A Synthetic Human Kinase Can Control Cell Cycle Progression in Budding Yeast

Megan J. Davey,1 Heather J. Andrijichetti, Xiaoli Ma, and Christopher J. Brandl
Department of Biochemistry, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario, Canada N6A 5C1

ABSTRACT The DDK kinase complex, composed of Cdc7 and Dbf4, is required for S-phase progression. The two component proteins show different degrees of sequence conservation between human and yeast. Here, we determine that Saccharomyces cerevisiae bearing human CDC7 and DBF4 grows comparably to cells with yeast DDK under standard growth conditions. HsDbf1 (a second human Dbf4-like protein) does not support growth, suggesting that HsDbf4 is the true ortholog of ScDbf4. Both human subunits are required to complement yeast cdc7Δ or dbf4Δ due to the inability of human Cdc7 or Dbf4 to interact with the corresponding yeast protein. Flow cytometry indicates normal cell cycle progression for yeast containing human DDK. However, yeast containing human DDK is sensitive to long-term exposure to hydroxyurea and fails to sporulate, suggesting that human DDK substitutes for some, but not all, of yeast DDK functions. We mapped the region of Cdc7 required for species-specific function of DDK to the C-terminus of Cdc7 by substituting the yeast C-terminal 55 amino acid residues in place of the equivalent human residues. The resulting hybrid protein supported growth of a cdc7Δ strain only in the presence of ScDBF4. The strain supported by the hybrid Cdc7 was not sensitive to HU and formed tetrads. Together, our data indicate that DDK’s targeting of its essential substrate is conserved between species, whereas the interactions within DDK are species specific.

Dbf4-dependent kinase (DDK) is a serine/threonine kinase complex required for the initiation of DNA replication. Both the catalytic subunit (Cdc7) and the regulatory subunit (Dbf4) are essential for growth in budding and fission yeast (Brown and Kelly 1998; Brown and Kelly 1999; Hartwell et al. 1973; Kitada et al. 1992; Masai et al. 1995), acting throughout S-phase to fire origins (Boussat and Difley 1998; Donaldson et al. 1998). In mammalian cells, depleting Cdc7 or Dbf4 adversely affects DNA replication and cell proliferation (Jiang et al. 1999; Kumagai et al. 1999). DDK requires Cdc7’s kinase activity for function. Its essential substrate is Mcm2-7, the catalytic core of the replicative helicase (Bruck and Kaplan 2009; Ohtoshi et al. 1997; Sheu and Stillman 2006; Sheu and Stillman 2010; Tsuji et al. 2006). Because of its importance in cell cycle progression, Cdc7 is being exploited as a therapeutic target in cancer (Montagnoli et al. 2010; Sawa and Masai 2009; Swords et al. 2010). In addition to its essential role in DNA replication initiation, DDK functions in the S-phase checkpoint, (Costanzo et al. 2003; Dolan et al. 2010; Duncker and Brown 2003; Fung et al. 2002; Gabrielse et al. 2006; Matsumoto et al. 2010; Njagi and Kilbey 1982; Ogi et al. 2008; Pessoa-Brandao and Scelfani 2004; Tsuji et al. 2008; Weinreich and Stillman 1999), mitotic exit (Miller et al. 2009), and meiosis (Katis et al. 2010; Lo et al. 2008; Marston 2009; Nakamura et al. 2002; Valentin et al. 2006; Wan et al. 2008).

Yeast and human Cdc7 are well conserved within the kinase family subdomains but much less so in the insertions between the subdomains [Figure 1A; (Hanks et al. 1988; Masai et al. 1995)]. Dbf4 (also called ASK for activator of S-phase kinase in human cells) contains only three short conserved regions, termed N, M, and C (Masai and Araki 2000; Ogino et al. 2001). A second Dbf4-like subunit found in many metazoans, Drf1 (Dbf4-related factor 1, also called DBF4B or ASK1) forms an independent kinase complex with Cdc7 (Montagnoli et al. 2002; Takahashi and Walter 2005; Yoshizawa-Sugata et al. 2005). Drf1 (DBF4B) should not be confused with DIA1, also referred to as DRF1 (diaphanous-related formin 1) in...
humans. Depletion of Drf1 perturbs the cell cycle in human cells, but the phenotype is less severe than depletion of Cdc7 or Dbf4 (Yoshizawa-Sugata et al. 2005). In Xenopus, Drf1 and Dbf4 are developmentally regulated, with each being essential at different life stages (Takahashi and Walter 2005). A second Dbf4 subunit is not developmentally regulated, with each being essential at different life stages (Takahashi and Walter 2005). A second Dbf4 subunit is not found in S. cerevisiae.

To evaluate the degree of functional conservation between human and budding yeast DDK with the goal of providing a genetically amenable system to study the structure and function of human DDK, we tested whether human Cdc7, Dbf4, and/or Drf1 substitute for S. cerevisiae Cdc7 in place of the C-terminal 52 amino acid residues that do not form tetrads. By substituting the C-terminal 55 amino acid residues of yeast Cdc7 in place of the C-terminal 52 amino acid residues of human Cdc7, we generated a hybrid Cdc7 molecule that functions instead (Takahashi and Walter 2005). A second Dbf4 subunit is not found in S. cerevisiae.

Figure 1 Human Cdc7 and Dbf4/Drf1. A) Comparison of the primary structure of Cdc7 from human (HsCdc7) and yeast (ScCdc7). The positions of conserved subdomains found in the eukaryotic protein kinase superfamily are indicated by black boxes. Grey boxes mark the positions of the conserved Cdc7 motifs. Black boxes mark the positions of the conserved Cdc7 motifs. The percent identity (similarity) in each region is indicated. B) Comparison of human Dbf4, yeast Dbf4, and human Drf1 primary structures. Black boxes mark the positions of the conserved Dbf4 motifs. The percent identity (similarity) of HsDbf4 and HsDrf1 with ScDbf4 in the motif regions is indicated under the schematics.

Figure 2 Human CDC7 and DBF4 do not interact with their yeast counterparts. A) Haploid cdc7 (rows 1, 2, and 5) or dbf4 (rows 3 and 4) deletion strains [BY23713, cdc7Δ (BY23988, dbf4Δ (Giaever et al. 2002)] transformed with the cognate yeast gene on a URA3 encoding plasmid (Gietz and Sugino 1988) were transformed with the indicated CEN or 2 μm plasmids (Gietz and Sugino 1988) encoding copies of HsCDC7, HsDbf4, or ScCDC7. Cell suspensions were diluted and then spotted on YPD (left) or 5-FOA–containing media (right) and grown at 30°C for two and three days, respectively. B) TAP-ScDbf4–associated proteins from extracts containing the indicated proteins were isolated using rabbit IgG coupled Epoxy M270 Dynabeads and then probed for the presence of ScDbf4 or myc9-HsDbf4 by Western blotting. "R" indicates recombinant yeast Cdc7 [700 ng; (Stead et al. 2011)], "I" the input extract (~1/100 of total), and "B" bound protein. C) A similar experiment to probe for the association of ScDbf4 and myc9-HsDbf4 with TAP-ScCdc7. D and E) Control experiments showing that the TAP-tagged proteins are enriched by the IgG beads (left panels) and that binding of ScCdc7 (D) or ScDbf4 (E) to the beads depends on the presence of TAP-ScDbf4 or TAP-ScCdc7, respectively. The “no tag” strain is BY4741. "W" is the wash.
transferred to the 2 μ LEU2 episomal plasmid YEplac181 (Gietz and Sugino 1988) using SacI-SfiI (HsCDC7, HsDBF4) or Smal-BamHI (DRFI). The LEU2 markers of YCplac111-HsCDC7 and YEp181-HsCDC7 were switched to HIS3 with pLH7 (Cross 1997), yielding YCplac111h-HsCDC7 and YEp181h-HsCDC7. The URA3 marker on YCplac33-ScDBF4 was switched to LEU2 with pUL9 (Cross 1997). Myc9-tagged versions of the human proteins were inserted into YEp88-URA3 (Hoke et al. 2008) as NotI-SacI or NotI-EcoRI fragments. A plasmid encoding both ScCDC7 and ScDBF4 (YCplac33-ScCDC7-ScDBF4) was constructed by inserting a SmaI fragment containing the ScCDC7 promoter and ORF (amplified using MD405 and MD438) into YCplac33-ScDBF4. C-terminal fragments of ScCdc7 were substituted in place of the corresponding HsCdc7 regions using gene splicing by overlapping extension PCR (Horton et al. 1990) and inserted as Ndel-SfiI fragments into YEp181 with the ScCDC7 promoter to yield YEp181-CD7-S1, YEp181-CD7-S2, and YEp181-CD7-S3. The LEU2 marker on these plasmids was switched to HIS3 using pLH7 (Cross 1997).

**Yeast strains**

Strains are listed in Table S2. Heterozygous deletion strains (BY23713, cdc7Δ0, BY23988, dbf4Δ0) (Giaever et al. 2002) and TAP-tagged Cdc7 and Dbf4 strains (Ghaemmaghami et al. 2003) were purchased from Open Biosystems. To generate haploid deletion strains complemented by plasmid copies, BY23713 was transformed with YCplac33-ScCDC7, and BY23988 with YCplac33-ScDBF4. Transformants were sporulated and G418 resistant colonies selected (MDY95: MATa, cdc7Δ0, HsCDC7-ScDBF4, CY4104: MATa, dbf4Δ0, YCplac33-ScDBF4). To generate a diploid strain deleted for cdc7 and dbf4, KanMX in CY4104 was switched to NatMX by transformation with linearized pLS39 (Tong and Boone 2006), creating CY4178. CY4178 was mated to MDY95 and the URA3 plasmids shuffled out, creating CY4348 (cdc7::KanMX dbf4::NatMX). CY4348 was then transformed with YEp181-HsDbf4 and YCplac111-HsCDC7 or YEp181h-HsCDC7, sporulated and His+, Leu+, G418, and ClonNat resistant colonies identified to generate CY4240 and CY4242. To generate a double deletion strain complemented by ScCDC7 and ScDBF4, YCplac111-ScCDC7 was shuffled into MDY95 to yield MDY195. Diploids from a MDY195 and CY4178 mating were sporulated. Leu+, His+, and MATa spore colonies that were G418 and ClonNat resistant were identified (CY4481: cdc7Δ0 dbf4Δ0; YCplac111-ScCDC7, YCplac33-ScDBF4). A strain deleted for both cdc7 and dbf4 containing YCplac33-ScCDC7-ScDBF4 was created by transforming CY4240 (MATa cdc7::KanMX dbf4::NatMX YCplac111-HsCDC7 YCplac181-HsDBF4) with YCplac33-ScCDC7-ScDBF4. After several passages in YPD, colonies that were Ura+, Leu+, His+, and 5-FOA sensitive were screened to yield MDY214 (MATa cdc7::KanMX dbf4::NatMX YCplac33-ScCDC7-ScDBF4). For homozygous diploid strains deleted at cdc7 and dbf4, CY4242 (MATa cdc7::KanMX dbf4::NatMX YCplac181h-HsCDC7 YCplac181-HsDBF4) and MDY214 were mated to create MDY270. Treatment with 5-FOA generated a strain containing only human CDC7 (CY5628; MATa/MATα cdc7::KanMX dbf4::NatMX YCplac181h-HsCDC7 YCplac181-HsDBF4). After several passages of MDY270 through YPD, Ura+, Leu+, and His+ colonies were identified to generate CY5627 (MATa/MATα cdc7::KanMX dbf4::NatMX YCplac181h-HsCDC7 YCplac181-HsDBF4). After several passages in YPD, colonies that were Ura+, Leu+, His+, and 5-FOA sensitive were screened to yield MDY214 (MATa cdc7::KanMX dbf4::NatMX YCplac33-ScCDC7-ScDBF4). For homozygous diploid strains deleted at cdc7 and dbf4, CY4242 (MATa cdc7::KanMX dbf4::NatMX YCplac181h-HsCDC7 YCplac181-HsDBF4) and MDY214 were mated to create MDY270. Treatment with 5-FOA generated a strain containing only human CDC7 (CY5628; MATa/MATα cdc7::KanMX dbf4::NatMX YCplac181h-HsCDC7 YCplac181-HsDBF4). After several passages of MDY270 through YPD, Ura+, Leu+, and His+ colonies were identified to generate CY5627 (MATa/MATα cdc7::KanMX dbf4::NatMX YCplac181h-HsCDC7 YCplac181-HsDBF4). After several passages in YPD, colonies that were Ura+, Leu+, His+, and 5-FOA sensitive were screened to yield MDY214 (MATa cdc7::KanMX dbf4::NatMX YCplac33-ScCDC7-ScDBF4). For homozygous diploid strains deleted at cdc7 and dbf4, CY4242 (MATa cdc7::KanMX dbf4::NatMX YCplac181h-HsCDC7 YCplac181-HsDBF4) and MDY214 were mated to create MDY270. Treatment with 5-FOA generated a strain containing only human CDC7 (CY5628; MATa/MATα cdc7::KanMX dbf4::NatMX YCplac181h-HsCDC7 YCplac181-HsDBF4). After several passages of MDY270 through YPD, Ura+, Leu+, and His+ colonies were identified to generate CY5627 (MATa/MATα cdc7::KanMX dbf4::NatMX YCplac181h-HsCDC7 YCplac181-HsDBF4). After several passages in YPD, colonies that were Ura+, Leu+, His+, and 5-FOA sensitive were screened to yield MDY214 (MATa cdc7::KanMX dbf4::NatMX YCplac33-ScCDC7-ScDBF4). For homozygous diploid strains deleted at cdc7 and dbf4, CY4242 (MATa cdc7::KanMX dbf4::NatMX YCplac181h-HsCDC7 YCplac181-HsDBF4) and MDY214 were mated to create MDY270. Treatment with 5-FOA generated a strain containing only human CDC7 (CY5628; MATa/MATα cdc7::KanMX dbf4::NatMX YCplac181h-HsCDC7 YCplac181-HsDBF4). After several passages of MDY270 through YPD, Ura+, Leu+, and His+ colonies were identified to generate CY5627 (MATa/MATα cdc7::KanMX dbf4::NatMX YCplac181h-HsCDC7 YCplac181-HsDBF4). After several passages in YPD, colonies that were Ura+, Leu+, His+, and 5-FOA sensitive were screened to yield MDY214 (MATa cdc7::KanMX dbf4::NatMX YCplac33-ScCDC7-ScDBF4). For homozygous diploid strains deleted at cdc7 and dbf4, CY4242 (MATa cdc7::KanMX dbf4::NatMX YCplac181h-HsCDC7 YCplac181-HsDBF4) and MDY214 were mated to create MDY270. Treatment with 5-FOA generated a strain containing only human CDC7 (CY5628; MATa/MATα cdc7::KanMX dbf4::NatMX YCplac181h-HsCDC7 YCplac181-HsDBF4). After several passages of MDY270 through YPD, Ura+, Leu+, and His+ colonies were identified to generate CY5627 (MATa/MATα cdc7::
KanMX dbf4::NatMX YCplac33-ScCDC7-ScDBF4. CY5627 was transformed with YEplac181h-CDC7-S1, and YCplac33L-ScDBF4, His+, and Leu+ colonies selected, and then the strain (MDY317) was plated on 5-FOA to yield MDY318.

**Cell cycle arrest and flow cytometry**

Yeast strains CY4240 (HsDDK) and CY4481 (ScDDK) were grown in YPD to an OD_{600} of ~0.6, incubated in 100 mM hydroxyurea (Sigma-Aldrich) for 2 h at 30°C, washed twice, and then resuspended in fresh YPD. Aliquots were removed at the indicated times, pelleted, and resuspended in 70% ethanol. Cell sorting was performed on a FACSCalibur (BD Biosciences) by the London Regional Flow Cytometry Facility.

**Cdc7 and Dbf4 interactions**

Log phase cells (4 × 10^10 cells) were harvested by centrifugation, washed with ice-cold water, and resuspended in 3 ml lysis buffer [25 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.1 mM EDTA pH 8.0, and 0.1% (v/v) NP40]. Cell extracts were made as described by Schultz et al. (1991). After centrifugation, the supernatant (~4 ml, 15–20 mg protein/ml) was mixed with 50 μl Epoxy M270 Dynabeads (Invitrogen) coupled to rabbit IgG (~7 mg IgG/ml beads). After 2 h at 4°C, beads were collected and the supernatant removed. The beads were washed twice with 1 ml of lysis buffer, once in 200 ml lysis buffer, and then incubated in 50 ml SDS loading dye at 90°C for 5 min. The elutions were analyzed by Western blotting. Primary antibodies were anti-ScCdc7 yN-18 and anti-ScDbf4 yA-16 (Santa Cruz Biotechnology) and monoclonal anti c-myc (Sigma-Aldrich). Rabbit anti-goat and goat anti-mouse antibodies coupled to horseradish peroxidase were from Sigma-Aldrich. Detection using chemiluminescence followed the manufacturer’s instructions (SuperSignal West Pico Kit; Pierce Biotechnology).

**RESULTS**

**Functional complementarity of human and yeast DDK**

To address the degree of functional conservation between yeast and human DDK subunits, we tested whether the individual human subunits were able to substitute for their yeast counterparts. We expressed HsCDC7 or HsDBF4 from centromeric and 2 μm plasmids (Gietz and Sugino 1988) and examined their ability to support viability by plasmid shuffling. The initial analysis was performed with CDC7 in which a haploid yeast strain deleted for cdc7 (Giaever et al. 2002) and complemented by a URA3 plasmid encoding ScCDC7 was transformed with either low copy or multicopy plasmids encoding HsCDC7. Cells were plated on 5-FOA where viability requires that the human gene substitutes for its yeast ortholog. As shown in Figure 2A (rows 1, 2), neither the low copy nor the multicopy plasmid encoding HsCDC7 supported viability of the cdc7 strain. In similar tests, HsDBF4 did not support growth of dbf4 (Figure 2A, rows 3, 4). The lack of complementation could be explained by the inability of HsCdc7 and HsDbf4 to interact with their yeast counterparts. To test this possibility, we compared the interaction of ScCdc7 and myc-tagged HsCdc7 with ScDbf4 fused to a tandem affinity purification (TAP) tag (Rigaut et al. 1999). Proteins that interacted with affinity-purified TAP-ScDbf4 were detected by Western blotting. As expected, we detected a robust signal for ScCdc7 in the fraction eluting with TAP-ScDbf4 (Figure 2B, left, D). In contrast to ScCdc7, myc-HsCdc7 was not associated with TAP-ScDbf4, even though there was a strong signal for this protein in the cell lysate (Figure 2B, right). Similar experiments with TAP-ScCdc7 and myc-HsDbf4 did not detect an interaction between ScCdc7 and HsDbf4 (Figure 2C, E). A reciprocal experiment in which antibodies to the human proteins were used also

![Figure 4](image-url)
did not identify a complex of yeast and human proteins (supporting information, Figure S1). We therefore concluded that HsCdc7 and HsDbf4 fail to interact with ScDbf4 and ScCdc7, respectively.

Since a cross-species DDK complex did not form, we wondered whether supplying both human genes would allow complementation of cdc7Δ or dbf4Δ. To test this, we repeated the plasmid shuffling, supplying copies of both HsCDC7 and LEU2-HsDbf4 (Figure 3A). Strains containing HsCDC7 and multicopy HsDBF4 were viable regardless of whether the yeast strain was deleted in cdc7 or dbf4 (Figure 3A, rows 3, 4). HsCDC7 was functional when expressed from either centromeric or multicopy plasmids. In contrast, only the multicopy plasmid bearing HsDBF4 complemented.

To determine whether the presence of ScDBF4 or ScCDC7 contributed to the activity of human DDK, we constructed a diploid strain (CY4348) heterozygous for deletion of both CDC7 (cdc7::KanMX) and DBF4 (dbf4::NatMX) in which the deletions provided resistance to G418 and ClonNAT, respectively. CY4348 was transformed with plasmids encoding HsCDC7 and HsDBF4. After sporulation and tetrad dissection, spore colonies that were both G418 and ClonNAT resistant were obtained. These spore colonies were all His+, (Figure S3). Thus, we concluded that a complex of HsCdc7 and HsDbf4, comprising HsDDK, substitutes for ScDDK and that this complex is sufficient to support viability in a temperature-independent manner.

**Human Drf1 in yeast cells**

Many metazoans contain a second homolog to Dbf4 called Drf1 (Furukohri et al. 2003; Montagnoli et al. 2002; Yoshizawa-Sugata et al. 2005). Depletion of Drf1 from cultured human cells interferes with cell cycle progression, but the phenotype is not as severe as depletion of Cdc7 or Dbf4 (Yoshizawa-Sugata et al. 2005). To determine whether HsDRF1 would support viability in place of HsDBF4, we cotransformed HsDRF1 on either LEU2 2 μ or CEN plasmids with HsCDC7 into the cdc7Δ dbf4Δ strain maintained by a URA3 plasmid encoding ScCDC7 and ScDBF4. Spotting the transformants on 5-FOA determined that HsDRF1 (with HsCDC7) was unable to complement cdc7Δ dbf4Δ (Figure 3C) under conditions in which HsDBF4 supports viability. Western blotting of a strain containing myc-tagged Drf1 indicated that it was expressed (Figure S4). These results suggest that HsDBF4 is the true ortholog of ScDBF4.

**Human DDK supports yeast cell cycle progression**

To address whether the cell cycle proceeds normally in yeast supported by human DDK, we performed flow cytometry on asynchronous cultures and synchronized cells arrested in mid-S phase using HU, an inhibitor of ribonucleotide reductase that induces the S-phase checkpoint and prevents firing of late origins. In asynchronous cultures, there were similar ratios of replicated and unreplicated cells (2C and 1C, respectively) for the human DDK and yeast DDK strains (Figure 4, top panel). Flow cytometry was also performed on cells arrested with HU, and then at different times after release from the block. Strains bearing HsDDK showed a similar pattern, but they were slightly slower in progressing through the cell cycle than cells with ScDDK after release from HU, particularly in the initial recovery from HU (Figure 4, 30 min time point).

**Response of human DDK to genotoxic agents in yeast cells**

DDK is important for yeast to respond to replicative stress. Mutations of Dbf4 result in sensitivity to genotoxic agents (Fung et al. 2002; Gabrielse et al. 2006; Harkins et al. 2009; Njagi and Kilbey 1982; Varrin et al. 2005). To examine whether HsDDK substitutes for ScDDK in this regard, we grew yeast strains containing human or yeast DDK under conditions of replicative stress. As shown in Figure 5, strains containing HsDDK were more sensitive to HU compared with strains containing ScDDK. The sensitivity to HU was independent of the DDK source on 5-FOA determined that HsDDK were more sensitive to HU compared with strains containing ScDDK. The sensitivity to HU was independent of HsCDC7 copy number. Note that in these experiments, the exposure to HU was continuous, whereas in the flow cytometry experiments the exposure to HU was relatively brief. A similar phenotype was observed with S. cerevisiae Dbf4 mutants (Jones et al. 2010). No changes in growth were noted with HsDDK on plates containing methyl methanesulfonate (MMS), a DNA-damaging agent (Figure 5).

**Human DDK does not support sporulation of yeast**

DDK is required for recombination and segregation events during meiosis in yeast (Marston 2009). To examine whether human DDK can substitute for yeast DDK during meiosis, we generated diploid homozygous cdc7Δ dbf4Δ strains bearing either ScDDK (CY5627) or HsDDK (CY5628) on plasmids. Each of the diploid strains was incubated in 1% potassium acetate for 5 to 7 days, and then examined for tetrads. Ten out of 10 sporulation cultures of the ScDDK strain contained tetrads, whereas none of the HsDDK sporulation cultures contained tetrads. Furthermore, the HsDDK cells had an abnormal appearance (Figure 6). The lack of tetrads with HsDDK was consistent with previous studies using mutations in DDK (Lo et al. 2008; Matos et al. 2008). The inhibition of sporulation by HsDDK was recessive as a strain with both human and yeast DDK (MDY270) contained tetrads after incubation in potassium acetate (Figure 6).
Region of Cdc7 required for species-specific formation of functional DDK

In S. cerevisiae, the region of Cdc7 that interacts with ScDbf4 maps to a 55 amino acid residue region at the C-terminal of ScCdc7 using yeast two-hybrid analysis (Jackson et al. 1993). While this region interacts with Dbf4, no information is available on whether the C-terminal 55 amino acid residues of ScCdc7 are sufficient to direct formation of a functional DDK complex. To address this, we created an allele in which amino acid residues 523-574 of HsCdc7 were replaced with residues 450-507 of ScCdc7 (Figure 7A). This chimeric allele was expressed on a LEU2 2 μ plasmid that was then shuffled into MDY99 (cdc7::KanMX ScCDC7-URA3). Cells containing the hybrid protein (Cdc7-S1) grew as well as cells containing ScCdc7 on 5-FOA (Figure 7B). In contrast, when shorter regions of the C-terminus (Cdc7-S2 and Cdc7-S3 in Figure 7A) were substituted, the hybrid proteins were unable to support viability of a cdc7Δ strain. Western blotting of the swapped constructs, tagged with myc9, indicated that the proteins were expressed (Figure S5). These results thus identify the C-terminal 55 amino acid residues as critical in defining the species specificity of S. cerevisiae Cdc7.

To determine whether replacement of the C-terminal 52 amino acid residues of HsCdc7 with yeast sequence interferes with the interaction between HsCdc7 and HsDbf4, we tested whether CDC7-S1 forms functional DDK with HsDbf4. As shown in Figure 7C, CDC7-S1 did not support growth of a dbf4Δ HsDbf4 strain, identifying a key role of the C-terminal 52 amino acid residues of HsCdc7 in species-specific formation of functional DDK, consistent with the requirement of amino acid residues 566-572 for interaction with HsDbf4 (Kitamura et al. 2011). We were unable to examine whether the shorter swaps (Cdc7-S2 and Cdc7-S3) support viability of dbf4Δ HsDbf4 because we did not obtain transformants with these plasmids.

As shown above, strains containing HsDbf4 are sensitive to HU. To address whether this HU sensitivity is due to HsCDC7 or HsDBF4, we compared the growth of the cdc7Δ strains containing plasmid-encoded ScCDC7 or CDC7-S1 and ScDBF4 on YPD and HU. The CDC7-S1 strain grew comparably to the ScCDC7 strain on both media, suggesting that the Dbf4 subunit provides the target specificity for the response to HU.

We also examined whether CDC7-S1 supports sporulation; recall that the diploid strain with HsDDK does not form tetrads. We transformed CDC7-S1 HIS3 and ScDBF4 LEU2 into CY5627 (cdc7Δ/cdc7Δ dbf4Δ/dbf4Δ ScCDC7-ScDBF4-URA3), treated the cells with 5-FOA to remove ScCDC7, and then incubated the resulting strain (MDY318) in potassium acetate. As seen in Figure 7E, tetrads were detected, suggesting that the defect in sporulation with HsDDK was in HsDbf4.

DISCUSSION

We established that the essential function of DDK is conserved between yeast and humans despite limited sequence conservation between the Dbf4 orthologs. These findings also demonstrate that it is their function as a complex rather than independent subunits that is essential for viability. Of note, in this two-component enzyme, the structure required for targeting of DDK to its essential substrates is conserved, but the regions required for interaction of the component proteins, Cdc7 and Dbf4, are not.

In yeast, the Mcm4 subunit of Mcm2-7, the replicative helicase, is the essential substrate of DDK. Phosphomimetic substitutions at the DDK target sites of Mcm4 bypass the requirement for CDC7 and/or DBF4 (Randell et al. 2010; Sheu and Stillman 2010). Targeting of Mcm4 is thought to occur through the Cdc7 subunit, which recognizes a “docking” site in a conserved region of Mcm4 (Sheu and Stillman 2006). Other Mcms are also substrates for DDK, including...
Mcm2 and Mcm6. Phosphorylation of Mcm6, with phosphorylation of Mcm4, is important for the initiation of DNA replication (Randell et al. 2010). Mutation of DDK-phosphorylation sites in Mcm2 does not affect viability; however, phosphorylation of Mcm2 by DDK may be important in the response to DNA damage (Randell et al. 2010; Stead et al. 2011).

**Checkpoint functions of DDK**

The sensitivity of the HsDDK-containing strain to HU but not MMS indicates that HsDDK can perform some, but not all, of ScDDK’s functions in response to stress and is consistent with reports indicating that different regions of Dbf4 are required for the response to HU and MMS (Fung et al. 2002; Gabrielse et al. 2006). Interestingly, the strain maintained by Cdc7-S1 and ScDbf4 was resistant to HU, implicating Dbf4 as the important subunit within DDK for providing resistance to HU, although it is still formally possible that the C-terminal 55 residues of ScCdc7 are important for resistance to HU.

The ability of ScDbf4 to confer resistance to HU suggests that it may have important roles in defining target molecules during the response to HU. Indeed, recognition of Mcm2 by DDK is thought to occur through the Dbf4 subunit in yeast. Mutations in Dbf4 that interfere with Mcm2 interaction include those that lead to sensitivity to HU (Jones et al. 2010). Interestingly, the sensitivity of HsDDK strains to HU is suppressed by a version of Mcm2 with phosphomimetic glutamic acid residue substitutions at the DDK target sites S164 and S170 (Stead et al. 2011). The targeting of Mcm2 by Dbf4 contrasts with the observation that Mcm4 recognition occurs through Cdc7 (Sheu and Stillman 2006; Bernard Duncker, personal communication) and provides an explanation for conservation of the sequences required to target Mcm4 but not Mcm2. Interestingly, ScDbf4 (with Cdc7-S1) also rescued the defect in sporulation with HsDDK. Not surprisingly, interaction of ScDDK with Cdc5, required for meiosis, is mediated by ScDbf4 (Chen and Weinreich 2010; Matos et al. 2008; Miller et al. 2009).

**Species-specific Cdc7-Dbf4 interaction**

Jackson et al. (1993) found the C-terminal 55 amino acid residues of ScCdc7 sufficient for interaction with Dbf4 in a yeast two-hybrid assay. We determined that the C-terminal 55 amino acid residues of yeast Dbf4 are necessary and sufficient for species-specific function of functional DDK. Swapping shorter portions of the C-terminus did not support formation of a functional DDK with ScDbf4, suggesting...
that the region encompassing amino acid residues 449-561 of ScCdc7 is required for interaction with ScDbf4. The C-terminus of HsCdc7 is likely required for formation of functional DDK with HsDbf4, as replacement of the human C-terminal 52 amino acid residues with yeast sequence was not functional with HsDbf4.

To examine whether the C-terminal residues of yeast and human Cdc7 are conserved through fungi and animals, we compared the ~55 C-terminal residues from various organisms (Figure 8). The human sequence was strongly conserved among many metazoan species, particularly in the C-terminal proximal residues (residues 534-573 of human). Note that this region includes Motif XI of the eukaryotic kinase domain (Hanks et al. 1988; Hess et al. 1998). The strong similarity of the C-terminal region in multicellular eukaryotes suggests there is a selective pressure to maintain this sequence at this position of the protein. Many of these species also contain a second Dbf4 subunit (Drf1) or, in the case of mouse, two Dbf4 isoforms. Therefore, one pressure to maintain the C-terminal sequence may be its requirement to interact with two different subunits. Of note, the C-terminus of the D. melanogaster Cdc7 protein (NP 727103) showed less similarity to human Cdc7 than the Cdc7 C-termini from other metazoans, even though D. melanogaster is predicted to encode two Dbf4 isoforms (NP 723965.1 and NP 523583.2). We speculate that this result from the fruit fly encoding a second Cdc7-like protein (NP 609876.2). The similarity among fungal species was not as strong as the similarity between most metazoan species. Interestingly, the strongest region of similarity within the fungal species corresponded to residues 441-523 of ScCdc7, which includes Motif XI and is consistent with the requirement of residues 449-561 for interaction with ScDbf4. With the exception of S. pombe, which encodes a second sporation-specific Cdc7-Dbf4 complex (Nakamura et al. 2002), the fungal species contain only single Cdc7 and Dbf4 subunits. Our data suggest that Motif XI of Cdc7 encodes species-specific interaction with Dbf4.

Implications for synthetic biology

To our knowledge, this is the first instance where complementation of a kinase deletion in yeast by the human ortholog requires the regulatory subunit. The complementation of a S. pombe cdc2 deletion was famously used to clone human cdc2, a cyclin-dependent kinase, but it did not require expression of human cyclins to function (Lee and Nurse 1987). In addition, the human gene encoding the catalytic subunit of casein kinase II (CK2α) complements deletion of the S. cerevisiae genes (there are two different catalytic subunits in budding yeast) without need of the regulatory subunit (Dotan et al. 2001). With DDK, the requirement of the regulatory subunit to achieve complementation serves as a guide for creation of hybrid synthetic genomes where consideration of the structure/function of multicomponent enzymes will be required. Of note, the component genes of DDK are found on different arms of the same chromosome (IV) in S. cerevisiae but on different chromosomes (1 and 7) in humans.

The species specificity of Cdc7-Dbf4 interaction is a potential area to exploit for the development of anti-mycotic drugs. Additionally, this genetically amenable system can be used to rapidly probe the key structure/function relationships of HsCdc7 and its interactions with potential cancer therapeutics. Human DDK in yeast may also prove useful for study of DDK’s nonessential roles in DNA-damage response and meiosis.

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LITERATURE CITED


Jiang, W., D. McDonald, T. J. Hope, and T. Hunter, 1999 Mammalian Cdc7-Dbf4 protein kinase complex is essential for initiation of DNA replication. EMBO J. 18: 5703–5713.


Steinberg, F. E., C. J. Brandl, and M. J. Davey, 2011 Phosphorylation of Mcm2 modulates Mcm2-7 activity and affects the cell's response to DNA damage. Nucleic Acids Res. DOI: 10.1093/nar/gkr371.


Weinreich, M., and B. Stillman, 1999 Cdc7-Dbf6p kinase binds to chromatin during S phase and is regulated by both the APC and the RAD53 checkpoint pathway. EMBO J. 18: 5334–5346.


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