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

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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. HsDrf1 (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's 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 (Bousset and Diffley 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 Sclafani 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 Arai 2000; Ogino *et al.* 2001). A second Dbf4-like subunit found in many metazoans, Drf1 (Dbf4-related factor 1, also called DBF4B or ASKL1) 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 DIAP1, also referred to as DRF1 (diaphanous-related formin 1) in
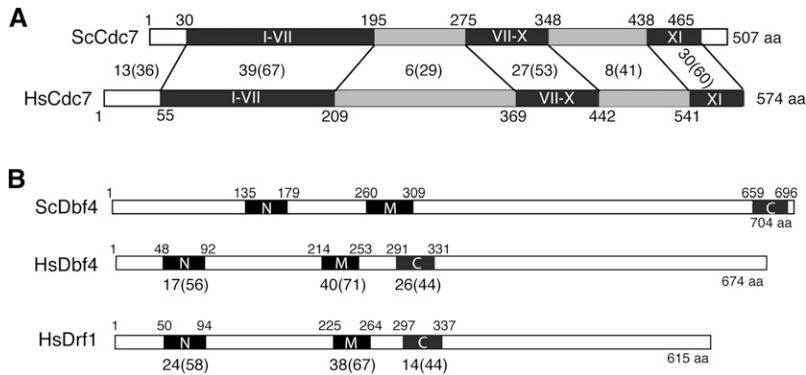
Figure 1 Human and yeast Cdc7 and Dbf4/Drf1. A) Comparison of the primary structure of Cdc7 from human (HsCdc7) and yeast (ScCdc7). The positions of conserved subdomains found in the eukaryotic protein kinase superfamily are indicated by black boxes. Kinase inserts, characteristic of the Cdc7 family, are indicated by gray boxes. 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.

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 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 DDK's essential activity in *S. cerevisiae*. We find that human (Hs) *CDC7* and *DBF4*, but not *DRF1*, complement deletion of the yeast genes. Both *HsCDC7* and *HsDBF4* must be present for complementation, a result that agrees with a lack of interaction between the yeast and human proteins. Yeast strains with human DDK are sensitive to hydroxyurea (HU), a phenotype associated with some Dbf4 mutations (Gabrielse *et al.* 2006; Harkins *et al.* 2009; Jones *et al.* 2010), and 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 with ScDbf4 but not HsDbf4. Interestingly, changing the Dbf4 specificity of HsCdc7 to ScDbf4 relieves the HU sensitivity, suggesting that

resistance to HU is provided by Dbf4. We thus demonstrate that the recognition of essential targets is conserved between DDK of different species despite the lack of cross-species interaction between the subunits. The results have implications for the study of Cdc7 and Dbf4 as targets for drug therapies and in the development of synthetic genomes.

MATERIALS AND METHODS

Plasmids

All molecules were amplified by PCR using *Pwo* polymerase (Roche) and the primers in Table S1. A 3 kb *Sal*I-*Sbf*I fragment containing the promoter and coding regions of *ScDBF4* was cloned into the *URA3* centromeric plasmid YCplac33 (Gietz and Sugino 1988). *ScCDC7*, *HsCDC7*, and *HsDBF4* were expressed from the *ScCDC7* promoter, inserted as *Sma*I-*Sal*I fragments into YCplac33 (*URA3-CEN*) or YCplac111 [*LEU2-CEN*; (Gietz and Sugino 1988)]. Coding sequences were inserted using an *Nde*I site placed at the ATG start codon and downstream *Hin*dIII (*ScCDC7*), *Sbf*I (*HsCDC7*, *HsDBF4*), or *Nde*I sites (*HsDRF1*). cDNA clones for human *CDC7*, *DBF4*, and *DRF1* were purchased from Open Biosystems (accession numbers BC11044, BC047693, and MHS1011-74961). Human genes were
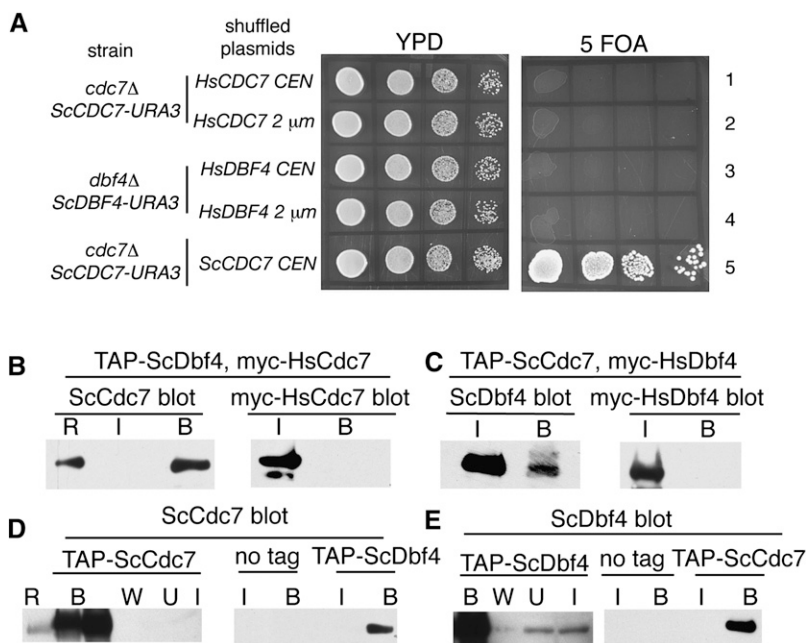

Figure 2 Human *CDC7* and *DBF4* do not interact with their yeast counterparts. A) Haploid *cdc7* (rows 1, 2, and 5) or *dbf4* (rows 3 and 4) deletion strains [BY23713, *cdc7Δ0*; BY23988, *dbf4Δ0*; (Giaever *et al.* 2002)] transformed with the cognate yeast gene on a *URA3* encoding plasmid (Gietz and Sugino 1988) were transformed with the indicated *CEN* or 2 μ 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° 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 ScCdc7 or *myc*^{9}-HsCdc7 by Western blotting. "R" indicates recombinant yeast Cdc7 [∼100 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 *myc*^{9}-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.
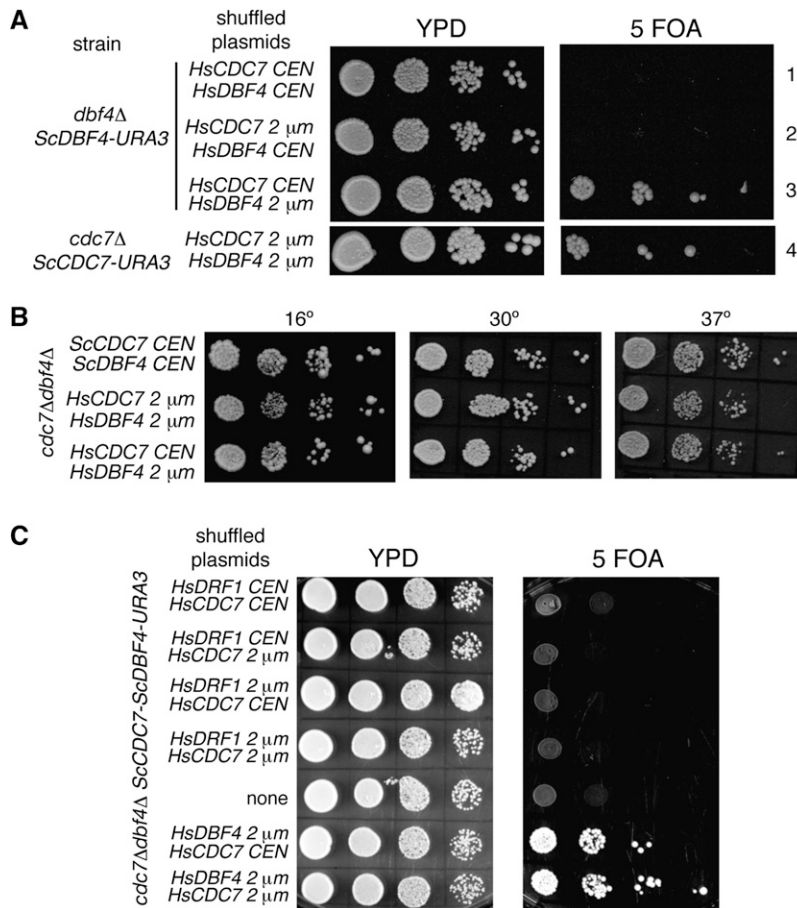
Figure 3 Complementation of *cdc7Δdbf4Δ* by human DDK. A) Haploid strains deleted for *dbf4* or *cdc7* (Giaever *et al.* 2002) and bearing the indicated plasmids encoding yeast and human *CDC7* and/or *DBF4* were spotted on YPD and 5-FOA plates and then grown at 30° for two and three days, respectively. B) Haploid strains deleted for *cdc7* and *dbf4* (*cdc7::KanMX dbf4:: NatMX*) and bearing the indicated plasmids encoding yeast or human *CDC7* and *DBF4* were spotted on YPD plates and then grown at 16° for seven days, or 30° or 37° for two days. C) MDY214 was transformed with CEN or multicopy plasmids encoding *HsDRF1* and *HsCDC7* as indicated. Five thousand cells and 10-fold serial dilutions were plated on YPD and 5-FOA and grown at 30° for one and three days. As a control, MDY214 transformed with *HsCDC7* and *HsDBF4* is also shown (bottom rows).

transferred to the 2 μ *LEU2* episomal plasmid YEplac181 (Gietz and Sugino 1988) using *SacI-Sbf*I (*HsCDC7*, *HsDBF4*) or *SmaI-Bam*HI (*DRF1*). The *LEU2* markers of YCplac111-*HsCDC7* and YEplac181-*HsCDC7* were switched to *HIS3* with pLH7 (Cross 1997), yielding YCplac111h-*HsCDC7* and YEplac181h-*HsCDC7*. The *URA3* marker on YCplac33-*ScDBF4* was switched to *LEU2* with pUL9 (Cross 1997). *Myc*[9]-tagged versions of the human proteins were inserted into YCp88-*myc*[9] (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-XbaI* 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 *NdeI-Sbf*I fragments into YEplac181 with the *ScCDC7* promoter to yield YEplac181-*CDC7-S1*, YEplac181-*CDC7-S2*, and YEplac181-*CDC7-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: *MAT*a, *cdc7Δ0*,

YCplac33-*ScCDC7*; CY4104: *MAT*α, *dbf4Δ0*, YCplac33-*ScDBF4*). To generate a diploid strain deleted for *cdc7* and *dbf4*, *KanMX* in CY4104 was switched to *NatMX* by transformation with linearized p4339 (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 YEplac181-*HsDBF4* and YCplac111h-*HsCDC7* or YEplac181h-*HsCDC7*, sporulated and His+, Leu+, Ura−, 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+, Ura+, and *MAT*a 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 (*MAT*α *cdc7::KanMX dbf4::NatMX* YCplac111h-*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 (*MAT*α *cdc7::KanMX dbf4:: NatMX* YCplac33-*ScCDC7-ScDBF4*). For homozygous diploid strains deleted at *cdc7* and *dbf4*, CY4242 (*MAT*a *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 DDK (CY5628; *MAT*a/*MAT*α *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 (*MAT*a/*MAT*α *cdc7::*
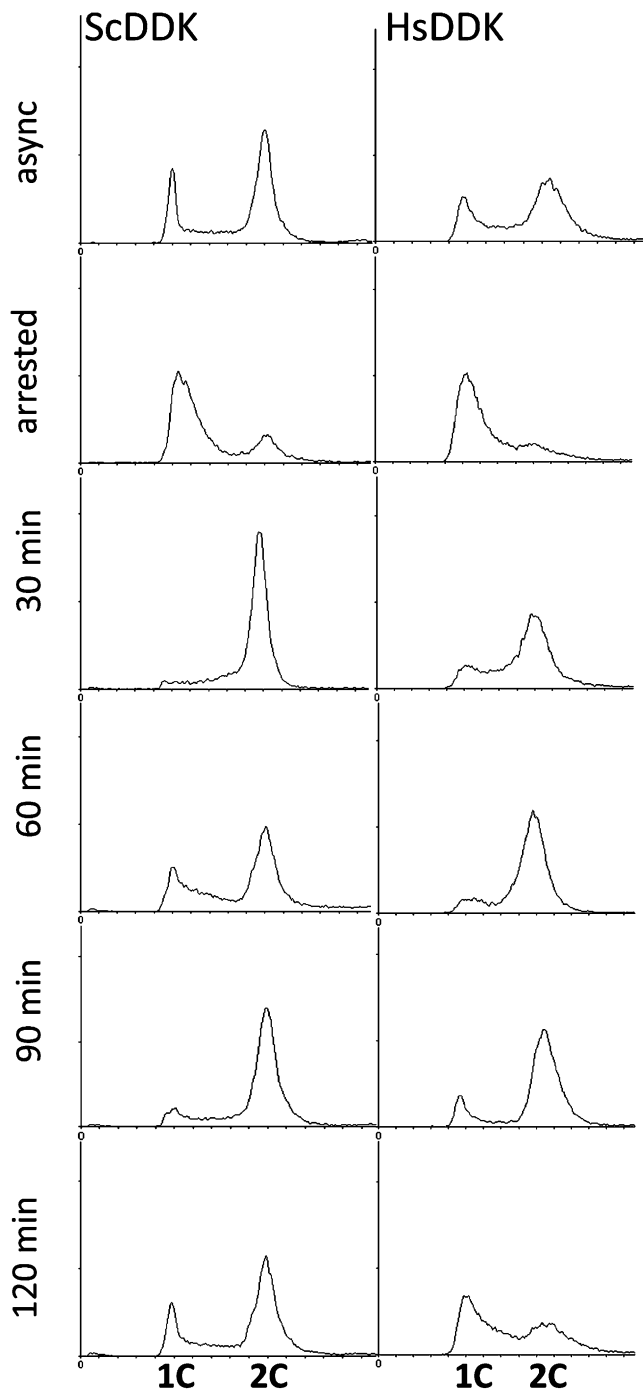
Figure 4 Effect of human DDK on the cell cycle progression of yeast. The progression of the cell cycle after arrest and release with HU was examined for a strain containing HsDDK (CY4240; cdc7::KanMX dbf4:: NatMX YCplac111h-HsCDC7 YCplac181-HsDBF4) or plasmid-encoded ScDDK (CY4481; cdc7::KanMX dbf4::NatMX, YCplac111-ScCDC7, YCplac33-ScDBF4). Log phase cells were incubated in 100 mM hydroxyurea (Sigma-Aldrich) for 2 h at 30°, washed twice, and then resuspended in fresh YPD. Aliquots were removed at the indicated times and subsequently examined for DNA content by FACS. The top panel is the analysis of asynchronously growing cells. The positions of cells containing 1C and 2C DNA content are indicated below.

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°, 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°, beads were collected and the supernatant removed. The beads were washed twice with 1 ml of lysis buffer, once in 200 μl lysis buffer, and then incubated in 50 μl SDS loading dye at 90° 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 chemiluminscence 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 μ 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 *cdc7Δ* strain. In similar tests, *HsDBF4* did not support growth of *dbf4Δ* (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^9-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^9-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^9-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
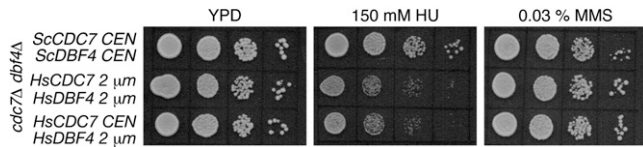
Figure 5 Effects of human DDK on the response of yeast to genotoxic agents. The haploid strain (cdc7::KanMX dbf4::NatMX) deleted for both cdc7 and dbf4 and bearing the indicated plasmids encoding yeast or human CDC7 and DBF4 were spotted (5000 cells and 10-fold serial dilutions) on YPD plates as well as YPD plates containing 150 mM HU or 0.03% MMS. Cells were grown for two days at 30°.

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 HIS3-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+ (HsCDC7) and Leu + (HsDBF4), indicating that HsDDK substitutes for ScDDK's essential function. PCR analysis confirmed that neither ScCDC7 nor ScDBF4 were present in the HsDDK strains (Figure S2). No major differences in growth between cells containing plasmid-encoded human or yeast DDK were noted at 16°, 30°, or 37° (Figure 3B). In addition, cells bearing HsDDK grew at the same rate in liquid media as strains containing plasmid-encoded ScDDK (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
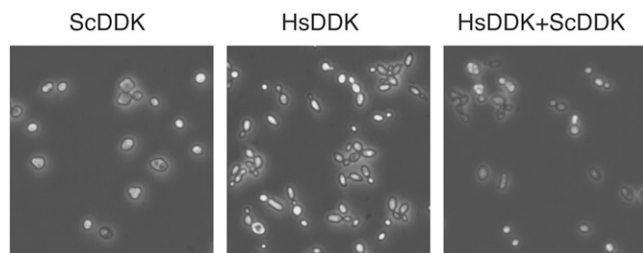

Figure 6 HsDDK diploids do not support meiosis. Light microscope images (400×) of diploid strains deleted at cdc7 and dbf4 with plasmid-encoded HsDDK and/or ScDDK. Images are of strains after at least five days incubation in 1% potassium acetate at 30°.

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 of yeast 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 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).
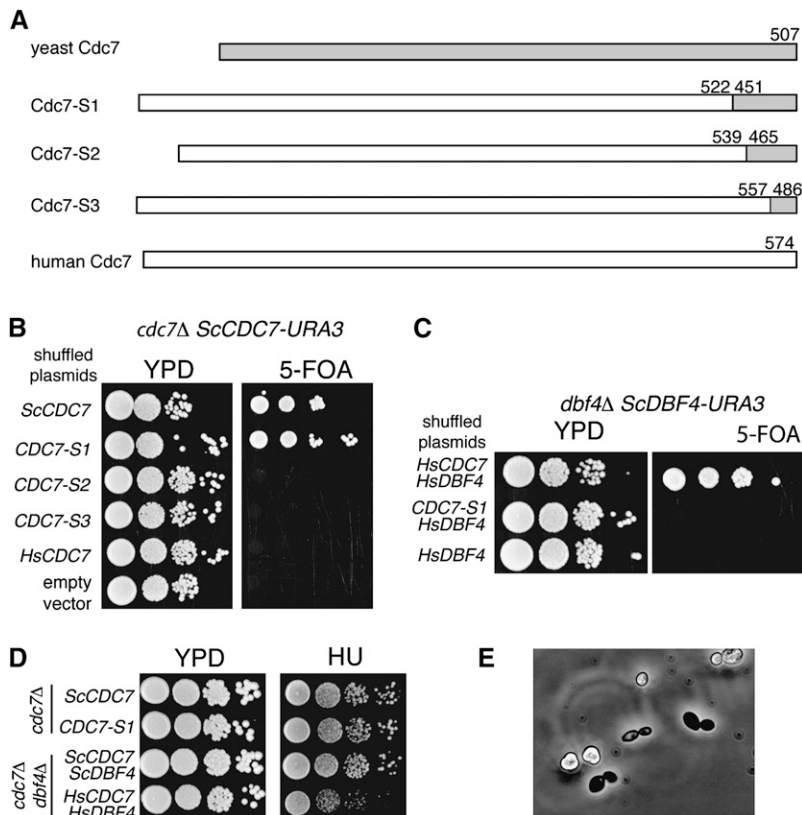
Figure 7 Definition of the species-specific interaction region in Cdc7. A) Positions of each swap are shown, as are schematics of the full-length proteins, with human sequences in white and yeast sequences in gray. B) Haploid *cdc7Δ* strains supported with *URA3-ScCDC7* were transformed with *LEU2* plasmids encoding *HsCDC7*, *ScCDC7*, or the indicated yeast-human *CDC7* chimeras. The strains were diluted serially and then spotted on 5-FOA. C) Haploid *dbf4* deletion strain with *URA3-ScDBF4* was transformed with both *LEU2-HsDBF4* and a *HIS3* 2 μ plasmid encoding wild-type *HsCDC7* or a chimera. The strains were serially diluted and spotted on YPD and 5-FOA. D) Strains deleted at *cdc7* complemented by *ScCDC7* or *CDC7-S1* were diluted and spotted on YPD or YPD containing 150 mM HU. For comparison, strains deleted at both *cdc7* and *dbf4* containing either ScDDK or HsDDK were also spotted. E) Image of a tetrad after incubation of MDY318 in potassium acetate (400×).

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-terminus 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 myc[9], 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 HsDDK 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

A metazoans

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D. melanogaster  649  -EAEQTEQDQLANGTPRKMRRQRYQSHRTVAASCEQVKQQVALDLQQAAINKL--
P. pectinifera   516  SSRSPPPSRVQKQTG-KDCVPDSAYDLLRRLLDLNPKTRITADEALKHPEIVEC--
X. laevis        432  -STPDNMEQSNHNG-WDRVPNEAYHLLDRLLDMNPATRITAEEALIHPLFKNMR-
D. ranio         408  --LCRSSAELKEIG-WDRVPDEVYDLLDKLLDLNPATRITASTALQHPLFKDLSE
A. melanoleuca   524  --GSLNTDATNSEG-WNEVPDEAYDLLDKLLDLNPASRITAGEALLHPLFKDMSL
B. taurus        525  --GNFEESATNLEG-WDEVPDEAYDLLDKLLDLNPASRITAEEALLHPFFKDMSL
H. sapiens       523  --HCFDEYNTNLEG-WNEVPDEAYDLLDKLLDLNPASRITAEEALLHPFFKDMSL
P. abellii       495  --HCFDEYNTNLEG-WNEVPDEAYDLLDKLLDLNPASRITAEEALWHPFFKDMSL
M. mulatta       495  --CCFDEYTTNLEG-WNEVPDEAYDLLDKLLDLNPASRITAEEALLHPFFKDMSL
C. jacchus       496  --RCFDEYTTNLEG-WNDVPDEAYDLLDKLLDLNPASRITAEEALLHPFFKDMSL
R. norvegicus    514  -RRHPRDRTANPEG-WDSVPDDAYDLLDKLLDLNPASRITAEAAVHPFFKDVC-
C. griseus       521  --SRPRECTTNLEG-WDSVPDEAYDLLDKLLDLNPASRITAEAALLHPFFKDMSS
M. musculus      474  --SHPKDCTSNSEG-WDSVPDEAYDLLDKLLDLNPASRITAEAALLHAFFKDMCS
A. carolinensis  510  --VRITEKGADPKG-WDNVPDEAYDLLDKLLDLNPATRITAKDALLHPFFRNMKQ
M. gallopavo     505  --GASEIKVTDMKG-WDQVPDEAYDLLDKLLDLNPATRITADALLHPFFKDMRL
consensus                 .  .. *  .. ......*...................*........
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Motif XI

B fungi

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S. pombe         449  FDNSFGETSFEKDED---LTAKHLSHILDFK-----EQE-ETDEPTSLSKRKR--------------SIDEILPNDALQDGA--------
T. rubrum        411  -----------PSAL-LRWSADGALYHEFFTNPQGADLPWGGEDPANLEHHSD--------HGDDNDDTACDAGDEVKLI----------
A. gypseum       417  -----------LDISER-TRCTAARALQHDFFNNPEGDDLPWGGDPEQLQHHAT--------QHHDETACDAGDDVKLI----------
C. albicans      598  ----LLYGCFKMDPS-KRLSAREILQLPFFH-----EILQISEDDTQDEFMQQ-----------NQTQTQEEEEDDEVILS----------
P. pastoris      643  NALELLKACFQLNPN-KRILANDAMNMPFFD-----AIR------------------------GENEENDEIILD----------
C. glabrata      445  -------QCFKLDPS-KRSSADELLRSAFFN-----ELNVGAGESTEGELTDE--------DVSDTASEAASSDEEVMLIA----------
S. kluyveri      419  WCFQLLENCFRLDPH-KRSTAGELLASAFFD-----EYH-ESNATEEGS---------------DSISARGSGDSDVMIIEG--------
L. thermotolerans 423 WCFEVLEQCFALNPH-KRSIADELLSSTFFN-----ELI-ESDCTEE--------------------NDEDDVLTIEN----------
K. waltii        423  WCFEVLNQCFNLNPR-KRSIADELLSSTFFN-----ELI-ESDCTEE--------------------NDEDDVLVIED----------
K. polysporus    441  -----QQCFEMDBQ-KRSSADELIHSAFFY-----ELT-EAGETTEGEMTDD---------DLNNTDYSGVEDNDVLLISG--------
Z. rouxii        435  -----LEQCLEFDPQ-KRSSASELLQSAFFN-----ELN-EADIYTDVETTDE---------DQMSEEDQAVDDDLLLIPG--------
S. castellii     483  --------CFEMDPL-KRSSADELLHSQFFS-----ELN-ENGDETELEDTDDDFTNGASQDDVISSSQGDAVDDDVLLISDK-------
S. cerevisiae    441  --------CFEMDPQKKRSSAAEDLLKIQFFN-----ELN-ENTYLLDGESTDE--------DDVVSSSEADLLDKDVLLISE--------
S. bayanus       448  --------QCFEMDPQ-KRSSAEDLLKNPFFN-----ELN-ENAYLQDGDSTDE--------DDVVSSSEGDLLDKDVLLISE--------
K. lactis        424  WCFDLLAKCFELNSY-KRPSASDLLEHLWFK-----ELN-EDLLDRD-----------------QRREDHFTGVVE-------
A. gossypii      444  WCFDVLERCFEMDER-KRSSAEELLQHLLFS-----ELT-EHHEPDA-----------------EVQDEVITLESQPELKLHR
consensus              .  .......  ....*.... . *      ..  .E  PDA                          .......
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Motif XI

Figure 8 Alignment of Cdc7 C-terminal sequences. The C-terminal regions of Cdc7 orthologs from multicellular euakaryotes (A) and fungi (B) and were aligned using MUSCLE (Edgar 2004a, 2004b). Accession numbers of the full-length protein sequences are in Table S3. Black boxes indicate conserved residues and gray boxes similar residues in at least 50% of the sequences at the same position. The position of Motif XI is indicated for each alignment.

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 formation 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 results 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 sporulation-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.

LITERATURE CITED

Bousset, K., and J. F. Diffley, 1998 The Cdc7 protein kinase is required for origin firing during S phase. Genes Dev. 12: 480–490.

Brown, G. W., and T. J. Kelly, 1998 Purification of Hsk1, a minichromosome maintenance protein kinase from fission yeast. J. Biol. Chem. 273: 22083–22090.

Brown, G. W., and T. J. Kelly, 1999 Cell cycle regulation of Dfp1, an activator of the Hsk1 protein kinase. Proc. Natl. Acad. Sci. USA 96: 8443–8448.

Bruck, I., and D. Kaplan, 2009 Dbf4-Cdc7 phosphorylation of Mcm2 is required for cell growth. J. Biol. Chem. 284: 28823–28831.

Chen, Y. C., and M. Weinreich, 2010 Dbf4 regulates the Cdc5 Polo-like kinase through a distinct non-canonical binding interaction. J. Biol. Chem. 285: 41244–41254.

Costanzo, V., D. Shechter, P. J. Lupardus, K. A. Cimprich, M. Gottesman *et al.*, 2003 An ATR- and Cdc7-dependent DNA damage checkpoint that inhibits initiation of DNA replication. Mol. Cell 11: 203–213.

Cross, F. R., 1997 'Marker swap' plasmids: convenient tools for budding yeast molecular genetics. Yeast 13: 647–653.

Dolan, W. P., A. H. Le, H. Schmidt, J. P. Yuan, M. Green *et al.*, 2010 Fission yeast Hsk1 (Cdc7) kinase is required after replication initiation for induced mutagenesis and proper response to DNA alkylation damage. Genetics 185: 39–53.

Donaldson, A. D., W. L. Fangman, and B. J. Brewer, 1998 Cdc7 is required throughout the yeast S phase to activate replication origins. Genes Dev. 12: 491–501.

Dotan, I., E. Ziv, N. Dafni, J. S. Beckman, R. O. McCann *et al.*, 2001 Functional conservation between the human, nematode, and yeast CK2 cell cycle genes. Biochem. Biophys. Res. Commun. 288: 603–609.

Duncker, B. P., and G. W. Brown, 2003 Cdc7 kinases (DDKs) and checkpoint responses: lessons from two yeasts. Mutat. Res. 532: 21–27.

Edgar, R. C., 2004a MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5: 113.

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

Fung, A. D., J. Ou, S. Bueler, and G. W. Brown, 2002 A conserved domain of Schizosaccharomyces pombe dfp1+ is uniquely required for chromosome stability following alkylation damage during S phase. Mol. Cell. Biol. 22: 4477–4490.

Furukohri, A., N. Sato, H. Masai, K. Arai, A. Sugino *et al.*, 2003 Identification and characterization of a Xenopus homolog of Dbf4, a regulatory subunit of the Cdc7 protein kinase required for the initiation of DNA replication. J. Biochem. 134: 447–457.

Gabrielse, C., C. T. Miller, K. H. McConnell, A. DeWard, C. A. Fox *et al.*, 2006 A Dbf4p BRCA1 C-terminal-like domain required for the response to replication fork arrest in budding yeast. Genetics 173: 541–555.

Ghaemmaghami, S., W. K. Huh, K. Bower, R. W. Howson, A. Belle *et al.*, 2003 Global analysis of protein expression in yeast. Nature 425: 737–741.

Giaever, G., A. M. Chu, L. Ni, C. Connelly, L. Riles *et al.*, 2002 Functional profiling of the Saccharomyces cerevisiae genome. Nature 418: 387–391.

Gietz, R. D., and A. Sugino, 1988 New yeast-Escherichia coli shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene 74: 527–534.

Hanks, S. K., A. M. Quinn, and T. Hunter, 1988 The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science 241: 42–52.

Harkins, V., C. Gabrielse, L. Haste, and M. Weinreich, 2009 Budding yeast Dbf4 sequences required for Cdc7 kinase activation and identification of a functional relationship between the Dbf4 and Rev1 BRCT domains. Genetics 183: 1269–1282.

Hartwell, L. H., R. K. Mortimer, J. Culotti, and M. Culotti, 1973   Genetic control of the cell division cycle in yeast: V. Genetic analysis of cdc mutants. Genetics 74: 267–286.

Hess, G. F., R. F. Drong, K. L. Weiland, J. L. Slightom, R. A. Sclafani *et al.*, 1998   A human homolog of the yeast CDC7 gene is overexpressed in some tumors and transformed cell lines. Gene 211: 133–140.

Hoke, S. M., J. Genereaux, G. Liang, and C. J. Brandl, 2008   A conserved central region of yeast Ada2 regulates the histone acetyltransferase activity of Gcn5 and interacts with phospholipids. J. Mol. Biol. 384: 743–755.

Horton, R. M., Z. L. Cai, S. N. Ho, and L. R. Pease, 1990   Gene splicing by overlap extension: tailor-made genes using the polymerase chain reaction. Biotechniques 8: 528–535.

Jackson, A. L., P. M. Pahl, K. Harrison, J. Rosamond, and R. A. Sclafani, 1993   Cell cycle regulation of the yeast Cdc7 protein kinase by association with the Dbf4 protein. Mol. Cell. Biol. 13: 2899–2908.

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.

Jones, D. R., A. A. Prasad, P. K. Chan, and B. P. Duncker, 2010   The Dbf4 motif C zinc finger promotes DNA replication and mediates resistance to genotoxic stress. Cell Cycle 9: 2018–2026.

Katis, V. L., J. J. Lipp, R. Imre, A. Bogdanova, E. Okaz *et al.*, 2010   Rec8 phosphorylation by casein kinase 1 and Cdc7-Dbf4 kinase regulates cohesin cleavage by separase during meiosis. Dev. Cell 18: 397–409.

Kitada, K., L. H. Johnston, T. Sugino, and A. Sugino, 1992   Temperature-sensitive cdc7 mutations of saccharomyces cerevisiae are suppressed by the DBF4 gene, which is required for the G1/S cell cycle transition. Genetics 131: 21–29.

Kitamura, R., R. Fukatsu, N. Kakusho, Y. S. Cho, C. Taniyama *et al.*, 2011   Molecular mechanism of activation of human Cdc7 kinase: bipartite interaction with Dbf4/ASK stimulates ATP binding and substrate recognition. J. Biol. Chem. 286: 23031–23043.

Kumagai, H., N. Sato, M. Yamada, D. Mahony, W. Seghezzi *et al.*, 1999   A novel growth- and cell cycle-regulated protein, ASK, activates human Cdc7-related kinase and is essential for G1/S transition in mammalian cells. Mol. Cell. Biol. 19: 5083–5095.

Lee, M. G., and P. Nurse, 1987   Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2. Nature 327: 31–35.

Lo, H. C., L. Wan, A. Rosebrock, B. Futcher, and N. M. Hollingsworth, 2008   Cdc7-Dbf4 regulates NDT80 transcription as well as reductional segregation during budding yeast meiosis. Mol. Biol. Cell 19: 4956–4967.

Marston, A. L., 2009   Meiosis: DDK is not just for replication. Curr. Biol. 19: R74–R76.

Masai, H., and K. Arai, 2000   Dbf4 motifs: conserved motifs in activation subunits for Cdc7 kinases essential for S-phase. Biochem. Biophys. Res. Commun. 275: 228–232.

Masai, H., T. Miyake, and K. Arai, 1995   hsk1+, a Schizosaccharomyces pombe gene related to Saccharomyces cerevisiae CDC7, is required for chromosomal replication. EMBO J. 14: 3094–3104.

Matos, J., J. J. Lipp, A. Bogdanova, S. Guillot, E. Okaz *et al.*, 2008   Dbf4-dependent CDC7 kinase links DNA replication to the segregation of homologous chromosomes in meiosis I. Cell 135: 662–678.

Matsumoto, S., M. Shimmoto, N. Kakusho, M. Yokoyama, Y. Kanoh *et al.*, 2010   Hsk1 kinase and Cdc45 regulate replication stress-induced checkpoint responses in fission yeast. Cell Cycle 9: 4627–4637.

Miller, C. T., C. Gabrielse, Y. C. Chen, and M. Weinreich, 2009   Cdc7p-dbf4p regulates mitotic exit by inhibiting Polo kinase. PLoS Genet. 5: e1000498.

Montagnoli, A., R. Bosotti, F. Villa, M. Rialland, D. Brotherton *et al.*, 2002   Drf1, a novel regulatory subunit for human Cdc7 kinase. EMBO J. 21: 3171–3181.

Montagnoli, A., J. Moll, and F. Colotta, 2010   Targeting Cdc7 kinase: a new approach for cancer therapy. Clin. Cancer Res. 16: 4503–4508.

Nakamura, T., M. Nakamura-Kubo, and C. Shimoda, 2002   Novel fission yeast Cdc7-Dbf4-like kinase complex required for the initiation and progression of meiotic second division. Mol. Cell. Biol. 22: 309–320.

Njagi, G. D. E., and B. J. Kilbey, 1982   cdc7-1 a temperature sensitive cell-cycle mutant which interferes with induced mutagenesis in Saccharomyces cerevisiae. Mol. Gen. Genet. 186: 478–481.

Ogi, H., C. Z. Wang, W. Nakai, Y. Kawasaki, and H. Masumoto, 2008   The role of the Saccharomyces cerevisiae Cdc7-Dbf4 complex in the replication checkpoint. Gene 414: 32–40.

Ogino, K., T. Takeda, E. Matsui, H. Iiyama, C. Taniyama *et al.*, 2001   Bipartite binding of a kinase activator activates Cdc7-related kinase essential for S phase. J. Biol. Chem. 276: 31376–31387.

Ohtoshi, A., T. Miyake, K. Arai, and H. Masai, 1997   Analyses of Saccharomyces cerevisiae Cdc7 kinase point mutants: dominant-negative inhibition of DNA replication on overexpression of kinase-negative Cdc7 proteins. Mol. Gen. Genet. 254: 562–570.

Pessoa-Brandao, L., and R. A. Sclafani, 2004   CDC7/DBF4 functions in the translesion synthesis branch of the RAD6 epistasis group in Saccharomyces cerevisiae. Genetics 167: 1597–1610.

Randell, J. C. W., A. Fan, C. Chan, L. I. Francis, R. C. Heller *et al.*, 2010   Mec1 is one of multiple kinases that prime the Mcm2-7 helicase for phosphorylation by Cdc7. Mol. Cell 40: 353–363.

Rigaut, G., A. Shevchenko, B. Rutz, M. Wilm, M. Mann *et al.*, 1999   A generic protein purification method for protein complex characterization and proteome exploration. Nat. Biotechnol. 17: 1030–1032.

Sawa, M., and H. Masai, 2009   Drug design with Cdc7 kinase: a potential novel cancer therapy target. Drug Des. Devel. Ther. 2: 255–264.

Schultz, M. C., S. Y. Choe, and R. H. Reeder, 1991   Specific initiation by RNA polymerase I in a whole-cell extract from yeast. Proc. Natl. Acad. Sci. USA 88: 1004–1008.

Sheu, Y. J., and B. Stillman, 2006   Cdc7-Dbf4 phosphorylates MCM proteins via a docking site-mediated mechanism to promote S phase progression. Mol. Cell 24: 101–113.

Sheu, Y. J., and B. Stillman, 2010   The Dbf4-Cdc7 kinase promotes S phase by alleviating an inhibitory activity in Mcm4. Nature 463: 113–117.

Stead, B. 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.

Swords, R., D. Mahalingam, M. O'Dwyer, C. Santocanale, K. Kelly *et al.*, 2010   Cdc7 kinase - a new target for drug development. Eur. J. Cancer 46: 33–40.

Takahashi, T. S., and J. C. Walter, 2005   Cdc7-Drf1 is a developmentally regulated protein kinase required for the initiation of vertebrate DNA replication. Genes Dev. 19: 2295–2300.

Tong, A. H., and C. Boone, 2006   Synthetic genetic array analysis in Saccharomyces cerevisiae. Methods Mol. Biol. 313: 171–192.

Tsuji, T., S. B. Ficarro, and W. Jiang, 2006   Essential role of phosphorylation of MCM2 by Cdc7/Dbf4 in the initiation of DNA replication in mammalian cells. Mol. Biol. Cell 17: 4459–4472.

Tsuji, T., E. Lau, G. G. Chiang, and W. Jiang, 2008   The role of Dbf4/Drf1-dependent kinase Cdc7 in DNA-damage checkpoint control. Mol. Cell 32: 862–869.

Valentin, G., E. Schwob, and F. Della Seta, 2006   Dual role of the Cdc7-regulatory protein Dbf4 during yeast meiosis. J. Biol. Chem. 281: 2828–2834.

Varrin, A. E., A. A. Prasad, R. P. Scholz, M. D. Ramer, and B. P. Duncker, 2005   A mutation in Dbf4 motif M impairs interactions with DNA replication factors and confers increased resistance to genotoxic agents. Mol. Cell. Biol. 25: 7494–7504.

Wan, L., H. Niu, B. Futcher, C. Zhang, K. M. Shokat *et al.*, 2008   Cdc28-Clb5 (CDK-S) and Cdc7-Dbf4 (DDK) collaborate to initiate meiotic recombination in yeast. Genes Dev. 22: 386–397.

Weinreich, M., and B. Stillman, 1999   Cdc7p-Dbf4p kinase binds to chromatin during S phase and is regulated by both the APC and the RAD53 checkpoint pathway. EMBO J. 18: 5334–5346.

Yoshizawa-Sugata, N., A. Ishii, C. Taniyama, E. Matsui, K. Arai *et al.*, 2005   A second human Dbf4/ASK-related protein, Drf1/ASKL1, is required for efficient progression of S and M phases. J. Biol. Chem. 280: 13062–13070.

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