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Cdc7l1

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Cdc7 (Cell division cycle 7), also known as Hsk1 in fission yeast, is an important serine/threonine kinase, whose sequence is conserved from yeasts to mammals. The kinase activity of Cdc7 is regulated during the cell cycle by an activation subunit Dbf4 (also known as Dfp1/Him1 in fission yeast and ASK in mammals,) via heterodimer formation between the two. Cdc7 was first identified in budding yeast as a temperature-sensitive mutant (cdc7^{ts}) defective in cell cycle progression. The budding yeast cdc7^{ts} cells arrest immediately before the onset of S phase at the non-permissive temperature, but resume growth and complete S phase in the absence of ongoing protein synthesis upon return to the permissive temperature. Cdc7 plays a conserved, pivotal role in triggering origin firing through phosphorylation of MCM (mini-chromosome maintenance) proteins. It facilitates the loading of Cdc45 and other replisome factors onto the pre-replicative complex, to generate active replication forks. In addition, it regulates other chromosomal transactions including DNA damage checkpoint, meiotic recombination, bypass DNA synthesis and histone functions. Selective induction of apoptosis in human cancer cells, but not in normal fibroblasts, after Cdc7 inhibition has provoked the effort in the development of Cdc7 inhibitors as potential anti-cancer drugs. Indeed, studies to date have suggested human Cdc7 as a new promising target in cancer therapy.

PROTEIN FUNCTION

Cell-division-cycle 7 (Cdc7), also known as Hsk1 (in fission yeast), is an important serine/threonine kinase conserved from yeasts to mammals (Hartwell 1970, 1971; Patterson *et al*. 1986; Hollingsworth and Sclafani 1990; Masai *et al*. 1995; Jiang and Hunter 1997; Sato *et al*. 1997; Kim *et al*. 1998; Faul *et al*. 1999; Guo and Lee 1999; Johnston *et al*. 2000). During cell cycle, kinase activity of Cdc7 is regulated by heterodimeric complex formation with a Dbf4-related subunit, Dbf4/Dfp1(Him1)/ASK (Johnston and Thomas 1982; Kitada *et al*. 1992; Jackson *et al*. 1993; Brown and Kelly 1998; Jiang *et al*. 1999; Kumagai *et al*. 1999; Takeda *et al*. 1999). A second Dbf4-related subunit, Drf1/ASKL1, has been identified in human and *Xenopus* (Montagnoli *et al*. 2002; Yanow *et al*. 2003; Takahashi and Walter 2005; Yoshizawa-Sugata *et al*. 2005). Role of Cdc7 in initiation of DNA replication has been the focus of many studies since 1970s. Recently, crucial roles of Cdc7 in other cell cycle events including meiotic recombination, checkpoint regulation, DNA damage repair (bypass DNA synthesis) and mitosis have also been shown, in keeping with some of the old genetic observations (Sclafani 2000; Bell and Dutta 2002; Masai and Arai 2002; Masai *et al*. 2010). A second Hsk1-like protein kinase, Spo4, together with its regulator, Spo6, have also been identified in fission yeast (Nakamura *et al*. 2000, 2002). The Spo4-Spo6 kinase complex is shown to function specifically during late stages of meiosis, although its specific targets and mode of actions remain unclear.

1. Cdc7 during mitotic growth

1.1 Cdc7 in mitotic DNA replication

DNA replication initiates at specific sites on a genome called replication origins. Generally, DNA replication proceeds in two temporally regulated steps during cell cycle: origin licensing at the late mitosis-early G1 transition and origin activation at the G1/S-S phase. During origin licensing, chromatin-bound origin recognition complex (ORC) recruits Cdc6 and Cdt1 followed by loading of the minichromosome maintenance 2-7 (MCM2-7) complex to form a pre-replicative complex (pre-RC) at replication origins. Subsequent origin activation involves recruitment of some additional replication factors (such as Cdc45, MCM10, Sld2-Sld3, GINS and Dpb11 in budding yeast) to the replication origins and rearrangement of the pre-RC to form a pre-initiation complex (pre-IC).

Licensed origins are activated at different times during the S phase. Genome-wide studies of the replication timing program indicates the presence of domains (i.e. replication timing domains) which may dictate the timing of DNA replication. Replication timing program dynamically changes during development and differs between cell-types (Hiratani *et al*. 2008; Hansen *et al*. 2010), suggesting the regulation of this program at the chromatin level. However, the precise nature of determinants used to define the replication timing domains remains to be elucidated. Studies in yeast show suppression of the late-firing origins in a checkpoint-dependent manner, indicating that the timing of origin firing is regulated by the replication checkpoints (Santocanale and Diffley 1998; Shirahige *et al*. 1998; Zegerman and Diffley 2010). Although Cdc7 has been speculated to play roles in temporal regulation of origin firing, how it recognizes early and late replication origins in a differential timing remains unclear (Bousset and Diffley 1998; Donaldson *et al*. 1998; Walter 2000; Patel *et al*. 2008; Wu and Nurse 2009).

The MCM2-7 complex plays important role in both the initiation and the elongation phases during DNA replication (Tye 1999; Kelly and Brown 2000). Subunits of the MCM2-7 complex are the major Cdc7 phosphorylation targets identified thus far (Lei *et al*. 1997; Sato *et al*. 1997; Brown and Kelly 1998; Takeda *et al*. 1999; Masai *et al*. 2000; Sheu and Stillman 2006). In budding yeast, Cdc7 phosphorylates all MCM

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subunits except MCM5 *in vitro* (Weinreich and Stillman 1999). Recent data shows that Cdc7-dependent phosphorylation in MCM4 and MCM6 subunits is mediated by prior phosphorylation of these subunits by Mec1 and a proline-directed kinase (Randell *et al.* 2010). Besides, budding yeast Cdc7 shows preference in associating with and phosphorylating origin-bound MCM2-7 complex (Sheu and Stillman 2006; Francis *et al.* 2009). Notably, Cdc7 is not required for viability in the budding yeast *mcm5-bob1* mutant (Hardy *et al.* 1997). Further analyses suggested that this bypass of Cdc7 function may be permitted by a conformation change in the MCM5 subunit which plausibly mimics the active conformation of MCM5 in the Cdc7-phosphorylated MCM2-7 complex (Hoang *et al.* 2007).

In fission yeast, purified Hsk1 specifically phosphorylates Cdc19/MCM2 and Cdc21/MCM4 subunits of the MCM2-7 complex purified from fission yeast (Brown and Kelly 1998; Lee *et al.* 2003). Hsk1-dependent phosphorylation of MCM2 in the MCM complex has been shown to be facilitated by MCM10/Cdc23 (Lee *et al.* 2003). In human, Cdc7 phosphorylates MCM2, MCM4 and MCM6 subunits of the MCM2-7 complex (Masai *et al.* 2000, 2006; Cho *et al.* 2006; Montagnoli *et al.* 2006; Tsuji *et al.* 2006; Charych *et al.* 2008). A recent study showed that Cdc7-mediated phosphorylation at the N-terminus of human MCM2 is required for chromatin loading of the MCM2-7 complex during cell cycle re-entry from quiescent phase (Chuang *et al.* 2009).

Cdc7-dependent phosphorylation of Cdt1 and Cdt1-binding protein, Geminin, *in vitro*, suggested a possible role of Cdc7 in regulating origin licensing (Masai *et al.* 2000; Ballabeni *et al.* 2009). However, the role of Cdc7 in pre-replicative complex (pre-RC) formation during cell cycle has not been described to date. Indeed, Hsk1 mutations showed no effects on the genome-wide distribution of the pre-RC formation in fission yeast (Kanoh *et al.* personal communication).

The crucial role of Cdc7 during initiation of DNA replication may be to facilitate the association of Cdc45 with the pre-RC (Jares and Blow 2000; Walter 2000; Zou and Stillman 2000; Dolan *et al.* 2004; Masai *et al.* 2006; Yabuuchi *et al.* 2006). In fission yeast, Sld3 is loaded onto the pre-RC in an Hsk1-dependent manner and may facilitate subsequent Cdc45 loading (Nakajima and Masukata 2002; Yamada *et al.* 2004; Yabuuchi *et al.* 2006). Loading of Sld3 is dependent on both Cdc7 and CDK in budding yeast (Tanaka *et al.* 2007; Zegerman and Diffley 2007; Araki *et al.* unpublished data). In human, Cdc7 facilitates loading of Cdc45 by phosphorylating MCM2 and MCM4 subunits (Masai *et al.* 2006). Besides, interaction between Cdc7 and Cdt1 has also been suggested to contribute to Cdc45 loading (Ballabeni *et al.* 2009).

Although both Cdc7 and CDK are known to regulate Cdc45 loading in various organisms, their sequence of actions remains controversial (Zou and Stillman 1998; Jares and Blow 2000; Walter 2000; Dolan *et al.* 2004; Yabuuchi *et al.* 2006). While Cdc7 or Hsk1 acts before CDK in *Xenopus* or fission yeast, respectively (Jares and Blow 2000; Walter 2000; Yabuuchi *et al.* 2006), Cdc7 acts after CDK in budding yeast (Nougarede *et al.* 2000). On the contrary, both Cdc7 and CDK may be dispensable for Cdc45 chromatin loading in starfish after fertilization (Tachibana *et al.* 2010). No delay in Cdc45 chromatin loading was observed when fertilized eggs incurred both Cdk inhibition and morpholino-mediated Cdc7 knockdown.

Cdc7, in concert with S-CDKs and MCM complex, has also been shown to facilitate association of Replication Protein A (RPA) with the replication origins (Tanaka and Nasmyth 1998). This most likely reflects the conversion of the double-stranded DNA (dsDNA) at replication origins to single–stranded DNA (ssDNA) after the action of CDK and Cdc7. Besides, Cdc7 is also known to phosphorylate other replication proteins including DNA polymerase alpha p180 (Weinreich and Stillman 1999; Masai *et al.* 2000), Cdc45 (Nougarede *et al.* 2000), ORC4 subunit of the ORC1-6 complex and SV40 T antigen *in vitro* (Masai *et al.* 2000). A Cdk2 interacting protein, CINP, is also known to be phosphorylated by Cdc7 *in vitro* (Grishina and Lattes 2005). Although significance of this phosphorylation remains unknown, it may be possible that CINP acts as a functional and physical link between Cdk2 and Cdc7 complexes during DNA replication (Grishina and Lattes 2005).

1.2 Cdc7 in S-phase checkpoint

The ATR-Chk1 signaling is the major pathway for the intra S-phase checkpoint which can be activated by a broad spectrum of DNA lesions and replication blocks (Nyberg *et al.* 2002; Osborn *et al.* 2002; Paulsen and Cimprich 2007). Upon ATR-Chk1 checkpoint activation, DNA replication forks are stalled and activation of the late replication origins is suppressed to prevent further DNA replication (Bousset and Diffley 1998). Experimental results have implicated Cdc7 as both a final target inactivated by the S-phase checkpoint (see section 1.2.1) as well as an upstream regulator of the checkpoint responses (see section 1.2.2).

1.2.1 Cdc7-Dbf4 as a checkpoint target

Dbf4 subunit is hyperphosphorylated in a checkpoint-dependent manner upon S-phase checkpoint activation, suggesting a possibility that Cdc7-Dbf4 is a downstream target in the checkpoint pathway (Brown and Kelly 1999; Pasero *et al.* 1999; Takeda *et al.* 1999; Weinreich and Stillman 1999; Snaith *et al.* 2000; Duncker and Brown 2003; Ogi *et al.* 2008). Indeed, downregulation of the Cdc7-Dbf4 kinase activity upon genotoxic stress has been reported in some studies (Bousset and Diffley 1998; Pasero *et al.* 1999; Weinreich and Stillman 1999; Ogi *et al.* 2008). Recently, it was shown that alanine substitution of the putative Rad53-dependent phosphorylation sites in budding yeast Dbf4 in combination with similar mutation in Sld3 resulted in an abrogated checkpoint response (Lopez-Mosqueda *et al.* 2010; Zegerman and Diffley 2010), demonstrating that Dbf4 is a target of the S-phase checkpoint.

Cdc7-Dbf4 activity has been shown to be downregulated in different ways depending on the genotoxic agent used, i.e. hydroxyurea (HU), etoposide (ETO) and ionizing radiation (IR). In HU-treated budding yeast, Dbf4 subunit undergoes Rad53-dependent phosphorylation and is displaced from chromatin, leading to reduced Cdc7 kinase action (Pasero *et al.* 1999; Weinreich and Stillman 1999; Ogi *et al.* 2008). It was also reported that Cdc7 kinase activity decreases in the budding yeast cells treated with HU (Weinreich and Stillman 1999). Furthermore, purified Rad53 was shown to inactivate the kinase activity of purified Cdc7-Dbf4 kinase *in vitro* (Kihara *et al.* 2000). Likewise, human Dbf4/ASK and fission yeast Dfp1/Him are phosphorylated following HU treatment in a manner dependent on Chk1 and Cds1, respectively (Brown and Kelly 1999; Snaith *et al.* 2000; Kim *et al.* 2008). Fission yeast Hsk1 also undergoes Cds1-dependent phosphorylation in response to HU (Snaith *et al.* 2000). However, there is no report that shows

inhibition of human Cdc7 or fission yeast Hsk1 kinase activity after HU treatment so far. In a recent study, human Cdc7 kinase activity was shown to be unaffected by HU treatment (Tenca *et al.* 2007).

ETO treatment in *Xenopus* and human leukemic cells has been shown to reduce Cdc7 kinase activity by disrupting the complex formation and the chromatin association of the Cdc7-Dbf4/ASK complex (Costanzo *et al.* 2003; Dierov *et al.* 2004). However, several other studies showed that neither HU nor ETO affected the formation and stability of the Cdc7 heterodimeric complexes (i.e. Cdc7-Dbf4/ASK and Cdc7-Drf1/ASKL1) in *Xenopus* and human cell lines, even when high concentration of ETO was used (Tenca *et al.* 2007; Tsuji *et al.* 2008). In Chinese hamster ovary (CHO) cells, ionizing radiation (IR)-induced S-phase checkpoint downregulates Cdc7 function through reduction of the Dbf4/ASK mRNA levels (Guo and Lee 2001). At present, it remains controversial whether the Cdc7 kinase activity is affected after treatment with genotoxic agents. On the other hand, two p53-responsive microRNAs, miR-192 and miR-215, which are activated during genotoxic stress, were shown to downregulate Cdc7 expression in human cells (Georges *et al.* 2008)(see also Section 3.4).

1.2.2 Cdc7-Dbf4 as a checkpoint regulator

Recent studies suggested that Cdc7 may regulate the S-phase checkpoint pathway through the upstream mediator protein, Claspin/Mrc1 (Kakusho *et al.* 2008; Kim *et al.* 2008; Gold and Dunphy 2010; Matsumoto *et al.* 2010). By using the budding yeast *cdc7Δ* or fission yeast *hsk1Δ* bypass mutant, Cdc7 or Hsk1 was shown to be required for HU-induced Rad53 or Cds1 activation, respectively (Ogi *et al.* 2008; Matsumoto *et al.* 2010; Sheu and Stillman 2010). In fission yeast *hsk1*[ts] mutant, Cds1 activation and Mrc1 hyperphosphorylation were impaired, although Rad3 kinase activity was not affected (Takeda *et al.* 2001; Matsumoto *et al.* 2010).

Likewise, Cdc7-depleted human cells showed impaired Chk1 activation but intact ATR activation (ATR, the human homologue of Rad3; Kim *et al.* 2008). Hyperphosphorylation and chromatin association of Claspin, the human homologue of Mrc1, also decreased in these cells (Kim *et al.* 2008). Further studies in fission yeast indicated that the persistent activation of Cds1 and Mrc1 in the presence of HU requires intact Hsk1 activity (Matsumoto *et al.* 2010).

Fission yeast Hsk1 has also been shown to both genetically and physically interact with the Swi1-Swi3 replication fork protection complex, suggesting its role in stabilizing stalled forks during checkpoint activation (Matsumoto *et al.* 2005; Sommariva *et al.* 2005; Shimmoto *et al.* 2009). Furthermore, it was shown that HU-induced Mrc1/Cds1 activation required Cdc45, but not MCM or other pre-RC components, leading to the proposal that chromatin loading of Cdc45 may be crucial during checkpoint activation (Matsumoto *et al.* 2010).

1.3 Cdc7 in gene silencing

Regulatory role of Cdc7 in gene silencing was initially suggested in budding yeast, when a new allele of *cdc7* temperature-sensitive mutant, *cdc7-90*, was identified as a suppressor of defective gene silencing at the mating-type locus HMR (Axelrod and Rine 1991). Later in fission yeast, Hsk1-Dfp1/Him1 was shown to regulate heterochromatin-mediated

silencing through interaction with and phosphorylation of the heterochromatin protein 1 (Swi6/HP1; Bailis *et al.* 2003; Bailis and Forsburg 2004; Hayashi *et al.* 2009). Human and *Xenopus* Cdc7 may regulate the heterochromatin-mediated silencing through chromatin assembly factor 1 (CAF1; see section 1.5).

1.4 Cdc7 in sister chromatin cohesion

Genetic data in fission yeast has suggested a role of Hsk1 in sister chromatid cohesion (Snaith *et al.* 2000; Takeda *et al.* 2001). Later studies showed that in addition to gene silencing, Hsk1 may also regulate heterochromatin-mediated cohesion through heterochromatin protein 1 (Swi6/HP1; Bailis *et al.* 2003; Bailis and Forsburg 2004). However, it remains unclear whether Hsk1 regulates cohesion directly or indirectly through its role in DNA replication.

Recently, interaction between *Xenopus* Cdc7-Drf1 and the Scc2-Scc4 cohesin loading complex was reported, highlighting a direct regulatory role for Cdc7 in promotion of sister chromatid cohesion (Takahashi *et al.* 2008). Whether similar mechanisms operate in other eukaryotes is still unclear.

1.5 Cdc7 in chromatin assembly

Human and *Xenopus* Cdc7 play a role in chromatin assembly by phosphorylating p150 subunit of chromatin assembly factor 1 (CAF1), which subsequently stabilizes the CAF1 monomer and enhances interaction between CAF1 and proliferating cell nuclear antigen (PCNA) during DNA synthesis (Gerard *et al.* 2006). CAF1 is recruited to the single-strand DNA (ssDNA) break sites and plays a major role in histone deposition onto newly replicated DNA. Besides, CAF1 may also be required for heterochromatin-mediated silencing (see section 1.3).

1.6 Cdc7 in mitotic regulation

Genetic interaction between budding yeast Cdc7-Dbf4 and Cdc5 polo-like kinase has been described (Kitada *et al.* 1993; Hardy and Pautz 1996). Expression of Cdc5 on a multicopy plasmid was shown to suppress growth defects of *dbf4*[ts] mutants (Kitada *et al.* 1993). Recently, interaction between Cdc7-Dbf4 and Cdc5 in budding yeast was shown to be important in regulating the mitotic exit network (MEN) and the monopolin attachment in meiosis I (Matos *et al.* 2008; Miller *et al.* 2009; Chen and Weinreich 2010).

1.7 Cdc7 in histone modification

Histone H3 T45 phosphorylation is a replication-associated histone modification in budding yeast. Recently, purified native Cdc7-Dbf4 complex has been shown to facilitate this H3-T45 phosphorylation *in vitro* (Baker *et al.* 2010), suggesting the involvement of Cdc7 in post-translational modification of histones. Prolonged replication stress results in accumulation of H3-T45 phosphorylation over time, whilst loss of this phosphorylation causes phenotypes consistent with replicative defects.

2. Cdc7 during meiotic cell cycle

Budding yeast Cdc7 and fission yeast Hsk1 have been shown to play various roles during the meiotic cell cycle (Sclafani *et al.* 1988; Ogino *et al.* 2006; Wan *et al.* 2006, 2008; Lo *et al.* 2008; Sasanuma *et al.* 2008). Earlier genetic analyses in budding yeast suggested the role of Cdc7 in meiotic recombination but not in premeiotic DNA replication (Simchen 1974; Schild and Byers

1978; Sclafani *et al.* 1988). Subsequently, involvement of budding yeast Cdc7 in synaptonemal complex formation was suggested (Sclafani *et al.* 1988). The function of Cdc7/Hsk1 during meiosis was more clearly defined later in fission yeast. A second pair of Hsk1-Dfp1/Him complex, Spo4-Spo6, is present in the fission yeast and has been shown to play important roles in sporulation (Nakamura *et al.* 2000, 2002). In mouse, characterization of the Cdc7 hypomorphic mice showed defective testis and ovary development as a result of an early premeiotic arrest (Kim *et al.* 2003). These observations strongly suggest the conservation of Cdc7 function during meiosis across species.

2.1 Cdc7 in premeiotic DNA replication

Importance of the Cdc7-Dbf4 complex in premeiotic DNA replication remains controversial (Hardy *et al.* 1997; Valentin *et al.* 2006; Wan *et al.* 2006). Studies utilizing budding yeast *cdc7*^{ts} or fission yeast *hsk1*^{ts} cells suggested that Cdc7 or Hsk1 is dispensable for premeiotic DNA replication (Simchen 1974; Schild and Byers 1978; Sclafani *et al.* 1988; Ogino *et al.* 2006), although slight delay in initiation of DNA replication was observed in the *hsk1*^{ts} mutant (Ogino *et al.* 2006). However, other studies in budding yeast showed that premeiotic DNA replication was repressed upon Dbf4 depletion in the *mcm5-bob1* mutant or Cdc7 inactivation in the *cdc7-as* strain, suggesting a positive role of Cdc7 in premeiotic DNA replication (Valentin *et al.* 2006; Wan *et al.* 2006).

2.2 Cdc7 in meiotic recombination

Roles of Cdc7 in meiotic recombination and synaptonemal complex formation were initially suggested in budding yeast (Schild and Byers 1978; Sclafani *et al.* 1988). Recently, fission yeast Hsk1 and budding yeast Cdc7 were shown to be required for induction of DNA double-strand breaks (DSBs) formation which is important for the initiation of meiotic recombination (Ogino *et al.* 2006; Wan *et al.* 2006; Matos *et al.* 2008). In budding yeast, Mer2, a factor that promotes DSB formation through recruitment of Spo11 to the DSB sites (Henderson *et al.* 2006), was identified as a crucial substrate of Cdc7 kinase (Sasanuma *et al.* 2008; Wan *et al.* 2008). Cdc7 phosphorylates Mer2 at Ser29, and this process is facilitated by a prior CDK-dependent phosphorylation of Mer2 at Ser30. This is a situation similar to phosphorylation of MCM2 by Cdc7 and CDK (Masai *et al.* 2000; Montagnoli *et al.* 2006). These results indicate that Cdc7, in concert with CDK, regulates initiation of meiotic recombination through Mer2 in budding yeast (Matos *et al.* 2008; Sasanuma *et al.* 2008; Wan *et al.* 2008).

2.3 Cdc7 in meiotic prophase I

Cdc7 has been shown to play a role in the pachytene stage of meiotic prophase I in budding yeast (Simchen 1974; Schild and Byers 1978; Matos *et al.* 2008) and mouse (Kim *et al.* 2003). In the absence of Cdc7, budding yeast *mcm5-bob1 cdc7Δ* mutant strain arrested at prophase I, although DNA replication was completed (Sasanuma *et al.* 2008). Likewise, *Cdc7-/-*tg mice displayed disrupted spermatogenesis prior to pachytene exit which caused infertility (Kim *et al.* 2003).

Recent study in budding yeast revealed that transcription of the NDT80, a meiosis-specific transcriptional activator that functions in the pachytene exit and meiotic progression, is regulated by Cdc7-Dbf4 (Lo *et al.* 2008). This finding suggests that the prophase I arrest observed may be a result of the compromised NDT80 transcription in the absence of Cdc7 (Lo *et al.* 2008; Sasanuma *et al.* 2008). On the other hand, it has been suggested that prophase I arrest may also be provoked by checkpoint activation following replication defects (Matos *et al.* 2008). However, this possibility remains vague since inactivation of various checkpoint genes failed to relieve the arrest (Lo *et al.* 2008).

2.4 Cdc7 in monopolin attachment and monoorientation of sister kinetochores

The observation that *hsk1* mutant is arrested uniformly with one nuclei in meiosis I suggested a role of fission yeast Hsk1 kinase in meiotic cell division (Ogino *et al.* 2006). Budding yeast *cdc7* or *dbf4* mutants also failed to undergo segregation of homologue chromosomes during meiosis I (Matos *et al.* 2008). Recently, budding yeast Cdc7-Dbf4 complex, in collaboration with Cdc5-Spo13, has been shown to play an essential role in regulating monopolin localization through phosphorylation of Lrs4, a component in the monopolin complex (Lo *et al.* 2008; Matos *et al.* 2008). Monopolar attachment of the monopolin complex on sister kinetochores is the key determinant to the successful homologue segregation in meiosis I.

2.5 Cdc7 in cohesion cleavage

Budding yeast Cdc7 plays a regulatory role in cohesin cleavage during meiosis by phosphorylating a cohesin alpha-kleisin subunit, Rec8 (Katis *et al.* 2010). Cohesin cleavage is essential in promoting destabilization of the kinetochore-microtubule attachments to allow chromosome segregation.

3. Cdc7 in embryogenesis

Cdc7 is essential during embryogenesis in mouse (Kim *et al.* 2002, 2003) and *Xenopus* (Silva *et al.* 2006). In mouse, embryos were non-viable in the absence of Cdc7 and died between day 3.5 and day 6.5 during embryogenesis (Kim *et al.* 2002). Although expression of a transgene-encoded Cdc7 (*Cdc7-/-*tg) was able to rescue this lethality, the pups displayed retarded growth and infertility (Kim *et al.* 2003; see also Section 8, 4.1). In *Xenopus* egg extract, Cdc7-Drf1 complex is present in excess over Cdc7-Dbf4 and plays a major role during early embryogenesis (Yanow *et al.* 2003; Takahashi and Walter 2005; Silva *et al.* 2006). Roles of *Xenopus* Dbf4 in embryogenesis were suggested based on the facts that morpholino-induced Dbf4 knockdown resulted in defective heart and eye development in the embryos (Brott and Sokol 2005). However, the role of Dbf4 here may be independent of its role as an activator of the Cdc7 kinase, since the Dbf4 mutant lacking motif-M and -C essential for Cdc7 activation was still able to rescue the growth defects. Instead, Dbf4 may act as a negative regulator of the Wnt signaling pathways during embryogenesis through interaction with Frodo, an inhibitory factor for this pathway (Brott and Sokol 2005).

4. Cdc7 in DNA damage-induced mutagenesis

Cdc7-Dbf4 plays an important role in DNA damage-induced mutagenesis in budding yeast (Njagi and Kilbey 1982; Kilbey 1986; Sclafani *et al.* 1988; Hollingsworth *et al.* 1992; Ostroff and Sclafani 1995). Frequency of the induced mutagenesis in the budding yeast *cdc7*^{ts} mutant was greatly reduced when the cells were treated with different DNA-damaging agents, including UV light, methyl methanesulfonate (MMS) and N-

Methyl-N'-Nitro-N-Nitrosoguanidine (MNNG; Njagi and Kilbey 1982; Kilbey 1986). Ectopic overexpression of Cdc7 was able to rescue this defect and showed an increase in the frequency of induced mutation following UV treatment (Sclafani *et al*. 1988; Ostroff and Sclafani 1995). In fission yeast, *hsk1* and *dfp1* mutants also showed defects in induced mutagenesis when treated with methyl methanesulfonate (Dolan *et al*. 2010). See the heading 5.

5. Cdc7 in Translesion DNA Synthesis (TLS), a post-replication DNA repair mechanism

The requirement of Cdc7 in DNA repair was first suggested in budding yeast when the involvement of Cdc7 in DNA-damaged induced mutagenesis was noted (see section 4; Njagi and Kilbey 1982; Kilbey 1986; Sclafani *et al*. 1988; Ostroff and Sclafani 1995). DNA damage-induced mutagenesis is one of the several post-replication DNA damage tolerance mediated by the RAD6 epistasis group. Cdc7 has been shown to function in the Translesion Synthesis (TLS) branch of the Rad6 epistasis group of DNA repair genes in budding yeast (Njagi and Kilbey 1982; Pessoa-Brandao and Sclafani 2004). Besides, recent characterization showed that BRCT domain (Dbf4-motif-N) of the budding yeast Dbf4 could be uniquely substituted with the BRCT domain from Rev1, a translesion DNA polymerase. This finding suggests that Cdc7-Dbf4 complex and Rev1 may interact with common proteins during translesion synthesis through the BRCT domains, although the actual targets remain elusive (Harkins *et al*. 2009).

In human, the Cdc7-Dbf4/ASK complex was recently shown to phosphorylate the C-terminus of Rad18 *in vitro* and in human cells (Day *et al*. 2010). Rad18 is an E3 ubiquitin ligase which plays important roles in TLS. Cdc7-mediated phosphorylation promotes complex formation between Rad18 and DNA polymerase η, a translesion polymerase, thereby facilitating Rad18-dependent recruitment of DNA polymerase η to the stalled replication forks, i.e. DNA lesions.

6. Bypass of Cdc7 function

Mutations in the MCM subunits (i.e. MCM2, 4 or 5) have been shown to allow bypass of the Cdc7 function during initiation of DNA replication in budding yeast (Hardy *et al*. 1997; Sclafani *et al*. 2002; Chuang *et al*. 2009; Sheu and Stillman 2010). Among them, *mcm5-bob1* (bypass of block 1) mutant carrying a proline to leucine substitution at amino acid 83 (P83L) in MCM5 has been well characterized (Hardy *et al*. 1997). The MCM5 P83L mutation is able to rescue the growth defects of *cdc7*[ts] and *cdc7* null cells. However, it is unable to suppress the meiotic defects in these cdc7 mutants (Hardy *et al*. 1997). Recent studies suggested that the MCM5 P83L mutation may cause a conformation change in the MCM5 subunit, which plausibly mimics the active conformation of MCM5 in the Cdc7-phosphorylated MCM2-7 complex, thereby allowing bypass of the Cdc7 function. However, the *mcm5-bob1* mutant shows reduced replication origin efficiency as compared to the wild type. It was proposed that MCM5 P83L mutation results in several MCM5 conformations, only one of which is active for origin activation (Hoang *et al*. 2007). Substitution of the MCM5 P83 residue to other larger amino acids (i.e. P83K and P83W), but not to smaller amino acids (i.e. P83G and P83A), also allowed bypass in the *cdc7*[ts] mutant to some extent (Fletcher *et al*. 2003).

In addition to the MCM5 mutation, phosphomimetic mutations

at the N-terminus of human MCM2 (Chuang *et al*. 2009) as well as deletion at the N-terminus of budding yeast MCM4 (Sheu and Stillman 2010) have also been shown to facilitate bypass of the Cdc7 function. It was proposed that the N-terminus of MCM4 is phosphorylated by Cdc7 to antagonize the inhibitory effect of this segment during initiation of DNA replication. Hence, deletion of this segment would permit DNA replication and cell growth in the *cdc7* null mutant (Sheu and Stillman 2010). Taken together, these findings suggest that the roles of Cdc7 for cell viability may be explained by its action on the MCM subunits in budding yeast. However, how Cdc7-mediated phosphorylation affects the functions of the MCM complex remains unclear. Besides, it is also not known whether similar mutations in these MCM subunits will allow bypass of Cdc7 functions in other organisms.

In fission yeast, *mrc1* deletion has been shown to bypass the requirement of Hsk1 function for growth (Matsumoto *et al*. unpublished data). Mrc1 is an integral component of the replication fork machinery which would negatively regulate the initiation of DNA replication through both checkpoint-dependent and -independent manners. This may suggest that the requirement of Hsk1 in initiation of DNA replication could be lessened by the loss of "negative" factors which may restrain the process.

REGULATION OF ACTIVITY
Activity of Cdc7 has been shown to be regulated in several ways as elaborated below.

1. Regulation by Dbf4-like activation subunits (See Molecule Page for Dbf4)

In general, Cdc7 is inactive on its own and is active only in a complex with Dbf4-related activation subunits, Dbf4/Dfp1/Him1/ASK or Drf1/ASKL1 (Yoon *et al*. 1993; Takeda *et al*. 1999; Montagnoli *et al*. 2002; Yanow *et al*. 2003; Yoshizawa-Sugata *et al*. 2005). However, the fission yeast Cdc7 homologue, Hsk1, was shown to be significantly active on its own; although the presence of its Dbf4-related activation subunit, Dfp1/Him1, increases its substrate affinity (Brown and Kelly 1998; Takeda *et al*. 1999). Recently, a homotetramer of budding yeast Cdc7 has also been shown to be partially active (Bruck and Kaplan 2009).

1.1. Regulation by Dbf4 (dumbbell former-4)/ASK (activator of S-phase kinase)/Dfp1 (Dbf four in pombe)/ Him1 (Hsk1-interacting molecule 1)

Budding yeast *dbf4* (dumbbell former-4) was originally isolated as a temperature-sensitive (ts) mutant which is defective for initiation of DNA replication (Johnston and Thomas 1982). Later, the *dbf4*[+] on a multicopy plasmid was shown to restore the growth of a budding yeast cdc7[ts] mutant at a non-permissive temperature (Kitada *et al*. 1992). Subsequent characterization suggested that Dbf4 is an activation subunit for Cdc7 kinase (Kitada *et al*. 1992; Jackson *et al*. 1993; Dowell *et al*. 1994; Dixon and Campbell 1997; Oshiro *et al*. 1999). Dbf4 homologues have been identified in fission yeast and in mammalian cells, known as Dfp1/Him1 and Dbf4/ASK, respectively. Each Dbf4 homologue is known to form complexes with its respective Cdc7 kinase *in vivo*, and regulates Cdc7 kinase activity during the cell cycle (Yoon *et al*. 1993; Brown and Kelly 1998, 1999; James *et al*. 1999; Jiang *et al*. 1999; Kumagai *et al*. 1999; Lepke *et al*. 1999; Takeda *et al*. 1999).

Dbf4/Dfp1/Him1/ASK is a cell-cycle regulated protein the expression of which peaks at the G1-S boundary through S phase during cell cycle (Jackson *et al*. 1993; Brown and Kelly 1999; Kumagai *et al*. 1999; Takeda *et al*. 1999; Weinreich and Stillman 1999; Ogino *et al*. 2001; Wu and Lee 2002; Yamada *et al*. 2002; Matos *et al*. 2008; Nambiar *et al*. 2008). Dbf4 was found to be expressed at constant levels during cell cycle progression in the egg extract of the *Xenopus* egg extracts (Furukohri *et al*. 2003). A degradation signal identified in the N-terminus of budding yeast Dbf4 suggests that Dbf4 may be degraded through the APC-dependent pathway at the mitotic exit (Cheng *et al*. 1999; Weinreich and Stillman 1999; Ferreira *et al*. 2000). In mammalian cells, Dbf4/ASK protein disappears in G1 phase and is likely to be degraded through the APC/Cdh1-dependent proteasome degradation pathway (Kumagai *et al*. 1999; Yamada *et al*. 2002).

Mammalian Dbf4/ASK carries a long C-terminal tail sequence which is absent in the yeast counterparts and is non-essential for kinase activation (Kumagai *et al*. 1999). The last 50 amino acid residues at the C-terminus, which are rich in serines and threonines, were shown to be responsible for the auto-phosphorylation of Dbf4/ASK observed in mammalian cells which causes its mobility-shift on SDS-PAGE (Sato *et al*. 2003; Hughes *et al*. 2010). Deletion of this 50-amino acid segment resulted in a hyperactive kinase, suggesting an autoinhibitory role of this segment in regulating Cdc7 kinase activity (Hughes *et al*. 2010). Interaction between this C-terminal segment of Dbf4/ASK and the lens epithelium-derived growth factor (LEDGF) *in vitro* was shown to relieve the autoinhibitory effect, and consequently stimulated Cdc7 kinase activity by more than 10-fold, as indicated by the phosphorylation level of MCM2 *in vitro* (Hughes *et al*. 2010).

1.2. Regulation by Drf1 (Dbf4-related factor 1)/ASKL1 (activator of S phase kinase like-1)

Drf1/ASKL1 is the second Cdc7 regulatory subunit identified only in human and *Xenopus* so far (Montagnoli *et al*. 2002; Yanow *et al*. 2003; Takahashi and Walter 2005; Yoshizawa-Sugata *et al*. 2005). In human, Drf1/ASKL1 is also a cell-cycle regulated protein. However, unlike Dbf4/ASK, expression of Drf1/ASKL1 peaks at G2/M phase. Degradation of Drf1/ASKL1 protein may occur slightly later than Dbf4/ASK at the end of mitosis, although the mechanisms involved remain undefined (Montagnoli *et al*. 2002). Human Cdc7-Drf1/ASKL1 complex plays a role in efficient progression of S and M phases of a cell cycle. Its roles in regulating G2/M phase have also been suggested (Yoshizawa-Sugata *et al*. 2005). In *Xenopus*, the Cdc7-Drf1 complex is known to be essential for DNA replication during early embryogenesis, whilst the Cdc7-Dbf4 complex is required for the later stages (Takahashi and Walter 2005; Silva *et al*. 2006).

2. Regulation by phosphorylation

Functions of the Cdc7/Hsk1 kinase complex can also be regulated through phosphorylation. Several proteins have been shown to phosphorylate Cdc7-Dbf4/ASK and Hsk1-Dfp1/Him complexes thus far.

2.1. Phosphorylation by Cyclin-dependent kinases (CDKs)

Cdc7 contains several CDK-dependent phosphorylation sites (Ser-Pro-Gln-Arg) and has been shown to undergo CDK-dependent phosphorylation (e.g. by Cdc28 in budding yeast; Cdk2-CyclinE, Cdk2-CyclinA and Cdc2-CyclinB1 in mammalian cells) *in vitro* and *in vivo* (Yoon *et al*. 1993; Ohtoshi *et al*. 1996; Toone *et al*. 1997; Faul *et al*. 1999; Masai *et al*. 2000). Among the potential CDK phosphorylation sites, the T376 residue located close to the T-loop of human Cdc7 was suggested to be phosphorylated by Cdc2-CyclinB (Masai *et al*. 2000). Subsequent characterization of this T376 residue showed that alanine substitution (T376A) resulted in a hypomorphic Cdc7 kinase, whereas glutamic acid substitution (T376E) resulted in a completely dead Cdc7 kinase (Masai *et al*. 2000). This finding is consistent with the data from a previous mutation analysis of the T279 residue in budding yeast Cdc7 (corresponding to the T376 residue in human Cdc7), whereby the T279A mutant was partially defective, whilst the T279E mutant was completely defective in terms of complementation of cdc7[ts] mutants (Ohtoshi *et al*. 1996). Although significance of this phosphorylation remains elusive, the T376-phosphorylated human Cdc7 is strongly localized in the cytoplasm during M phase of a cell cycle, suggesting that this phosphorylation may regulate the cellular localization of Cdc7 kinase.

On the other hand, the recombinant human Cdc7-Dbf4/ASK complexes produced in *E. coli* and insect cells possess identical specific activity, indicating that kinase activity of Cdc7 may not be affected by phosphorylation. This notion suggested that Cdc7 kinase activity is unlikely to be regulated by CDK-mediated phosphorylation, although Cdc7 is a phosphorylation substrate of CDK (Masai *et al*. 2000).

2.2. Phosphorylation by Cdc5/Polo-kinase I

During meiosis I, budding yeast Cdc5/Polo-kinase I phosphorylates the Dbf4 subunit of the Cdc7-Dbf4 complex *in vivo* and subsequently lead to an enhanced Cdc7-Dbf4 kinase activity (Matos *et al*. 2008).

2.3. Phosphorylation by Rad53/Cds1/Chk1

In response to hydroxyurea (HU) treatment (i.e. depletion of nucleotide pools for DNA replication), budding yeast Dbf4, fission yeast Dfp1 and human Dbf4/ASK undergo hyperphosphorylation in a manner dependent on checkpoint kinase Rad53, Cds1 and Chk1, respectively (Brown and Kelly 1999; Takeda *et al*. 1999; Weinreich and Stillman 1999; Snaith *et al*. 2000; Kim *et al*. 2008; Ogi *et al*. 2008). The Rad53-mediated phosphorylation of Dbf4 was shown to be important for suppression of origin firing in response to replication stress in budding yeast. Alanine substitutions of potential Rad53-dependent phosphorylation sites in Dbf4 along with that in Sld3, another putative Rad53 checkpoint target, lead to abrogated replication checkpoint-mediated origin suppression (Lopez-Mosqueda *et al*. 2010; Zegerman and Diffley 2010). In fission yeast, Hsk1 was also reported to undergo Cds1-dependent phosphorylation in response to HU (Snaith *et al*. 2000).

Downregulation of the Cdc7 kinase activity in response to replication block was reported in budding yeast and fission yeast (Weinreich and Stillman 1999; Kihara *et al*. 2000; Snaith *et al*. 2000). In *Xenopus* egg extracts, treatment with Etoposide was shown to induce dissociation of Dbf4 from Cdc7, thereby inactivating the Cdc7 kinase activity (Constanzo *et al*. 2003). On the contrary, another study showed that complex formation and kinase activities of purified Cdc7-Dbf4 and Cdc7-Drf1 complexes added to the *Xenopus* egg extracts were unaffected

by similar genotoxic agents (Tsuji *et al.* 2008). Similarly, the activity of human Cdc7-Dbf4/ASK complex, both in the untreated and in the Dbf4/ASK-overproduced cells, was not affected by genotoxic stress (Montagnoli *et al.* 2004; Tenca *et al.* 2007; Tsuji *et al.* 2008). Taken together, more precise experiments are needed to clarify how genotoxic stress may affect the functions of the Cdc7-Dbf4 kinase during replication checkpoint responses.

3. Stimulators and inhibitors of Cdc7 kinase

3.1. Stimulation and inhibition by charged polymers

Activity of human Cdc7 and fission yeast Hsk1 kinase can be stimulated by positively charged polymers, such as polyamines (e.g. spermine and spermidines) and polylysine, whilst it can be inhibited by negatively charged polymers, such as polyglutamic acid and nucleic acid (Kakusho *et al.* 2008). Further characterization suggested that polyamines may specifically target at the Dbf4-related activation subunit in the Cdc7/Hsk1 kinase complex for stimulation. Polyamines may modulate the Cdc7/Hsk1 kinase-substrate interactions because the extent of stimulation was affected by the nature of substrates used (Kakusho *et al.* 2008).

3.2. Stimulation by histones

Histones can potently stimulate Cdc7 kinase activity *in vitro* (Kakusho *et al.* 2008). This may be due to the basic nature (or positively charged surface) of histones. Such observation highlighted the potential roles of histones in facilitating Cdc7-dependent phosphorylation of the chromatin-bound replication factors. In fact, human MCM protein complex, the prime phosphorylation substrate of Cdc7 kinase, is known to bind to histone H3 (Ishimi *et al.* 1996).

3.3. Inhibition by ATP-competitive compounds

Cdc7 kinase is a promising target of cancer therapy because inhibition of Cdc7 induces potent cell death in cancer cells but does not significantly affect the cell viability in normal cells (Montagnoli *et al.* 2004, 2008; Ito *et al.* 2008; Kim *et al.* 2008; Sawa and Masai 2009). To date, several classes of ATP-competitive Cdc7 inhibitors such as pyrrolopyridinones (Montagnoli *et al.* 2008; Vanotti *et al.* 2008; Menichincheri *et al.* 2009), indazoles (Shafer *et al.* 2008), pyrido-thieno-pyrimidines (Zhao *et al.* 2009), 1H-pyrrolo[2,3-b]pyridines (Ermoli *et al.* 2009), 5-heteroaryl-3-carboxamido-2-aryl pyrroles (Menichincheri *et al.* 2010) and benzofuropyrimidinone (Robertson 2008, conference poster) have been identified.

Among them, a pyrrolopyridinones derivative, PHA-767491 (2-heteroaryl-pyrrolopyridinones), inhibits Cdc7 kinase activity with an IC_{50} of 10 nM (in the presence of 1.5 µM ATP) *in vitro* and exhibits selective anti-proliferative and cell death-inducing effects on cancer cells over normal fibroblasts (Montagnoli *et al.* 2008). Similar to the effects observed in siRNA-induced Cdc7-depleted cells, PHA-767491-induced Cdc7 inhibition results in p53-independent apoptosis in cancer cells, whereas it induces p53-dependent checkpoint arrest in normal cells. Furthermore, PHA-767491 shows anti-tumor property in rats and nude mice carrying human tumors(Montagnoli *et al.* 2004).

Further optimization of the pyrrolopyridinones derivatives lead to the development of NMS-1116354, an orally available Cdc7 inhibitor (IC_{50} of 3 nM; Montagnoli *et al.* 2008, conference poster), which inhibits cell proliferation and induces apoptosis in a broad panel of cancer cell lines. *In vivo*, NMS-1116354 shows anti-tumor effects in xenograft models and in a rat mammary carcinogenic-induced tumor model (DMBA). NMS-1116354 entered Phase I clinical trials in year 2009 and is currently being evaluated for the treatment of advanced/metastatic solid tumors (ClinicalTrials.gov, as of April 2011). BMS-863233/XL-413, a benzofuropyrimidinone class of Cdc7 inhibitor (Robertson 2008, conference poster), is another orally available compound which entered Phase I-II clinical trials in year 2009 (ClinicalTrials.gov Database, as of April 2011). These trials involved patients with either advanced and/or metastatic solid tumors or heamatological malignancies and have completed in early year 2010. BMS-863233/XL-413 treatment shows potent and selective Cdc7 inhibitory activity, leading to the S/G2 phase arrest in most cell lines and an eventual cell death in some lines. BMS-863233/XL-413 also inhibits hydroxyurea-induced Chk1 phosphorylation, thereby attenuates the checkpoint signaling induced by DNA damaging agents. In xenograft models, oral dosing of BMS-863233/XL-413 inhibits phosphorylation of MCM2 *in vivo* and shows potent tumor growth inhibition.

3.4. Inhibition by micro-RNA

Expression of Cdc7 can be suppressed by two p53-inducible homologous mircoRNAs, miR-192 and miR-215 (Georges *et al.* 2008), although the precise mechanisms remain unclear. Both miR-192 and miR-215 are upregulated by genotoxic stress in a p53-dependent manner, inhibiting the expression of many regulators for DNA synthesis including Cdc7 and enhancing the expression of p21. These contribute to the G1 and G2/M cell cycle checkpoints induced by DNA damages (Braun *et al.* 2008; Georges *et al.* 2008).

INTERACTIONS

1. Interaction for nuclear-cytoplasmic translocation

Human Cdc7 interacts with importin-α and importin-β for nuclear import, and interacts with CRM1/Exportin for nuclear export (Kim and Lee 2006; Kim *et al.* 2007). For details, see section "Subcellular Localization".

2. Interaction with chromatin

Chromatin loading of Cdc7 can be facilitated in a Dbf4-dependent manner in yeast (Pasero *et al.* 1999; Weinreich and Stillman 1999), *Xenopus* (Duncker *et al.* 2002; Jares *et al.* 2004) and human (Kim *et al.* 2003; Sato *et al.* 2003). Chromatin-bound MCM 2-7 complex has also been shown to facilitate chromatin loading of Cdc7 in *Xenopus* (Walter 2000). Recently, a Nuclear Retention Signal (NRS; amino acid 306-326) which may be required for binding of Cdc7 to chromatin at the replication origin was identified in human Cdc7 (Kim *et al.* 2007).

3. Interaction for kinase activation

In all the organisms examined, Cdc7 interacts with Dbf4/ASK activation subunit to become a fully active kinase. Budding yeast Cdc7 or fission yeast Hsk1 interacts with Dbf4 or Dfp1/Him1 through its C-terminal acidic tail segment (Patterson *et al.* 1986; Jackson *et al.* 1993; Dowell *et al.* 1994; Masai *et al.* 1995; Masai and Arai 2000; Varrin *et al.* 2005). Although

mammalian Cdc7 lacks a similar C-terminal acidic region (Sato *et al*. 1997; Guo and Lee 1999), a short conserved C-terminal sequence (DAM-1, Dbf4/ASK association motif-1) which is essential for interaction with human Dbf4/ASK has recently been identified. Furthermore, DAM-2, another motif also required for Cdc7-Dbf4/ASK interaction, was identified in the Kinase Insert III of human Cdc7. Further analyses showed that DAM-1 and DAM-2 interact with motif-M and motif-C of Dbf4/ASK, respectively; both motif-M and motif-C are known to be required for interaction with Cdc7 (Kitamura *et al*. JBC, in press; Sato *et al*. 2003).

Besides Dbf4/ASK, human and *Xenopus* Cdc7 can be activated by interacting with Drf1/ASKL1 (Montagnoli *et al*. 2002; Yanow *et al*. 2003; Takahashi and Walter 2005; Yoshizawa-Sugata *et al*. 2005). Although interaction domain for this interaction has not yet been defined, it is plausible that Cdc7 interacts with Drf1/ASKL1 in a manner similar to that utilized for its interaction with Dbf4/ASK, since both Drf1/ASKL1 and Dbf4/ASK share three highly conserved motifs (motif-M, -C and -N; Masai and Arai 2000).

4. Interaction with pre-RC components (MCMs, ORCs, Cdt1)

MCM2-7 complex is the major substrate of the Cdc7-Dbf4/ASK kinase complex. Budding yeast Dbf4 interacts with MCM2 subunit both *in vitro* and *in vivo* (Jackson *et al*. 1993; Lei *et al*. 1997; Bruck and Kaplan 2009). Although Cdc7 alone also interacts with MCM2, the interaction is weaker compared to the interaction between Dbf4 and MCM2 (Bruck and Kaplan 2009). Similarly, murine Dbf4/ASK interacts with MCM2 subunit *in vivo* (Lepke *et al*. 1999). In yeast two-hybrid assays, interaction between murine Dbf4/ASK and murine MCM2, 3, 4 and 7 subunits, as well as that between murine Cdc7 and murine MCM2, 4, 5 and 7 subunits were observed (Kneissl *et al*. 2003).

Several subunits of the ORC1-6 complex have been shown to interact with Cdc7-Dbf4/ASK complex. Interaction between budding yeast Cdc7 and the ORC2 subunit was observed in yeast two-hybrid assay (Hardy 1996). On the other hand, budding yeast Dbf4 was shown to interact with the ORC subunits through its motif-N (Weinreich and Stillman 1999; Duncker *et al*. 2002; Masai and Arai 2000). Yeast two-hybrid assays also showed interaction between murine Dbf4/ASK and ORC1, 2, 5 and 6 subunits, as well as that between murine Cdc7 and ORC1 and 6 subunits (Kneissl *et al*. 2003).

In human, N-terminus of Cdt1 interacts with and is phosphorylated by Cdc7-Dbf4/ASK complex *in vitro* (Ballabeni *et al*. 2009). Although Cdc7 alone can interact with Cdt1, substrate affinity of Cdc7 increased upon heterodimeric complex formation of Cdc7-Dbf4/ASK, possibly due to conformational change in Cdc7.

5. Interaction with other proteins

Cdc7 is able to interact with several other proteins either directly or through Dbf4/ASK or Drf1/ASKL1 in the active kinase complex. In budding yeast, Cdc7-Dbf4 complex interacts with Cdc5 Polo-box Domain (PBD) through Dbf4 *in vitro* (Kitada *et al*. 1993; Hardy and Pautz 1996) and *in vivo* (Matos *et al*. 2008). Recently, a Polo-box Interaction Region (PIR; amino acid 67-109) required for this interaction was identified in Dbf4. Alongside, Cdc7-Dbf4 dependent phosphorylation of Cdc5 PBD *in vitro* was observed (Miller *et*

al. 2009). On the other hand, Cdc7-Dbf4 complex also interacts with and phosphorylates monopolin subunit Lrs4 (Matos *et al*. 2008) and cohesin subunit Rec8 (Katis *et al*. 2010), highlighting the importance of Cdc7 in regulating monopolin localization and cohesin cleavage during meiosis I. Genetic interaction between Cdc7-Dbf4 complex and Cdc28 was also observed, although precise mode of interaction remains unknown (Ohtoshi *et al*. 1996). Budding yeast Cdc7-Dbf4 complex also interacts with Rad53 through Dbf4. It was shown by yeast two-hybrid assays and immunoprecipitation that Dbf4 interacts with FHA1 and FHA2 domains of Rad53 through its motif-N (Dohrmann *et al*. 1999; Weinreich and Stillman 1999; Duncker *et al*. 2002). Recently, budding yeast Cdc7-Dbf4 was shown to form a complex with Histone H3/H2B (i.e. histone in a complex with TAP-tagged Cdc7 and histone kinase complex purified from whole cell extract) (Baker *et al*. 2010.)

In fission yeast, Hsk1-Dfp1/Him1 complex interacts with the N-terminus of Cdc23/MCM10 through Dfp1/Him1 *in vitro* (Lee *et al*. 2003). This interaction has been suggested to be essential for efficient Hsk1-Dfp1/Him1-dependent phosphorylation in the MCM complex. In addition, Hsk1-Dfp1/Him1 complex interacts with and phosphorylates HP1/Swi6 *in vitro* through a HP1-binding motif identified near the C-terminus of Dfp1/Him1 (MIR domain; Bailis *et al*. 2003; Hayashi *et al*. 2009). This HP1-binding motif was recently shown to be required for the recruitment of Hsk1-Dfp1/Him1 complex to the centromeric heterochromatin structure (Hayashi *et al*. 2009). Either Hsk1-Dfp1/Him1 complex or Hsk1 alone is able to interact with Swi1/Tim1 (Matsumoto *et al*. 2005; Sommariva *et al*. 2005; Shimmoto *et al*. 2009). Mrc1 has also been shown to interact with Hsk1 through its central segment (378–879) containing a SQ/TQ cluster (Shimmoto *et al*. 2009).

In *Xenopus*, the Cdc7-Dbf4 complex interacts with and phosphorylates the Cdk2 Interacting protein (CINP), a component of the active Cyclin E/Cdk2 and Cyclin A/Cdk2 complexes, *in vitro* (Grishina and Lattes 2005). CINP may act as a docking protein to target Cdk2 and Cdc7 kinases to the replication origin, thereby enabling them to work together in regulating DNA replication. In addition, active *Xenopus* Cdc7-Dbf4 and Cdc7-Drf1 complexes, but not Cdc7 alone, interact with Scc2-Scc4 complex, as indicated by the lack of the interaction in Dbf4 or Drf1 depleted low speed supernatant of *Xenopus* egg cytoplasm (Takahashi *et al*. 2008). Low binding affinity was also noted when Scc2-Scc4 complex interacts with either Dbf4 or Drf1 alone. Interaction between Cdc7-Drf1 (or Cdc7-Dbf4) and the Scc2-Scc4 complex may facilitate recruitment of cohesin to chromatin (Takahashi *et al*. 2008).

In human as well as in *Xenopus*, Cdc7-Dbf4/ASK complex interacts with the p150 subunit of Chromatin Assembly Factor 1 (CAF1) *in vivo* and *in vitro*. Cdc7-Dbf4/ASK complex phosphorylates the p150 subunit of CAF1, thereby facilitating its interaction with proliferating cell nuclear antigen (PCNA) *in vitro* (Gerard *et al*. 2006). In addition, human Cdc7-Dbf4/ASK complex interacts with Rad18 and phosphorylates this protein both in vivo and in vitro, and this phosphorylation was shown to promote recruitment of DNA polymerase η to the sites of DNA damages (Day *et al*. 2010). As shown in fission yeast (see above, Shimmoto *et al*.), Cdc7 was also shown to interact with Claspin (ortholog of Mrc1) in human cells as well as in *Xenopus* egg extracts (Kim *et al*. 2008; Gold and Dunphy 2010). The Dbf4/ASK subunit of the human Cdc7-Dbf4/ASK complex also interacts weakly with Chk1 *in vivo* (Heffernan *et al*. 2007) and

with LEDGF (Lens epithelium-derived growth factor) *in vitro* (Hughes *et al.* 2010). The latter interaction was shown to stimulate the Cdc7 kinase activity by relieving the inhibitory effect of C-terminal tail of the ASK subunit (Hughes *et al.* 2010).

PHENOTYPES
1. Budding Yeast

1.1. Effects of Cdc7 null

Cdc7 and its activation subunit, Dbf4, are both required for mitotic growth. Null mutants are non-viable. Viability in both *cdc7* null or *dbf4* null cells can be restored by the *mcm5-bob1* mutation (Hardy *et al.* 1997). The *mcm5-bob1* mutation is able to rescue DNA replication, but not other defects including meiotic recombination, in the *cdc7* null and *dbf4* null cells. Although meiotic spores could be generated in the *mcm5-bob1 cdc7* null background, the spores failed to complete cell division with anaphase arrest during meiosis (Hardy *et al.* 1997; Matos *et al.* 2008).

1.2. Effects of Cdc7 mutation

A series of *cdc7* temperature-sensitive (*cdc7*^ts^) mutants (i.e. *cdc7-1, -2, -4, -5, -7*) and *cdc7* analogue-sensitive (*cdc7-as*) mutants (i.e. *cdc7-as1, -as2, -as3*) have been generated and characterized so far (Culotti and Hartwell 1971; Hartwell 1973; Schild and Byers 1978; Hardy *et al.* 1997; Wan *et al.* 2006).

1.2.1. *cdc7* temperature-sensitive (*cdc7*^ts^) strain

Generally, *cdc7*^ts^ mutants grow at 23°C (permissive temperature) and 27°C (semi-permissive temperature) but not at or above 36°C (non-permissive temperature). At the non-permissive temperature, the mutants arrest at the G1/S boundary with defective initiation of DNA synthesis (Hartwell 1973; Diffley *et al.* 1994; Ohtoshi *et al.* 1997; Donaldson *et al.* 1998). This arrest can be reversed by lowering the temperature (i.e. to the permissive temperature) and the cells resume the cell cycle in a synchronous manner (Donaldson *et al.* 1998). Although the mutants are arrested at the G1/S boundary, further studies examining Cdc7 function during the course of S phase indicated that Cdc7 is required throughout the S phase to act on each replication origin (Diffley *et al.* 1995; Donaldson *et al.* 1998). At the semi-permissive temperature, S phase progression in the *cdc7*^ts^ mutants (*cdc7-1* and *cdc7-4* strain) is delayed due to inefficient firing of both early and late replication origins (Bousset and Diffley 1998; Donaldson *et al.* 1998). Cdc7 activity is also required for conversion of the pre-replication complex into post-replication complex on the chromatin.

Combining *cdc7*^ts^ with *dbf4*^ts^ or *rad53-31* mutation causes lethality (Sclafani and Jackson 1994; Dohrmann *et al.* 1999). On the other hand, *cdc7*^ts^ (*cdc7-1* strain) is able to suppress lethality in *mec1* null but not that in *rad53* null mutant (Desany *et al.* 1998). Temperature-sensitive growth defects in the *cdc7*^ts^ mutants can be partially complemented by alanine substitution in the T281 residue of Cdc7 (T281A) (Shellman *et al.* 1998).

A *cdc7*^ts^ or *cdc7* null mutant (suppressed by *mcm4ΔN*) shows defective intra-S phase checkpoint with impaired Rad53 kinase activation in the presence of hydroxyurea (Ogi *et al.* 2008; Sheu and Stillman 2010). *cdc7*^ts^ mutants also exhibit reduced level of induced mutagenesis when treated with UV light, methyl methanesulfonate (MMS) or N-Methyl-N'-Nitro-N-Nitrosoguanidine (MNNG) at the permissive temperature (Njagi and Kilbey 1982; Kilbey 1986). Ectopic overexpression of Cdc7 could rescue this defect and increased the frequency of induced mutation following UV treatment (Sclafani *et al.* 1988; Hollingsworth and Sclafani 1990; Ostroff and Sclafani 1995). Another paper also showed that *cdc7*^ts^ mutants are hypersensitive to UV and diepoxybutane (Baranowska *et al.* 1982). More recent reports show *cdc7*^ts^ is defective in translesion synthesis (TLS) (Pessoa-Brandao and Sclafani 2004).

In meiotic cell cycle, the *cdc7*^ts^ mutants show intact premeiotic DNA replication but reduced frequency in meiotic recombination leading to cell cycle arrest at prophase I at non-permissive temperature (Simchen 1974; Schild and Byers 1978; Matos *et al.* 2008; Sasanuma *et al.* 2008). At 31°C (semi-permissive temperature), the mutant cells replicated DNA normally but formed only two spores which are mostly viable (Matos *et al.* 2008).

1.2.2. *cdc7* analogue-sensitive (*cdc7-as*) strain

A series of the analogue-sensitive (as) version of *cdc7*, *cdc7-as*, was created and analyzed for its phenotypes (Wan *et al.* 2006). The *cdc7-as3* allele contains L120A and V181A mutation, whilst the *cdc7-as1* or *cdc7-as2* allele contains L120G or L120A mutation, respectively. The enlarged ATP-binding pocket in the *cdc7-as* mutants allows conditional inactivation of Cdc7 by using purine analogues, PP1 or 1-NM-PP1 (Bishop *et al.* 2001). In the presence of the purine analogues, *cdc7-as* strongly resembles *cdc7*^ts^ mutants at the restrictive temperature. Cdc7 inactivation in *cdc7-as* mutants results in growth inhibition in the mitotic cells. In the meiotic cells, it results in delayed pre-meiotic DNA replication and cell cycle arrest at prophase (Wan *et al.* 2006). No recombination was observed due to an impaired double-strand breaks (DSBs) formation. Removal of the purine analogues inhibitor after DNA replication shows a highly synchronized cell population that rapidly undergoes DSB formation and repair (Wan *et al.* 2006). However, in another report using *mcm5-bob1* strain, downregulation of Cdc7 activity by Dbf4 depletion at the time of meiotic induction resulted in halted DNA replication, suggesting a positive role of Cdc7-Dbf4 in initiation of premeiotic DNA replication. Dbf4 depletion after initiation of S phase allowed S phase progression but cells arrested at anaphase I (Valentin *et al.* 2006).

1.3. Effects of Cdc7 overexpression

Overexpression of wild-type Cdc7 alone did not show any significant morphological changes (Hollingsworth and Sclafani 1990). However, overexpression of kinase-dead forms of Cdc7 in mitotic cells caused strong G1 arrest, presumably due to sequestration of Dbf4 protein by the kinase-dead Cdc7 (Ohtoshi *et al.* 1997). On the other hand, co-expression of Cdc7 along with its activation subunit, Dbf4, resulted in cell lethality (Nougarede *et al.* 2000). Cdc7-Dbf4 activity on MCM2 phosphorylation increased by 3.5-fold *in vivo* under this condition (Nougarede *et al.* 2000). Overexpression of Dbf4 alone in an APC mutant also showed some level of cell lethality (Nougarede *et al.* 2000).

2. Fission Yeast

2.1. Effects of Hsk1 null

Absence of *hsk1* is lethal at 30°C (Masai *et al.* 1995; Brown and Kelly 1998). Germinating spores of the *hsk1* null cells arrested with predominantly 1C DNA content at 30°C, although some proceeded to abortive S phase and aberrant mitosis with "cut" (cell untimely torn) phenotype (Masai *et al.* 1995).

2.2. Effects of Hsk1 downregulation

Cells were viable even when the level of Hsk1 protein is reduced by expressing *hsk1*[+] under the repressed pRPE81 nmt1 promoter in *hsk1* null cells or by inducing degradation through a degron construct. Such observations suggested that a very low level of Hsk1 is sufficient to support mitotic growth in fission yeast (Masai *et al.* 1995; Matsumoto *et al.* unpublished data).

2.3. Effects of Hsk1 overexpression

Overexpression of either wild-type Hsk1 or Hsk1 kinase-attenuated mutant (Hsk1 K129A or Hsk1 T291A) showed cell viablility with normal phenotype in fission yeast (Brown and Kelly 1998; Takeda *et al.* 1999, 2001). However, overexpression of Hsk1 D216N or Hsk1 T291E mutant resulted in an accumulation of G1 phase cells and abnormal morphology with "cut" phenotype, presumably due to inhibition of the endogenous Hsk1 kinase activity by titrating out its activation subunit, Dfp1/Him (Brown and Kelly 1998). The latter two mutants displayed significantly lower (more than 10x) kinase activity *in vitro* and loss of function *in vivo* (Brown and Kelly 1998). Recently, overexpression of Dfp1/Him1 in fission yeast was shown to cause late origin firings and increased firing efficiency, providing further support for the roles of Hsk1 kinase in regulating the timing and site determination for firing of replication origins (Patel *et al.* 2006, 2008; Wu and Nurse 2009).

2.4. Effects of Hsk1 mutation

Two *hsk1* temperature-sensitive (*hsk1*[ts]) mutants, namely *hsk1-1312* and *hsk1-89*, have been isolated and characterized so far (Snaith *et al.* 2000; Takeda *et al.* 2001; Bailis *et al.* 2003; Dolan *et al.* 2004; Matsumoto *et al.* 2005; Sommariva *et al.* 2005; Ogino *et al.* 2006). The *hsk1-1312* mutant grows at 25°C (permissive temperature) and 29°C (semi-permissive temperature) but not at or above 32°C (non-permissive temperature; Snaith *et al.* 2000). At the non-permissive temperature, *hsk1-1312* suffers from abnormal S phase and exhibited abnormal nuclear morphology. In release from hydroxyurea-induced early S phase arrest, *hsk1-1312* completes S phase, but accumulates nuclear damages by entering aberrant mitosis. On the other hand, the *hsk1-89* mutant grows at 25°C (permissive temperature) but not at 30°C (non-permissive temperature; Takeda *et al.* 2001). At the non-permissive temperature, the *hsk1-89* cells initially arrest at G1/S with 1C DNA content and eventually proceed into an abortive S phase with concomitant loss of viability. Cells accumulated nuclear damages and showed broken chromosomes as well as "cut" phenotypes (Takeda *et al.* 2001). The temperature-sensitive phenotypes in both mutants could be partially suppressed by Cds1 deletion (Snaith *et al.* 2000; Matsumoto *et al.* 2005). The *hsk1-89* mutant also grows at 37°C, albeit at a slower rate compared to that at 25°C. The reason for this apparent suppression of the *hsk1* mutation at a higher temperature is currently unknown (Matsumoto *et al.* 2005).

Both *hsk1-1312* and *hsk1-89* showed hypersensitivity to genotoxic agents such as hydroxyurea (HU), methyl methane-sulfonate (MMS) or Thiabendazole (TBZ), consistent with the role of Hsk1 in replication checkpoint regulation as well as in M phase regulation (Takeda *et al.* 2001; Bailis *et al.* 2003; Sommariva *et al.* 2005). Defective intra-S phase checkpoint with impaired Cds1 kinase activation was observed in *hsk1-89* in the presence of genotoxic agent (Takeda *et al.* 2001; Matsumoto *et al.* 2005; Shimmoto *et al.* 2009). Disrupted Mcl1p (Ctf4 homologue) chromatin association and Mcl1p-Pol1p protein interaction were reported in the HU-treated *hsk1-1312* cells (Williams and McIntosh 2005).

On the other hand, both *hsk1-1312* and *hsk1-89* showed centromere specific defect in cohesion. Both mutants showed synthetic lethality in combination with *rad21-K1* (cohesin subunit) mutation, suggesting a role of Hsk1 in sister chromatid cohesion (Takeda *et al.* 2001; Bailis *et al.* 2003). Deletion of *rad3* or *chk1* also resulted in synthetic lethality in both mutants (Snaith *et al.* 2000; Takeda *et al.* 2001). In addition, *hsk1-1312* showed synthetic lethality in combination with *hus2* deletion (Snaith *et al.* 2000), whereas *hsk1-89* showed synthetic lethality or synthetic growth defects in combination with *cdc19-P1/mcm2* mutation at 25°C or with *swi1* deletion at 37°C, respectively (Takeda *et al.* 2001; Matsumoto *et al.* 2005).

Characterization of the *hsk1-89* mutant in meiosis showed defective meiosis especially in the meiotic recombination. Premeiotic DNA replication was delayed but completed. However, cells were arrested before meiosis I with one nucleus and formation of DNA double-strand breaks (DSBs) for meiotic recombination was largely impaired in the *hsk1-89* cells (Ogino *et al.* 2006).

3. *Xenopus*

3.1. Effects of Cdc7 null/depletion

Immunodepletion of Cdc7 in *Xenopus* egg extracts resulted in reduced DNA replication (Jares and Blow 2000; Walter 2000; Takahashi and Walter 2005; Silva *et al.* 2006). Depletion of *Xenopus* Cdc7 also inhibited Cdc45 chromatin loading and reduced DNA replication by 5-6 folds (Walter 2000; Jares and Blow 2000). Similarly, immunodepletion of Cdc7 along with Drf1 in egg extracts lead to inhibited DNA replication, whilst little effect was observed when Cdc7-Dbf4 was depleted, suggesting that Drf1 is a major Cdc7-activating partner in *Xenopus* egg extracts (Furukohri *et al.* 2003; Takahashi and Walter 2005; Silva *et al.* 2006). It was noted that inhibition of DNA replication was incomplete after depletion of Cdc7 in *Xenopus* egg extracts, but it remains unclear whether this is due to the existence of a Cdc7-independent DNA replication or the incomplete depletion of the proteins (Takahashi and Walter 2005; Silva *et al.* 2006). Chromatin association of Scc2-Scc4 complex, a component essential for cohesin loading, was also impaired upon immunodepletion of Cdc7-Drf1 (Takahashi *et al.* 2008), implicating a direct regulatory role of Cdc7 in sister chromatid cohesion.

4. Mouse

4.1. Effects of Cdc7 null/depletion

Cdc7 knockout mice showed early embryonic lethality, and the *Cdc7* null embryos died between embryonic day 3.5 and day 6.5 (Kim *et al.* 2002). In murine embryonic stem (ES) cells,

conditional knockout of both *Cdc7* alleles (i.e. *Cdc7-/-* mutant) resulted in cessation of DNA synthesis and S-phase arrest, although *Cdc7+/-* mutant displayed a wild-type phenotype (Kim *et al*. 2002). Recombinational repair pathway was activated upon the loss of Cdc7, as shown by accumulation of the nuclear damages and the Rad51 foci in the *Cdc7-/-* ES cells. G2/M checkpoint was also induced, consistent with the persistent phosphorylation status of the Cdc2/Cdk1 at Tyr-15. In addition, expression of p53 and its downstream targets was elevated, causing an eventually p53-dependent apoptotic cell death in the *Cdc7-/-* ES cells. Similar p53-dependent apoptotic cell death was also observed in the *Cdc7-/-* early embryos (Kim *et al*. 2003; see below).

Expression of a Cdc7 transgene (tg) could rescue such cell lethality completely in the *Cdc7-/-tg* ES cells but not as completely in the *Cdc7-/-tg* embryos. Most of the *Cdc7-/-tg* embryos died during later stages of embryogenesis, whilst *Cdc7-/-tg* pups died within 3 days post partum with only less than 25% survived to adulthood. These surviving *Cdc7-/-tg* mice showed intact immune responses but retarded growth (only half of the size of the wild-type) and with tail flexion anomalies. They were infertile with testical or ovarian atrophy and impaired spermatogenesis or oogenesis, respectively. The phenotypes observed could be due to the low levels of Cdc7 as a result of silencing of the Cdc7 transgene expression in differentiated cells (Kim *et al*. 2003). Expression of an additional copy of Cdc7 transgene in the *Cdc7-/-tg* mice (resulting in *Cdc7-/-tg/tg*) showed restoration of normal development and fertility, although the size of the testis could not be completely restored (Kim *et al*. 2003).

On the other hand, the early lethality in the *Cdc7-/-* embryos could be partially rescued by p53 knockdown. The *Cdc7-/-p53-/-* double knockout embryos showed an extended survival up to embryonic day 9.5. Examination of the *in vitro* cultured blastocysts derived from the *Cdc7-/-* embryos showed no inner cell mass (ICM), whereas that from the *Cdc7-/-p53-/-* double knockout embryos showed significant ICM development.

Cdc7+/-tg mutant murine embryonic fibroblasts (MEFs) exhibited normal phenotype whilst *Cdc7-/-tg* mutant MEF displayed reduced DNA replication, delayed S phase entry and slow S phase progression. These defects were restored in the *Cdc7-/-tg/tg* MEFs, suggesting that sufficient level of Cdc7 is required for normal growth and differentiation/development of somatic cells (Kim *et al*. 2003).

5. Human

5.1. Effects of Cdc7 depletion/downregulation

Cdc7 depletion by siRNA leads to the inhibition of DNA synthesis and impaired S-phase progression but not to the induction of the p53-dependent checkpoint response in human cancer cell lines (Montagnoli *et al*. 2004; Yoshizawa-Sugata *et al*. 2005; Kulkarni *et al*. 2009; Rodriguez-Acebes *et al*. 2010). Instead, these cells eventually die through p53-independent apoptosis or aberrant mitosis. The p38-MAPK-dependent pathway was recently shown to be responsible for the apoptotic cell death in Cdc7-depleted HeLa cells (Im and Lee 2008).

Cdc7 siRNA treatment in primary normal human dermal fibroblasts results in the inhibition of DNA synthesis and the p53-dependent cell cycle arrest at G1 or early-S phase

(Montagnoli *et al*. 2004). In diploid human fibroblasts, siRNA-induced Cdc7-depletion leads to the activation of three inhibitory axes regulating cell growth: p15^{INK4B} upregulation, ARF-HDM-p53-mediated p21 pathway activation and Dkk3 upregulation, which downregulates Myc and CyclinD1. These three axes are coordinated by the transcription factor FoxO3a and their activation leads to a reversible G1 arrest. Disruption of any of these axes in the Cdc7-depleted fibroblasts results in an abortive DNA synthesis and eventual cell death (Tudzarova *et al*. 2010). Similar G1 arrest was observed also in primary human breast and bronchial epithelial cells upon Cdc7 depletion, whereas abortive S phase and apoptotic cell death was observed in p53-deficient, Her2-overexpressing, triple-negative breast cancer cells after Cdc7 downregulation (Rodriguez-Acebes *et al*. 2010). Inhibition of human Cdc7 by Cdc7 inhibitor (PHA-767491) also results in p53-independent apoptosis in human cancer cells and p53-dependent G1 arrest in normal fibroblasts, similar to that observed in the Cdc7 siRNA-treated cells (Montagnoli *et al*. 2008).

On the other hand, indirect downregulation of Cdc7 activity through siRNA-induced Drf1/ASKL1 depletion in cancer cells results in an increase of the multinucleated cell population, slower cell cycle progression and mitotic delay (Yoshizawa-Sugata *et al*. 2005). Although there was an increase in the late S or G2/M cell population, G2/M checkpoint was not activated in these cells.

5.2 Effects of Cdc7 overexpression

Overexpression of Cdc7 is a common feature in many human cancer cells and tumor tissues (see section "Major Sites of Expression" and references therein). Recent studies show that elevated Cdc7 expression level is related to genomic instability, accelerated cell cycle progression and advanced clinical stage in human breast and ovarian epithelial carcinomas and results in reduced disease-free survival in cancer patients (Kulkarni *et al*. 2009; Rodriguez-Acebes *et al*. 2010). However, overexpression of Cdc7-Dbf4 complex through a tetracycline-regulated promoter in human cancer cell line did not significantly alter the cell cycle progression, although an increase in the phosphorylation level of MCM2 subunit, a Cdc7-Dbf4 substrate, was observed (Sato *et al*. 2003; Tsuji *et al*. 2008). Cdc7 overexpression was reported to be correlated with p53 inactivation in breast tumors and many human cancer cells (Bonte *et al*. 2008).

6. Chinese hamster (CHO cell line)

6.1. Effects of Cdc7 overexpression

Overexpression of hamster Cdc7 and/or Dbf4 in the Chinese hamster ovarian (CHO) cells at low levels did not result in multiple rounds of DNA replication or stimulation of S phase entry in CHO cells. However, a moderate increase in the levels of Dbf4, but not Cdc7, induced G2/M arrest and an eventual cell death. This observation coincides with the hyperphosphorylation status of the Cdc2/Cdk1 at Tyr-15, suggesting that high levels of Dbf4 may induce G2/M checkpoint activation. Further increase in the levels of Cdc7 and/or Dbf4 (by 2-4 folds) leads to G1 phase arrest and retarded S phase progression (Guo *et al*. 2005).

MAJOR SITES OF EXPRESSION
Murine Cdc7 is a ubiquitously expressed protein. In adult mouse tissues, Cdc7 mRNA is expressed at high levels in testis,

spleen and thymus; at moderate levels in lung, stomach and brain; and at low levels in liver, kidney, muscle and small intestine (Kim *et al*. 1998). In cell cultures, Cdc7 mRNA is expressed in embryonic stem (ES) cells and embryonic fibroblast (MEF) at almost similar levels (Kim *et al*. 2003). During embryogenesis, Cdc7 mRNA is actively transcribed and peaks at embryonic day-11 (Kim *et al*. 1998). Refer to UniGene (Mm.20842) for more information on mouse Cdc7 mRNA expression profile.

Similarly, human Cdc7 is ubiquitously expressed and its expression levels differ depending on cell types. In normal adult tissues, Cdc7 mRNA is expressed at high levels in testis, placenta and brain, and at low levels in kidney and liver (Sato *et al*. 1997; Hess *et al*. 1998). Overexpression of Cdc7 is a common occurrence in malignancy, as observed in various types of human tumor tissues and transformed cell lines (Sato *et al*. 1997; Hess *et al*. 1998; Bonte *et al*. 2008). For instance, Cdc7 expression is highly elevated in tumor tissues of primary colon, breast and lung cancers when compared to their matched normal tissues (Bonte *et al*. 2008). Similarly, invasive melanomas and atypical Spitz nevi also show increased Cdc7 expression in comparison to that of normal skin (Clarke *et al*. 2009). Although there is no correlation between Cdc7 overexpression and hyperproliferation in tumors, Cdc7 overexpression may be related to neoplastic transformation in some tumors (Hess *et al*. 1998; Bonte *et al*. 2008). Recent studies have suggested Cdc7 as a good molecular predictor of survival in ovarian cancer, and as a potent anti-cancer target in ovarian and breast carcinomas (Kulkarni *et al*. 2009; Rodriguez-Acebes *et al*. 2010).

Extensive studies have been conducted to examine levels and patterns of Cdc7 expression in tissues and cell lines of human origin. UniGene (Hs.533573) and Human Protein Atlas (Cdc7) are two of the useful and easily accessible databases which provide comprehensive references to human Cdc7 mRNA and protein expression profiles, respectively. Data in UniGene (Hs.533573) show that human Cdc7 is transcribed at high levels in bone marrow, embryonic tissues, lymph nodes, testis and thymus, and at moderate levels in adrenal gland, ascites, bladder, blood, brain, heart, pharynx, thyroid and uterus. Data in Human Protein Atlas (Cdc7), on the basis of immunohistochemistry, indicate that Cdc7 protein is expressed at high levels in lymphoid tissues in appendix, lymph nodes, rectum, stomach, testis, and tonsil. It should be noted that slight differences in Cdc7 expression have been observed from one study to another, probably due to the differences in the experimental procedures or the detection sensitivity.

SPLICE VARIANTS

Mouse expresses a number of Cdc7 splice variants. Northern blotting has revealed four murine Cdc7 mRNA transcript variants sized 2.9 kb, 4.0 kb, 4.4 kb and 7.5 kb (Kim *et al*. 1998). All of these transcript variants, except the 7.5kb transcript, are expressed in developing embryos and adult tissues. The 7.5kb transcript is solely expressed in developing embryos with a maximum expression level at embryonic day-11 (Kim *et al*. 1998). On the other hand, seven Cdc7 protein isoforms sized between 35 kDa and 70 kDa were detected in mouse testis through western blotting, further supporting the presence of multiple splice variants of murine Cdc7. Only two of these isoforms, sized 43 kDa and 58 kDa, are expressed in mouse spleen and thymus. Sequence analysis of murine complementary DNA (cDNA) revealed two splice variants sized 1.6 kb and 1.7 kb with open reading frames, which

encode proteins with 532 and 564 amino acids, respectively (Kim *et al*. 1998). The 1.6 kb (532 amino acids) variant is the result of exon 8 skipping and it correlates with the 58 kDa isoform observed in the western blotting. This variant lacks a part of the Kinase Insert II sequence but it is functionally indistinguishable from the 564-amino acid form (Kim *et al*. 1998).

Human Cdc7 also undergoes high rate of alternative splicing. Northern blot analyses from two studies have revealed the presence of three Cdc7 mRNA transcript variants sized 2.4 kb (or 2.0 kb), 3.5 kb (or 3.4 kb) and 4.4 kb in human cells (Sato *et al*. 1997; Hess *et al*. 1998). Among them, two of these variants are of slightly different sizes (as written in brackets), plausibly due to the difference in the experimental settings. The 2.4 kb (or 2.0 kb) transcript is a C-terminally truncated variant expressed only in human testis and a panel of cancer cell lines, but not in other normal tissues (Sato *et al*. 1997; Hess *et al*. 1998). On the other hand, the 3.5 kb (or 3.4 kb) and the 4.4 kb transcripts are expressed at high and low levels, respectively, in all human tissues and cancer cell lines examined. Further characterization of the 3.5 kb (or 3.4 kb) transcript confirmed an open reading frame which encodes a mature Cdc7 protein with 574 amino acids (64 kDa; Sato *et al*. 1997). To date, sequences of seven Cdc7 transcript variants have been deposited in the EnsEMBL database, two of which represent processed transcripts which do not contain any open reading frame. Three of these variants differ in terms of their lengths and sequences within 5'- and/or 3'- non-coding regions but all encode a 574-amino acid Cdc7 protein. Meanwhile, each of the remaining two Cdc7 transcript variants encodes a protein with only 157 amino acids or 187 amino acids, respectively (EnsEMBL database, as of Jan 2011).

In *Xenopus*, two Cdc7 mRNA transcripts with open reading frames sized 1.4 kb and 1.5 kb have been identified (Sato *et al*. 1997). The 1.5 kb variant contains additional eight amino acids in the N-terminus including a putative ATG. In another study, two transcript variants sized 1.7 kb and 3.0 kb were detected in late stages of embryogenesis, and two Cdc7 protein isoforms sized above 50 kDa were detected in maturing oocytes (Roberts *et al*. 1999).

No additional transcript variants have been reported in hamster cells (3.0 kb; Guo and Lee 1999), budding yeast (1.7 kb; Meddle *et al*. 1985; Patterson *et al*. 1986) or fission yeast (2.0 kb; Masai *et al*. 1995) thus far. The budding yeast Cdc7 mRNA contains two translational initiation sites at codon 1 and 19, potentially generating 56 kDa and 58 kDa proteins, respectively (Patterson *et al*. 1986; Bahman *et al*. 1988; Ham *et al*. 1989; Yoon and Campbell 1991). Both proteins appear to be functionally identical.

For more information on Cdc7 transcript variants in other species, please refer to the UniGene and/or EnsEMBL databases.

REGULATION OF CONCENTRATION

The promoter of the budding yeast Cdc7 gene lacks a functional TATA box but carries a 30 bp-element which shares significant sequence homology with the basal promoter element in the mammalian *c-fos* promoter (Ham *et al*. 1989). This segment may bind to a sequence-specific transcription factor that regulates the basal promoter activity of the *c-fos* promoter *in vivo* (Gilman *et al*. 1986). It is not known whether similar transcription factor binds to this element in budding yeast. Budding yeast Cdc7 mRNA and protein are expressed at

constant levels during mitotic cell cycle (Sclafani *et al*. 1988; Weinreich and Stillman 1999). This is in contrast to the expression of Dbf4 and Dfp1/Him1 which undergoes dramatic oscillation at both mRNA and protein levels during the mitotic cell cycle in budding yeast and fission yeast, respectively (Sclafani *et al*. 1988, Brown and Kelly 1999; Takeda *et al*. 1999). On the other hand, expression of the Cdc7 mRNA is temporally regulated during the meiotic cell cycle, with the lowest levels in early meiosis and a gradual increase at the time of premeiotic S phase and meiotic recombination. Expression of Cdc7 mRNA is maintained at high levels even at the time of sporulation in budding yeast (Sclafani *et al*. 1988).

In fission yeast, the Hsk1 mRNA expression levels slightly oscillate, whereas the protein levels remain mostly constant during mitotic cell cycle (Takeda *et al*. 1999). On the other hand, both Hsk1 mRNA and protein expression undergo biphasic oscillation during meiotic cell cycle. The levels increase at the time of premeiotic DNA replication through initiation of meiotic recombination, followed by a temporal decrease and increase again later at meiosis II or sporulation (Ogino *et al*. 2006). Similar biphasic oscillation has also been observed for the Hsk1 activation subunit, Dfp1/Him1, during meiotic cell cycle (Ogino *et al*. 2006).

Transcription of murine Cdc7 may be regulated by several factors. The promoter of murine Cdc7 also lacks a functional TATA box, but contains binding sites for several transcription factors including E2F, YY1 and Sp1 (Kim *et al*. 1998). In addition, murine Cdc7 promoter activity could be activated by growth factors whilst repressed by cell differentiation signals (Kim *et al*. 1998). The promoter activity of murine Cdc7 is repressed during quiescent (G0) phase, and is activated once cells enter the mitotic cell cycle, leading to a gradual increase in the Cdc7 mRNA levels until the cells reach the G1/S phase border. The mRNA levels remain relatively constant in the cycling cells throughout the cell cycle (Kim *et al*. 1998). Likewise, promoter activity of the Chinese hamster Cdc7 is also repressed at quiescent (G0) phase and is activated once cells enter the mitotic cell cycle (Guo and Lee 1999).

In human, Cdc7 mRNA and protein were initially reported to be expressed at constant levels throughout the cell cycle in the cycling cells (Jiang and Hunter 1997; Sato *et al*. 1997; Jiang *et al*. 1999; Chuang *et al*. 2009). However, later studies indicated that Cdc7 protein level may oscillate during the cell cycle (see below). Activity of the human Cdc7 promoter could be repressed by periphilin, an interactor with a precursor of the cornified cell envelope, periplakin (Kurita *et al*. 2004, 2007). Besides, Cdc7 transcription may be positively regulated by Cdk2 during cell cycle entry from quiescence, since induction of human Cdc7 mRNA expression from the quiescent phase was repressed by a dominant-negative form of Cdk2 (Chuang *et al*. 2009). However, the precise mechanism for this regulation remains unknown.

In *Xenopus*, although transcriptional regulation of Cdc7 is unclear, it has been suggested that the abundance of Cdc7 mRNA may be regulated by mRNA stability (Roberts *et al*. 1999). Cdc7 mRNA expression is maintained at a relatively constant levels in the resting oocytes and through early embryogenesis, but the levels gradually decrease in midblastomere, possibly due to destabilization of the maternal mRNA (Roberts *et al*. 1999).

Studies in various organisms have shown that Cdc7 protein levels gradually increase during the mitotic cell cycle re-entry, but the protein levels do not oscillate significantly in the cycling cells (Sclafani *et al*. 1988; Jiang and Hunter 1997; Sato *et al*. 1997; Kim *et al*. 1998; Guo and Lee 1999). However, a recent study with probably a better synchronization protocol indicated that human Cdc7 protein levels indeed oscillate during the mitotic cell cycle (Masai *et al*. 2006). In the human HeLa cells, expression of the Cdc7 protein was at the lowest level in G1, elevated during G1/S transition, and remained high until G2/M phase. This observation suggested that human Cdc7 protein may be degraded at the mitotic exit in an APC/Cdh1-dependent manner (Masai *et al*. 2006; Toh *et al*. unpublished data). Further studies are required to understand the precise mechanism regulating Cdc7 abundance during mammalian cell cycle.

ANTIBODIES

Commercially available anti-Cdc7 antibodies are as listed below. The description is based on the suppliers' brochure and may not be necessarily experimentally validated by a third party.

I. Mouse monoclonal antibodies

a. Clone Cdc7 (DSC-341)

1. Abcam, catalog number ab10535; GenWay Biotech Inc., catalog number 20-272-190246. Directed against full-length human Cdc7. It has been tested for immunocytochemistry, immunoprecipitation, ELISA and western blotting.

2. Affinity BioReagents, catalog number MA1-24746; Novus Biologicals, catalog number NB120-10535. Directed against full-length human Cdc7. It has been tested for immunoprecipitation and western blotting.

3. Sigma-Aldrich, catalog number C6613. Directed against full-length human Cdc7. It has been tested for immunocytochemistry, immunoprecipitation, microarray, indirect ELISA and western blotting.

4. Santa Cruz Biotechnology Inc., catalog number sc-56274. Directed against full-length human Cdc7. It has been tested for immunoprecipitation, immunofluorescence and western blotting.

5. Thermo Fisher Scientific Inc, catalog number MS-1888 (various packing available; e.g. different quantity, with or without BSA, and ready-to-use). Directed against GST-fused full-length recombinant human Cdc7. It has been tested for immunoprecipitation, immunohistology using formalin/paraffin-embedded sections and western blotting.

b. Clone Cdc7 (SPM171)

1. Abcam, catalog number ab17880; GenWay Biotech Inc., catalog number 20-272-191570; Novus Biologicals, catalog number NB120-17880. Directed against full-length recombinant human Cdc7. It has been tested for immunoprecipitation, immunohistochemistry using formalin/PFA-fixed paraffin-embedded sections and western blotting.

2. Santa Cruz Biotechnology Inc., catalog number sc-56275. Directed against full-length recombinant human Cdc7. It has been tested for immunoprecipitation, immunofluorescence, immunohistochemistry using formalin/PFA-fixed paraffin-embedded sections and western blotting.

3. Abcam, catalog number ab53919. Cdc7 (SPM171; prediluted). Directed against full-length recombinant human Cdc7. It has been tested for immunohistochemistry using formalin/PFA-fixed paraffin-embedded sections.

c. others

1. Lifespan Biosciences, catalog number LS-C14413 or LS-C14414. Directed against GST-fused full-length recombinant human Cdc7. It has been tested for immunoprecipitation, immunohistochemistry and western blotting.

2. MBL International Corporation, catalog number K0070-3 (or K0070-3S for smaller quantity). Cdc7 (DSC-342). Directed against full-length human Cdc7 fusion protein. It has been tested for immunoprecipitation, immunohistochemistry and western blotting.

3. MBL International Corporation, catalog number CY-M1021. Phospho-Cdc7 (Thr376). Directed against phosphorylated form (at residue Threonine 376) of human Cdc7. It has been tested for ELISA, immunofluorescence and western blotting.

II. Polyclonal antibodies

a. Host: Rabbit

1. GenWay Biotech Inc., catalog number 18-461-10828; Imgenex, catalog number IMG-71931; LifeSpan BioSciences, catalog number LS-A7979; MBL International Corporation, catalog number LS-A7979; Novus Biologicals, catalog number NLS7979. Directed against the kinase domain of human Cdc7; with 63% identity and 79% similarity to mouse Cdc7 sequence. It has been tested for immunohistochemistry using formalin fixed paraffin embedded sections.

2. GenWay Biotech Inc., catalog number 18-461-10829; Imgenex, catalog number IMG-71932; LifeSpan BioSciences, catalog number LS-A7980; MBL International Corporation, catalog number LS-A7980; Novus Biologicals, catalog number NLS7980. Directed against the kinase domain of human Cdc7; with 94% identity and 100% similarity to mouse Cdc7 sequence. It has been tested for immunohistochemistry using formalin fixed paraffin embedded sections.

3. Abgent, catalog number AP7515a. Cdc7L1 (N-term). Directed against the N-terminal region (amino acids 1-100) of human Cdc7. It has been tested for flow cytometry, ELISA and western blotting.

4. Abgent, catalog number AP7515b. Cdc7L1 (C-term). Directed against the C-terminal region (amino acids 500-600) of human Cdc7. It has been tested for immunohistochemistry, ELISA and western blotting.

5. Santa Cruz Biotechnology Inc., catalog number sc-13010. Cdc7 (H-110). Directed against the N-terminal (amino acids 61-170) region of human Cdc7. Also recommended for detection of Cdc7 of mouse and rat origin. It has been tested for immunoprecipitation, immunofluorescence and western blotting.

b. Host: Goat

1. Santa Cruz Biotechnology Inc., catalog number sc-7518. Cdc7 (C-20). Directed against the C-terminal region of human Cdc7. Also recommended for detection of Cdc7 of mouse and rat origin. It has been tested for immunofluorescence and western blotting.

2. Santa Cruz Biotechnology Inc., catalog number sc-7519. Cdc7 (N-19). Directed against the N-terminal region of human Cdc7. It has been tested for immunofluorescence and western blotting

3. Santa Cruz Biotechnology Inc., catalog number sc-11964. Cdc7 (yN-18). Directed against the N-terminal region of Saccharomyces cerevisiae Cdc7. It has been tested for western blotting.

Table 1: Functional States

STATE DESCRIPTION	LOCATION	REFERENCES
Cdc7	nucleus	Faul T *et al.* 1999; Guo B and Lee H 1999; Hartwell LH *et al.* 1971; Hartwell LH *et al.* 1970; Hollingsworth RE and Sclafani RA 1990; Jiang W and Hunter T 1997; Johnston LH *et al.* ; Kim JM *et al.* 1998; Masai H *et al.* 1995; Patterson M *et al.* 1986; Sato N *et al.* 1997
Cdc7/CRM1	cytoplasm	Kim BJ *et al.* 2007
Cdc7/Importin-alpha	nucleus	Kim BJ *et al.* 2007; Kim BJ and Lee H 2006
Cdc7/Importin-beta	nucleus	Kim BJ and Lee H 2006; Kim BJ *et al.* 2007
Cdc7/Dbf4	nucleus	Brown GW and Kelly TJ 1999; Dixon WJ and Campbell JL ; Dowell SJ *et al.* 1994; Jackson AL *et al.* 1993; James SW *et al.* 1999; Jiang W *et al.* 1999; Kitada K *et al.* 1992; Kumagai H *et al.* 1999; Lepke M *et al.* 1999; Oshiro G *et al.* 1999; Takeda T *et al.* 1999; Yoon HJ *et al.* 1993; Brown GW and Kelly TJ 1998
Cdc7-P/Dbf4-P	nucleus	Dixon WJ and Campbell JL ; Brown GW and Kelly TJ 1998; Dowell SJ *et al.* 1994; Guo B and Lee H 1999; Guo B and Lee H 2001; Jackson AL *et al.* 1993; Johnston LH and Thomas AP ; Kitada K *et al.* 1992; Masai H and Arai K 2000; Masai H *et al.* 1995; Oshiro G *et al.* 1999; Patterson M *et al.* 1986; Sato N *et al.* 1997; Varrin AE *et al.* 2005
Cdc7-P/Dbf4-P/αprimase-P	nucleus	Masai H *et al.* 2000; Weinreich M and Stillman B 1999
Cdc7-P/Dbf4-P/CAF1-P	nucleus	Gérard A *et al.* 2006
Cdc7-P/Dbf4-P/CAF1-P/PCNA	nucleus	Gérard A *et al.* 2006
Cdc7-P/Dbf4-P2/Cdc5-P	nucleus	Hardy CF and Pautz A 1996; Kitada K *et al.* 1993; Matos J *et al.* 2008; Miller CT *et al.* 2009
Cdc7-P/Dbf4-P/Cdc28	Unknown	Ohtoshi A *et al.* 1996
Cdc7-P/Dbf4-P/Cdc45-P	Unknown	Nougarède R *et al.* 2000
Cdc7-P2/Dbf4-P/Cdk2	nucleus	Martin L *et al.* 2007; Masai H *et al.* 2000
Cdc7-P/Dbf4-P/Cdt1-P	nucleus	Ballabeni A *et al.* 2009
Cdc7-P/Dbf4-P2/Chk1	nucleus	Brown GW and Kelly TJ 1999; Dohrmann PR *et al.* 1999; Duncker BP *et al.* 2002; Kihara M *et al.* 2000; Kim JM *et al.* 2008; Lopez-Mosqueda J *et al.* 2010; Ogi H *et al.* 2008; Pasero P *et al.* 1999; Snaith HA *et al.* 2000; Weinreich M and Stillman B 1999; Zegerman P and Diffley JF 2010
Cdc7-P/Dbf4-P/CINP-P	nucleus	Grishina I and Lattes B 2005
Cdc7-P/Dbf4-P/Claspin-P2	nucleus	Gold DA and Dunphy WG 2010; Kakusho N *et al.* 2008; Kim JM *et al.* 2008; Matsumoto S *et al.* 2010; Ogi H *et al.* 2008; Sheu YJ and Stillman B 2010; Shimmoto M *et al.* 2009; Takeda T *et al.* 2001
Cdc7-P/Dbf4-P/Geminin-P	Unknown	Masai H *et al.* 2000
Cdc7-P/Dbf4-P/HP1-P	nucleus	Bailis JM *et al.* 2003; Hayashi MT *et al.* 2009; Yabuuchi H *et al.* 2006
Cdc7-P/Dbf4-P/LEDGF-P	Unknown	Hughes S *et al.* 2010
Cdc7-P/Dbf4-P/MCM2-P	nucleus	Brown GW and Kelly TJ 1998; Bruck I and Kaplan D 2009; Charych DH *et al.* 2008; Cho WH *et al.* 2006; Francis LI *et al.* 2009; Jackson AL *et al.* 1993; Jares P and Blow JJ 2000; Kneissl M *et al.* 2003; Lee JK *et al.* 2003; Lei M *et al.* 1997; Lepke M *et al.* 1999; Masai H *et al.* 2000; Montagnoli A *et al.* 2006; Sheu YJ and Stillman B 2006; Takeda T *et al.* 1999; Takeda T *et al.* 2001; Tsuji T *et al.* 2006; Weinreich M and Stillman B 1999
Cdc7-P/Dbf4-P/MCM3-P	nucleus	Kneissl M *et al.* 2003; Lei M *et al.* 1997; Weinreich M and Stillman B 1999
Cdc7-P/Dbf4-P/MCM4-P	nucleus	Kneissl M *et al.* 2003; Lee JK *et al.* 2003; Lei M *et al.* 1997; Masai H *et al.* 2000; Masai H *et al.* 2006; Randell JC *et al.* 2010; Sheu YJ and Stillman B 2010; Weinreich M and Stillman B 1999
Cdc7-P/Dbf4-P/MCM5	nucleus	Hardy CF *et al.* 1997; Hoang ML *et al.* 2007; Kneissl M *et al.* 2003; Sclafani RA *et al.* 2002; Fletcher RJ *et al.* 2003
Cdc7-P/Dbf4-P/MCM6-P	nucleus	Lei M *et al.* 1997; Masai H *et al.* 2000; Masai H *et al.* 2006; Randell JC *et al.* 2010; Weinreich M and Stillman B 1999
Cdc7-P/Dbf4-P/MCM7-P	nucleus	Kneissl M *et al.* 2003; Weinreich M and Stillman B 1999
Cdc7-P/Dbf4-P/MCM10	nucleus	Lee JK *et al.* 2003
Cdc7-P/Dbf4-P/ORC1	nucleus	Kneissl M *et al.* 2003
Cdc7-P/Dbf4-P/ORC2	nucleus	Duncker BP *et al.* 2002; Hardy CF *et al.* 1996; Kneissl M *et al.* 2003
Cdc7-P/Dbf4-P/ORC3	nucleus	Duncker BP *et al.* 2002
Cdc7-P/Dbf4-P/ORC4-P	nucleus	Masai H *et al.* 2000
Cdc7-P/Dbf4-P/ORC5	nucleus	Kneissl M *et al.* 2003
Cdc7-P/Dbf4-P/ORC6	nucleus	Kneissl M *et al.* 2003
Cdc7-P/Dbf4-P/Rad18-P	nucleus	Day TA *et al.* 2010
Cdc7-P/Dbf4-P/Rec8-P	nucleus	Katis VL *et al.* 2010
Cdc7-P/Dbf4-P/Tim-Tipin	nucleus	Sommariva E *et al.* 2005; Shimmoto M *et al.* 2009; Matsumoto S *et al.* 2005
Cdc7/Drf1	nucleus	Montagnoli A *et al.* 2002; Takahashi TS and Walter JC 2005; Yanow SK *et al.* 2003; Yoshizawa-Sugata N *et al.* 2005

Cdc7-P/Drf1-P	nucleus	Montagnoli A *et al.* 2002; Takahashi TS and Walter JC 2005; Yanow SK *et al.* 2003; Yoshizawa-Sugata N *et al.* 2005; Masai H and Arai K 2000
Cdc7-P/Drf1-P/Scc2-Scc4	nucleus	Takahashi TS *et al.* 2008
Cdc7-P/Dbf4-P/H3-P	nucleus	Baker SP *et al.* 2010

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This molecule exists in 39 states , has 38 transitions between these states and has 19 enzyme functions.(Please zoom in the pdf file to view details.)