

# Complementation used to clone a human homologue of the fission yeast cell cycle control gene *cdc2*

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*A human homologue of the cdc2 gene has been cloned by expressing a human cDNA library in fission yeast and selecting for clones that can complement a mutant of cdc2. The predicted protein sequence of the human homologue is very similar to that of the yeast cdc2 gene. These data indicate that elements of the mechanism by which the cell cycle is controlled are likely to be conserved between yeast and humans.*

THE *cdc2* gene plays an important role in controlling the cell cycle of the fission yeast *Schizosaccharomyces pombe*<sup>1,2</sup>. In this article we describe the isolation and characterization of a *cdc2* homologue from human cells. The gene was cloned by expressing a human cDNA library in *S. pombe* and selecting those clones which could complement a mutation in the *cdc2* gene. This method is applicable for isolating other mammalian genes for which mutants in equivalent genes are available in yeast. The human *CDC2* gene has been sequenced, and potentially encodes a protein of the same molecular weight as *S. pombe cdc2* with a 63% identity of amino-acid residues. This functional and structural similarity indicates that elements of cell cycle control are likely to be conserved between yeast and humans, and therefore will probably be found in all eukaryotic cells.

## Cell cycle control

The *cdc2* gene encodes a function which is required at the two major control points during the *S. pombe* mitotic cell cycle<sup>1,2</sup>. The first control is called start and is located in G1 at the point where the cell becomes committed to the mitotic cell cycle<sup>3</sup>. Once past start, the cell cannot undergo the alternative developmental fate of conjugation until the cycle in progress is completed, and the cell also begins the programme of events which lead to S-phase<sup>4</sup>. The second control is located in G2 and determines when the cell initiates mitosis<sup>1,2,5,6</sup>. Recessive temperature sensitive lethal *cdc2* mutants which lack *cdc2* function are unable to proceed past either the G1 or G2 control points<sup>7</sup>. Dominant *cdc2* mutants which contain a *cdc2* function with altered regulatory properties traverse G2 more rapidly and initiate mitosis and cell division at a reduced cell size compared with wild type cells<sup>8</sup>. The *cdc2* gene has been shown to encode a phosphoprotein of relative molecular mass 34,000 (*M<sub>r</sub>* 34K) which has protein kinase activity<sup>9</sup>. In nutrient-deprived cells the protein is dephosphorylated and has no kinase activity. On readdition of nutrients the protein gains kinase activity and becomes phosphorylated. This suggests that entry into the mitotic cell cycle could be regulated by modulating *cdc2* protein kinase activity, possibly by phosphorylation<sup>9</sup>. Three genes are important in controlling the *cdc2* gene function at the initiation of mitosis; *cdc25*<sup>10,11</sup> and *nim1*<sup>12</sup> act as activators and *wee1*<sup>8,13</sup> acts as an inhibitor. A fourth gene *suc1* probably also interacts with *cdc2* gene function<sup>14,15</sup>.

A gene analogous to *cdc2* is found in the evolutionarily divergent budding yeast, *Saccharomyces cerevisiae*, and is called *CDC28*<sup>16</sup>. This gene function is required at start<sup>17</sup> and probably later in the cycle for mitosis<sup>18</sup>. It encodes a 36K phosphoprotein with protein kinase activity<sup>19</sup>, and has 62% identity in protein sequence to the *cdc2* gene product<sup>20</sup>. The similarities in structure and function of the *cdc2* and *CDC28* genes indicate that aspects of cell cycle control are conserved in the two yeasts.

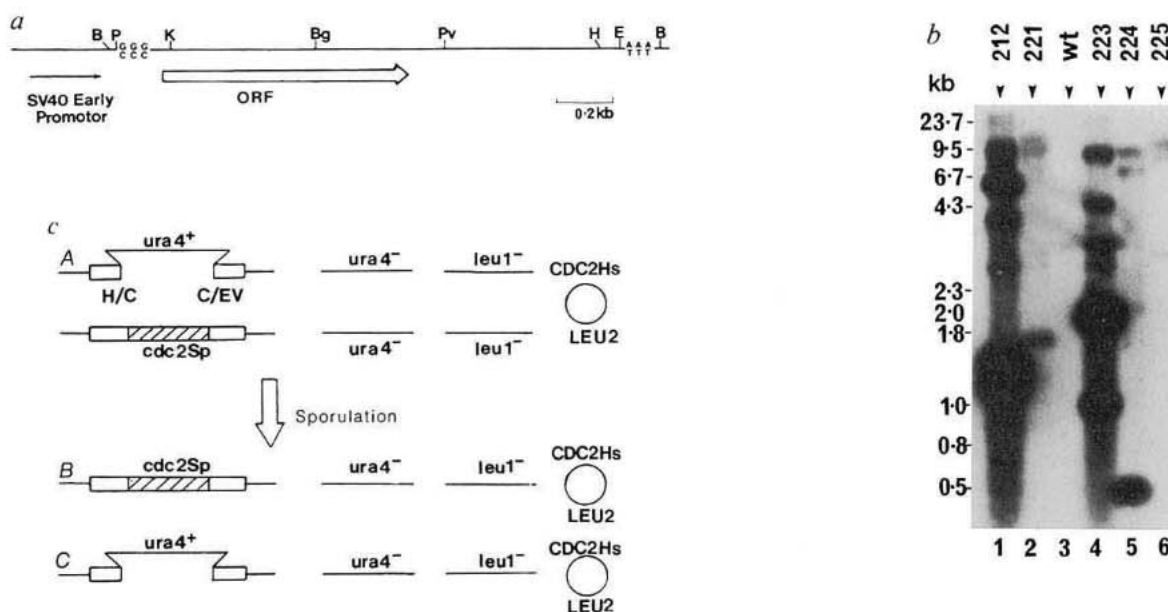
## Strategy for cloning

Three strategies can be employed to clone human homologues of known yeast genes: 1, low stringency hybridization to detect shared nucleotide sequences; 2, antibody screening of expression libraries to detect shared structural features; 3, complementation of mutants to detect genes with similar functions. We have used the third of these strategies, employing a human cDNA library to select for a gene that can complement a defective *cdc2* function. This approach enables genes to be isolated not because of their structural similarities but because they can provide a similar function<sup>21,22</sup>. A human cDNA library was used because all mammalian introns cannot be expected to be spliced out in *S. pombe*. Because plasmids do not require an autonomously replicating sequence (ARS) for *S. pombe* transformation<sup>23</sup> and the SV40 early promoter works well in *S. pombe*, we were able to use the excellent full-length Okayama and Berg human cDNA library made in an SV40 expression vector<sup>24</sup>. This library was co-transformed with another vector containing a selectable marker allowing cells that had taken up plasmid to be tested for their ability to grow in the absence of the *cdc2* gene function. The vector used was pDB262 which transforms *S. pombe* very efficiently and contains the *S. cerevisiae* *LEU2* gene able to complement *S. pombe* *leu1-32* (ref. 25).

## Cloning *CDC2Hs*

The *S. pombe* temperature sensitive mutant strain *cdc2-33 leu1-32* was co-transformed with the plasmid vector pDB262 and the human cDNA library in the Okayama and Berg expression vector. A total of 10<sup>6</sup> leucine prototrophic transformants were grown for 24 h at the permissive temperature of 29 °C and were then shifted to the restrictive temperature of 36 °C. Five transformants were found to form colonies. All of these were unstable for growth at 36 °C, indicating that the complementing activity was located on a plasmid. Two to four library plasmids were recovered into *E. coli* from each of the original five transformants. Each of these was tested for the ability to complement *cdc2-33* on retransformation with pDB262 back into the *cdc2-33 leu1-32 S. pombe* strain. Two plasmids, pOB231 and pOB245, isolated from two different original yeast transformants, were found to complement the *cdc2-33* mutation. Restriction mapping demonstrated that the plasmids were identical, each containing a similar 2-kilobase (kb) insert. A restriction map of this is shown in Fig. 1a. *Bam*HI sites are located just outside the GC and AT tails in the vector<sup>24</sup>, and thus a *Bam*HI digest of pOB231/245 removes the cDNA insert together with a small amount of flanking vector sequence. To establish if the other three original yeast transformants contained sequences related to pOB231/245, a Southern blot was made of *Bam*HI digested DNA prepared from the five yeast strains and was probed with





**Fig. 1** *a*, Restriction map of the pOB231/245 insert. The restriction sites are B, *Bam*HI; P, *Pst*I; K, *Kpn*I; Bg, *Bgl*II; PV, *Pvu*II; H, *Hind*III; E, *Eco*RI. The positions of the SV40 early promoter, the sites of the GC and AT tails used in the library construction, and the open reading frame (ORF) are shown. *b*, Southern blot of *Bam*HI-digested DNA made from wild-type cells and the five transformants probed with the cDNA insert of pOB231. Lane 1, strain 212; lane 2, 221; lane 3, wild type; lane 4, 223; lane 5, 224; lane 6, 225. The plasmids pOB231 and pOB245 were derived from strains 223 and 224 respectively. The arrow marks the size of the cDNA insert. *c*, Scheme illustrating the construction of the *cdc2* deletion strain containing pSAB2Hs. *a*, The original diploid strain containing one chromosome homologue with an intact *cdc2* gene (called *cdc2*Sp) showing the ORF as hatched, and a second homologue with the deleted gene and an insertion of *ura4*<sup>+</sup>. The entire *cdc2*Sp ORF has been deleted between the *Hind*III (H) and *Eco*RV (EV) sites and the *ura4*<sup>+</sup> gene inserted after addition of *Cla*I (C) linkers. *b*, On sporulating the diploid, half of the spores contained the homologue with the intact *cdc2*Sp gene and thus were uracil auxotrophs. *c*, The other half of the spores contained the homologue deleted for the *cdc2*Sp gene with the *ura4*<sup>+</sup> insert. Those spores also containing pSAB2Hs formed uracil and leucine prototrophic colonies. *d*, Cells of the *cdc2*Sp deletion strain containing pSAB2Hs. Cells containing the plasmid continue to divide, forming normal sized cells. On plasmid loss the cells become highly elongated and no longer divide.

**Methods.** *a*, The *S. pombe* strain *cdc2-33 leu1-32* was transformed as described in refs 37 and 38 using 100 µg of the Okayama Berg cDNA library<sup>24</sup> and 100 µg of pDB262 mixed with  $3 \times 10^9$  sphaeroplasts. Library plasmids were recovered back into *E. coli* strain JA226, selecting for ampicillin resistance as described in ref. 38. *b*, DNA was prepared from the *S. pombe* strains as described<sup>39</sup> *Bam*HI digested DNA was probed with the purified 2-kb *Bam*HI fragment from pOB231 made radioactive by oligolabelling using hexadeoxynucleotide primers<sup>40</sup>. *c*, The gene replacement was carried out using the one step procedure<sup>41</sup> as modified for *S. pombe*<sup>11</sup>. The plasmid pSAB2Hs containing the pOB231 insert (called CDC2Hs) and LEU2 was transformed into this diploid.

the insert prepared from pOB231. Hybridizing sequences were detected in all five transformants (Fig. 1b, tracks 1, 2, 4, 5, 6) whereas no hybridization was seen in an untransformed strain (Fig. 1b, track 3). Considerable plasmid rearrangement appears to have taken place in all five yeast strains. Only the two yeast transformants which yielded pOB231 and 245 (Fig. 1b, tracks 4, 5) contained a band corresponding in size to the 2-kb insert found in pOB231/245.

These experiments establish that all of the original yeast transformants contained sequences related to the insert of pOB231 and that this plasmid could complement the *cdc2-33* mutation. Our failure to recover complementary plasmids from the three other yeast transformants was probably due to plasmid rearrangements occurring in yeast which prevented their recovery in *E. coli*.

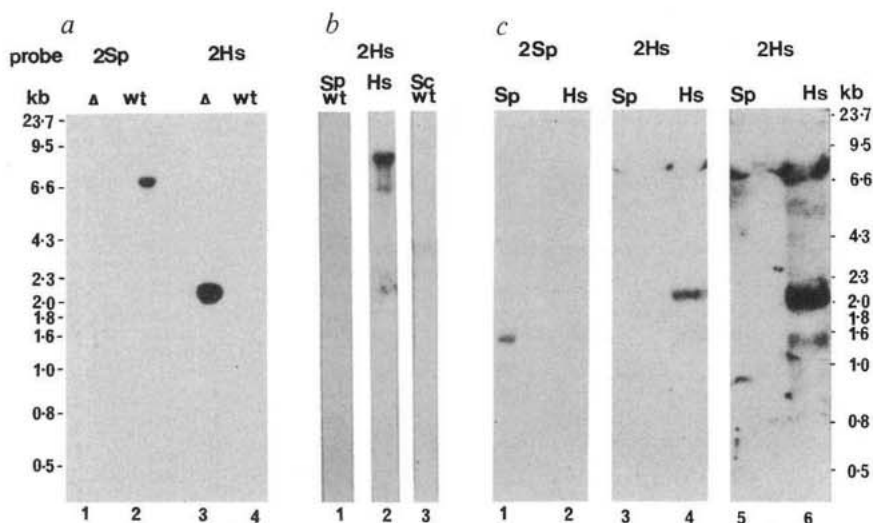
To prove that the insert in pOB231 could provide all the

functions encoded by *cdc2*, the 2-kb *Bam*HI insert was transferred to the LEU2 and 2 µm-containing *S. pombe* plasmid vector pSAB1 (pDB248X<sup>25</sup>, with a *Sal*I site deleted), to generate plasmid pSAB2Hs. This plasmid complemented all five *cdc2*<sup>ts</sup> mutants tested, *cdc2-56*, L7, M26, M55 and M63, and also enabled an *S. pombe* strain deleted for *cdc2* to grow normally. The deletion strain was constructed as shown in Fig. 1c. Initially a diploid strain was made which contained *cdc2*<sup>+</sup> on one chromosome homologue, and a deletion of the entire *cdc2* open reading frame with an insertion of the marker *ura4*<sup>+</sup> on the other homologue. After transformation with pSAB2Hs the diploid was sporulated. Those spores which contained the chromosome deleted for *cdc2* germinated but failed to undergo cell division, forming elongated cells. But if the spores contained pSAB2Hs, cells lacking *cdc2*<sup>+</sup> could divide like wild-type cells and formed colonies. Because the cells lose the plasmid



**Fig. 2** *a*, Southern blots of DNA prepared from a wild-type strain and the *cdc2Sp* deletion strain containing pSAB2Hs. Lane 1, deletion strain probed with *cdc2* (2Sp); lane 2, wild type probed with 2Sp; lane 3, deletion strain probed with pOB231 cDNA insert (2Hs); lane 4, wild-type probed with 2Hs. *Bam*HI digestion generates a 7-kb fragment containing the *cdc2Sp* gene and a 2-kb fragment containing the pOB231 insert. *b*, Southern blots of DNA prepared from *S. pombe*, *H. sapiens* and *S. cerevisiae* probed with pOB231 insert (2Hs). Lane 1, *S. pombe* DNA; lane 2, *H. sapiens* DNA; lane 3, *S. cerevisiae* DNA. No significant hybridization is seen in *S. pombe* or *S. cerevisiae*, whereas a strong band of 8 kb is seen in *H. sapiens*. *c*, Northern blots of RNA prepared from *S. pombe* wild-type cells and *H. sapiens* cell line HT29. Lane 1, *S. pombe* RNA (Sp) probed with *cdc2Sp* (2Sp); lane 2, *H. sapiens* RNA (Hs) probed with 2Sp; lanes 3, 5, *S. pombe* RNA (Sp) probed with pOB231 cDNA insert (2Hs); lanes 4, 6, *H. sapiens* RNA (Hs) probed with 2Hs. Lanes 5, 6 are longer exposures of lanes 3, 4.

**Methods.** Polyadenylated RNA was prepared from *S. pombe* wild-type cells as described<sup>42</sup> and probed with purified fragments of the pOB231 cDNA insert and *cdc2Sp* made radioactive by oligolabelling using hexadeoxynucleotide primers<sup>40</sup>.



frequently these colonies constantly produce highly elongated cells which divide no further (Fig. 1d). To establish that cells from these colonies had all their *cdc2* sequences deleted and contained the pOB231 insert, DNA was prepared and compared with DNA from wild type cells on a Southern blot. Probing with the pOB231 insert showed hybridizing sequences in the growing deletion clone but not in wild type (Fig. 2a, tracks 3, 4), whilst probing with *cdc2* showed the gene in wild type but not in the growing deletion strain (Fig. 2a, tracks 1, 2). Therefore the pOB231 human cDNA insert can provide all the functions of *S. pombe cdc2*. We shall call the gene from which this cDNA is derived the human *CDC2* gene. When necessary to avoid ambiguity we will use the suffix Hs (*CDC2Hs*) to indicate its *Homo sapiens* origin and Sp (*cdc2Sp*) when referring to the *S. pombe cdc2* gene.

### Human origin of *CDC2Hs*

It was important to demonstrate that *CDC2Hs* was of human origin. To do this DNA was prepared from *H. sapiens*, *S. pombe* and *S. cerevisiae* and digested with *Bam*HI. *S. cerevisiae* was also included in this experiment as it contains the gene *CDC28* which can complement mutants of *S. pombe cdc2*. After preparing Southern blots the DNAs were probed with *CDC2Hs*. No significant hybridization was observed with *S. pombe* or *S. cerevisiae*, but a major band of 8 kb and a minor band of 6 kb were observed with *H. sapiens* (Fig. 2b). From the band intensity the gene does not appear to be dispersed throughout the human genome and is likely to be single or low copy. To establish whether the gene is expressed in human cells polyadenylated RNA was prepared from the human colonic carcinoma cell line HT29, together with *S. pombe* polyadenylated RNA. Both RNAs were Northern blotted and probed with *cdc2Sp* and *CDC2Hs*. A 1.6-kb transcript hybridizing to *cdc2Sp* was seen in *S. pombe* but not in *H. sapiens* (Fig. 2c, tracks 1, 2), whereas a 2.0-kb transcript hybridizing to *CDC2Hs* was seen in *H. sapiens* (Fig. 2c, tracks 4, 6) but not in *S. pombe*, even after over-exposure of the autoradiograph (Fig. 2c, tracks 3, 5). This confirms that *CDC2Hs* is of human origin and is not derived from *S. pombe* or *S. cerevisiae*, and that it is derived from a 2-kb polyadenylated transcript. As this is the length of the cDNA clone isolated it can be assumed that this clone is full length or almost so.

### Sequence homology of *CDC2Hs*

The 2-kb *Bam*HI fragment containing *CDC2Hs* was sequenced by subcloning into pTZ18 and pTZ19 (Pharmacia Ltd.,

Molecular Biology Division) using exonuclease III deletion and *Alu*I and *Hae*III digestion. An extensive open reading frame (ORF) was located in the 1-kb fragment passing through the *Kpn*I and *Bgl*II sites. Sequencing both strands in this region confirmed that *CDC2Hs* potentially codes for a protein of 297 amino acids. The nucleotide sequence and its translation are given in Fig. 3.

The predicted ORF shares extensive homology with the fission yeast gene *cdc2Sp* and the budding yeast gene *CDC28* (Fig. 4). Amino-acid identities throughout the proteins are 63% between *CDC2Hs* and *cdc2Sp*<sup>20</sup> and 58% between *CDC2Hs* and *CDC28*<sup>26</sup>. If only those regions which are identical between *cdc2Sp* and *CDC28* are compared with *CDC2Hs* the identity rises to around 80%. The overall length of the three proteins are almost identical at 297 amino acids for *CDC2Hs*, *cdc2Sp* and 298 amino acids for *CDC28*. The predicted ORF of *CDC2Hs* also contains the two consensus regions surrounding the ATP binding (Fig. 3, nucleotides 170–239) and phosphorylation sites (Fig. 3, nucleotides 519–728) found in protein kinases<sup>1</sup> suggesting that it encodes a protein kinase. Despite the conservation at the protein level, there are no stretches longer than 14 nucleotides which are the same between *CDC2Hs* and *cdc2Sp*.

The protein sequences of *CDC2Hs* and *cdc2Sp* are remarkably similar. Comparisons of histone H2A/B and  $\alpha/\beta$  tubulin proteins, which are very closely related proteins, show identities between *S. pombe* and mammalian cells of between 68%–76%<sup>27–29</sup>, not much greater than the 63% we have observed here. We propose that *CDC2Hs* encodes a functional and structural homologue of the fission yeast *cdc2Sp* gene. The human gene *CDC2Hs* is also likely to be the functional homologue of the budding yeast *CDC28* gene, given its structural similarity to this gene.

A *cdc2Sp* like gene has been isolated from a human HeLa cDNA library using synthetic oligonucleotide probes corresponding to regions of expected similarity based on the sequences of conserved stretches within the catalytic domain of serine kinases<sup>30</sup>. This cDNA has been partially sequenced and this shows 47% identity in protein sequence to *cdc2Sp*. But it is different from *CDC2Hs* and its overall similarity to *cdc2Sp* is less than that found for *CDC2Hs*.

### Human protein of *M*<sub>r</sub> 34K

To investigate the *CDC2Hs* gene product in human cells, antibodies were raised against a peptide represented by the single letter amino-acid code EGVPTAIRELLKE, which



56  
GGGGG GGGGGGACT TUGCTTCAAA GCTGGCTCTT GGAATNAG CGGAGACGAG  
SacI  
CGGCTTCTT TAGCTGCGCT GGGGGGGG CGGAATAATA AGCGGGATC TACCATACCA TTAGTAATC  
140

KpnI  
ATG GAA GAT TAT ACC AAA ATA GAG AAA ATT GAA GAA GGT ACC TAT GGA GTT GTG  
M E D Y T K I K K I G E G T Y G V V  
194

AccI  
TAT AAG GGT AGA CAC AAA ACT ACA GGT CAA GTG GTA GCC ATG AAA AAA ATC AGA  
Y K G R H K T T G Q V V A M K K I R  
248

ScaI  
CTA GAA AGT GAA GAG GAA GGG GTT COT AGT ACT GCA ATT CGG GAA ATT TCT CTA  
I E S E E K G V P S T A I R E I S I  
302

XbaI  
TFA AAG GAA CTT CGT CAT CCA AAT ATA GTC AGT CTT CAG GAT GTG CTT ATG CAG  
I K E I R H P N I V S I Q D V I M Q  
356

GAT TCC AGG TTA TAT CTC ATC TTT GAG TTT CTT TCC ATG GAT CTG AAG AAA TAC  
D S R I Y I I F E P I S M D I K K Y  
410

TTG GAT TCT ATC CCT CCT GGT CAG TAC ATG GAT TCT TCA CTT GTT AAG AGT TAT  
I D S I P P G Q Y M D S S I V K S Y  
464

XbaI  
TFA TAC CAA ACT CTA CAG GGT ATT GTC TTT TTT CAC TCT AGA AGA GTT CTT CAC  
I Y Q I I Q G I V F C H S R R V I H  
518

AGA GAC TTA AAA CCT CAA AAT CTC TTG ATT GAT GAC AAA GGA ACA ATT AAA CTG  
R D I K P Q N I I I D D K G T I K I  
572

GCT GAT TTT GGC CTT GCC AGA GCT TTT GGA ATA COT ATC AGA GTA TAT ACA CAT  
A D F G I A R A F G I P I R V Y T H  
626

HgIII  
GAG GTA GTA ACA CTC TGG TAC AGA TAT CCA GAA GTA TTG CTG GGG TTA GCT COT  
E V V T I W Y R S P E V I I G S A R  
680

HindIII  
TAC TCA ACT CCA GTT GAC ATT TAT ACT ATA GGC ACC ATA TTT GCT GAA CTA GCA  
Y S T P V D I W S I G T I F A E I A  
734

HgII  
ACT AAG AAA CCA CTT TTT CAT GGT GAT TCA GAA ATT GAT CAA CTG TTG AGG ATT  
T K K P I F H G D S K I D Q I F R I  
788

TTG AAT GGT TTT GGC ACT CCC AAT AAT GAA GTG TGG CCA GAA GTG GAA TCT TTA  
F R A I G T P N N E V W P E V E S I  
842

CAG GAC TAT AAG AAT ACA TTT CUC AAA TGG AAA CCA GGA AGC CTA GCA TCC CAT  
Q D Y K N T P P K W K P G S I A S H  
896

GTC AAA AAG TTG GAT GAA AAT GGC TTG GAT TTG CTC TGG AAA ATG TTA ATC TAT  
V K N I D E N G I D I I S K M I I Y  
950

GAT CCA GGC AAA CCA ATT TTT GGC AAA ATG GCA CTG AAT CAT CCA TAT TTT AAT  
D P A K R I S G K M A I N H P Y P N  
1004

GAT TTG GAC AAT CAG ATT AAG AAG ATG TAG CTCTCTGACA AAAAGTTTCC ATATGTTATG  
I I D N Q I K K M  
1064

Fig. 3 DNA sequence and its translation of pOB231 insert. Some 6-base recognition restriction enzyme sites are shown above the sequence. The ORF from the first initiator codon to the stop codon is given.

corresponds to nucleotide positions 264–311 (Fig. 3) and is not conserved in other protein kinases (Fig. 3). The serum detected a protein of 34K relative molecular mass on Western blots of protein prepared from the human colonic carcinoma cell line HT29 (Fig. 5a, track 1; Fig. 5b, track 1). A similar-sized protein was also detected in *S. pombe* cells (Fig. 5b, track 2 and Fig. 5c, track 1). This protein was shown to be the *cdc2Sp* gene product by demonstrating that in a yeast strain overproducing *cdc2Sp* transcript the 34K band is increased in intensity (Fig. 5b, track 3). The serum was also shown to detect the *CDC2Hs* protein by Western blot analysis of protein made from the yeast strain deleted for *cdc2Sp* but containing pSAB2Hs. This strain contained no *cdc2Sp* sequences and so could not contain any *cdc2Sp* protein. A 34K protein of similar size to that seen in human cells was detected in this strain (Fig. 5c, track 3; Fig. 5d, track 1). Cells deleted for *cdc2Sp* and not containing pSAB2Hs generated by germinating spores harbouring the chromosome with the deletion, had no 34K protein detectable by the serum (Fig. 5c, track 2). The anti-peptide serum detected a similar-sized protein

2Hs  
2Sp  
28  
M S Q E L A N Y K H L E K V G E G T Y G V V Y K A L D L R P Q Q

2Hs  
2Sp  
28  
G Q R V V A M K K I R L E S E R E G V P S T A I R E I S L L K E  
G R I V A H K K I R L E D E S E G V P S T A I R E I S L L K E  
G Q R V V A L K K I R L E S E D E G V P S T A I R E I S L L K E

2Hs  
2Sp  
28  
L R H P N I V S L Q D V L M Q D S R L Y L I F E F L S  
V N D E N N R S N C V R L L D I L H A K S K L Y L V F E F L D  
L K D D N I V R L Y D I V H S D A H K L Y L V F E F L D

2Hs  
2Sp  
28  
M D L K K Y L D S I P P G Q Y M D S S L V K S Y L T Q I L Q  
H D L K K Y M D R I S E T G A T S L D P R L V Q A P T Y Q L V N  
L D L E R Y M E G I P E D Q P L G A D I V K F P M H Q L C K

2Hs  
2Sp  
28  
G I V F C H S R R V L H R D L K P Q N L L I D D K G T I K L A D  
G V N F C H S R R I I H R D L K P Q N L L I D K E G N L K L A D  
G I A Y C H S R R I L H R D L K P Q N L L I N K D G N L K L G D

2Hs  
2Sp  
28  
F O L A R A F G I F I R V Y T H E V V T L W Y R S P E V L L G S  
F G L A R S F G V P L H N Y T H E I V T L W Y R A P E V L L G S  
F G L A R A F G V P L R A Y T H E I V T L W Y R A P E V L L G G

2Hs  
2Sp  
28  
A R Y S T F V D I W S I G T I P A E L A T K A P L F H G D S E I  
R H Y S T G V D I W S V G C I P A E M I R R S P L P F G D S E I  
K Q Y S T G V D T W S I G C I P A E M C N K A P T P S G D S E I

2Hs  
2Sp  
28  
D Q L P R I P R A L G T P N N E V W P E V E S L Q D Y K N T P P  
D E I P E I F Q V L G T P N E E V W P G V T L L Q D Y K S T P P  
D Q I P E I F R V L G T P N E A T W P D I V Y L P D F K P S P P

2Hs  
2Sp  
28  
K W K P G S L A S H V K N L D E W G L D L L S K M L I T D P A K  
K W K R M D L H K V V P H G E E D A I E L L S A M L V Y D P A H  
Q W K R K D L S Q V Y P S L D P R G I D L L D E L L A Y D P I N

2Hs  
2Sp  
28  
R I S O K M A L H H P Y P N D L D N Q I K K M  
R I S A E K A L Q Q N Y L R H P F H  
R I S A R R A A I H P Y P Q E S

Fig. 4 Comparison of the predicted amino-acid sequences of *H. sapiens CDC2* (2Hs), *S. pombe cdc2* (2Sp) and *S. cerevisiae CDC28* (28). Identities between *CDC2Hs* and *cdc2Sp* are boxed. When residues in *CDC28* are also identical to the other two sequences these are also boxed.

in three other human cell lines, J6 T cells, Heb7a HeLa-derived cells and Daudi B cells (Fig. 5d, tracks 2–4). A second serum raised against the carboxy-terminal 99 amino-acid residues of *cdc2Sp* (provided by V. Simanis) also detected a 34K protein in these three cell lines (Fig. 5e, tracks 1–3). We have also detected a 34K protein in untransformed Butler human embryo fibroblast cells using the anti-peptide serum (data not shown). These data suggest that the 34K protein is likely to be the *CDC2Hs* gene product.

The level of 34K protein produced in the *cdc2Sp* deletion strain containing pSAB2Hs (Fig. 5c, track 3) is similar in level to that produced by *cdc2Sp* in wild-type cells (Fig. 5c, track 1). This suggests that complementation of the fission yeast *cdc2Sp* gene function can be achieved without overproducing the human *CDC2Hs* protein.

## Human genes in yeast

The experiments we have described provide an alternative approach for isolating homologous genes to using reduced stringency DNA hybridization or antibodies with a bacterial expression library. For the complementation approach to be successful the cDNA probably must be full length and should be made in a vector that can transform and express the cDNA in yeast. The Okayama and Berg library met these criteria in fission yeast but probably new vectors would have to be developed if other simple eukaryotes such as *S. cerevisiae* or *Aspergillus nidulans* were to be used instead<sup>22</sup>.

In addition to allowing the isolation of human genes by complementation, *S. pombe* can be used as an experimental system for investigating human gene function. The power of *in vitro* mutagenesis can be coupled with the ease of yeast genetics to isolate mutants in the human gene. Of particular interest are mutants with a dominant phenotype that can be tested for their effects after introduction back into human cells. For example, conditional dominant lethals could be isolated which prevent function of the wild type gene by competing out positive effectors



**Fig. 5** Western blot analysis of *S. pombe* and *H. sapiens* protein. Equivalent protein samples loaded: *a*, lane 1, human colonic carcinoma cell line HT29; *b*, lane 1, human colonic carcinoma cell line HT29; lane 2, *S. pombe* wild type; lane 3, *S. pombe*, *cdc2* overproducer; *c*, lane 1, *S. pombe* wild type; lane 2, *S. pombe* germinated spores deleted for *cdc2Sp*; lane 3, *S. pombe* deleted for *cdc2Sp* containing pSAB2Hs. *d*, lane 1, *S. pombe* deleted for *cdc2Sp* containing pSAB2Hs; lane 2, human J6 T cell; lane 3, human Heb7a, HeLa-derived; lane 4, human Daudi B cell; *e*, lane 1, human J6 T cell; lane 2, human Heb7a, HeLa-derived; lane 3, Human Daudi B cell. Blots *a*, *b*, *c*, *d* were made using affinity purified antibodies against the peptide EGVSTAIKELLKE and *e* using serum against the carboxy-terminal 99 amino-acid residues of *cdc2Sp*. The arrowhead marks the position of the 34K protein.

**Methods.** Extraction of proteins and preparation of peptides conjugates for rabbit immunization were as described in ref. 9. Western blot procedures were performed using GeneScreen and GeneScreen Plus membranes according to the manufacturer's (NEN) instructions.

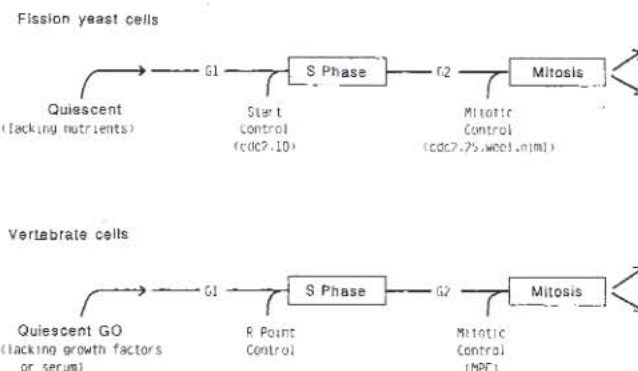
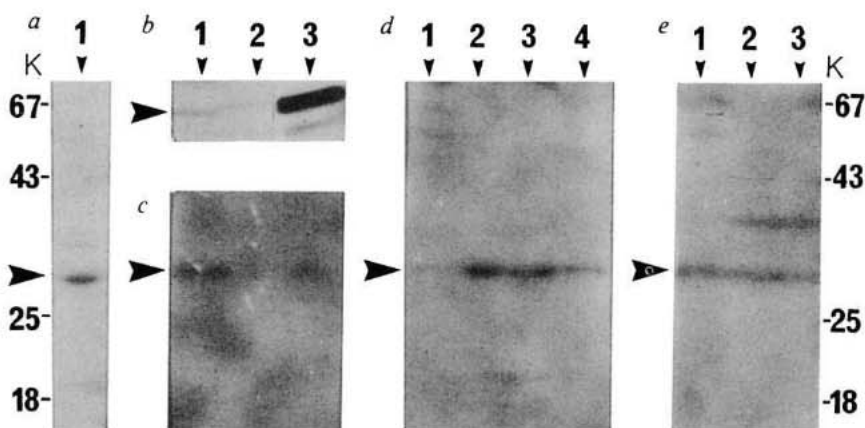
or substrates. After isolation in yeast these could be used for knocking out gene function in mammalian cells providing an alternative procedure to present techniques such as antibody injection or anti-sense message.

## Cell cycle implications

Because the human *CDC2Hs* gene can provide all of the functions of *cdc2Sp* in fission yeast it is reasonable to assume that it performs a similar role to *cdc2Sp* in controlling the human cell cycle. This conclusion is supported by the structural similarity between the two genes both in overall homology and size of the proteins. But it will be important to establish that *CDC2Hs* does have this role in human cells.

The most likely points of action of *CDC2Hs* during the human cell cycle, analogous to start and the mitotic control in *S. pombe*, in late G1 at the R-point and in late G2 at the initiation of mitosis (Fig. 6). At the R-point, cells undergo a transition which leads to the cell becoming less dependent on the presence of growth factors and the maintenance of high rates of protein synthesis for completion of the cell cycle<sup>31-33</sup>. This commitment to the mitotic cell cycle could correspond to the passage of start in the yeasts. Initiation of mitosis has been shown to involve maturation promotion factor (MPF) in vertebrate eggs<sup>34</sup>, and similar activities have been recovered from mammalian mitotic cells<sup>35</sup> and the budding yeast<sup>36</sup>. The action of MPF is quite analogous to the mitotic control which operates in *S. pombe*.

Given the homology of *CDC2Hs* to protein kinases in general and to the *cdc2Sp* protein kinase specifically it is likely that passage through the two control points in the human cell cycle will involve protein phosphorylation. The identification of a



**Fig. 6** Comparison of cell cycle control in fission yeast and vertebrate cells. The *S. pombe* start commitment control is analogous to the vertebrate R-point control, and the *S. pombe* mitotic control to the G2/M transition regulated by MPF in vertebrate cells.

*cdc2*-like function in human cells suggests that elements of the mechanism by which the cell cycle is controlled will probably be found in all eukaryotic cells.

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