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The genomic repertoire for cell cycle control and DNA metabolism in *S. purpuratus*

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Abstract

A search of the *Strongylocentrotus purpuratus* genome for genes associated with cell cycle control and DNA metabolism shows that the known repertoire of these genes is conserved in the sea urchin, although with fewer family members represented than in vertebrates, and with some cases of echinoderm-specific gene diversifications. For example, while homologues of the known cyclins are mostly encoded by single genes in *S. purpuratus* (unlike vertebrates, which have multiple isoforms), there are additional genes encoding novel cyclins of the B and K/L types. Almost all known cyclin-dependent kinases (CDKs) or CDK-like proteins have an orthologue in *S. purpuratus*; CDK3 is one exception, whereas CDK4 and 6 are represented by a single homologue, referred to as CDK4. While the complexity of the two families of mitotic kinases, Polo and Aurora, is close to that found in the nematode, the diversity of the NIMA-related kinases (NEK proteins) approaches that of vertebrates. Among the nine NEK proteins found in *S. purpuratus*, eight could be assigned orthologues in vertebrates, whereas the ninth is unique to sea urchins. Most known DNA replication, DNA repair and mitotic checkpoint genes are also present, as are homologue, whereas the INK4 and ARF families of tumor suppressors. Interestingly, the p21/p27 family of CDK inhibitors is represented by one homologue, whereas that, while the cell cycle control mechanisms known from other animals are generally conserved in sea urchin, parts of the machinery have diversified within the echinoderm lineage. The set of genes uncovered in this analysis of the *S. purpuratus* genome should enhance future research on cell cycle control and developmental regulation in this model.

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Introduction

The eukaryotic cell cycle orchestrates cell growth, the replication and segregation of genomic DNA, and cell division. While its core mechanisms are highly conserved, with the evolution of animals, the cell cycle became contingent on intercellular mitogenic signaling and thereby subjugated to the variety of ontogenetic programs found in different animal clades.

Progression through the eukaryotic cell cycle is controlled by a complex network of kinase activities. Central to this

machinery is the family of cyclin-dependent kinases (CDKs), which phosphorylate a set of regulatory factors. The activity of these serine/threonine kinases is in turn controlled at multiple levels: by synthesis of the regulatory cyclin subunits, by phosphorylation of both the catalytic and cyclin subunits, by binding to CDK inhibitors (CKIs), by subcellular localization, and by specific ubiquitylation targeting of cyclins and CKIs to proteasome-mediated proteolysis (Obaya and Sedivy, 2002). Cyclins involved in cell cycle control have been classified as G1-, S-, or M-phase regulators, based on when their activities are required in the cell cycle (Pines, 1999). Cyclin D-dependent kinases CDK4/CDK6 collaborate with cyclin E/CDK2 to promote phosphorylation of the retinoblastoma protein (pRB) and its relatives p107 and p130, relieving the inhibition of E2F family transcription factors and thus stimulating progression from G1 to S phase. The G1 phase of the cell cycle is characterized by low CDK activity and replication origins being assembled in a pre-replicative state competent to undergo DNA replication in the subsequent S phase. Assembly of prereplicative complexes (pre-RC) is marked by the sequential binding of ORC (for "origin recognition complex"), Cdc6, Cdt1, and finally the DNA helicase MCM2-7 (for "mini chromosome maintenance") complex to the origin of replication (reviewed in Bell, 2002; Spradling, 1999). Activation of CDK2 and of the heterodimeric kinase Cdc7/Dbf4 at the G1-S transition drives the cell into a state where replication can be initiated but new pre-RC cannot be assembled, ensuring that chromosomal DNA replicates only once per cell cycle (reviewed in Blow and Dutta, 2005; Woo and Poon, 2003). Completion of S phase triggers the activation of mitotic kinases which fully activate cyclin B/CDK1, initiating the complex program of chromosome segregation. Exit from mitosis is marked by the shutdown of CDK activity that follows the rapid degradation of cyclin B. Cyclin B, with other critical substrates for cell cycle progression, is targeted for degradation by the ubiquitin-dependent pathway.

The core genes involved in these biological processes have been found in animals, plants, and unicellular organisms such as yeast. However, some regulatory mechanisms have evolved differently. For example, multiple gene isotypes (e.g. those encoding cyclins) are present in certain lineages, the precise number varying between different organisms. Here we surveyed the genome of *Strongylocentrotus purpuratus* to obtain an insight into how echinoderms either conserve or have modified these fundamental systems.

The sea urchin has been an important model for many years in the study of basic biology. Crucial in developmental biology, it has also been essential in cell cycle studies, a key example being the identification of the first cyclin. It was in *Arbacia punctulata* that Tim Hunt first noticed the "unexpected behavior" of a protein synthesized early after fertilization that almost disappeared before cleavage and was "henceforth called cyclin" (Evans et al., 1983). Together with studies on amphibians, data obtained in marine invertebrates, i.e. the detection in starfish oocytes of a cytoplasmic activity which mediates maturation-inducing hormonal action and brings about germinal vesicle breakdown (Kishimoto and Kanatani, 1976), the demonstration of the universality of this M-phase promoting factor (MPF) (Kishimoto et al., 1982), the sequencing of Clam cyclin A (Swenson et al., 1986), and the purification of cyclin B/CDK1 from starfish (Labbe et al., 1989) were decisive for understanding the mechanism underlying meiotic and mitotic onset (MPF reviewed in Kishimoto, 1999; Masui, 2001).

A key feature of sea urchins is that their gametes are stored as haploid eggs. Thus, fertilization directly triggers entry into mitosis rather than resumption of meiosis as in most animal eggs. After one cycle in which short G1 and G2 phases precede DNA replication and mitosis respectively, a rapid succession of S and M phases marks the cleavage stages. These early divisions rely on translation of maternally stored mRNA or post translational modifications of existing proteins stored in the eggs until the cell cycle becomes dependent on zygotic transcription and develops into a more conventional somatic cycle with gap phases.

The sea urchin occupies an important evolutionary position with respect to vertebrates because the echinoderms and their sister group hemichordates are the only other deuterostome animals beside the chordates. The sea urchin is thus more closely related to humans than are the other major invertebrate models *Drosophila* and *C. elegans*. The sequenced genome of *S. purpuratus* strengthens the sea urchin as a model organism for studying the regulatory networks that control cell proliferation, with potentially important implications for understanding their human counterparts.

In this paper, we describe the sea urchin repertoire for cell cycle control and DNA metabolism as identified in the genome sequence of *S. purpuratus* and supplement this description with currently available functional data.

Materials and methods

Identification of cell-cycle- and DNA-replication-related genes

To identify cell-cycle-related genes in the *S. purpuratus* genome, BLAST searches were performed against the Baylor College of Medicine (BCM) sea urchin database. Sequences that lack a cyclin domain according to Pfam domain analysis were eliminated. Computationally predicted models of other cell cycle genes were collected by searching the GLEAN3 gene set at Baylor, either by using BLASTP homologous amino acid sequences from known cell cycle control genes or by using the PFAM search engine set up on the Baylor annotation site. Domains were analyzed through the SMART (simple modular architectural research tool) web interface from http://smart.embl-heidelberg.de/ or the ScanProsite search engine from http://www.expasy.ch/. Nek Alignment was done using ClustalW at http://www.ebi.ac.uk/clustalW/.

For phylogenetic trees, protein sequences were aligned with MAFFT (Katoh et al., 2005, 2002) using E-INS-i strategy. The conserved domains were selected and manually improved using Seaview (Galtier et al., 1996). In order to make a Maximum Likelihood analysis, Prottest (Abascal et al., 2005) was used to determine the best amino acid substitution matrix, RtREV+I+G+F was the most suitable for our data. This matrix was used to run the local version of PHYML (Guindon et al., 2005) to infer the ML tree. Trees were validated with a bootstrap procedure using 1000 replicates. Treeillustrator (Trooskens et al., 2005) was used to draw the final tree.

Identification of DNA repair genes

The Gene Ontology database at Mouse Genome Informatics (MGI) (http:// www.informatics.jax.org/) was searched with the term "DNA repair". Under DNA repair ([GO:0006281]; {Biological process{DNA metabolism{DNA repair}}}), the subgroups base excision repair [GO:0006284] (17 genes, 20 annotations), double-strand break repair [GO:0006302] (8 genes, 10 annotations), nucleotide excision repair [GO:0006289] (19 genes, 21 annotations) and mismatch repair [GO:0006298] (7 genes, 8 annotations) were followed to retrieve all of the individual MGI entries. The peptide sequence for each retrieved entry was used to query the *S. purpuratus* GLEAN3 peptide sequence predictions through the BLAST server at Baylor. The results of that search are displayed in Table S2. Some mouse genes with repair annotations had no clear homolog in the GLEAN3 set and in that case the assembled *S. purpuratus* genome sequence and available ESTs were directly searched to confirm the absence. Xrcc1, not present as a base excision repair factor at MGI, was added to the base excision repair list.

Results and discussion

The kinase network

The cell cycle clock is operated by a network of kinase activities, at the core of which are the cyclin and CDK families. The regulatory cyclin subunits not only activate CDKs by changing the conformation at their catalytic sites, but also contribute to the selection of CDK substrates, subcellular localization, and regulation of protein stability (reviewed in Malumbres and Barbacid, 2005; Murray, 2004; Sanchez and Dynlacht, 2005; Sherr and Roberts, 2004). On top of this core machinery, additional kinases, often monomeric, regulate various events during the cell cycle. These include the mitotic kinases, aurora, polo, the NimA-related kinases (NEK), or the checkpoint kinases Chk (reviewed in Nigg, 2001). The mitogen-activated protein kinases (MAPK) signaling cascades which regulate meiotic and mitotic cycles are described in an accompanying paper (Bradham et al., 2006).

Cyclin/CDK complexes

Cyclins were among the first characterized cell cycle regulators (Evans et al., 1983) and since their identification a large number of cyclin genes have been identified in various organisms. On the basis of sequence similarity, expression pattern, and protein activity during the cell cycle, cyclins have been grouped into several classes. In animals, at least 13 classes (A to L and T) of cyclins have been described (Pines, 1995), and the human genome encodes a total of 22 cyclins. Not all cyclins in animals are demonstrated to be involved in cell cycle regulation, and only cyclins A, B, D, and E are commonly considered to be part of the core cell cycle control machinery. A second set of cyclins, cyclins K, L and T, are involved in transcription or mRNA processing (Loyer et al., 2005), while a third set, cyclin C and H, have been implicated in regulation of both cell cycle and gene expression. The functions of the remaining cyclins, cyclin F, G, I, and J, remain somewhat unclear.

All the distinctive classes of cyclins were found in the *S. purpuratus* genome (Fig. 1), which contains at least 16 genes encoding proteins with a cyclin domain. However, in contrast to the human genome, which includes various isotypes of several of these cyclins, the sea urchin genome contains a single gene for each defined subfamily, with the exception of cyclins of the B and K/L types.

The S. purpuratus genome sequence confirmed that the previously identified G1-S cyclins, cyclin D and E (Moore et al., 2002; Sumerel et al., 2001), are indeed the only members of these respective subfamilies in sea urchins. Cyclin D mRNA is barely detectable in eggs and early embryos but increases dramatically at the early blastula stage, remaining thereafter at a constant level throughout embryogenesis (Moore et al., 2002). Premature expression of cyclin D in cleavage embryos is lethal; however, while cyclin D expression is required for normal development, it may not be necessary for cell cycle control. Cyclin E is synthesized during oogenesis, and the level of cyclin E protein is maintained constant throughout early sea urchin embryogenesis. This implies that fluctuations in cyclin E are not required for cell cycle progression through the early embryonic cell cycles. Instead, it has been postulated that cyclin E may play a role in the remodeling of the sperm chromatin, a prerequisite for subsequent DNA replication (Schnackenberg and Marzluff, 2002). After the blastula stage, cyclin E mRNA and protein levels are very low, and cyclin E expression is predominant only in cells that are actively dividing, suggesting that cyclin E contributes to cell cycle control at these stages.

Mitotic cyclins include three distinct gene families, cyclins A, B, and B3 (Jacobs et al., 1998), that appear to have overlapping functional properties. As in other invertebrates including starfish (Table S1), surf clam, limpet, and fly (Lehner and O'Farrell, 1989; Okano-Uchida et al., 1998), a single cyclin A has been found in sea urchin. Cyclin A is present in sea urchin oocytes, but its synthesis is not required for meiotic maturation (Voronina et al., 2003). Fertilization increases synthesis of cyclin A which associates with CDK1 and CDK2 (Moreau et al., 1998). The function of cyclin A during S phase has not yet been demonstrated in sea urchin, but with cyclin B, it drives the G2–M transition by potentiating MPF activation during early embryogenesis (Genevière A.M. et Picard A., unpublished data), as is the case in Xenopus (Devault et al., 1992) or starfish (Okano-Uchida et al., 2003). Moreover, cyclin A can compensate for an absence of cyclin B in early sea urchin embryos, demonstrating a functional overlap between the two mitotic cyclins (Voronina et al., 2003). Three B type cyclins were identified in S. purpuratus: the previously characterized sea urchin cyclin B (Lozano et al., 1998; Thatcher et al., 1995; Voronina et al., 2003), an orthologue of cyclin B3 (SPU_15285), and a novel cyclin B related to mitotic cyclins of plants (SPU_20986). A whole-genome analysis in Caenorhabditis elegans also revealed two novel B type cyclins that are closely related to the previously known cyclin B (Nieduszynski et al., 2002). A phylogenetic tree of B type cyclins (Fig. 2) shows that the novel S. purpuratus cyclin B is not related to any other cyclin B subfamily, a feature shared with a previously classified cyclin A-like protein in Ciona intestinalis (Kawashima et al., 2003), which is in fact more closely related to the sea urchin novel cyclin B than to A type cyclins (Figs. 1 and 2). These data reinforce evidence for multiple cyclin B gene duplication events (Nieduszynski et al., 2002). An early duplication event in animal evolution may have given rise to the B and B3 type cyclins, identified in all animals to date. More recent duplication events



Fig. 1. Phylogenetic tree of cyclins, reconstructed by the Maximum Likelihood method (ML), with bootstrap values higher than 50% shown for each clade. Sequence Ids for the human (Hs), *Xenopus* sp. (Xl and Xt), *Drosophila melanogaster* (Dm), or *Caenorhabditis elegans* (Ce) cyclins (here noted CCN) are reported in Table S3. The *S. purpuratus* cyclins are noted with a star. The novel cyclins identified in *S. purpuratus* (Sp) which are not associated with an established cyclin subgroup are highlighted by two stars.

account for the variation in number of B type cyclins between organisms.

Cyclin C is one cyclin whose protein level does not vary significantly during the cell cycle or following growth factor stimulation. Recent analysis demonstrated that cyclin C, associated with CDK3, may play a role in the exit from quiescence, prior to pRb phosphorylation by CDK4/6/cyclin D1 in mammalian cells (Ren and Rollins, 2004). However, Cdk3 is mutated and thus non-functional in most laboratory strains of mice (Ye et al., 2001). While a potential cyclin C is present in the sea urchin, there is no apparent orthologue of the partner kinase, CDK3 (Table 1). Thus, the proposed mammalian function of cyclin C in the G0-G1 transition may not be conserved in all phyla. Cyclin C also associates with CDK8 (Leclerc and Leopold, 1996). An orthologue of CDK8 was identified in S. purpuratus (discussed below), which may be the only partner of cyclin C in the sea urchin. A second cyclin with a potential role both in cell cycle and transcription, cyclin H, is also present in the sea urchin genome. In vertebrates, cyclin H controls the activity of CDK7 as a Cdk activating kinase as well as its ability to phosphorylate the C-terminal domain of RNApolymerase II (reviewed in Fisher, 2005; Harper and Elledge, 1998).

Cyclin K, T, and L constitute a protein subfamily with high sequence conservation within the conserved cyclin box and are members of the "transcription/mRNA processing" cyclin family (Loyer et al., 2005). The three cyclins have single potential orthologues in the sea urchin genome as displayed in Fig. 1. In addition, another gene of the cyclin K/L sub-type was identified in sea urchins (SPU_023343). This gene has potential orthologues in humans (XP_291577), mice (AAH07232), nematodes (NP_506615), and *Drosophila* (CG31232), the encoded proteins being uncharacterized to date. This suggests that new functions of cyclin proteins remain to be discovered.

Potential orthologues of cyclin F, G, I, and J were also found in the sea urchin genome. The expression of cyclin F in sea urchin embryo, as indicated by transcriptome tiling array data (Samanta et al., 2006), is in accordance with its proposed requirement for embryonic development (Tetzlaff et al., 2004).

Since their initial discovery in yeast, the CDKs have proven to be universal regulators of the cell cycle in all eukaryotes. It is generally admitted that cell cycle progression requires a different CDK/cyclin complex for each stage of the cell cycle. Thus, as mentioned in Introduction, progression through G1 is primarily under the control of CDK4/6 family members in association with cyclin D; CDK2, paired with



Fig. 2. Phylogenetic tree for selected B type cyclins. The tree was constructed by the Maximum Likelihood method (ML), with bootstrap values shown for each clade. The *S. purpuratus* cyclin B are noted with a star. Sequence Ids for the human (Hs), *Asterina pectinifera* (Ap), *Marthasterias glacialis* (Mg), *Xenopus laevis* (Xl), *Drosophila melanogaster* (Dm), *Caenorhabditis elegans* (Ce), and *Ciona intestinalis* (Ci) cyclin B (here noted CCNB) are reported in Table S3.

cyclin E and A, is required for G1–S transition and progression through S phase; while CDK1 together with cyclin A and B governs the G2–M transition. A second CDK network is critical for the regulation of transcription and mRNA processing (reviewed in Loyer et al., 2005). A few CDKs, such as CDK7, are involved in both regulatory networks. The sea urchin genome encodes most of the cell-cycle- or transcription-related CDKs encountered in mammalian cells, as displayed in Table 1. One exception is Cdk3, the absence of which was discussed above.

The core cell cycle genes Cdk1 and Cdk2 were previously identified in sea urchins (Moreau et al., 1998). Both kinases are constitutively expressed during early development. While the cyclin A and B/CDK1 activities peak in mitosis (Geneviere-Garrigues et al., 1995), CDK2 kinase activity is stable during early embryogenesis. In contrast to its role in somatic cells, CDK2 is dispensable for the activation of the first DNA replication after fertilization; however, CDK activities are required to maintain replication to one per cell cycle (Moreau et al., 1998).

Only one member of the CDK4/6 family was identified in the *S. purpuratus* genome. Similarly, a single homologue of Cdk4/6 is found in *D. melanogaster*, *C. elegans*, and *C. intestinalis*, supporting the view that the gene duplication is specific to vertebrates. Recent data from *D. melanogaster*, *C. elegans*, *S. purpuratus*, and mice suggest that cyclin D/CDK4 complexes

are not essential for cell proliferation or early development in these species and may be involved more in the regulation of cell growth and/or developmental patterning than in cell cycle progression (Datar et al., 2000; Malumbres et al., 2004; Meyer et al., 2000; Moore et al., 2002; Park and Krause, 1999).

The network of CDK/cyclin complexes implicated in transcription and mRNA processing is conserved in S. purpuratus. Mammalian CDK7/cyclin H and CDK8/cyclin C, which phosphorylate the C-terminal domain (CTD) of RNApolymerase II (RNAP II), regulate in an opposite manner the initiation of transcription, belonging respectively to the TFIIH and mediator complexes (reviewed in Lover et al., 2005). CDK9, the CTD kinase subunit of P-TEFb, associated with cyclins T or K, positively controls the elongation phase of RNAP II transcription, while CDK11 bound to cyclin L has been implicated in transcription and pre-mRNA splicing. The latest kinase in this large family to be identified, CDK12 (previously CrkRS) and its close relative CDC2L5, are the highest molecular mass CDKs identified to date (Chen et al., 2006; Ko et al., 2001; Marques et al., 2000). Recently, both proteins have been shown to regulate pre-mRNA splicing (Chen et al., 2006; Even et al., 2006). A full-length CDC2L5 cDNA was initially identified in the sea urchin Sphaerechinus granularis (Marques et al., 2000). In this species, the mRNA is ubiquitously expressed throughout development, and in

Table 1							
S. purpuratus	cell	cycle	genes	identified	in	this	study

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005850053500747RE++CDK regulatory subunitCyclin00544S-PCTAIRE00545S-pcyclin ADC-2-M++00644S-pcyclin AD(2-M+00713S-pcyclin CDCell cycletranscription00713S-pcyclin CDCell cycletranscription00714S-pcyclin CDCell cycletranscription00715S-pcyclin CDCell cycletranscription00716S-pcyclin CDCell cycletranscription00717S-pcyclin CDCell cycletranscription00718S-pcyclin CDCell cycletranscription00719S-pcyclin CDTanscription00719S-pcyclin CDTanscription00719S-pcyclin CDTanscription00719S-pcyclin CDTanscription00719S-pcyclin CDTanscription00719S-pcyclin CDTanscription00710S-pcyclin CD00719S-pcyclin CD00719S-pcyclin CD00719S-pcyclin CD00710S-pcyclin CD007110S-pcyclin CD0071111S-pcyclin CD00			028236	Sp-CDK12/CDC2L5	mRNA processing	+
CDK regulatory subunit005529-P2TAIRE			016385	Sp-PCTAIRE		+
CDK regulatory subunitCyclin003528003528003528003528003528003528003528003528003528003530			003654	Sp-PFTAIRE		-
15285/025/5659-cyclin B62M+003967Sp-cyclin B62M-003967Sp-cyclin CCell cycle/transcription-00713Sp-cyclin G00713Sp-cyclin G009985Sp-cyclin G009985Sp-cyclin G01658Sp-cyclin G016780Sp-cyclin G016780Sp-cyclin G016985Sp-cyclin G019985Sp-cyclin KLTranscription+019980Sp-cyclin KL02343Sp-cyclin KL-+02343Sp-cyclin KL-+19980/1123000238Sp-cyclin KL-+19980/1123000238Sp-cyclin KL-+19980/1123000238Sp-cyclin KL-+19980/1123000238Sp-cyclin KL-+19980/1123000238Sp-cyclin KL++19980/1123000238Sp-cyclin KL++19980/1123000238Sp-cyclin KL++19980/1123000238Sp-cyclin KL++19980/1123000238Sp-cyclin KL++19980/1123000238Sp-cyclin KL++19980/1123000238Sp-ChS-G2-M+19990/1123000238Sp-ChS-G2-M+19990/112300238Sp-PLK4G2-M+19990/11230103Sp-PLK4G2-M+<	CDK regulatory subunit	Cyclin	003528	Sp-cyclin A	S-G2-M	+
bookstam Sp-sycin B3 G2-M + bookstam Sp-sycin D G1-S + bookstam Sp-sycin D G1-S + bookstam Sp-sycin D G1-S + bookstam Sp-sycin B - - bookstam Sp-sycin F - - bookstam <td></td> <td></td> <td>015285/026576</td> <td>Sp-cyclin B</td> <td>G2-M</td> <td>+</td>			015285/026576	Sp-cyclin B	G2-M	+
NameNon-Specific NameCell cyclimscription-007013Specyclin CC1-S+00712Specyclin CC1-S+006781/007033Specyclin F009985Specyclin RCell cycle/transcription01158Specyclin ISpecyclin I01712Specyclin I01712Specyclin I01698Specyclin ITranscription+-014989011255000328Specyclin ITmRNaproteosing+014989011255000328Specyclin Br-+171 krinaseCC40414890Specyclin Br-171 krinaseCC40414890Specyclin Br-+171 krinaseCC5019568SpecDC25S-G2-M+171 krinaseCDC25019568SpelC24S-G2-M+171 krinasePLK019048SpelK1-+171 krinasePLK01957SpelK1-+171 krinasePLK019048SpelK2-+171 krinasePLK019021SpelK1-+171 krinasePLK019021SpelK1++172 krinasePLK022369SpelK1++173 krinasePLK010328SpelK1++174 krinasePLKSpelK1-++175 krinasePLKSpelK1- <td< td=""><td></td><td></td><td>006444</td><td>Sp-cyclin B3</td><td>G2-M</td><td>+</td></td<>			006444	Sp-cyclin B3	G2-M	+
Network007013Sp-cyclin DG1-S+00712Sp-cyclin F00718Sp-cyclin FCell cycle/transcription-011658Sp-cyclin ICell cycle/transcription-01712Sp-cyclin I01714Sp-cyclin I01704Sp-cyclin ITranscription+0105021018199Sp-cyclin ITranscription+0105021018199Sp-cyclin KLr-+020806Sp-cyclin KLr-+020807Sp-cyclin KLr-+17 rkinaseWel01418/07012Sp-cyclin KLr+17 rhosphataseCDC2019568Sp-cyclin KLr+17 rhosphataseCDC2019508Sp-CDC25S-C2-M+17 rhosphataseCDC25019568Sp-CDC25S-C2-M+161552Sp-PLK2G2-M++161552Sp-PLK2G2-M++161552Sp-Nek1-++161552Sp-Nek1-++161652Sp-Nek1-++1616502Sp-Nek1-++1616502Sp-Nek1-++1616502Sp-Nek1-++1616502Sp-Nek1-++1616502Sp-Nek1-++1616502Sp-Nek1-++1616502Sp-Nek1-+ <td< td=""><td></td><td></td><td>003967</td><td>Sp-cyclin C</td><td>Cell cycle/transcription</td><td>-</td></td<>			003967	Sp-cyclin C	Cell cycle/transcription	-
Network001712Sp-cyclin F+000985Sp-cyclin F-000985Sp-cyclin F-001518Sp-cyclin F-011658Sp-cyclin ICell cycle/transcription007312Sp-cyclin ITranscription01704Sp-cyclin ITranscription01999011295000328Sp-cyclin ITranscription01999011295000328Sp-cyclin ITranscription701312Sp-cyclin ITranscription7003210Sp-cyclin BP-711815027012Sp-cyclin BP-711815027012Sp-cyclin BP-711815027012Sp-cyclin BP-711815027012Sp-cyclin BP-711815027012Sp-cyclin BP-711815027012Sp-Crk-711815027012Sp-MyI1S-02-M7119568Sp-ClC2S-02-M119701410958Sp-ClC2S-02-M119701410958Sp-PLK1G2-M1197014107203Sp-Nk1-110702783Sp-Nk1-111702078Sp-Nk1-111702078Sp-Nk1-111702078Sp-Nk1-111702078Sp-Nk1-111702078Sp-Nk1-111875023408Sp-Nk1-111702078Sp-Nk1-111702078Sp-Nk1-111702078Sp-Nk1-111702078Sp-			007013	Sp-cyclin D	G1–S	+
006781/007933Sp-cyclin F+006781/007935Sp-cyclin HCell cycle/transcription-011688Sp-cyclin HCell cycle/transcription-00712Sp-cyclin ITranscription+021704Sp-cyclin ITranscription+014989/011295/00028Sp-cyclin ImRNA processing+021812Sp-cyclin TTranscription+021812Sp-cyclin TTranscription+2019Sp-cyclin TTranscription+2019Sp-cyclin TTranscription+Ser/Thr kinaseCAK004789Sp-cyclin T-Tyr hosphataseWest01418/02/012Sp-WestSc-Q-M+Mitotic kinaseUN48Sp-CDC25S-C2-M++106352Sp-PLK1C2-M+++106352Sp-PLK3C2-M+++106352Sp-PLK4C2-M+++106352Sp-PLK3C2-M+++106352Sp-PLK3C2-M+++106352Sp-PLK3C2-M++++107505Sp-PLK3C2-M+++++10865Sp-PLK3C2-M++++++++++++++++++++++++++++++ <td< td=""><td></td><td></td><td>001712</td><td>Sp-cyclin E</td><td>G1–S</td><td>+</td></td<>			001712	Sp-cyclin E	G1–S	+
009985Sp-cyclin G-011658Sp-cyclin I-007312Sp-cyclin I-017314Sp-cyclin I-017314Sp-cyclin ITranscription-014980011295000328Sp-cyclin ImRoscription-014980011295000328Sp-cyclin ITranscription-014980011295000328Sp-cyclin IITranscription-023946Sp-cyclin KLr203945Sp-cyclin KLr7p r kinaseCAK004789Sp-CcrkS-C2-M-Tyr kinaseCOC25019480Sp-Cl25S-C2-M-Tyr kinaseCOC25019480Sp-Cl25S-C2-M-Mitoic kinasePLK019540Sp-PLK1C2-M-Mitoic kinasePLK019540Sp-PLK3C2-M-Aurora023350Sp-PLK4C2-MAurora023350Sp-Nek301455Sp-Nek401459025176Sp-Nek3014590025176Sp-Nek101459025176Sp-Nek101459025176Sp-Nek101459025176Sp-Nek101459025176Sp-Nek101459025176Sp-Nek10145902			006781/007933	Sp-cyclin F		+
101658Sp-cyclin PCell cycle/ranscription-007312Sp-cyclin J017040Sp-cyclin JTranscription+010521/018199Sp-cyclin KTranscription+014889/011295/00328Sp-cyclin KmRNA processing+014889/011295/00328Sp-cyclin K/L-+021812Sp-cyclin K/L-+020860Sp-cyclin K/L-+SerThr kinaseCAK004789Sp-Cck-Tyr hosphataseWel001418/02/012Sp-Wel1S-G2-M+Tyr hosphataseDC250019568Sp-ClxMitotic kinasePLK00468Sp-PLK3C2-M+00527Sp-Nak0C2-M+Mitotic kinasePLK007352Sp-PLK4-+01532Sp-PLK3C2-M++-016352Sp-Nak0++01637Sp-Nak1-+++01875/023408Sp-Nak1-++01837/023408Sp-Nak1-+++01837/023408Sp-Nak1-+++Checkpoint kinaseATM01017/026783Sp-ATMManager/epilcation++01837/023408Sp-ATMDNA damager/epilcation++++Checkpoint kinaseGirsfitGirsfitGirsfit-+++C			009985	Sp-cyclin G		-
view 07312 Sp-cyclin J - 021704 Sp-cyclin KL maxCription - 014989011295000328 Sp-cyclin KL maxCription - 021812 Sp-cyclin KL maxCription - 023343 Sp-cyclin KL - - Ser/Thr kinase CAC 00789 Sp-cyclin KL - Tyr kinase CAC 00789 Sp-cyclin KL - Tyr kinase CAC 004789 Sp-cyclin KL - - Tyr kinase CDC2 019568 Sp-Velx - - - Tyr honsphate CDC2 019568 Sp-Mk1 G2-M - - Mitotic kinase PLK 019020 Sp-PLK4 G2-M - - Mitotic kinase PLK 019021 Sp-Nk1 G2-M - - Mitotic kinase PLK 019021 Sp-Nk1 G2-M - - Mitotic kinase 1019021 Sp-Nk1 G2-M			011658	Sp-cyclin H	Cell cycle/transcription	-
bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit bit<			007312	Sp-cyclin I		+
Normal Normal Sp-cyclin KL mRNA processing H 04989/01295/00328 Sp-cyclin KLr mRNA processing H 021812 Sp-cyclin KLr Transcription H 020986 Sp-cyclin KLr F H Ser/Thr kinase CK 04789 Sp-Cyclin KLr H Ser/Thr kinase CK 04789 Sp-Cyclin KLr S-G2-M H Tyr kinase UPC4 014850/2012 Sp-Pt KL G2-M H Tyr honsphatase UPLK 01956 Sp-Pt KL G2-M H Nitotic kinase UPLK 01968 Sp-Pt KL G2-M H 016352 Sp-Pt KL G2-M H H 016352 Sp-Pt KL G2-M H H 016352 Sp-Pt KL G2-M H H 016352 Sp-Nekl G2-M H H 016352 Sp-Nekl G2-M H H 01921 Sp-Nekl Sp-Nek			021704	Sp-cyclin J		-
picklightbicklightSpecyclin L picklightmRNA processing+021812Sp-cyclin K/Lr+020360Sp-cyclin K/Lr+200966Sp-cyclin K/Lr+Ser/Thr kinaseCAK04/780Sp-Crk-Tyr kinaseCAK04/870Sp-Crk-Myl08220Sp-Myl1S-G2-M+Tyr phosphataseCDC25019568Sp-ClC25S-G2-M+Mitotic kinasePLK07949Sp-PLK1G2-M+000468Sp-PLK3G2-M++01632Sp-PLK4G2-M++01632Sp-Nek1G2-M++01632Sp-Nek3G2-M++01632Sp-Nek4G2-M++01632Sp-Nek1G2-M++028348Sp-Nek67+++028348Sp-Nek67+++01052176Sp-Nek8+++01052176Sp-Nek1+++0107205176Sp-Nek1+++0107205176Sp-Nek1-++0107205176Sp-Nek1-++0107205176Sp-Nek1-++0107205176Sp-Nek1-++0107205176Sp-Nek1-++0107205176Sp-Nek1-++01107205176Sp-Nek1+<			005021/018199	Sp-cyclin K	Transcription	+
921812Sp-cyclin TTranscription+023340Sp-cyclin K/Lr+020986Sp-cyclin K/Lr+Tyr hinaseCAK04789Sp-Cck+Tyr hinaseWeel014185/027012Sp-WelS-G2-M+Tyr hinaseCDC25019568Sp-CDC25S-G2-M+Mitotic kinaseCDC25019568Sp-PLK1G2-M+016352Sp-PLK2G2-M++016352Sp-PLK2G2-M++016352Sp-PLK2G2-M++016352Sp-PLK3G2-M++016352Sp-PLK3G2-M++016352Sp-PLK3G2-M++016352Sp-PLK4G2-M++016352Sp-PLK3G2-M++016352Sp-Nek6-++016352Sp-Nek7-++016352Sp-Nek6-++01790Sp-Nek7-++01790Sp-Nek8-++01790Sp-Nek8-++01790Sp-Nek1-++01790Sp-Nek1-++01790Sp-Nek1-++01790Sp-Nek1-++01790Sp-Nek1-++01790Sp-Nek1+01761O17505Sp-ATM			014989/011295/000328	Sp-cyclin L	mRNA processing	+
Ser/Thr kinaseCAK033343Sp-cyclin KLr+Ser/Thr kinaseCAK004789Sp-Cyclin Br+Tyr kinaseCAK004789Sp-Cyclin S-C2-M+Myt10008200Sp-Myt1S-G2-M+Tyr phosphataseCDC25S-G2-M+Mitotic kinaseCDC3Sp-PLK1G2-M+004668Sp-PLK3G2-M+Auroa027333/027666Sp-Auroa+00527Sp-Nek1C2-M+00527Sp-Nek1-+00527Sp-Nek3-+005271Sp-Nek67++005271Sp-Nek7++005271Sp-Nek7++005271Sp-Nek7++01790Sp-Nek7++01790Sp-Nek7++01790Sp-Nek1++01790Sp-Nek1++01790Sp-Nek1++Checkpoint kinaseATM0107/025176Sp-Nek1+Checkpoint kinaseATM01017/026783Sp-Chi1DNA damage/replication+ChK100751Sp-Ck1DNA damage/replication+ChK2004975Sp-Ck1I-S++ChK2004975Sp-Ck1DNA damage/replication+ChK2004975Sp-Ck1Cl-S++ChK2004975Sp-Ck1G1-S++ChK2004			021812	Sp-cyclin T	Transcription	+
Ser/Thr kinaseCAK004789Sp-Crk+Tyr kinaseWeel014185/027012Sp-VeckS-G2-M+Tyr phosphataseCDCS0195680Sp-DLCG-G2-M+Mitotic kinaseCDCS0195680Sp-PLK1G2-M+Mitotic kinasePLK017949Sp-PLK2G2-M+16352Sp-PLK2G2-M++16352Sp-PLK2G2-M++16352Sp-PLK2G2-M++16352Sp-Nek1-++10352Sp-Nek1+++10357Sp-Nek4+++1035703408Sp-Nek67+++10459/025176Sp-Nek67+++10459/025176Sp-Nek1+++10459/025176Sp-Nek1+++10459/025176Sp-Nek1+++10459/025176Sp-Nek1-++10459/025176Sp-Nek1NA damage/replication++10459/025176Sp-Nek1NA damage/replication++10459/025176Sp-Nek1NA damage/replication++10459/025176Sp-Nek1NA damage/replication++10459/025176Sp-Nek1NA damage/replication++1050Sp-Ck1ONA damage/replication+++10510ONS56Sp-Ck1G1-SC+++ </td <td></td> <td></td> <td>023343</td> <td>Sp-cyclin K/Lr</td> <td></td> <td>+</td>			023343	Sp-cyclin K/Lr		+
Ser/Thr kinase CAK 004789 Sp-Cerk + Tyr kinase Weil 014185/027012 Sp-Cerk S-G2-M + Tyr phosphatase Myti 00280 Sp-Myti S-G2-M + Mitotic kinase PLK 019568 Sp-PLX G2-M + Mitotic kinase PLK 00468 Sp-PLX G2-M + Auroa 027333/027666 Sp-Auroa G2-M + Auroa 027333/027666 Sp-Neki - + NEK 019021 Sp-Neki + + 023369 Sp-Neki + + + 0457023048 Sp-Neki + + + 01458/0025176 Sp-Neki + + + + 01459/025176 Sp-Neki + <td></td> <td></td> <td>020986</td> <td>Sp-cyclin Br</td> <td></td> <td>+</td>			020986	Sp-cyclin Br		+
Tyr kinaseWe1014185/027012Sp-We1S-G2-M+Tyr phosphataseCDC25019568Sp-DC25S-G2-M+Mitotic kinasePLK017949Sp-PLK1G2-M+016352Sp-PLK2G2-M++016352Sp-PLK4G2-M++016352Sp-NetAG2-M++016352Sp-NetAG2-M++016352Sp-NetAG2-M++02366Sp-NetAHitosis++02376Sp-NetAHitosis++02376Sp-NetAHitosis++02376Sp-NetAHitosis++014559/025176Sp-NetA+++014559/025176Sp-NetAHitosis-+Checkpoint kinaseATM01107/026783Sp-ATRDNA damagereplication+Checkpoint kinaseATM01107/026783Sp-Ck1DNA damagereplication+ChEC/Cyclin inhibitorCip/Kip005856Sp-QtA1ONA damagereplication+CDK/Cyclin regulatorMth008616Sp-MytG1-S+Tanseription factor/cofactorMth008516Sp-E2/HG1-S+Run008616Sp-MycG1-S++104292/025615Sp-E2/HG1-S++104292/025615Sp-E2/HG1-S++104292/025615Sp-Rb-IG1-S++ <td>Ser/Thr kinase</td> <td>CAK</td> <td>004789</td> <td>Sp-Ccrk</td> <td></td> <td>+</td>	Ser/Thr kinase	CAK	004789	Sp-Ccrk		+
Myrl008280Sp-MyrlS-G2-M+Tyr phosphataseCDC25019568Sp-CDC25S-G2-M+Mitotic kinasePLK01940Sp-PLK1G2-M+Mitotic kinasePLK02-M-+Aurora027833/027666Sp-AuroraG2-M+Aurora027833/027666Sp-Nek1-+NEK019021Sp-Nek1-+023369Sp-Nek67-+023484Sp-Nek7-+018375/023408Sp-Nek7++018375/023408Sp-Nek8-+01456Sp-Nek8-+01456/025176Sp-Nek1-+017900Sp-Nek1-+017900Sp-Nek1-+Checkpoint kinaseATM011072/025176Sp-Nek1-Checkpoint kinaseMTM011072/025176Sp-Nek1-+Checkpoint kinaseMTM01107/02783Sp-ATMDNA damage/replication+Checkpoint kinaseMTM011072/025176Sp-Chk1DNA damage/replication+Checkpoint kinaseMTM011072/025176Sp-Chk1DNA damage/replication+Checkpoint kinaseMt1011072/02513Sp-Chk1DNA damage/replication+Checkpoint kinaseGipK1-+++Checkpoint kinaseGipK1-++Checkpoint kinaseGipK1Sp-Chk1GipK1 <td>Tyr kinase</td> <td>Wee1</td> <td>014185/027012</td> <td>Sp-Wee1</td> <td>S-G2-M</td> <td>+</td>	Tyr kinase	Wee1	014185/027012	Sp-Wee1	S-G2-M	+
Tyr phosphataseCDC25019568Sp-CDC25S-G2-M+Mitotic kinasePLK017949Sp-PLK2G2-M+016352Sp-PLK2G2-M++NEK010352Sp-Nek1++NEK01901Sp-Nek1++023369Sp-Nek4+++023366Sp-Nek67++018375/023408Sp-Nek67++018375/023408Sp-Nek1++018375/023408Sp-Nek10++017970Sp-Nek1++018375/023408Sp-Nek10++017070Sp-Nek3++Checkpoint kinaseATM01107/025176Sp-AtMDNA damage/replication+Checkpoint kinaseATM01107/025176Sp-AtMDNA damage/replication+Checkpoint kinaseATM011017/026733Sp-Ch1DNA damage/replication+ChK204975Sp-Ch2DNA damage/replication++CDK/Cyclin inhibitorCip/Kip005856Sp-Ch2DNA damage/replication+Tanseription factor/cofactorMyb000861Sp-MybG1-S+Rb01954Sp-E14G1-S++04392/025615Sp-Rb1-1G1-S++04392/025615Sp-Rb1-1G1-S++04392/025615Sp-Rut-1G1-S++Ubiquitin-proteolysis pathwayCullin0093		Myt1	008280	Sp-Myt1	S-G2-M	+
Minotic kinase MItotic kinase PLK 017949 Sp-PLK1 G2-M + 000468 Sp-PLK4 G2-M + 016552 Sp-PLK4 G2-M + Aurora 027833/027666 Sp-Aurora G2-M + NEK 019021 Sp-Nek1 - + 023369 Sp-Nek2 Mitosis + 023369 Sp-Nek67 - + 023369 Sp-Nek67 - + 023369 Sp-Nek67 - + 023369 Sp-Nek67 - + 018375/023408 Sp-Nek67 - + 014659/025176 Sp-Nek11 - + 017900 Sp-Nek11 - + 014659/025176 Sp-Nat DNA damage/replication + 014659/025176 Sp-Chk12 DNA damage/replication + CDk/Cyclin inhibitor ChK1 007530 Sp-Ckip1 ONA damage/replication + CDK/Cyclin regul	Tyr phosphatase	CDC25	019568	Sp-CDC25	S-G2-M	+
000468Sp-PLK2G2-M+016352Sp-PLK4G2-M+016352Sp-PLK4G2-M+NEW019021Sp-NatoraG2-M+NEK019021Sp-Nek1-+03369Sp-Nek4-+023369Sp-Nek4-+07456Sp-Nek9-+018375/023408Sp-Nek9-+014659/025176Sp-Nek1-+014659/025176Sp-Nek1-+01790Sp-Nek1-+01679/025176Sp-NcH1-+Checkpoint kinaseATM0107/2025176Sp-ATMDNA damage/replication+ChK100753Sp-ATRDNA damage/replication+ChK2004975Sp-ATRDNA damage/replication+CDK/Cyclin inhibitorGip/Kip005856Sp-Chk1DNA damage/replication+CDK/Cyclin regulatorMat014250Sp-Mat-+Tanscription factor/cofactorMgc03166Sp-MybG1-S+Mgc03166Sp-MybG1-S++06312Sp-Rb1-1G1-S++06312Sp-Rb1-1G1-S++04292/025612Sp-Rb1-1G1-S++04292/025612Sp-Rb1-1G1-S++01954Sp-Rb1-1G1-S++01954Sp-Rb1-1G1-S++01954 <td>Mitotic kinase</td> <td>PLK</td> <td>017949</td> <td>Sp-PLK1</td> <td>G2-M</td> <td>+</td>	Mitotic kinase	PLK	017949	Sp-PLK1	G2-M	+
Nek016352Sp-PLK4G2-M+Aurora027833/027666Sp-NachG2-M+NEK010021Sp-Nek1-+005277Sp-Nek2Mitosis+02369Sp-Nek6/7-+023848Sp-Nek6/7++07456Sp-Nek6-+07456Sp-Nek10-+1875/023408Sp-Nek10-+016459/025176Sp-Nek10-+0101072/025176Sp-Nek1++0101072/025176Sp-Nek1-+0101072/025176Sp-Nek1-+0101072/025176Sp-Nek1-+0101072/025176Sp-NtRDNA damago/replication+Checkpoint kinaseATM01101/026783Sp-ATRDNA damago/replication+Chil007530Sp-Chil2DNA damago/replication+CDK/Cyclin inhibitorCip/Kip005856Sp-Ckip1G1-S+Transcription factor/cofactorMyb03166Sp-MybG1-S+Mybe003166Sp-MycG1-S++06312Sp-DPIG1-S++P53008978008979Sp-St-likeG1-S+194uitin-proteolysis pathwaCullin00753Sp-Run-1G1-S+195uitin-proteolysis pathwaCullin029233Sp-Run-1G1-S+195uitin-proteolysis pathwaCullin029233Sp-Run-1G1-S<			000468	Sp-PLK2	G2-M	+
			016352	Sp-PLK4	G2-M	+
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	Ubiquitin-proteolysis pathway	Cullin	002933	Sp-Cul-1	G1-S	+

Table	e 1	(continued))

Biochemical process/function	Family Spu Id		Assigned name	Role of homologues	Embryonic expression	
		026594	Sp-Cul-2	G1–S	+	
		019174/021121	Sp-Cul-3	G1–S	+	
		018555/001926/009731	Sp-Cul-4	DNA damage/replication	-	
		006755	Sp-Cul-5		+	
		018512	Sp-Apc2	Mitosis	+	
	Fbox	019951	Sp-Fbw7	G1–S	-	
		003152	Sp-Fbw7-like	G1–S	+	
		019391	Sp-Skp2	DNA replication	+	
		006781/007933	Sp-cyclin F	G2-M	+	
		088809	Sp-Emi1	Mitosis	+	
	Skp1	0024507	Sp-Skp1	G1–S	+	
	Fzr/Cdh1	019392	Sp-Cdh1	Mitosis	+	
	Fzy/Cdc20	004395	Sp-Cdc20	Mitosis	+	
	Apc1	024153/008018/012580	Sp-Apc1	Mitosis	-	
	Cdc27	022322	Sp-Apc3	Mitosis	+	
	Apc4	021195	Sp-Apc4	Mitosis	+	
	Apc5	011921	Sp-Apc5	Mitosis	+	
	Cdc16	002193	Sp-Apc6	Mitosis	+	
	Apc7	009240	Sp-Apc7	Mitosis	+	
	Apc8	012696	Sp-Cdc23	Mitosis	+	
	Apc10	025744	Sp-Apc10	Mitosis	+	
	Apc11	021695	Sp-Apc11	Mitosis	+	
Condensin/cohesin complexes	SMC1	021629	Sp-SMC1	Mitosis	+	
	SMC2	024546	Sp-SMC2	Mitosis	+	
	SMC3	019803	Sp-SMC3	Mitosis	+	
	SMC4	013617	Sp-SMC4	Mitosis	+	

^a The embryonic expression (up to the mid-late gastrula stage) was deduced from the transcriptome analysis (Samanta et al., 2006). When the signal in the activity profile was negative or positive only for a minority of exons of a gene, the mRNA was considered as absent.

agreement with its proposed role in pre-mRNA splicing, the protein is localized to the nucleus (Genevière A.M., unpublished data). CDC2L5 appears to be the only gene of this type in *S. purpuratus*, suggesting that the two human kinases CDC2L5 and CrkRS represent a chordate or vertebrate-specific duplication.

While the functions of CDK/cyclin complexes regulating transitions between phases of the cell cycle or transcription are quite well understood, the binding partners and cellular role of some CDK-related proteins have still not been established. The PCTAIRE and PFTAIRE protein kinases are a subfamily of CDC2-related serine/threonine protein kinases named for a cysteine/phenylalanine-for-serine substitution in the PSTAIRE motif conserved in the initially characterized CDK proteins. Three PCTAIRE family genes have been identified in humans (Meyerson et al., 1992; Okuda et al., 1994), and two are found in mice (Okuda et al., 1992), whereas only one is present in *S. purpuratus*, as is the case in the cellular slime mold (Michaelis and Weeks, 1993). Similarly, a single PFTAIRE gene was identified in sea urchins.

CDK activating kinases and phosphatases

The CDK7/cyclin H complex, which phosphorylates the CTD of RNA-polymerase II, also participates in the full activation of CDKs by promoting phosphorylation of the conserved threonine residue within the T-loop region of these kinases (Kaldis, 1999). In metazoans, CDK7/cyclin H, in complex with the ring finger protein Mat1, is the major CDK activating kinase (CAK, reviewed in Harper and Elledge, 1998;

Fisher, 2005). None of these proteins has been experimentally characterized in sea urchins; however, orthologues of cyclin H, CDK7 and Mat1 are found in the *S. purpuratus* genome, suggesting that the CAK complex is functional in sea urchins.

In yeasts, a second monomeric CAK activity has been reported (Espinoza et al., 1996; Kaldis et al., 1996; Lee et al., 1999; Thuret et al., 1996). Attempts to identify othologues of yeast CAK in the human genome were unsuccessful until the genome of Drosophila was used as intermediate in BLAST analysis, whereupon a protein (p42/Ccrk) with sequence similarity to both the CAK1 and CDK7 groups of CAK was discovered (Liu et al., 2004). However, its CAK activity in human remains controversial (Wohlbold et al., 2006). A homologue of p42/Ccrk is found in S. purpuratus. An analysis of S. purpuratus embryonic transcriptome (Samanta et al., 2006) indicates that this kinase is expressed during early embryogenesis, whereas neither Cdk7 nor cyclin H mRNA appears to be present. Given that Cdk7 inactivation causes embryonic lethality in C. elegans or Drosophila (Larochelle et al., 1998; Wallenfang and Seydoux, 2002), these results are intriguing. If the unexpected absence of CDK7/cyclin H mRNA in the embryo is verified, sea urchin embryogenesis may help to better understand the CAK activity requirement during embryonic cell cycles.

Cyclin B-associated CDK1 undergoes inhibitory phosphorylation at Thr 14 and Tyr 15 by the Wee1 family of protein kinases (Wee1, Mik1, and Myt1) and at entry into M phase, the inhibitory phosphates are removed by the activating phosphatase CDC25 (reviewed in O'Farrell, 2001). Structural orthologues of Wee1 and Myt1 have been identified in *S. purpuratus*, with high similarity with the corresponding starfish *Asterina pectinifera* genes (Table S1, Nemer and Stuebing, 1996; Okano-Uchida et al., 1998; Okumura et al., 2002). Vertebrates have three CDC25 homologues (CDC25A, B and C), whereas only one was found in *S. purpuratus*, similar to its homologue in *A. pectinifera* (Deshimaru et al., 2002). Of the three vertebrate CDC25 paralogues, sea urchin CDC25 is most similar to CDC25B.

Mitotic kinases

The process of mitosis is controlled by phosphorylation events performed not only by CDK1 but also by other serine/ threonine kinases, known as mitotic kinases. These kinases include three intensely studied proteins: the polo-like kinases (PLKs), the Aurora-related kinases and the NIMA-related kinases (NEK) (Nigg, 2001).

The founding member of the PLK family, Polo, was originally identified in *D. melanogaster* where it was shown to be required for mitosis (Fenton and Glover, 1993) and to have an activity peaking cyclically at anaphase–telophase (Glover et al., 1996).

Plks have since proven to be important regulators for centrosome duplication and maturation, DNA damage checkpoint activation, mitotic onset, bipolar spindle formation, Golgi fragmentation, chromosome segregation, and cytokinesis (Barr et al., 2004; Blagden and Glover, 2003; Dai and Cogswell, 2003). Polo remains the single gene product of this family described in *Drosophila*, while four proteins sharing significant homology with Polo were identified in mammalian cells (Dai, 2005). The *S. purpuratus* genome encodes three Polo homologues (Table 1) as does the *C. elegans* genome (Chase et al., 2000; Ouyang et al., 1999), the *S. purpuratus* Plk1 being highly similar to the starfish orthologue (Table S1, Okano-Uchida et al., 2003).

The Aurora kinases also play a crucial role in regulating G2– M phase progression (Andrews et al., 2003; Eyers and Maller, 2003; Marumoto et al., 2005). The *S. purpuratus* genome apparently contains a single kinase in this family. The *C. intestinalis* genome similarly includes a single gene (Brown et al., 2004), whereas two genes have been described in *D. melanogaster* and *C. elegans*. Mammals alone have three Aurora kinases, Aurora A, B, and C, the two latter having

		I		11			111 1	IV	
SnNekA	1	SYOKVI CMCNECKAW	U.VRSRESR-		ETNVVCMGEKERE-	RAVN DVA	TIGRUE		65
SpNek1	1	VIKKKOIGEGSEGKAI	LVKSKADG-	KEYVIK	EINTSKMKRKEKE-	ESKKEVA		HPNTVSVAE	65
SpNek2	1	YDILYTIGSGSYCKCF	RKVRRKSDG-	KILVSK	EIDYGTMGEVEKO-	MLVSINN	LIRELP	HEFTVRYYD	65
SpNek4	1	YEHLKVVGKGSYGEVW	LVRNKKDK-	KOFVIK	KMELLRASKREKK-	AAEOBAK	LISKLF	RHPNTVSYKD	65
SpNek7	1	FAIEKRICKCOFSEVY	RARCLIND-	RVTALK	KVOIFEMMDAKARN	DCIKEID	LIKSLI	HDHVIKYLA	66
SpNek8	1	YEKIKVVGRCAFCTVY	LCRRLHDM-	KLITIK	DIPVEOMTKDERO-	SALNEVK	VISMLI	HPNTIEYYE	65
SpNek9	1	YTPVRVLCKCAFCEAV	LYRKTEDN-	SLVVWK	EVNLTRCSERVMY-	DAONBIE	IISMLN	JHANTISYFN	65
SpNek10	1	YLVYEHLGTGAFCSVY	KVKKNAGG-	NFLALK	EISFNNPAVGRTAK	EKEKSIESI	IISELSIVKEHMF	RHPNVVRYYR	74
SpNek11	1	YRVEKKLGSCNFCTAF	LVFDMKANP	DKGEEEWKVLK	EISCGDLAPDETV-	DAMHEAK-	L I SKLH	HPNIVKFYD	71
NEK1 Hs	1	YVRLOKIGEGSFGKAI	LVKSTEDG-	ROYVIK	EINISRMSSKERE-	ESRREVA	VLANMF	HPNIVOYRE	65
NEK2 Hs	1	YEVLYTIGTGSYGRCC	KIRRKSDG-	KILVW <mark>K</mark>	ELDYGSMTEAEKQ-	MLVS E VN	L L RELF	HPNIVRYYD	65
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			VV	_		VIa		-	
SpNekA	66	AFVAGGGGILAIVMEY	(GD <mark>GG</mark> DLARK	IEEAKLSGQSI	S <u>A</u> PQ	I I	LNWFVQLCLALY	M <mark>H</mark> SE	123
SpNek1	66	SFEERGNLYIVMDY	CD <mark>GG</mark> DLYQA	INRRKGVLF	PBDE	• <u>I</u> •	MDWFVQICLALKF	IV <mark>H</mark> DR	119
SpNek2	66	RIVDRATSTIYIIMEY	CE <mark>GG</mark> DLGSL	ISKCKKDRKFL	E <mark>B</mark> SF	<u>A</u> V	WKIFQ <mark>Q</mark> LTI <mark>A</mark> LQE	ECHRRGKG-R	127
SpNek4	66	SFESEEG-FLYIVMGF	CD <mark>GG</mark> DLYNR	LKQQKNAALI	E <mark>E</mark> RQ	· V7	vewfv <mark>q</mark> iam <mark>a</mark> lqy	/MHER	120
SpNek7	67	SFIENNELNIVLEI	LADAGDLSRM	IKHFKKQKRLI	PERT	IV	WKYFV <mark>Q</mark> LCS <mark>A</mark> LEH	IM <mark>H</mark> QK	122
SpNek8	66	NFLEDKALMIVMEY	AE <mark>GC</mark> TVFEY	LQQRGNALII	E <mark>E</mark> DE	· VI	LRLMAQILL <u>S</u> MKH	IV <mark>H</mark> SK	119
SpNek9	66	HFLDDQSLFIEMEY	(ANGGTLYEK	IVHQDGTLF	EEKQLKQVNHCFN	IITFNLQLVI	lwylfotvsavay	II <mark>I</mark> QI	136
SpNek10	75	TFTEPQVEKMYIIMEL	LIEGAPLYEH	FNSLKEKNENF:	S E ER	· IV	WHIFIQIVL <mark>A</mark> LRY	IL <mark>H</mark> KE – – – – K	133
SpNek11	72	SFLDGEFFCIITEY	(CEGGDLDDK	ITAWKKAGKKFI	DQNV	·IN	MDWFVQLAL <mark>A</mark> VQF	IM <mark>H</mark> KR	127
NEK1_Hs	66	SHEENGSLYIVMDY	CEGGDLFKR	INAQKGVLF	2 B DQ	II	LDWFVQICLALKH	IVHDR	119
NEK2_Hs	66	RIIDRTNTTLY	CEGGDLASV	ITKGTKERQYLI	D B EF	VI	lrvmt oltl<u>a</u>lke	EC H RRSDGGH	128
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		VIb	VII		VIII		IX		
SoNekA	124		VII	FC ISKMDOHTL	VIII DHASTTI GTDVVI S			IVELVELTE	202
SpNekA	124 120	VID KVLHRDLKPSNLFLTS	VII SKG-LI <mark>KVGD</mark> SKG-LVKMGD	FGISKMLQHTLI ECIARVINNTMI	VIII DHASTTIGTPYYLS ELARTCIGTPYYLS	PEICQKQP	IX YNQKSDMWAAGCI YNNKSDTWALCO	ILYELVTLTR /LYETLTIKH	202
SpNekA SpNek1 SpNek2	124 120 128	VIb KVLHRDLKPSNLFLTS KILHRDIKSQNIFLTR AILHRDLKPANVFLDA	VII SKG-LIKVGD RKG-IVKMGD ADH-NVKLGD	FGISKMLQHTLI FGIARVLNNTM FGLARVLOHDT	VIII DHASTTIGTPYYLS ELARTCIGTPYYLS SFAKTFVGTPYYMS	PEICQKQP PEMCENKP PEOMNRLS	IX YNQKSDMWAAGCI YNNKSDIWALGCV YNDKSDIWSLGCI	LYELVTLTR /LYETLTLKH	202 198 206
SpNekA SpNek1 SpNek2 SpNek4	124 120 128 121	VID KVLHRDLKPSNLFLTS KILHRDIKSONIFLTF AILHRDLKPANVFLDA NILHRDLYIVGE	VII SKG-LIKVGD RKG-IVKMGD ADH-NVKLGD OTNOIIKVGD	FGISKMLQHTL FGIARVLNNTM FGLARVLQHDT IGIARVLGHNI	VIII DHASTTIGTPYYLS ELARTCIGTPYYLS SFAKTFVGTPYYMS DMATTLIGTPYYMS	PEICQKQPY PEMCENKPY PEQMNRLSY PELFSNRPY	IX YNQKSDMWAAGCI YNNKSDIWALGCV YNDKSDIWSLGCI YNHKSDWWALGCC	LYELVTLTR /LYETLTLKH LYELCSLSP /VYEMTTLKH	202 198 206 196
SpNekA SpNek1 SpNek2 SpNek4 SpNek7	124 120 128 121 123	VID KVLHRDLKPSNLFLTS KILHRDLKPSNLFLTS AILHRDLKPANVFLDA NILHRDLYIVGI RVMHRDLKPANVFITA	VII SKG-LIKVGD RKG-IVKMGD ADH-NVKLGD DTNQIIKVGD ADG-KVKLGD	FGISKMLQHTLJ FGIARVLNNTM FGLARVLQHDT LGIARVLQHDT LGIARVLEGNNI	VIII DHASTTIGTPYYLS ELARTCIGTPYYLS SFAKTFVGTPYYMS DMATTLIGTPYYMS FAAHSLVGTPYYMS	PEICQKQP PEMCENKP PEQMNRLS PELFSNRP PERIHETG	IX YNQKSDMWAAGCI YNNKSDIWALGCU YNDKSDIWSLGCI YNHKSDWWALGCO YNFKSDIWSLGCI	LYELVTLTR LYETLTLKH LYELCSLSP VYEMTTLKH LYEMAALOS	202 198 206 196 201
SpNekA SpNek1 SpNek2 SpNek4 SpNek7 SpNek8	124 120 128 121 123 120	VID KVLHRDLKPSNLFLTS KILHRDIKSONIFLTS AILHRDLKPANVFLDA NILHRDLYIVGE RVMHRDIKPANVFITA OILHRDLKTCNILINS	VII SKG-LIKVGD RKG-IVKMGD ADH-NVKLGD OTNQIIKVGD ADG-KVKLGD RDCDVIKIGD	FGISKMIQHTLI FGIARVLNNTMI FGLARVLQHDT: LGIARVLGGNNI LGLGRFFSSKT FGISKILS-SK:	VIII DHASTTIGTPYYLS ELARTCIGTPYYLS SFAKTFVGTPYYMS DMATTLIGTPYYMS TAAHSLVGTPYYMS SKAFTVVGTPSYIS	E PEICQKQP PEMCENKP PEQMNRLS PELFSNRP PERIHETG PELCEGKP	IX YNQKSDMWAAGCI YNNKSDIWALGC YNDKSDIWSLGCI YNHKSDWALGC YNFKSDIWSLGCI YNOKSDMWAIGC	LYELVTLTR /LYETLTLKH LYELCSLSP /VYEMTTLKH LYEMAALQS /LYELLTLRR	202 198 206 196 201 198
SpNekA SpNek1 SpNek2 SpNek4 SpNek7 SpNek8 SpNek9	124 120 128 121 123 120 137	VIb KVLHRDLRPSNLFLTS KILHRDLRPSNLFLTR AILHRDLRPANVFLDP NILHRDLRPANVFIDP OILHRDLRTNILLNR OVLHRDLRTINILFMTK	VII SKG-LIKVGD RKG-IVKMGD ADH-NVKLGD OTNQIIKVGD ADG-KVKLGD RDCDVIKIGD SSG-LIKVGD	FGISKMLOHTLI FGIARVUNNTMI FGLARVUOHDT: LGIARVUEGNNI LGLGRFFSSKT FGISKIUS-SK FGISKVUG-DD:	VIII DHASTTIGTPYYLS ELARTCIGTPYYLS SFAKIFVGTPYYMS DMATTLIGTPYMS SKAFTVVGTPSYIS SKAFTVVGTPSYIS	E SPEICQKQF SPEMCENKP SPEQMNRLS SPELFSNRP SPERIHETG SPELCEGKP SPELVKGQQ	IX XNNKSDIMALGG XNNKSDIMALGG XNNKSDIMSLGG XNKSDIMSLGGG XNFKSDIMSLGGG XNCKSDMALGG XNCKSDMALGG XNAKSDVMALGG	LYELVTLTR /LYETLTLKH LYELCSLSP /VYEMTTLKH LYEMAALQS /LYELLTLRR /LYELLTLRR	202 198 206 196 201 198 214
SpNekA SpNek1 SpNek2 SpNek4 SpNek7 SpNek8 SpNek9 SpNek10	124 120 128 121 123 120 137 134	VIb KVLHRDLKPSNLFLTS AILHRDLKSQNIFITF AILHRDLKPANVFLDA NILHRDLYIVGI RVMHRDLKPANVFITA QILHRDLKTCNILLNK DVLHRDLKTINIFMTK KIVHRDLTPNNIMLGG	VII KG-LIKVGD RKG-IVKMGD DH-NVKLGD DTNQIKVGD ADG-KVKLGD KSG-LIKVGD ERD-RVTITD	FGISKMLOHTLI FGIARVLOHDT FCLARVLOHDT LGLARVLEGNNI LGLGRFFSSKT FGISKILS-SK FGISKILS-SK FGLARAROPDA	UIII DHASTTICTPYMIS SFAKTFVGTPYMVS SFAKTFVGTPYMVS MATTLIGTPYMVS SKAFTVVGTPYMVS SKAFSVVGTPLYMS SKMTSVVGTPLMS SKMTSVVGTLLMS	PEICQKQP SPEMCENKP SPEQMNRLS SPELFSNRP SPERIHETG SPELCEGKP SPELVKGQQ PEIVQSTP	IX INOKSDIWALGG INNKSDIWALGG INFKSDIWALGG INFKSDIWALGG INFKSDIWALGG INOKSDWALGG INOKSDWALGG INOKSDWALGG GERADWAAGG	LYELVTUTR LYETLTLKH LYELCSUSS VYEMTTUKH LYEMAALQS JLYELLTURR JLYELTURR JLYELTURR	202 198 206 196 201 198 214 212
SpNekA SpNek1 SpNek2 SpNek4 SpNek7 SpNek8 SpNek9 SpNek10 SpNek11	124 120 128 121 123 120 137 134 128	VID KVLHRDLKPSNLFLTS KLHRDLKSONIFLTA AILHRDLKPANVFLDA NILHRDLYIVGT RVMHRDLKPANVFITA QILHRDLKTONILLNK DVLHRDLKTONILLNK KIVHRDLKTINIFMTK KIVHRDLTPNNIMLGB	UII SKG-LIKVGD RKG-IVKNGD DTNQIIKVGD ADG-KVKLGD RDCDVIKIGD (SG-LIKVGD SRD-RVTID CNMIKIGD	FGISKMIQHTLI FGIARVIQHDT: IGIARVIQHDT: IGIARVIGHDT: IGISKIIS-SK: FGISKIIS-SK: FGISKVIG-DD FGISRVIG-DD FGISRVIMGTTI	UIII DHASTTIGTEYNIS ELARTCIGTEYNIS SFAKTFVGTPYNS SMATTLIGTEYNS SKAFTVVGTPSVIS SKAFTVVGTPSVIS SKMTSVVGTPLMS SKMTSVVGTILVSC DMSTFTGTFYMS	PEICQKQP PEMCENKP PEQMNRLS PELFSNRP PERIHETG PELCEGKP PELVKGQQ PEIVQSTP PEVVLKHEG	IX INOKSDIWALGG VNDKSDIWALGG VNDKSDIWALGG VNFKSDIWALGG VNFKSDIWALGG VNAKSDWALGG VNAKSDWALGG VGEKADVWAAGO VGEKADVWAAGO	LYELVILIR LYELCSLSP WIMITLKH LYEMAALQS LYELLILR LYELLIR LYELLIR LYELCALQH	202 198 206 196 201 198 214 212 205
SpNekA SpNek1 SpNek2 SpNek4 SpNek7 SpNek8 SpNek9 SpNek10 SpNek11 NEK1_Hs	124 120 128 121 123 120 137 134 128 120	VID KVLHRDLKPSNLFLTS KILHRDLKSONIFLTR AILHRDLY IVGI RVMHRDLKPANVFIDA OILHRDLKTONIFLAN DVLHRDLKTINIFMTK KIVHRDLTPNNIMLGE RVLHRDLKTRNIFLTK KILHRDLKSONIFLTX	UII SKG-LIKVGD ADH-NVKLGD OTNQIIKVGD ADG-KVKLGD RDCDVIKIGD CSG-LIKVGD ERD-RVTITD CNMIKIGD CDG-TVQLGD	FCISKMLQHTLI FCLARVLQHTL ICLARVLQHT ICLARVLGHT ICLGRFFSSKT FCISKILS-SK FCISKILS-SK FCISKVLG-DD FCLARARQPDA FCISRVIMGTTI FCLARVLNSTVI	UIII DHASTTIGTEYMIS ELARTCIGTEYMIS SPAKIFVGTPYMS SPAKIFVGTPYMS SKAFTVGTPYMS SKAFTVGTPSMS SKAFTVGTPSMS SKAFTVGTPIMS DMASTFTGTEYMIS DMASTFTGTEYMIS LARTCIGTEYMIS	PEICQKQP PEMCENKP SPEQMNRLS SPELFSNRP SPERIHETG SPELCEGKP SPELVKGQQ PEIVQSTP SPEVLKHEG SPEICENKP	IX NKSDIMAAGCI NNKSDIMALGC NNKSDIMALGC NNKSDIMALGC NNKSDIMALGC NNKSDIMALGC NAKSDWALGC NAKSDWALGC NSSDWALGC NNKSDIMALGC	LYPLVTLTR LYPLCSLSP WYPMTLKH LYPMAALQS LYPLLTLRR LYPLLTLRR LYPLLTLRR LYPLCALQH LYPLCALQH	202 198 206 196 201 198 214 212 205 198
SpNekA SpNek1 SpNek2 SpNek4 SpNek7 SpNek8 SpNek9 SpNek10 SpNek11 NEK1_Hs NEK1_Hs	124 120 128 121 123 120 137 134 128 120 129	VIb KVLHRDL&PSNLFLTS KILHRDL&PSNLFLTS AILHRDL&PANVFIDA NILHRDL&PANVFIDA OILHRDL&PANVFITA OVLHRDL&TONILLNK KIVHRDLTPNNIMLGE RVLHRDL&TRNIFLKK KILHRDL&PANVFLDG	 SKG-LIKVGD ADH-NVKLGD DTNQIIKVGD RDCDVIKLGD SRDCDVIKLGD SG-LIKVGD SRD-RVTITD RNM-IKIGD CNG-TVQLGD SKQ-NVKLGD	FCISKMIQHTLI FCIARVLOHDT FCLARVLOHDT ICLORFFSSKT FCISKULG-DD FCISKULG-DD FCLARAROPDA FCIARVLGHUSTU FCIARVLMGTT FCIARVLMSTU	UIII DHASTTIGTPYNIS ELARTCIGTPYNIS SFAKIFVGTPYNIS SMATTLIGTPYNIS SKAFTVVGTPYNIS SKAFTVVGTPINIS SKAFSVGTILISC DMASTFIGTPYNIS ELARTCIGTPYNIS SFAKTFVGTPYNIS	PEICQKQP PEMCENKP SPEQMNRLS SPELFSNRP SPELFER SPELCEGKP SPELVKGQQ DEIVQSTP SPELVKHEG SPEVLKHEG SPEUCENKP SPEQMNRMS	IX VINKSDIWALGO VINKSDIWALGO VINKSDIWALGO VINKSDWALGO VINKSDWALGO VINKSDWALGO VIAKSDWALGO VIAKSDWALGO VINKSDIWALGO VINKSDIWALGO VINKSDIWALGO VINKSDIWALGO	LYELVILTR LYELCSISP VYPMTTLKH LYELCSISP VYPMTTLKH LYELLTR LYELLTR LYELLTR LYELCALOH LYELCALOH LYELCALMP	202 198 206 196 201 198 214 212 205 198 207
SpNekA SpNek1 SpNek2 SpNek2 SpNek4 SpNek4 SpNek8 SpNek8 SpNek10 SpNek10 SpNek11 NEK1_Hs	124 120 128 121 123 120 137 134 128 120 129	VID KVLHRDLKPSNLFITS KILHRDLKSONIFITK AILHRDLZPANVFLDA NILHRDLZIVGI QILHRDLKTONILLNK VLHRDLKTONILLNK KIVHRDLKTONIFITK KIVHRDLKTRNIFIKK KILHRDLKSONIFITY TVLHRDLKPANVFLDG	VII KG-LIKVGM ADH-NVKKGD DTNQIIKVGD DTNQIIKVGD RDCDVIKIGD SG-LIKVGC SG-LIKVG CDG-RVTITD CMM-IKIGD KQ-NVKLG	FGISKMLOHTLI FGLARVLOHDT: IGLARVLOHDT: IGLARVLEGNNI IGLARVISSKTLS-SK FGISKILS-SK FGISKVLG-DD FGLARARVINSTVI FGLARAVINSTVI FGLARVINSTVI FGLARILNHDT:	UIII DHASTTIGTEYMIS ELARTCIGTEYMIS SFAKTFVGTBYMS SKAFTVGTBYMS SKAFTVVGTBSYIS SKMSVVGTBYMS SKMSVVGTLMS DMASTFTGTFYMS ELARTCIGTFYMIS SFAKTCYGTPYMS	BPEICQKOP SPEMCENKP SPEQMNRLS SPELFSNRP SPELFSNRP SPELCEGKP SPELCEGKP SPELVQSTP SPEVLKHEG SPEIVQSTP SPEVLKHEG SPEICENKP SPEQMNRMS	IX NOKSDIMAAGC NNKSDIMAAGC NDKSDIMSIGG NHKSDVMAIGC NOKSDMAIGO NOKSDMAIGO NSKSDVMAIGC NSKSDVMSIAC NNKSDIMAAGC NNKSDIMAAGC NSKSDVMSIAC	LYELVILTR LYETLSISP WYEMTTLKH LYELSSISP WYEMTTLKH LYELLTLRR LYELLTRR LYELCALQH LYELCALQH LYELCALMP	202 198 206 196 201 198 214 212 205 198 207
SpNekA SpNek1 SpNek2 SpNek4 SpNek7 SpNek8 SpNek9 SpNek10 SpNek10 SpNek11 NEK1_Hs NEK2_Hs	124 120 128 121 123 120 137 134 128 120 129	VID KVLHRDLKPSNLFLTS KILHRDLKSQNIFLTF ALLHRDLYPANVFLDZ NILHRDLYIVGT RVMHRDLKPANVFITG QLHRDLKTQNILLNK DVLHRDLKTQNILLNK KIVHRDLTPNNIMLG RVLHRDLKTQNIFLKK KILHRDLKTQNIFLKK KILHRDLKTQNIFLKK	VII KKG-LIKVGD ADH-NVKLGD DTNQIRVGD ADG-KVKLGD ADG-KVKLGD SGC-LIKVGD SGC-LIKVGD CRD-RVTITD CDG-TVOLGD SKQ-NVKLGD	FGISKMLQHTLI FGLARVLOHDT: IGLARVLGHDT: IGLARVLGHDT: IGLARVLGHDT: IGLARFSSKT FGISKILS-SK FGISKULG-DD FGLARAROPDA: FGISRUMGTT FGIARVLNSTV; FGLARILNHDT:	UIII OHASTTIGTEYMIS ELARTCIGTEYMIS SFAKTFVGTPYMAS SKAFTVGTPYMAS SKAFTVVGTPYMAS SKMTSVVGTPIMAS SKMTSVVGTPIMAS SKMTSVVGTPIMAS ELARTCIGTPYMAS SFAKTFVGTPYMAS	BPEICQKOP SPEMCENKP SPEQMNRLS SPELFSNRP SPELHETG SPELCEGKP SPELVKGQO PEIVKGQO PEIVQSTP SPEVLKHEG SPEICENKP SPEICENKP SPEICENKP SPEQMNRMS	IX (NCKSDWWAAGG (NNKSDIWAIGG (NNKSDWAIGG (NFKSDIWSIGG (NCKSDWAIGG (MAKSDWAIGG (NAKSDWAIGG (NSKSDWSIAG) (NNKSDIWAIGG (NNKSDIWAIGG (NEKSDIWSIGG)	LYELVTUTR LYELCSLSP VYEMTIKH IYELALQS IYELTURR LYELLTURR LYELLTURR LYELCTIKH LYELCALMP LYELCALMP	202 198 206 196 201 198 214 212 205 198 207
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SpNekA SpNek1 SpNek2 SpNek4 SpNek7 SpNek8 SpNek8 SpNek10 SpNek10 SpNek11 NEK1_Hs NEK2_Hs	124 120 128 121 123 120 137 134 128 120 129	VID KVLHRDLKPSNLFLTS KILHRDLKSONIFLTA AILHRDLKPANVFLDA NILHRDLYIVGI QTHRDLKTONILLNF QTLHRDLKTONILLNF VLHRDLKTONIFLTK KIUHRDLKTRNIFLKK KILHRDLKSONIFITK TVLHRDLKPANVFLDO	VII KKG-LIKVGD KKG-LIKVGD ADH-NMKIGD DTNQIIKVGD DG-KVKIGD RDCDVIKIGD SCOVIKIGD SCOVIKIGD SCOVIKIGD SKQ-NVKIGD X - PTIIKIL	FGISKMLQHTLI FGLARVLQHDT: IGLARVLGNNTM FGLARVLGNNT IGLARVFSSKT FGISKITS-SKI FGISKUG-DD FGISKVLG-DD FGLARACPDA: FGLARACPDA: FGLARINNTV: FGLARINNTY: FGLARINNTY:	VIII DHASTTIGTFYMIS ELARTCIGTFYMIS SFAKTFVGTPYMS SKAFTVVGTPSMIS SKAFTVVGTPSMIS SKAFTVVGTPSMIS SKMTSVVGTPSMIS DHASTFTGTFYMIS ELARTCIGTFYMIS SFAKTFVGTPYMS MGTVIEELVTVLLS	SPEICQKQP SPEMCENKP SPEQMNRLS SPELFSNRP SPERIHETG SPELCEGKP SPELVKGQO SPELVKGQO SPELVKGQO SPELVKGQO SPELCENKP SPEJCENKP SPEJCENKP SPEJCENKP SPEJCENES	IX (N) (SSD MAAGC (N) (SSD M	LYELVTTR LYETTTKH LYETTTKH LYELCSISP WYEMTTKH LYELLTR LYELLTRR LYELLTRR LYELCALQH LYELCALQH LYELCALQH LYELCALMP	202 198 206 196 201 198 214 212 205 198 207
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Fig. 3. Amino acid comparison of the kinase domain of the nine *S. purpuratus* NIMA-related kinases, with human Nek2 and Nek1. The eleven kinase subdomains are indicated in roman numerals, and diagnostic motifs for Nek family (Lu and Hunter, 1995) are underlined with bold bars. The star indicates the key lysine residue for kinase activity. Arrow points to a conserved serine/threonine residue, whose phosphorylation is required for activation. Identical residues are in black, conserved residues are in gray.

recently evolved from an ancestral Aurora B/C found in coldblooded vertebrates (Brown et al., 2004). Aurora A is emerging as a critical regulator of centrosome and spindle function, while Aurora B mediates chromosome segregation by ensuring proper biorientation of sister chromatids. Aurora C, a chromosomal passenger protein similar to Aurora B, is specifically expressed in testis, indicating a role in spermatogenesis. The sequence of the sea urchin Aurora is very similar to that of its homologue in the starfish *Marthasterias glacialis* (Lapasset et al., 2005); however, like the two Auroras previously found in *C. elegans* and *D. melanogaster*, both sequences are highly divergent from the chordate counterparts (Brown et al., 2004).

The NEK proteins have been called the "third family of mitotic kinases" (O'Connell et al., 2003). The NEKs are represented in a wide variety of eukaryotic organisms, ranging from one to twenty or more representatives per genome. The NEK family possesses a highly conserved kinase domain at the N-terminus and a divergent C-terminus responsible for their specific roles in the cell. The founding member of the family,

the NIMA kinase, is required for mitotic entry and progression through mitosis in *Aspergillus nidulans*. In higher eukaryotes, NEK2, the closest relative to NIMA, plays important roles in centrosome regulation, and its overexpression leads to centriole splitting and its association with centrosome-bound proteins (Fry, 2002). The functions of other members of the NEK family are less well understood.

Nine members of the NEK family are encoded in the sea urchin genome, the kinase domains of which are aligned in Fig. 3. While eight of them clustered with the identified mammalian NEK subfamilies (Table 1, Fig. 4), no orthologue could be found for NEK3 and NEK5. The ninth sea urchin NIMA-related kinase, Sp-NEKA, displays similarity with NEK1 in its kinase domain (Fig. 4). Sp-NEKA is highly related to a sea urchin *S. granularis* cDNA (AJ841701), however, no orthologue was found in other organisms, suggesting that Sp-NEKA may constitute an echinoderm-specific innovation of the NEK family.

Quarmby and Mahjoub (2005) showed a correlation between the number of NEK kinases in the genome of an organism and



Fig. 4. Phylogenetic tree of NEK kinases. The kinase domains of NIMA-related kinases from *S. purpuratus* (Sp), human (Hs), mouse (Mm), *Drosophila melanogaster* (Dm), *Caenorhabditis elegans* (Ce), and *Aspergillus nidulans* (NIMA) were identified by ScanProsite program or retrieved from the kinome dataset at Kinase.com. (**) the sea urchin specific NEKA.

whether or not it has dividing ciliated cells. They proposed that the expansion of the NEK family has coevolved with centrioles, which serve both as basal bodies and microtubule-organizing centers. The complexity of NEK kinases found in *S. purpuratus* fits this hypothesis.

Checkpoint kinases

A complex network of checkpoint pathways helps ensure precise replication and segregation of the genome by delaying cell cycle progression in response to DNA damage or replication defects, facilitating repair of the defects. Genotoxic stress arising from unreplicated DNA or from DNA damage activates DNA damage checkpoint kinases such as ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3 related) (reviewed in Sancar et al., 2004). These proteins in turn activate Ser/Thr checkpoint kinases, Chk1 and Chk2, and the p53 tumor suppressor, leading to inactivation of cyclindependent kinases to inhibit cell progression from G1 to S (the G1-S checkpoint) or from G2 to mitosis (G2-M checkpoint) (Sancar et al., 2004; Zakian, 1995). Orthologues of the key proteins involved in both the G1-S and G2-M DNA damage checkpoints were found in S. purpuratus (Table 1), including one isoform each of ATM, ATR, Chk1, and Chk2. Chk1 mRNA has also been identified in S. purpuratus eggs by RT-PCR (Adams, unpublished data).

The CDC25 phosphatases, which trigger the final activation of the CDK/cyclin complexes, are important downstream effectors of the Chk1/Chk2 response. Depending on the cell cycle stage and the nature of the damaging agent, activated Chks phosphorylate one or the other human CDC25 isoform, promoting either ubiquitin/proteasome mediated degradation (CDC25A in G1-S or S phase checkpoint) or 14-3-3 mediated sequestration (CDC25A and C in G2-M checkpoint) of the targeted phosphatase, preventing the activation of the downstream CDK/cyclin complex (reviewed in Kristjansdottir and Rudolph, 2004; Sancar et al., 2004; Stanford and Ruderman, 2005). As mentioned above, sea urchins have a single CDC25 homologue. Three 14-3-3 genes were identified in the S. purpuratus genome, whereas mammals have seven (Boston and Jackson, 1980). The 14-3-3 proteins play a pivotal role in several checkpoints targeting not only the CDC25 phosphatase but also CDK2, CDK4, and the CDK inhibitor p27 at the G1-S transition, and CDK1, Wee1, Chk1, and p53 at G2-M (Hermeking and Benzinger, 2006). How specificity of interaction among 14-3-3 isoforms is generated is not entirely clear, and 14-3-3 function in the unperturbed cell cycle is still poorly understood. As the three 14-3-3 genes are expressed during early embryogenesis (as indicated by the transcriptome analysis; Samanta et al., 2006), the sea urchin embryo should provide a good model for investigating their function.

The control of DNA replication

During S phase, the many thousands of replication forks involved in replicating chromosomal DNA must be coordinated to ensure that, despite the very large quantities of DNA

involved, chromosomal DNA is replicated once and only once per cell cycle. Cells achieve this by having distinct stages that proceed sequentially. Prior to S phase, replication origins are "licensed" by loading pre-RC onto them. The licensing of replication origins depends on the sequential recruitment to DNA of ORC, Cdc6, Cdt1 and MCM2-7 proteins (Blow and Dutta, 2005; Forsburg, 2004; Nishitani and Lygerou, 2002) The six subunits of the ORC initiator (ORC1-6) were identified in the S. purpuratus genome, as were the six MCM helicases. Cdt1 and Cdc6 are also present (Table 2). Recently, two additional members of the MCM helicase family were identified: MCM8 and MCM9 (Gozuacik et al., 2003; Lutzmann et al., 2005; Yoshida, 2005). While MCM8 has been proposed to function as DNA helicase with a role distinct from that of the MCM2-7 complex, MCM9 function is unknown. MCM9 is more closely related to MCM8 than to the other MCM2-7 proteins and was hitherto known only in vertebrates, and thought to be a recent duplication of the MCM8 gene (Maiorano et al., 2006). The identification of MCM9 orthologues in the genome of S. purpuratus and the tunicate C. intestinalis (Fig. 5) demonstrates that the gene is not limited to vertebrates, suggesting that the MCM8/9 duplication occurred within deuterostomes prior to the split between chordates and echinoderms.

Table 2

S. purpuratus DNA replication genes identified in this study

Biochemical process/function	Family	Spu Id	Assigned name	Embryonic expression
Components of	ORC	019821	Sp-ORC1	+
pre-replication		010533	Sp-ORC2	+
complexes		000544	Sp-ORC3	+
(pre-RC)		024094	Sp-ORC4	+
· ·		018823	Sp-ORC5	+
		005343	Sp-ORC6	+
	MCM	06096/011491	Sp-MCM2	+
		012983/006848	Sp-MCM3	+
		024515	Sp-MCM4	+
		012431	Sp-MCM5	+
		023819	Sp-MCM6	+
		026450	Sp-MCM7	+
	CDC6	010595	Sp-CDC6	+
	Cdt1	002046	Sp-Cdt1	+
Inhibitor of pre-RC	Geminin	023715	Sp-Geminin	+
Components of	CDC45	023032/024816	Sp-CDC45	+
replisome	RPA	028925	Sp-RPA1	+
progression		026990	Sp-RPA2	+
complexes		019078	Sp-RPA3	+
*	GINS	012376	Sp-Sld5	+
		017817/017818	Sp-Psf-1	+
		021616	Sp-Psf2	-
		018457	Sp-Psf3	+
	MCM-10	005667	Sp-MCM10	+
Polymerases	Alpha-1	000210	Sp-Pol-alpha-1	+
(DNA-directed)	Alpha2	011199	Sp-Pol-alpha-2	+
	Epsilon-1	025749	Sp-Pol-epsilon-1	+
	Epsilon-2	014691	Sp-Pol-epsilon-2	+
Regulators of	CDC7	000046	Sp-CDC7	+
initiation	Dbf4	023982	Sp-Dbf4	+
Unknown	MCM	007007	Sp-MCM8	+
		003633	Sp-MCM9	+

Activation by protein kinases is required for the initiation of licensed origins (Bell and Dutta, 2002). In higher eukaryotes, the activities of CDK2/cyclin E and Cdc7/Dbf4 have been shown to result in changes in the pre-RC that lead to the binding of Cdc45 to the MCM complex followed by the unwinding of replication origins and the recruitment of RPA, DNA polymerase α and ε to initiation sites. Replication initiation is tightly coupled to removal of the license of origins (reviewed in Blow and Dutta, 2005). Metazoans mainly prevent re-licensing during S phase and G2 by the inhibitory binding of geminin to Cdt1. Homologues of all the components of this DNA replication regulatory network were found in the *S. purpuratus* genome as reported in Table 2.

Additional levels of cell cycle regulation

Proliferative disorders such as cancer are associated with somatic mutations and genomic instability, which are generally caused by errors in DNA replication or mitosis. Premature entry into either S or M phase increases the probability of error, and hence multiple levels of cell cycle control machinery are dedicated to ensuring that this does not occur. In animals, these control networks are linked to the transcriptional regulatory networks that control development (reviewed in Coffman, 2004). In addition to being under the control of the cyclins, CDKs, and other regulatory machinery discussed above, G1-S phase and G2-M phase transitions are regulated by CDK inhibitors such as mammalian $p27^{kip1}$ and $p21^{cip1/waf1}$ and the INK4 proteins and by expression levels and activities of transcription factors and cofactors such as E2F, pRB, and p53 family members (reviewed in Coffman, 2004; Sherr, 2000). Many of the CDKs discussed above function as switches in the proteolytic pathways that control levels of cyclins and other cell cycle control proteins, phosphorylated forms of which are targeted for proteolytic degradation by ubiquitin-ligases in the SCF and APC complexes (Reed, 2003). CDK inhibitors and regulators, transcription factors associated with cell cycle control, and proteins involved in ubiquitin-mediated proteolysis found encoded in the S. purpuratus genome are listed in Table 1. Notable absences include the INK4 CDK inhibitors, and the ARF tumor suppressor, which to date have been identified only in mammals and are probably vertebrate (and possibly mammalian) novelties. Moreover, the presence of two pRB family homologues, two E2Fs, a single p53 homologue, and one p21^{cip1}/p27^{kip1} homologue in sea urchins mirrors the situation in flies, suggesting that this is the primitive bilaterian



Fig. 5. Phylogenetic tree of MCM proteins. The tree was constructed by the Maximum Likelihood method (ML), with bootstrap values shown for each clade. Sequence Ids for the human (Hs), *Xenopus laevis* (Xl), *Danio rerio* (Dr), *Drosophila melanogaster* (Dm), *Caenorhabditis elegans* (Ce), and *Ciona intestinalis* (Ci) MCMs are reported in Table S3.

repertoire of these genes, which underwent expansion and diversification in the chordate-vertebrate lineage.

An extensive network of enzymes and other proteins that detect and repair damaged DNA provides another critical line of defense against mutation and genomic instability. Sea urchin genes associated with DNA repair functions (base excision, double-strand break, nucleotide excision, and mismatch repair) were identified using BLAST searches with mouse protein sequences retrieved by Gene Ontology searches (as described in Materials and methods); these are listed in Table S2. In general (and as might be expected), this set of genes is highly conserved between vertebrates and sea urchins. Homologues were found for all of the searched mammalian repair proteins, with the exception of one DNA base excision repair protein and two nucleotide excision repair proteins (Table S2).

Conclusions

The *S. purpuratus* genomic toolkit for cell cycle control is similar to, albeit somewhat simpler than, that of vertebrates. For most gene families, each subtype is represented by a single gene with some limited but notable exceptions. This holds particularly true for the CDK and cyclin families.

A comparison of sea urchin cell cycle genes with those from vertebrates, C. elegans, Drosophila melanogaster, as well as the tunicate C. intestinalis, provides new insight into the evolution of cell cycle control. While some gene subtypes have undergone lineage-specific expansions in vertebrates (i.e. the cyclins A, B, D, and E, the mitotic kinases aurora, polo, and NEK), others seem to be lost in that lineage (i.e. the novel cyclin B identified in S. purpuratus and C. intestinalis which are not found in zebrafish, mouse, or humans). On the other hand, the recently identified replicative helicase MCM9, which was previously thought to be a vertebrate innovation, is also found in sea urchins. Moreover, analysis of the sea urchin genome highlighted a few genes which either are specific to the echinoderm lineage, such as NekA, or were identified in S. purpuratus and found to have potential orthologues in other species, such as the novel family of cyclin K/L-related proteins. Most of the genes participating in the initiation and progression of DNA replication, in DNA repair, or in the tight control of cell cycle events, such as the checkpoint proteins, are highly conserved between echinoderms and vertebrates. The CDK inhibitors are a notable exception, with only one p21/p27 homologue, and an absence of the INK4 family, which is apparently confined to vertebrates.

Most of the genes included in this analysis (Tables 1 and 2) are expressed during embryogenesis (109/120) as deduced from the transcriptome analysis (Samanta et al., 2006), and those strictly related to cell cycle control are all being expressed at this stage. The genomic repertoire of cell cycle regulators uncovered in this survey will thus provide molecular tools that further enhance the physiological features that have made the early sea urchin embryo a productive model for understanding cell cycle control and its developmental regulation.

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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.2006.09.012.

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