein light chain, roadblock-type 1 | | 96 (59%) | SPU_003137 | hsDYNLRB1 (NP_054902.1) | |
| Sp-DYNLRB2 | Dynein light chain, roadblock-type 2 | | 96 (60%) | SPU_008699 | hsDYNLRB2 (NP_570967.1) | |
| Sp-DYNLT1 | Dynein light chain, Tctex-type 1 | | 113 (85%) | SPU_008471 | hsDYNLT1 (CAI95303.1) | |
| Sp-DYNLT2 | Dynein light chain, Tctex-type 2 | | 198 (35%) | SPU_006354 | hsDYNLT2 (AAN34631.1) | |
| Sp-DYNLT3 | Dynein light chain, Tctex-type 3 | | 116 (59%) | SPU_008471 | hsDYNLT3 (NP_006511.1) | |
| Sp-DNAI1 | Axonemal dynein intermediate chain 1 homolog | | 699 (60%) | SPU_019506 | hsDNAI1 (NP_036276) | |
| Sp-DNAI2 | Axonemal dynein intermediate chain 2 homolog | | 605 (68%) | SPU_006699 | hsDNAI2 (AAG38489.1) | |

Table 3 (*continued*)

| *Strongylocentrotus purpuratus* gene | | | | | Homologous genes | |
|---|---|---|---|---|---|---|
| Gene name | Common name | Synonyms (organism) | Predicted polypeptide length (%identity to Indexing Gene) | SPU gene ID | Indexing gene ID (Accn. number) | Other homologs used (Accn. number) |
| Sp-Txl-2 | Thioredoxin-like chain 2 homolog | | 330 (46%) | SPU_007092 | hsTx1-2 (AAG28497.1) | |
| Sp-DNALI1 | Axonemal dynein light intermediate polypeptide 1 | | 280 (75%) | SPU_015320 | hsDNALI1 (NP_003453.2) | |
| Sp-TCTEX1D1 | Axonemal outer arm dynein light chain 1 homolog | | 179 (54%) | SPU_013200 | hsTCTEX1D1 (NP_689878.1) | |
| Sp-DNAL1 | Axonemal outer arm dynein light chain 1homolog | | 190 (69%) | SPU_018854 | hsDNAL1 (AAQ11377.1) | |
| Sp-DNAL4 | Axonemal dynein light polypeptide 4 homolog | | 105 (77%) | SPU_004377 | hsDNAL4 (NP_005731.1) | |

Dyneins in the sea urchin genome assembly. Column headings are as in Table 1 and as described in the text. Genus species abbreviations are as follows. Cf: *Canis familiaris*, Hs: *Homo sapiens*, Tg: *Tripneustes gratilla*.

gene from any one scaffold from the sea urchin genome assembly. A sequence encoding a putative diaphanous auto-inhibitory domain (DAD) of formin was found isolated on a distinct scaffold from other predicted formin encoding exons. Other major actin-binding proteins identified include: ABP1, ABP278/filamin B, adducin, ankyrin, annexins (4, 5, 6, 7, 13), annilin, calmodulin, cofilin (1, 2), ezrin/radixin/moesin, fascin, filamin (A, B, C), protocadherin, drebrin, dystonin, gelsolin, villin, spectrin (alpha, beta, G), talin, and beta-thymosin. Multiple homologs corresponding to genes encoding vertebrate skeletal muscle proteins have been predicted to exist following analysis of *S. purpuratus* genome sequences, including: tropomyosin, troponin I, troponin C, troponin T, titin, minititin, nebulin and dystrophin.

### *Tubulins*

Because tubulin genes (like those for actins) are highly conserved, the search for tubulin-encoding genes by homology with known genes in other organisms and sea urchin ESTs is relatively straightforward. The genome of *S. purpuratus* carries multiple genes encoding alpha- and beta-tubulin (~9 and 6 genes, respectively), as well as gamma-tubulin (1 gene), delta-tubulin (1 gene) and epsilon-tubulin (2 genes) (Fig. 1b and Table 1). Only the alpha, beta, and gamma sequences were known previously in sea urchin. The nine expressed alpha-tubulin genes that we have identified fall into two groups based on the number and positions of their introns. Four of the genes (SPU_012679, SPU_021668, SPU_021669, and SPU_024615) contain 2 introns apiece, and five of the genes (SPU_006756, SPU_007984, SPU_016746, SPU_019990, and SPU_028221) contain 3 introns apiece. The structures of all nine identified alpha-tubulin genes share the two previously identified introns that seem to be specific to invertebrate alpha-tubulins (Perumal et al., 2005) at codon 1 (i.e., separating the initiator methionine codon from the second codon) and codon 75. Each of the nine genes encodes a slightly different alpha-tubulin sequence, with most of the diversity occurring near the carboxyl terminus.

There are at least six beta-tubulin genes (see Table 1), and each gene has three exons interrupted by two introns at (identical) conserved locations. Each of these genes encodes a distinct beta-tubulin sequence.

### *MAPs*

Homologs corresponding to microtubule plus-end stabilizing protein Adenomatous Polyposis Coli (APC) and its binding partner, End-binding protein 1 (EB1), were identified (Table 1). CLIP170 binds EB1 and associates with tubulin dimers at growing microtubule ends (Folker et al., 2005; Strickland et al., 2005). The dynactin complex regulates dynein interaction with vesicles and trafficking along microtubules and has been implicated in regulating the plus-end microtubule-based interaction with the cortex that leads to furrow induction and cytokinesis (Strickland et al., 2005). Putative homologs corresponding to dynactin/p150-*glued*, dynactin p25 and dynamitin have been determined.

The genome contains genes for a range of microtubule-associated proteins (MAPs), many of which were first discovered in mammalian tissues but had not been observed in sea urchin microtubules previously despite attempts in multiple laboratories. These include homologs of the heat-stable mammalian brain MAP Tau/MAP2/MAP4 family (SPU_013254, SPU_000651) which contain four of this family's hallmark SKXGSXDNXXHXPGGGXVXI microtubule-binding repeats, as well as two divergent repeats with the sequences SKCGSLGNSTHRAGGGNVKI and SKXGSXDNXXHXP-SGGXVXII; a homolog of MAP1 light chain 3 (SPU_009444); a ca. 200 kDa MAP1A/MAP1B homolog (SPU_004628) that includes the carboxyl-terminal domain which encodes the MAP1 light chain and the heavy chain/light chain junction (Hammarback et al., 1991; Langkopf et al., 1992; Togel et al., 1999); and TPX2 (SPU_008221). The sequence of the human genome was found to contain five homologs of the canonical sea urchin MAP, known as the 77 kDa MAP (Bloom et al., 1985) or "EMAP" (Li and Suprenant, 1994; Suprenant et al., 2000), for which we have found a single gene in *S. purpuratus*, SPU_006911. By inspection of the *S. purpuratus* genome, we have also found a family of EMAP-related genes in *S. purpuratus*, including SPU_011819, SPU_026643, and SPU_001991, which may reflect the five human EMAP-like

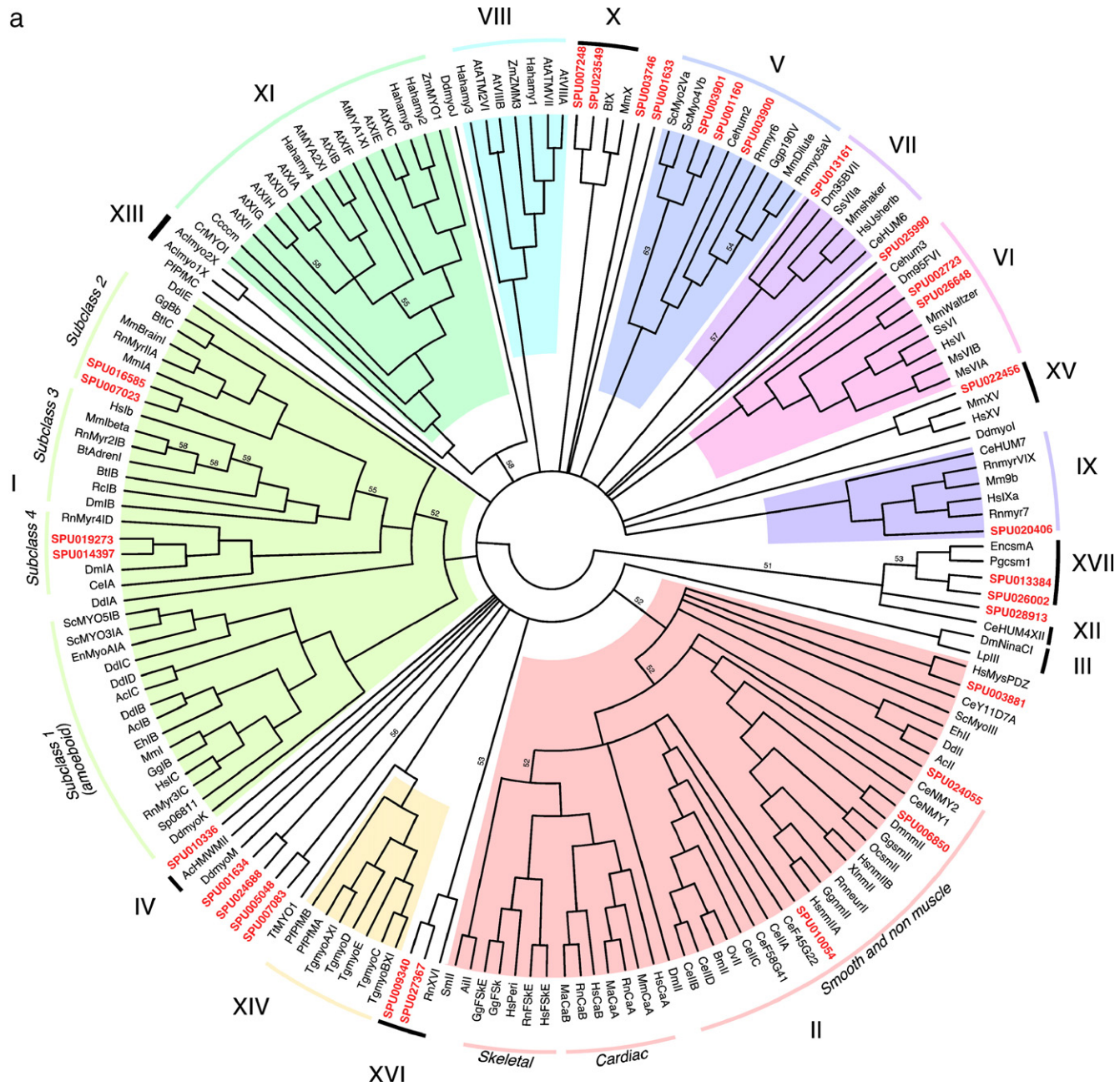


Fig. 1. Phylogenetic placement of predicted *S. purpuratus* myosin, tubulin, kinesin, and dynein sequences. (a) Myosin gene family. (b) Tubulin gene superfamily. (c) Kinesin gene superfamily. (d) Dynein gene family. All four trees are majority rule bootstrap consensus trees inferred as described in the text using full-length gene amino acid sequences wherever available. Only clades with >50% bootstrap support are shown for the myosin, tubulin, and dynein consensus trees. For the kinesin data, because we used the full-length gene sequences, the complexity of the gene product, and the method used to generate the multiple sequence alignment, the resulting 50% majority rule bootstrap consensus tree was uninformative. Therefore the bootstrap consensus tree for this tree was generated using the "extended majority rule" criteria. All clades with a bootstrap value of <20% were collapsed. In all four trees, branches have a bootstrap support of >70% unless indicated otherwise. The anomalous positioning of some *T. gratilla* sequences is due to their relatively short available lengths. A list of all sequences used in the four analyses as well as the multiple sequence alignments are available from the authors (SSM). Gene family designations and abbreviated gene names follow Hodge and Cope (2000) for the myosins, Dutcher (2003) for the tubulins, and Miki et al. (2005) for the kinesins. Nomenclature for dyneins follows FlyBase for *Drosophila*, Pazour et al. (2006) for *Chlamydomonas*, Asai and Wilkes (2004) for *Tetrahymena*, and the mammalian system (Wain et al., 2004) for other organisms. The predicted *S. purpuratus* sequences are in bold and colored red. Genus species abbreviations are as follows. Ac: *Acanthamoeba castellanii*, Ag: *Anopheles gambiae*, Ai: *Aequipecten irradians*, At: *Arabidopsis thaliana*, Bm: *Brugia malayi*, Bt: *Bos taurus*, Cc: *Cara coralline*, Ce: *Caenorhabditis elegans*, Cf: *Canis familiaris*, Ci: *Ciona intestinalis*, Cr: *Chlamydomonas reinhardtii*, Dd: *Dictyostelium discoideum*, Dm: *Drosophila melanogaster*, Dr: *Danio rario*, Ec: *E. coli*, Eg: *Eremothecium gossypii*, Eh: *Entamoeba histolytica*, En: *Emiricella nidulans*, Gg: *Gallus gallus*, Ha: *Helianthus annus*, Hs: *Homo sapiens*, Kl: *Kluyveromyces lactis*, Lp: *Limulus polyphemus*, Ma: *Mesocricetus auratus*, Mg: *Magnaporthe grisea*, Ml: *Macaca mulatta*, Mm: *Mus musculus*, Ms: *Morone saxatilis*, Nc: *Neurospora crassa*, Nv: *Nematostella ventensis*, Os: *Oryza sativa* (japonica cultivar-group), Ov: *Onchocerca volvulus*, Pf: *Plasmodium falciparum*, Pg: *Pyricularia grisea*, Rc: *Rana catesbeiana*, Rn: *Rattus norvegicus*, Sc: *Saccharomyces cerevisiae*, Scp: *Schizosaccharomyces pombe*, Sm: *Schistosoma mansoni*, Sp: *Strongylocentrotus purpuratus*, Ss: *Sus scrofa*, Tg: *Toxoplasma gondii*, Tt: *Tetrahymena thermophila*, Xl: *Xenopus laevis*, Zm: *Zea mays*.

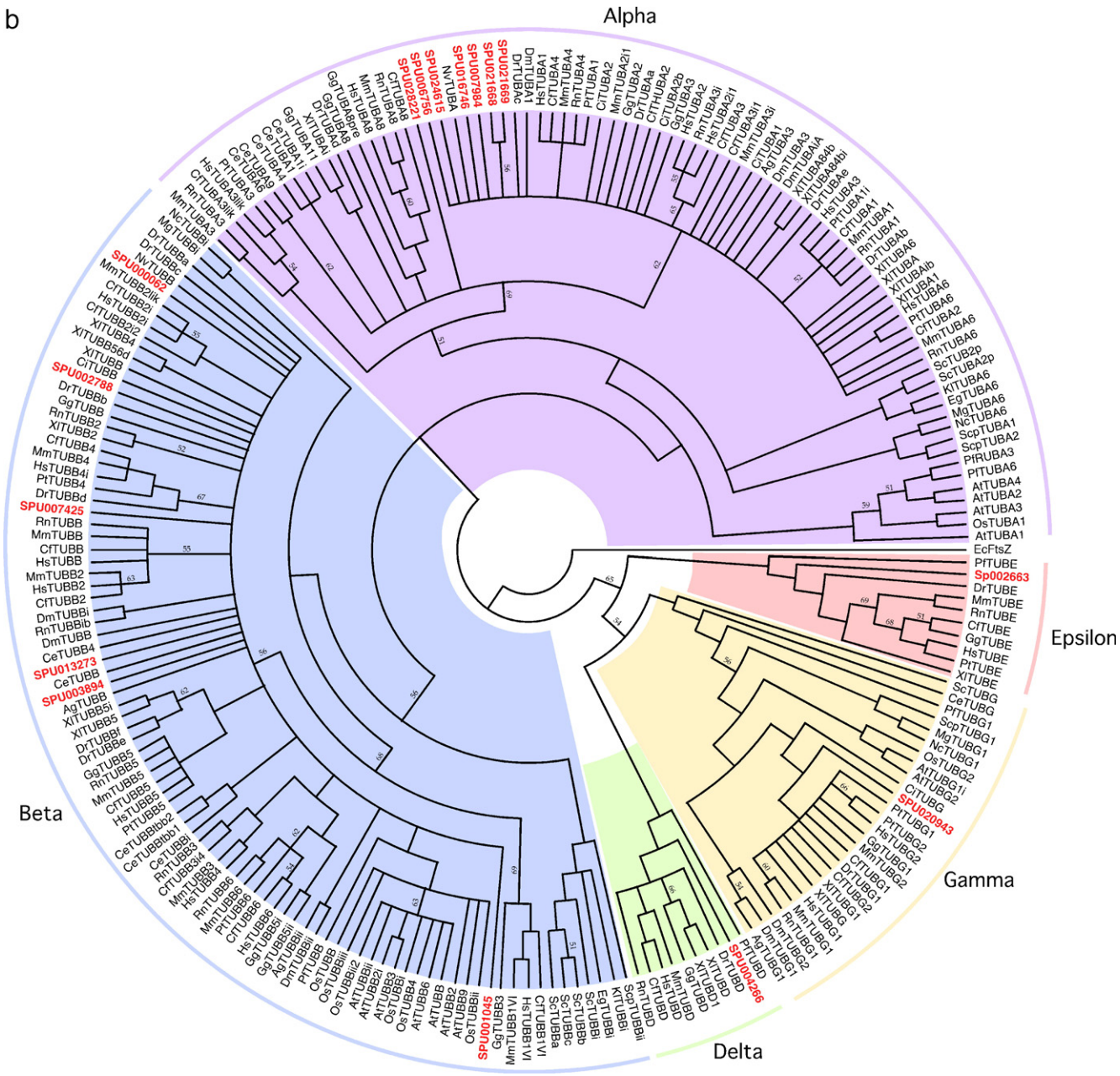


Fig. 1 (*continued*).

proteins termed EMLs1-5. Additional members of the well-studied MAP families and gene products that might correspond to other known sea urchin MAPs (see Vallee and Bloom, 1983; Maekawa et al., 1992, 1994) were not identified, and remain to be found. A gene (SPU_000096) of the MAST/orbit/CLASP family, which mediates microtubule attachment to kinetochores, was identified (Table 1).

*Kinesins*

The kinesins represent an extensive superfamily of plus end-directed microtubule-based motor proteins. The identification of kinesins in sea urchin eggs and embryos originally exploited the ability to purify microtubules from these cells in such tremendous quantity that one could biochemically analyze the MT-associated ATPases including the kinesins (Scholey et al., 1984, 1985; Collins and Vallee, 1986; Cole et al., 1992). Reverse genetics built upon this biochemical foundation to clone and sequence seven sea urchin kinesin motor polypeptides (Wright et al., 1991; Cole et al., 1993; Rashid et al., 1995; Rogers et al., 1999; Chui et al., 2000; Rogers et al., 2000) and non-motor-associated polypeptides (Wedaman et al., 1993, 1996). These kinesins participate in intracellular movements such as organelle transport (Bi et al., 1997; Wright et al., 1991), mitosis (Chui et al., 2000; Rogers et al., 2000; Sharp et al., 2000) and ciliogenesis (Morris and Scholey, 1997).

From the 147 GLEAN3 or NCBI GNOMON gene predictions that included a whole or partial kinesin motor domain, 35

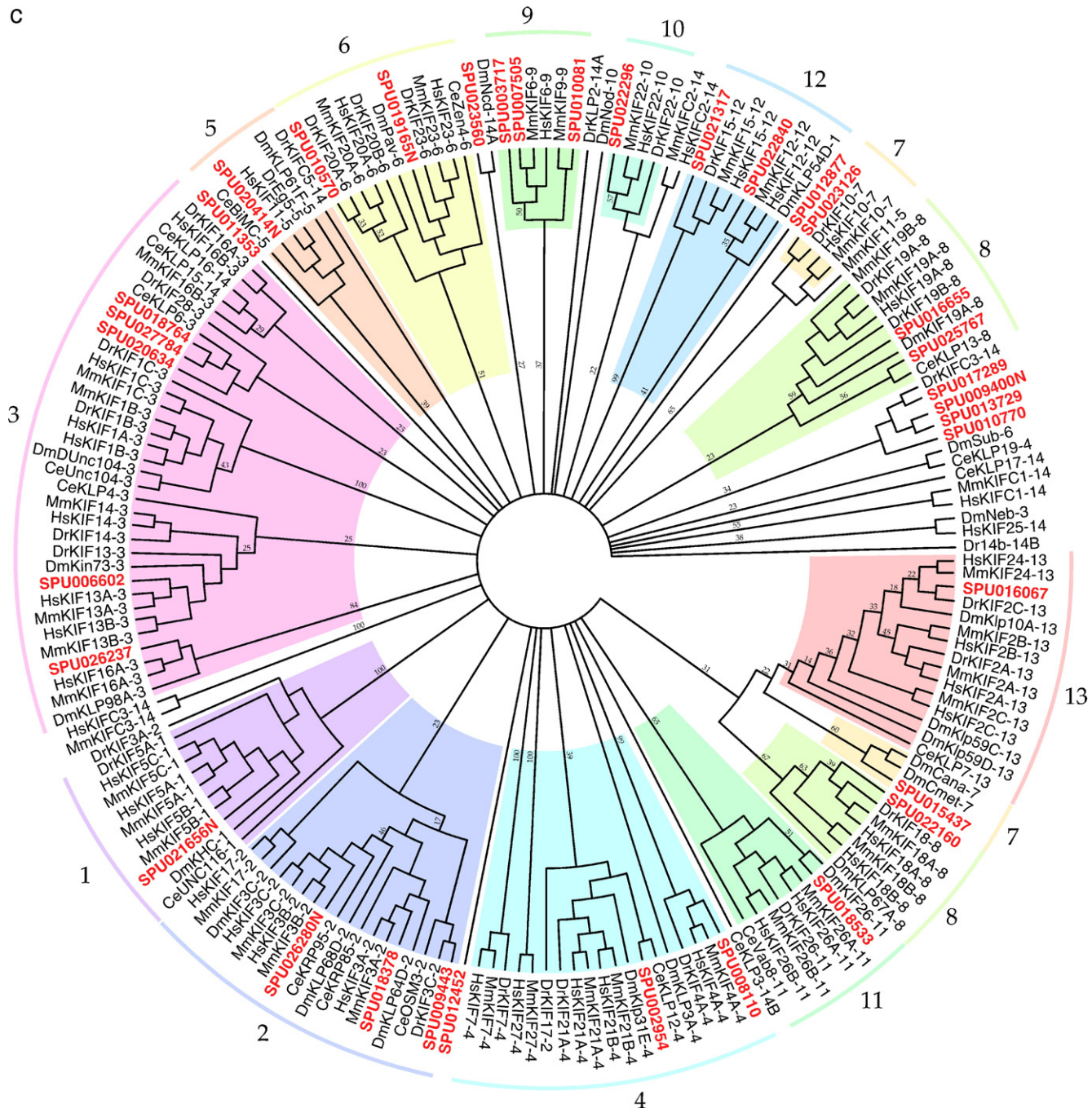


Fig. 1 (*continued*).

non-redundant kinesin genes and two kinesin-associated protein genes were identified in the *S. purpuratus* genome assembly including the 9 kinesin and kinesin-associated protein genes previously cloned from *S. purpuratus* (Chui et al., 2000; Cole et al., 1993; Rashid et al., 1995; Rogers et al., 1999, 2000; Wedaman et al., 1993, 1996; Wright et al., 1991), as well as members of all 14 families of kinesins identified by Lawrence et al. (2004) and 13 of 14 identified by Wickstead and Gull (2006) (Fig. 1c and Table 2). Not surprisingly, a total of 35 kinesins in echinoderms is intermediate between the 45 in human and the 25 of *Drosophila* (Miki et al., 2005), but more than the 31 predicted to exist in *Ciona* (Vale, 2003). With the 28 new kinesins discovered here, *S. purpuratus* possesses the same completeness of kinesin family representation seen in vertebrates (Miki et al., 2005; Wickstead and Gull, 2006), but more than seen in *Drosophila*. Interesting findings from the *S. purpuratus* kinesin repertoire include a third kinesin-2, Sp-Osm-3, that represents the homodimeric kinesin-2 proteins, as well as an orphan kinesin Sp-KIF27-like that is most similar to the kinesin-4s but almost as equally divergent from all other families. As found by Wickstead and Gull (2006), our analysis divided the kinesin-12 family of Lawrence et al. (2004) into two

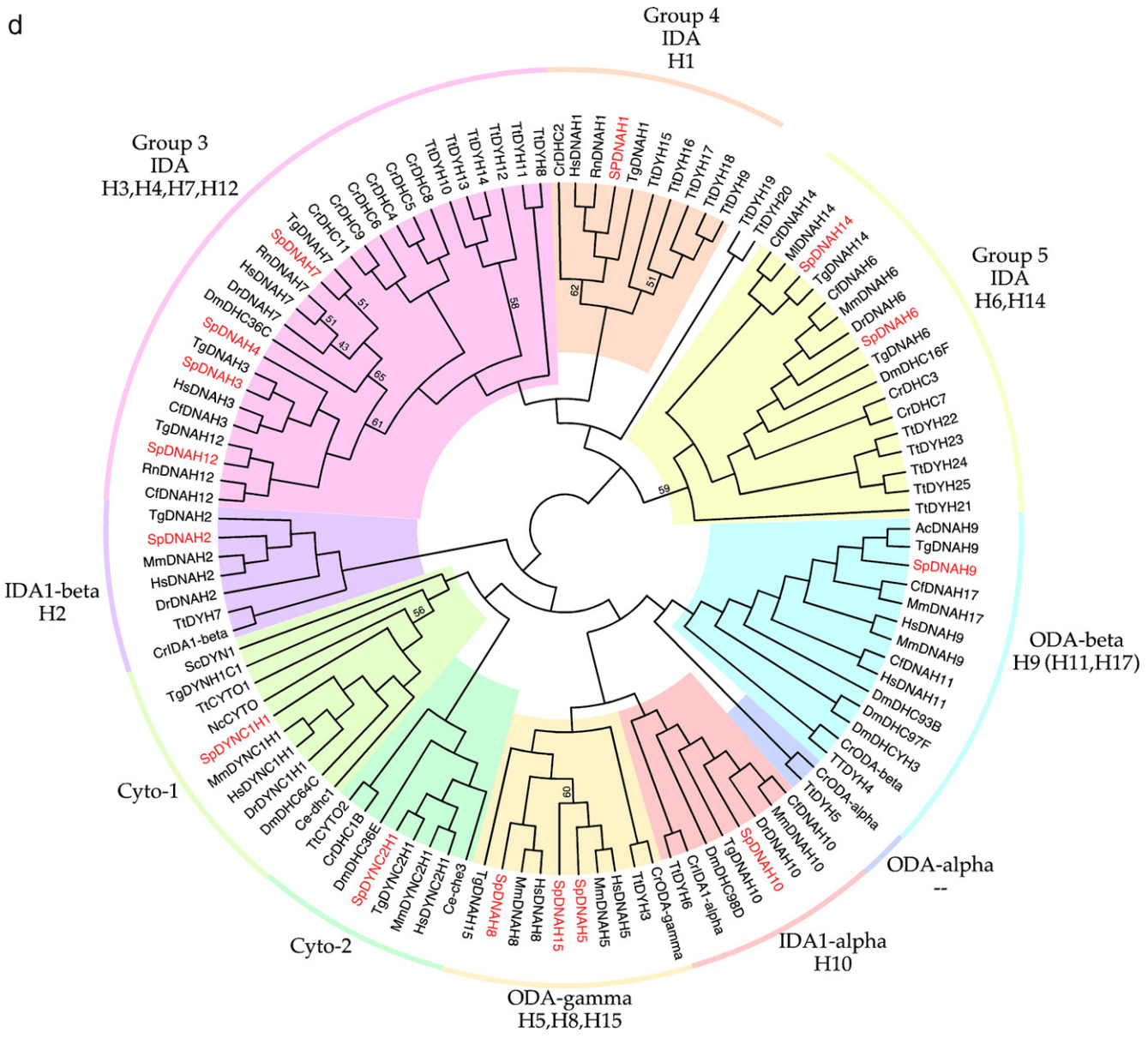


Fig. 1 (*continued*).

clades that Wickstead and Gull name the kinesin-15 and kinesin-16 families, both of which are represented in *S. purpuratus*, but unlike Wickstead and Gull (2006) who excluded HsKIF26, our analysis supports a grouping of Kif26s together in a kinesin-11 family (Fig. 1c) as proposed by Lawrence et al. (2004). Although the motor domain of SPU_015437 was KIF2-like (E value 6e-122), the full-length protein grouped with Dm CENP and Dm CENP meta which themselves usually group with the kinesin-7s (Miki et al., 2005; Wickstead and Gull, 2006), warranting further study. Only a few sequences designated as kinesin-14s in the analysis of Miki et al. (2005) formed clades with bootstrap values >50% in our analysis and so these sequences are neither grouped nor their family distinctly labeled in our phylogenetic tree (Fig. 1c). This dispersion results from the great complexity of the kinesin-14s described before (Miki et al., 2005; Wickstead and Gull, 2006).

Genes for the two known kinesin-associated non-motor polypeptides, kinesin-1-associated light chain KLC and kinesin-2-associated KAP (Wedaman et al., 1993, 1996) were also located (Table 2). Some notable absences from the urchin genome were additional kinesin-1s; Sp-KHC is more like Dm-KHC than like the three KIF5s found in mouse and human. Because of the typically long length of kinesin genes (averaging 20 exons per Sp gene in a sampling of 10 fully annotated sequences) some kinesin gene predictions remain incomplete and will require further analysis, eventually perhaps increasing the number of kinesins found in *S. purpuratus*.

To begin investigating the possibility that expression of some families of kinesins is up-regulated to play roles in early development, we investigated available EST libraries to estimate the relative abundance of different kinesins at different stages of development (Fig. 2). A variety of kinesins appear to

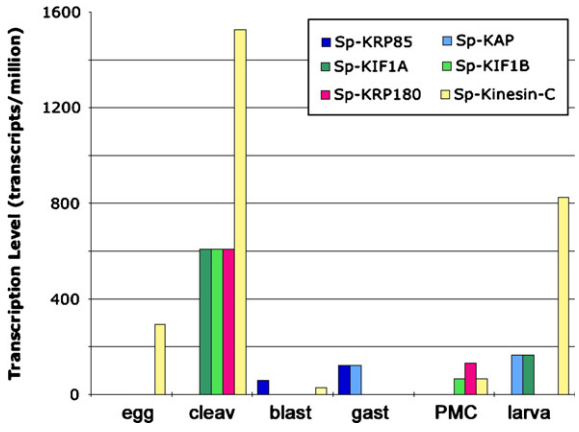


Fig. 2. Expression profiles of kinesins and associated proteins during *S. purpuratus* development. Transcript level as predicted by relative abundance of kinesin-specific ESTs is shown for a kinesin-2 (Sp-KRP85 and its associated protein Sp-KAP), two kinesin-3s (Sp-KIF1A subfamily and Sp-KIF1B subfamily), a kinesin-12 (KRP180), and a kinesin-14 (Sp-kinesin-C) in eggs, cleavage stage (cleav), blastula stage (blast), gastrula stage (gast), specifically in primary mesenchyme cells (PMCs) and larvae. Expression levels appeared to peak during cleavage stage for several of the kinesins.

be more actively expressed during cleavage stages than other stages, while some are expressed only later in development. The kinesin-2 subunits Sp-KR85 and Sp-KAP appear to increase in expression concomitant to the onset of ciliogenesis at the late blastula/early gastrula stages. These results support microinjection experiments showing that antibodies which block the function of kinesin-2 are capable of eliminating all kinesin-2 function through gastrulation (Morris and Scholey, 1997). While QPCR analysis is necessary to corroborate this evidence, these data illustrate the value of cataloging the entire complement of kinesins in a classic developmental model.

### *Dyneins*

The ample supplies of sperm flagella that can be obtained from sexually mature sea urchins have long made them favored material for studying the biochemistry and molecular biology of dynein, especially since the unusually high molecular mass of the dynein heavy chain (~550 kDa, corresponding to a 14 kb gene) places emphasis on quantity. The first dynein gene to be cloned and sequenced was that encoding one of the axonemal dynein heavy chains in sea urchin sperm flagella (Gibbons et al., 1991; Ogawa, 1991). Shortly thereafter, the genes of the motor domain for the other 13 axonemal and two cytoplasmic dyneins that together comprise the core of the dynein motor family in eukaryotes were cloned and sequenced (Gibbons et al., 1994; Rasmusson et al., 1994; Vaughan et al., 1996). Subsequent completion and annotation of the human and other mammalian genomes have resulted in the definition of an almost complete set of full-length dynein heavy chain sequences. Building upon this background, we decided to annotate the dynein genes in *S. purpuratus* by screening its genome assembly with dynein sequences from other sea urchin species where available, supplemented with full-length sequences from mammalian data banks as necessary. To encourage greater uniformity of dynein gene nomenclature in different species, we use a modestly extended version of the internationally recognized mammalian nomenclature for dynein genes (Wain et al., 2004) to describe our results.

All 13 of the axonemal dynein heavy chains and the two cytoplasmic dynein heavy chain genes that were previously identified in the sea urchin *Tripneustes gratilla* (Gibbons et al., 1994) are present in the *S. purpuratus* genome (Fig. 1d and Table 3). In most cases, we were able to align ~95% of the estimated full-length heavy chain gene to its indexing homolog. The greatest difficulty occurred in the N-terminal region, which is the least conserved region in all dynein heavy chains. Averaged over their full available lengths, most of the dynein heavy chains are 95% identical to the homologous gene in *T. gratilla* and 60–70% identical to their mammalian homologs. The major exception was Sp-DNAH14 which showed a substantially lower identity of only 41%. The majority of dynein heavy chain genes in *S. purpuratus* show an unambiguous one-to-one relationship to their mammalian homologs. One exception is the one-to-three relationship of Sp-DNAH9, corresponding to the beta heavy chain of axonemal outer arm dynein, in which the sequence is approximately equidistant between those of three closely similar genes DNAH9, DNA11 and DNAH17 in mammals. The only other exception is that two single genes in mammals, DNAH3 and DNAH5, each appear equivalent to a closely related gene pair in sea urchins, with the former corresponding to Sp-DNAH3 and Sp-DNAH4 and the latter corresponding to Sp-DNAH5 and Sp-DNAH15. In scanning the *S. purpuratus* genome, we encountered no new dynein heavy chain genes, additional to those previously identified in *T. gratilla* (Gibbons et al., 1994).

The three axonemal dynein intermediate chains previously identified in *Anthocidaris crassispina* (Ogawa et al., 1996; Kagami et al., 1998) match the three intermediate chains of *S. purpuratus* characterized here (Table 3). We also identified three axonemal lights chains. Among the other dynein subunits known from mammals (Pfister et al., 2005), we have identified one intermediate chain, 3 light intermediate chains and all six of the light chains known to be associated with cytoplasmic dynein in mammals. We have not, as yet, found orthologs of cytoplasmic dynein 1 intermediate chain 2 or of cytoplasmic dynein light intermediate chain 2. The *S. purpuratus* genome contains, at least 5 genes encoding a DLC-type light chain and 5 encoding an LC8-type light chain, genes en-coding two roadblock-type light chains and 3 kinds of Tctex-like light chains as well as an intermediate chain and 3 types of light intermediate chains (see above and Table 3).

### *Intermediate filaments*

Intermediate filament family proteins are a diverse group of proline-rich rod-shaped proteins that play roles in such diverse cellular processes as nuclear envelope structure, cellular structural support including functions of desmosomal junctions, cell-substrate adhesion, and cell structural support. Key intermediate filament family proteins have been identified in the sea urchin genome assembly, including: acidic keratin, desmin, lamin B and nestin. Genetic diversity within the lamin

gene family is likely to have arisen following the diversification of vertebrates from the chordates.

### *Phylogenetic analysis*

Using the data from the for CLIP170, fascin, gelsolin, lamin B, myosin II, and profilin genes, we inferred the relationship among human (*H. sapiens*), mouse (*M. musculus*), chicken (*G. gallus*), frog (*X. laevis/gilli*), zebrafish (*D. rario*), nematode (*C. elegans*), fruit fly (*D. melanogaster*), sea urchin (*S. purpuratus*), tunicate (*C. intestinalis*), and sea anemone (*N. vectensis*) using maximum likelihood methods with yeast (*S. cerevisiae*) as an outgroup (Fig. 3). Generally, the major groupings of Chordata, Deuterostomes, and Bilateria are upheld with the Cnidaria (represented by *C. intestinalis*) forming a basal lineage leading to the divergence of the Bilateria. The Protostomes (Ectozoa), as represented by *Drosophila* (Arthropoda) and *C. elegans* (Nematoda), do not form a monophyletic group. However, the relationship among nematode, fruit fly, and sea urchin, which defines the region where Protostomes and Deuterostomes diverged, is not particularly well supported in this analysis, with relatively low bootstrap and Bayesian support values in this region of the phylogeny. These results are consistent with recent studies on the relationship among eukaryotes based on differing suits of genes (Nei et al., 2001; Blair et al., 2005), demonstrating the potential of cytoskeletal and motility proteins in determining deep level relationships among organisms.

## Discussion

Annotation of the cytoskeletal genome of *S. purpuratus* has revealed the presence of representatives of essentially all known cytoskeletal proteins. Detailed evolutionary analysis of the genes of such functionally conserved proteins reveals an overall consistent trend of placing the genes most closely to those of the vertebrates. We conducted a detailed phylogenetic analysis of a broad spectrum of key cytoskeletal and motor proteins derived from the genome assembly and confirm what is becoming an accepted fact: *S. purpuratus*, being a deuterostome and representing echinoderms generally, is more closely related to chordates and vertebrates than are arthropods (i.e., *D. melanogaster*), or nematodes (i.e., *C. elegans*).

Perhaps more notable is the apparent absence of homologs for several vertebrate cytoskeletal and motor proteins. For instance phylogenetic analysis of the myosin family revealed several homologs that encode the motor domain of smooth and non-muscle myosin II, whereas no clear matches were identified for the motor domain of skeletal muscle myosin II. Sea urchins

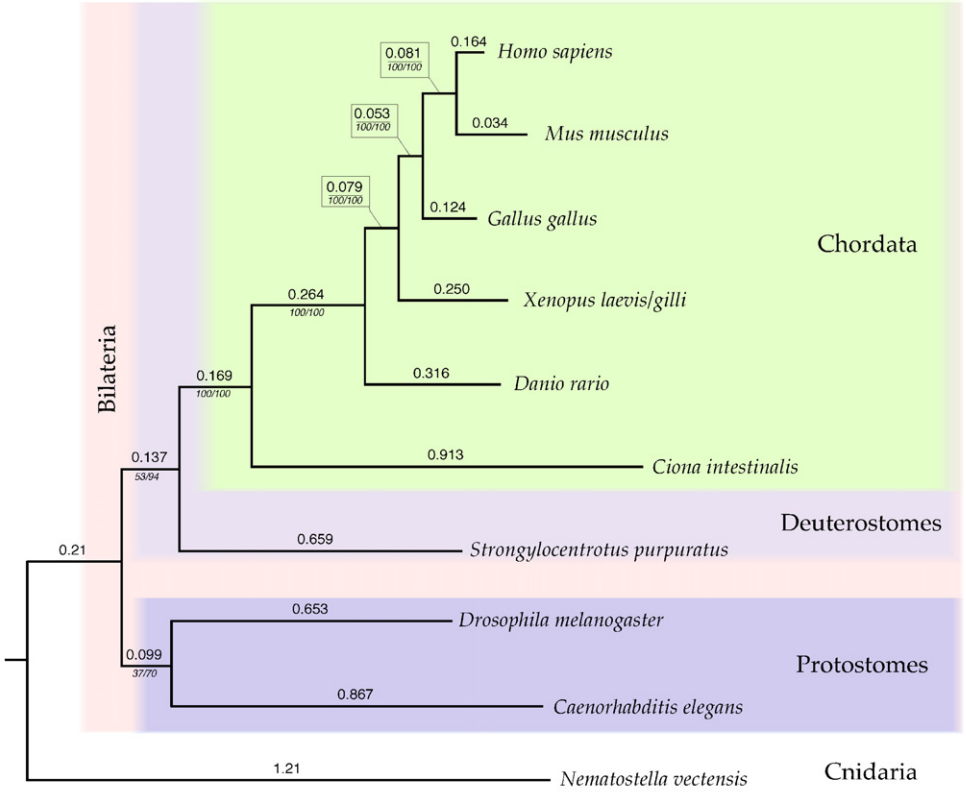


Fig. 3. Phylogenetic relationship among the ten species of eukaryotes based on CLIP170, fascin, gelsolin, lamin, myosin II, and profilin amino acid sequences. Values above each branch are the branch lengths based on a maximum likelihood analysis as described in the text. The values below each branch are the nonparametric bootstrap confidence values from a maximum likelihood analysis and the prior probabilities from a Bayesian analysis. All branches are shown regardless of the bootstrap or Bayesian support values. The basal portion of the tree showing the relationship among *D. melanogaster*, *S. purpuratus*, and *C. elegans* is relatively unresolved.

move using tube feet that are controlled by striated muscles and homologs of essentially all skeletal sarcomeric proteins were identified. It is thus likely that skeletal myosin II exists and the annotation has failed to discriminate its presence from other closely related myosin IIs. We were surprised to find two predicted myosin motor domains that corresponded to a new class of myosin, XVI, recently identified in rat brain development (Patel et al., 2001). The absence of a predicted myosin XIV and III, may indicate either a failed annotation or that *S. purpuratus* lacks these genes. The identification of such a wide variety of myosin classes makes possible their functional dissection in the many motile properties of cells from sea urchins.

The sea urchin actin gene family has been previously characterized in depth and the annotation has identified a full complement of the actin genes found in most vertebrates. Based on their highly conserved nature, comparison of actin gene sequences at the nucleotide level has been used historically as a basis for understanding evolutionary relationships between organisms. Over the past two decades, researchers have used biochemistry and cloning from cDNA libraries to reveal at least eight *S. purpuratus* actin genes, including a single skeletal muscle gene and two groups of five cytoskeletal actin genes: cytoskeletal actin (Cy) I, CyIIa, and CyIIb are closely linked as one group that is distinct from CyIIIa and CyIIIb, which are closely linked to each other (Fang and Brandhorst, 1996; Lee et al., 1984).

In both the case of the alpha-tubulins and the case of the beta-tubulins we have identified, there is relatively little heterogeneity in either gene structure or derived protein sequence. The alpha-tubulin genes, in particular, appear to be minor variations on a single theme, and 8 of the 9 identified genes cluster closely together phylogenetically (see Fig. 1b). This indicates that much of the functional diversity and selective patterns of expression of the tubulin proteins of the sea urchin (Gianguzza et al., 1989) comes as a result of post-translational modifications of the tubulin proteins which have been widely documented in these families (see McKean et al., 2001 for a review). The conserved intron locations and general structures of the genes are consistent with the "introns-early" hypothesis (see Perumal et al., 2005), and the occurrence of truncated or otherwise divergent pseudogenes is also well known in the alpha- and beta-tubulin gene families.

Identification of the entire complement of kinesins in the sea urchin will allow a thorough analysis of roles of individual kinesins and their families in development. With the discovery of a complete repertoire of 35 kinesin sequences, this study validates the use of sea urchin for studying the kinesins. With 35 gene sequences, we have identified a complex but not insurmountable set of kinesins in a model deuterostomre well suited for investigating the expression of all individual gene products during early developmental stages and the functions of whole kinesin families for which functions are not well understood. It will also allow characterization of the entire kinesin ensemble required for ciliogenesis since all kinesin families proposed to play roles in ciliogenesis (Wickstead and Gull, 2006) are represented in this organism where the ciliogenic cycle has been so well characterized (Masuda, 1979; Stephens, 1995).

The phylogenetic tree in Fig. 1d compares the predicted amino acid sequences of the dynein heavy chains genes in *S. purpuratus* to each other and to the corresponding genes in model organisms of other phyla. The largely monophyletic branching observed for the heavy chain subunits of outer arm dynein (ODA-beta and ODA-gamma), for the alpha and beta subunits of inner arm 1 dynein (IDA1-alpha and IDA1-beta) and for both cytoplasmic dynein 1 (Cyto1) and cytoplasmic dynein 2 (Cyto2) indicates that the conserved pattern of these subunits extends back to the earliest eukaryotic cells and is perhaps related to the extraordinary evolutionary stability of the 9+2 axonemal structure of cilia and eukaryotic flagella. On the other hand, the putative inner arm dynein components in other branches of the dynein tree (IDA Groups 3, 4, and 5) appear to be somewhat less tightly constrained and they have radiated into distinct subfamilies in such organisms as *Chlamydomonas* and *Tetrahymena* that possibly utilize more varied patterns of axonemal movement. An apparent example of more recent gene duplication in dynein is the splitting of the single ODA-beta gene present in organisms from *Tetrahymena* to sea urchin into the triad of related genes found in mammals, a divergence that might possibly have been favored by substantial thickening of the 9+2 structure of ciliary axonemes into the 9+9+2 organization present in mammalian spermatozoa.

With the *S. purpuratus* genome now available for analysis, it is interesting to note that different pre-genomic strategies to systematically explore the kinesin and dynein superfamilies in sea urchin produced qualitatively and quantitatively different outcomes. The PCR-based protocol used to identify dynein genes was particularly effective in finding most or all of the dynein heavy chain genes present in sea urchins (Gibbons et al., 1994), *Drosophila* (Rasmusson et al., 1994) and mammals (Vaughan et al., 1996) yet did not reveal associated proteins directly. In comparison, the microtubule-affinity/pan-kinesin antibody/reverse genetic approach was particularly effective at revealing the multi-subunit makeup of kinesin holoenzymes while avoiding the complications of pseudogenes (Cole and Scholey, 1995), but it uncovered only one-fifth of the kinesin genes present in sea urchins (Table 2). With genomic catalogs of dynein and kinesin genes as well as sequences of their associated proteins now available, it may be possible to bring to light sea urchin motor protein complexes with the completeness of the dynein heavy chain discoveries and the thoroughness of the kinesin holoenzyme discoveries.

Examples of most classes of intermediate filament proteins are present although in a very limited variety. The absence of such genes for key vertebrate cytoskeletal proteins, such as lamin A and vimentin, is consistent with their absence in other invertebrates and new appearance in the vertebrates. The rather simple fleet of intermediate filament family proteins is also consistent with the absence of intermediate filament-linked cadherins (Whittaker et al., 2006). The duplication event splitting lamin A from lamin B1 and lamin B2 occurred sometime after the divergence of complex vertebrates from primitive chordates. There appears to be only a single lamin in *C.*

*intestinalis*, so the duplication event likely occurred after the split of *Ciona* from the ancestor leading to higher vertebrates.

The initial characterization of the sea urchin cytoskeleton and motility genome provides new tools with which further analysis of cell structure and motility will be made possible. Cells and tissues of developing sea urchins are excellent models for analysis of such events as ciliogenesis, mitosis, cytokinesis, morphogenetic movements, lamellipod extension, filopod movements, and organelle translocation among others. Having the sea urchin cytoskeleton and motility genome available makes possible design of inhibitory antibodies, morpholinos, fluorescently tagged peptides and proteins, inhibitory peptides, constitutively active or inactive kinase domains and other such probes. These tools, combined with the ability to microinject into fertilized eggs at specific times during mitosis allows for functional dissection of the roles of specific cytoskeletal and motility proteins and their regulators. This experimental manipulation is unique in the study of cell division to the use of dividing echinoderm eggs. Thus, cells of sea urchins remain as critical for analysis of the roles of the cytoskeleton today as they were over 100 years ago.

## Note added in proof

Mutations in the cytoskeletal genes DNAH9, MYH1, MAP2, and TTL3 have been shown to have a highly significant pattern of occurrence in human breast and colorectal cancers (Sjöblom, et al., in press).

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## References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.

Asai, D.J., Wilkes, D.E., 2004. The dynein heavy chain family. J. Eukaryot. Microbiol. 51, 23–29.

Begg, D.A., Rebhun, L.I., 1979. pH regulates the polymerization of actin in the sea urchin egg cortex. J. Cell Biol. 83, 241–248.

Bi, G.Q., Morris, R.L., Liao, G., Alderton, J.M., Scholey, J.M., Steinhardt, R.A., 1997. Kinesin- and myosin-driven steps of vesicle recruitment for $Ca^{2+}$-regulated exocytosis. J. Cell Biol. 138, 999–1008.

Blair, J.E., Shah, P., Hedges, S.B., 2005. Evolutionary sequence analysis of complete eukaryote genomes. BMC Bioinformatics 6, 53.

Bloom, G.S., Luca, F.C., Collins, C.A., Vallee, R.B., 1985. Use of multiple monoclonal antibodies to characterize the major microtubule-associated protein in sea urchin eggs. Cell Motil. 5, 431–446.

Brokaw, C.J., Gibbons, I.R., 1973. Localized activation of bending in proximal, medial and distal regions of sea-urchin sperm flagella. J. Cell Sci. 13, 1–10.

Bryan, J., Edwards, R., Matsudaira, P., Otto, J., Wulfkuhle, J., 1993. Fascin, an echinoid actin-bundling protein, is a homolog of the *Drosophila* singed gene product. Proc. Natl. Acad. Sci. U. S. A. 90, 9115–9119.

Carlini, D.B., Reece, K.S., Graves, J.E., 2000. Actin gene family evolution and the phylogeny of coleoid cephalopods mollusca, cephalopoda. Mol. Biol. Evol. 17, 1353–1370.

Chui, K.K., Rogers, G.C., Kashina, A.M., Wedaman, K.P., Sharp, D.J., Nguyen, D.T., Wilt, F., Scholey, J.M., 2000. Roles of two homotetrameric kinesins in sea urchin embryonic cell division. J. Biol. Chem. 275, 38005–38011.

Cohen, W.D., Rebhun, L.I., 1970. An estimate of the amount of microtubule protein in the isolated mitotic apparatus. J. Cell Sci. 6, 159–176.

Cole, D.G., Scholey, J.M., 1995. Purification of kinesin-related protein complexes from eggs and embryos. Biophys. J. 68, 158s–162s.

Cole, D.G., Cande, W.Z., Baskin, R.J., Skoufias, D.A., Hogan, C.J., Scholey, J.M., 1992. Isolation of a sea urchin egg kinesin-related protein using peptide antibodies. J.Cell Sci. 101, 291–301.

Cole, D.G., Chinn, S.W., Wedaman, K.P., Hall, K., Vuong, T., Scholey, J.M., 1993. Novel heterotrimeric kinesin-related protein purified from sea urchin eggs. Nature 366, 268–270.

Collins, C.A., Vallee, R.B., 1986. A microtubule-activated ATPase from sea urchin eggs, distinct from cytoplasmic dynein and kinesin. Proc. Natl. Acad. Sci. U. S. A. 83, 4799–4803.

D'Andrea, L., Danon, M.A., Sigourdas, G.P., 1994. Identification of coelomocyte unconventional myosin and its association with in vivo particle/vesicle motility. J. Cell. Sci. 107 (Pt. 8), 2081–2094.

Dutcher, S.K., 2003. Long-lost relatives reappear, identification of new members of the tubulin superfamily. Curr. Opin. Microbiol. 6, 634–640.

Edds, K.T., 1980. The formation and elongation of filopodia during transformation of sea urchin coelomocytes. Cell Motil. 1, 131–140.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.

Fang, H., Brandhorst, B.P., 1996. Expression of the actin gene family in embryos of the sea urchin *Lytechinus pictus*. Dev. Biol. 173, 306–317.

Felsenstein, J., 1985. Confidence limits on phylogenies, an approach using the bootstrap. Evolution 39, 783–791.

Felsenstein, J., 2005. PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Fishkind, D.J., Bonder, E.M., Begg, D.A., 1987. Isolation and characterization of sea urchin egg spectrin, calcium modulation of the spectrin-actin interaction. Cell Motil. Cytoskeleton 7, 304–314.

Folker, E.S., Baker, B.M., Goodson, H.V., 2005. Interactions between CLIP-170, tubulin, and microtubules: implications for the mechanism of Clip-170 plus-end tracking behavior. Mol. Biol. Cell 16, 5373–5384.

Gianguzza, F., DiBernardo, M.G., Sollazzo, M., Palla, F., Ciaccio, M., Carra, E., Spinelli, G., 1989. DNA sequence and pattern of expression of the sea urchin (*Paracentrotus lividus*) alpha-tubulin genes. Mol. Reprod. Dev. 1, 170–181.

Gibbons, I.R., Gibbons, B.H., Mocz, G., Asai, D.J., 1991. Multiple nucleotide-binding sites in the sequence of dynein beta heavy chain. Nature 352, 640–643.

Gibbons, B.H., Asai, D.J., Tang, W.J., Hays, T.S., Gibbons, I.R., 1994. Phylogeny and expression of axonemal and cytoplasmic dynein genes in sea urchins. Mol. Biol. Cell 5, 57–70.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Hammarback, J.A., Obar, R.A., Hughes, S.M., Vallee, R.B., 1991. MAP1B is encoded as a polyprotein that is processed to form a complex N-terminal microtubule-binding domain. Neuron 7, 129–139.

Harvey, E.B., 1956. The American Arbacia and other Sea Urchins. Princeton University Press, Princeton.
Hiramoto, Y., 1968. The mechanics and mechanism of cleavage in the sea-urchin egg. Symp. Soc. Exp. Biol. 22, 311–327.
Hodge, T., Copefs, M.J.T.V., 2000. A myosin family tree. J. Cell Sci. 113, 3353–3354 (http://www.mrc-lmb.cam.ac.uk/myosin/trees/txalign.html).
Holleran, E.A., Holzbaur, E.L., 1998. Speculating about spectrin: new insights into the Golgi-associated cytoskeleton. Trends Cell Biol. 8, 26–29.
Jones, D.T., Taylor, W.R., Thornton, J.M., 1992. The rapid generation of mutation data matrices from protein sequences. Comp. Appl. Biosci. (CABIOS) 8, 275–282.
Kagami, O., Gotoh, M., Makino, Y., Mohri, H., Kamiya, R., Ogawa, K., 1998. A dynein light chain of sea urchin sperm flagella is a homolog of mouse Tctex 1, which is encoded by a gene of the t complex sterility locus. Gene 211, 383–386.
Kane, R., 1967. The mitotic apparatus: identification of the major soluble component of the glycerol-isolated mitotic apparatus. J. Cell Biol. 32, 243–253.
Kane, R.E., 1983. Interconversion of structural and contractile actin gels by insertion of myosin during assembly. J. Cell Biol. 97, 1745–1752.
Kane, R.E., 1986. Components of the actin-based cytoskeleton. Methods Cell Biol. 27, 229–242.
Katoh, K., Kuma, K., Toh, H., Miyata, T., 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res. 33, 511–518.
Kelleher, J.F., Atkinson, S.J., Pollard, T.D., 1995. Sequences, structural models, and cellular localization of the actin-related proteins Arp2 and Arp3 from Acanthamoeba. J. Cell Biol. 131, 385–397.
Kent, W.J., 2002. BLAT—The BLAST-like alignment tool. Genome Res. 12, 656–664.
Krause, M., Dent, E.W., Bear, J.E., Loureiro, J.J., Gertler, F.B., 2003. Ena/VASP proteins, regulators of the actin cytoskeleton and cell migration. Annu. Rev. Cell Dev. Biol. 19, 541–564.
Langkopf, A., Hammarback, J.A., Muller, R., Vallee, R.B., Garner, C.C., 1992. Microtubule-associated proteins 1A and LC2. Two proteins encoded in one messenger RNA. J. Biol. Chem. 267, 16561–16566.
Lawrence, C.J., Dawe, R.K., Christie, K.R., Cleveland, D.W., Dawson, S.C., Endow, S.A., Goldstein, L.S., Goodson, H.V., Hirokawa, N., Howard, J., Malmberg, R.L., McIntosh, J.R., Miki, H., Mitchison, T.J., Okada, Y., Reddy, A.S., Saxton, W.M., Schliwa, M., Scholey, J.M., Vale, R.D., Walczak, C.E., Wordeman, L., 2004. A standardized kinesin nomenclature. J. Cell Biol. 167, 19–22.
Lee, J.J., Shott, R.J., Rose III, S.J., Thomas, T.L., Britten, R.J., Davidson, E.H., 1984. Sea urchin actin gene subtypes. Gene number, linkage and evolution. J. Mol. Biol. 172, 149–176.
Li, Q., Suprenant, K.A., 1994. Molecular characterization of the 77-kDa echinoderm microtubule-associated protein. Homology to the beta-transducin family. J. Biol. Chem. 269, 31777–31784.
Mabuchi, I., 1973. A myosin-like protein in the cortical layer of the sea urchin egg. J. Cell. Biol. 59, 542–547.
Mabuchi, I., 1986. Biochemical aspects of cytokinesis. Int. Rev. Cytol. 101, 175–213.
Mabuchi, I., Okuno, M., 1977. The effect of myosin antibody on the division of starfish blastomeres. J. Cell Biol. 74, 251–263.
Mabuchi, I., Spudich, J.A., 1980. Purification and properties of soluble actin from sea urchin eggs. J. Biochem. (Tokyo) 87, 785–802.
Maekawa, S., Toriyama, M., Sakai, H., 1992. A novel 24-kDa microtubule-associated protein purified from sea urchin eggs. Eur. J. Biochem. 205, 1195–1200.
Maekawa, S., Mishima, M., Toriyama, M., Sakai, H., 1994. Purification of a low molecular weight microtubule binding protein from sea urchin eggs. Biochim. Biophys. Acta 1207, 194–200.
Masuda, M., 1979. Species specific pattern of ciliogenesis in developing sea urchin embryos. Dev. Growth Differ. 21, 545–552.
McKean, P.G., Vaughan, S., Gull, K., 2001. The extended tubulin superfamily. J. Cell Sci. 114, 2723–2733.
Miki, H., Okada, Y., Hirokawa, N., 2005. Analysis of the kinesin superfamily: insights into structure and function. Trends Cell Biol. 15, 467–476.
Morris, R.L., Scholey, J.M., 1997. Heterotrimeric kinesin-II is required for the assembly of motile 9+2 ciliary axonemes on sea urchin embryos. J. Cell Biol. 138, 1009–1022.
Nei, M., Xu, P., Glazko, G., 2001. Estimation of divergence times from multiprotein sequences for a few mammalian species and several distantly related organisms. Proc. Natl. Acad. Sci. 98, 2497–2502.
Ogawa, K., 1991. Four ATP-binding sites in the midregion of the beta heavy chain of dynein. Nature 352, 643–645.
Ogawa, K., Takai, H., Ogiwara, A., Yokota, E., Shimizu, T., Inaba, K., Mohri, H., 1996. Is outer arm dynein intermediate chain 1 multifunctional? Mol. Biol. Cell 7, 1895–1907.
Patel, K.G., Liu, C., Cameron, P.L., Cameron, R.S., 2001. Myr 8, a novel unconventional myosin expressed during brain development associates with the protein phosphatase catalytic subunits 1alpha and 1gamma1. J. Neurosci. 21, 7954–7968.
Pazour, G.J., Agrin, N., Leszyk, J., Witman, G.B., 2005. Proteomic analysis of a eukaryotic cilium. J. Cell Biol. 170, 103–113.
Pazour, G.J., Agrin, N., Walker, B.L., Witman, G.B., 2006. Identification of predicted human outer dynein arm genes: candidates for primary ciliary dyskinesia genes. J. Med. Genet. 43, 62–73.
Perumal, B.S., Sakharkar, K.R., Chow, V.T., Pandjassarame, K., Sakharkar, M.K., 2005. Intron position conservation across eukaryotic lineages in tubulin genes. Front. Biosci. 10, 2412–2419.
Pfister, K.K., Fisher, E.M., Gibbons, I.R., Hays, T.S., Holzbaur, E.L., McIntosh, J.R., Porter, M.E., Schroer, T.A., Vaughan, K.T., Witman, G.B., King, S.M., Vallee, R.B., 2005. Cytoplasmic dynein nomenclature. J. Cell Biol. 171, 411–413.
Poustka, A.J., Groth, D., Hennig, S., Thamm, S., Cameron, A., Beck, A., Reinhardt, R., Herwig, R., Panopoulou, G., Lehrach, H., 2003. Generation, annotation, evolutionary analysis, and database integration of 20,000 unique sea urchin EST clusters. Genome Res. 13, 2736–2746.
Rappaport, R., 1996. Cytokinesis in Animal Cells. Cambridge University Press, Cambridge.
Rashid, D.J., Wedaman, K.P., Scholey, J.M., 1995. Heterodimerization of the two motor subunits of the heterotrimeric kinesin, KRP85/95. J. Mol. Biol. 252, 157–162.
Rasmusson, K., Serr, M., Gepner, J., Gibbons, I., Hays, T.S., 1994. A family of dynein genes in *Drosophila melanogaster*. Mol. Biol. Cell 5, 45–55.
Rogers, G.C., Hart, C.L., Wedaman, K.P., Scholey, J.M., 1999. Identification of kinesin-C, a calmodulin-binding carboxy-terminal kinesin in animal (*Strongylocentrotus purpuratus*) cells. J. Mol. Biol. 294, 1–8.
Rogers, G.C., Chui, K.K., Lee, E.W., Wedaman, K.P., Sharp, D.J., Holland, G., Morris, R.L., Scholey, J.M., 2000. A kinesin-related protein, KRP(180), positions prometaphase spindle poles during early sea urchin embryonic cell division. J. Cell Biol. 150, 499–512.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Saitou, N., Nei, M., 1987. The neighbor joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.
Salmon, E.D., 1975. Spindle microtubules: thermodynamics of in vivo assembly and role in chromosome movement. Ann. N. Y. Acad. Sci. 253, 383–406.
Scholey, J.M., Neighbors, B., McIntosh, J.R., Salmon, E.D., 1984. Isolation of microtubules and a dynein-like MgATPase from unfertilized sea urchin eggs. J. Biol. Chem. 259, 6516–6525.
Scholey, J.M., Porter, M.E., Grissom, P.M., McIntosh, J.R., 1985. Identification of kinesin in sea urchin eggs, and evidence for its localization in the mitotic spindle. Nature 318, 483–486.
Schroeder, T.E., 1968. Cytokinesis: filaments in the cleavage furrow. Exp. Cell Res. 53, 272–276.
Sharp, D.J., Rogers, G.C., Scholey, J.M., 2000. Microtubule motors in mitosis. Nature 407, 41–47.
Shen, X., Ranallo, R., Choi, E., Wu, C., 2003. Involvement of actin-related proteins in ATP-dependent chromatin remodeling. Mol. Cell. 12, 147–155.
Sirotkin, V., Seipel, S., Krendel, M., Bonder, E.M., 2000. Characterization of sea urchin unconventional myosins and analysis of their patterns of expression during early embryogenesis. Mol. Reprod. Dev. 57, 111–126 (Oct.).

Sjöblom, T., Jones, S., Wood, L.D., Parsons, D.W., Lin, J., Barber, T., Mandelker, D., Leary, R.J., Ptak, J., Silliman, N., Szabo, S., Buckhaults, P., Farrell, C., Meeh, P., Markowitz, S.D., Willis, J., Dawson, D., Willson, J.K., Gazdar, A.F., Hartigan, J., Wu, L., Liu, C., Parmigiani, G., Park, B.H., Bachman, K.E., Papadopoulos, N., Vogelstein, B., Kinzler, K.W., Velculescu, V.E., in press. The consensus coding sequences of human breast and colorectal cancers. Science.

Sluder, G., 1979. Role of spindle microtubules in the control of cell cycle timing. J. Cell Biol. 80, 674–691.

Smith, L.C., Britten, R.J., Davidson, E.H., 1992. SpCoel1: a sea urchin profilin gene expressed specifically in coelomocytes in response to injury. Mol. Biol. Cell 3, 403–414.

Stephens, R.E., 1995. Ciliogenesis in sea urchin embryos—A subroutine in the program of development. BioEssays 17, 331–340.

Strickland, L.I., Wen, Y., Gundersen, G.G., Burgess, D.R., 2005. Interaction between EB1 and p150glued is required for anaphase astral microtubule elongation and stimulation of cytokinesis. Curr. Biol. 15 (24), 2249–2255.

Suprenant, K.A., Tuxhorn, J.A., Daggett, M.A., Ahrens, D.P., Hostetler, A., Palange, J.M., VanWinkle, C.E., Livingston, B.T., 2000. Conservation of the WD-repeat, microtubule-binding protein, EMAP, in sea urchins, humans, and the nematode *C. elegans*. Dev. Genes Evol. 210, 2–10.

Terasaki, A.G., Ohnuma, M., Mabuchi, I., 1997. Identification of actin-binding proteins from sea urchin eggs by F-actin affinity column chromatography. J. Biochem. (Tokyo) 122, 226–236.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Tilney, L.G., Gibbins, J.R., 1969. Microtubules and filaments in the filopodia of the secondary mesenchyme cells of *Arbacia punctulata* and *Echinarachnius parma*. J. Cell Sci. 5, 195–210.

Tilney, L.G., Hatano, S., Ishikawa, H., Mooseker, M.S., 1973. The polymerization of actin, its role in the generation of the acrosomal process of certain echinoderm sperm. J. Cell Biol. 59, 109–126.

Togel, M., Eichinger, R., Wiche, G., Propst, F., 1999. A 45 amino acid residue domain necessary and sufficient for proteolytic cleavage of the MAP1B polyprotein precursor. FEBS Lett. 451, 15–18.

Vale, R.D., 2003. The molecular motor toolbox for intracellular transport. Cell 112, 467–480.

Vallee, R.B., Bloom, G.S., 1983. Isolation of sea urchin egg microtubules with taxol and identification of mitotic spindle microtubule-associated proteins with monoclonal antibodies. Proc. Natl. Acad. Sci. U. S. A. 80, 6259–6263.

Vaughan, K.T., Mikami, A., Paschal, B.M., Holzbaur, E.L., Hughes, S.M., Echeverri, C.J., Moore, K.J., Gilbert, D.J., Copeland, N.G., Jenkins, N.A., Vallee, R.B., 1996. Multiple mouse chromosomal loci for dynein-based motility. Genomics 36 (1), 29–38.

Wain, H.M., Lush, M.J., Ducluzeau, F., Khodiyar, V.K., Povey, S., 2004. Genew: the Human Gene Nomenclature Database, 2004 updates. Nucleic Acids Res. 32, D255–D257 (Database issue).

Wang, C.C., Chan, D.C., Leder, P., 1997. The mouse formin (Fmn) gene: genomic structure, novel exons, and genetic mapping. Genomics 39, 303–311.

Wedaman, K.P., Knight, A.E., Kendrick-Jones, J., Scholey, J.M., 1993. Sequences of sea urchin kinesin light chain isoforms. J. Mol. Biol. 231, 155–158.

Wedaman, K.P., Meyer, D.W., Rashid, D.J., Cole, D.G., Scholey, J.M., 1996. Sequence and submolecular localization of the 115-kD accessory subunit of the heterotrimeric kinesin-II (KRP85/95) complex. J. Cell Biol. 132, 371–380.

Whittaker, C.A., Bergeron, K.-F., Whittle, J., Brandhorst, B.P., Burke, R.D., Hynes, R.O., 2006. The echinoderm adhesome. Dev. Biol. 300, 252–266.

Wickstead, B., Gull, K., 2006. A "holistic" kinesin phylogeny reveals new kinesin families and predicts protein functions. Mol. Biol. Cell 17, 1734–1743.

Wright, B.D., Henson, J.H., Wedaman, K.P., Willy, P.J., Morand, J.N., Scholey, J.M., 1991. Subcellular localization and sequence of sea urchin kinesin heavy chain: evidence for its association with membranes in the mitotic apparatus and interphase cytoplasm. J. Cell Biol. 113, 817–833.

Wright, B.D., Terasaki, M., Scholey, J.M., 1993. Roles of kinesin and kinesin-like proteins in sea urchin embryonic cell division: evaluation using antibody microinjection. J. Cell Biol. 123, 681–689.

Yokota, E., Mabuchi, I., Sato, H., 1987. Activation of sea urchin sperm flagellar dynein ATPase activity by salt-extracted axonemes. J. Biochem. (Tokyo) 102, 31–41.

Zhu, X., Mahairas, G., Illies, M., Cameron, R.A., Davidson, E.H., Ettensohn, C.A., 2001. A large-scale analysis of mRNAs expressed by primary mesenchyme cells of the sea urchin embryo. Development 128, 2615–2627.