Functionally homologous cell cycle control genes in budding and fission yeast

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Functionally homologous cell cycle control genes in budding and fission yeast

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The cdc *2 (previously called* wee *2) cell cycle start gene of* Schizosaccharomyces pombe, *which is required for start and the control of mitosis, has been isolated from an* S. pombe *gene bank by complementation of a* cdc *2 mutation. A functionally homologous sequence which complements the* cdc *2 mutation has also been isolated from a* Saccharomyces cerevisiae *gene bank and this sequence has been shown to contain the* cdc *28 cell cycle start gene of* S. cerevisiae. *It is concluded that the* cdc *2 and* cdc *28 genes perform homologous cell cycle control functions in the two organisms.*

THE fission yeast *Schizosaccharomyces pombe* and the budding yeast *Saccharomyces cerevisiae* have both been used as model organisms for studying the eukaryotic cell cycle[1,2]. This work has made extensive use of temperature-sensitive *cdc* (cell division cycle) mutants which become specifically arrested during the cell cycle when incubated at their restrictive temperatures; over 40 *cdc* genes have been described in *S. cerevisiae* and over 25 in *S. pombe*. All of these are required for normal cell division but only a few are expected to be involved directly in cell cycle controls such as commitment to the mitotic cycle and regulation of the rate of cell division. In *S. cerevisiae*, four genes (*cdc* 28, 36, 37 and 39) have been shown to have roles in a major cell cycle control called 'start'[3-6]. Cells accumulate in G_1 at start in poor nutritional conditions and under the influence of the mating hormones. Mutants of the four *cdc* genes block cell cycle progress at start and are able to conjugate from their point of cell cycle arrest. Once start has been completed the cell becomes committed to the mitotic cycle and is unable to undergo the alternative developmental pathway of conjugation. Completion of start is also the major rate-limiting step of the cell cycle regulating the rate of cell division[3,4]. In *S. pombe* there are two start genes, *cdc* 2 and *cdc* 10 (ref. 7). Mutants of these two genes block in G_1 and are able to conjugate from that point of arrest. In poor nutritional conditions, *S. pombe* cells also accumulate before start. Thus the cell cycles of both organisms can be divided operationally into an uncommitted pre-replicative phase before start and a committed replicative phase consisting of S-phase, mitosis and cell division after start (Fig. 1).

Although start divides the cell cycles of the two yeasts into two similar phases this does not necessarily mean that the two start events have the same molecular basis in the two organisms, especially as they are not closely related. To establish whether the molecular basis of start is indeed similar we have made use of the fact that *cdc* start genes can be physically isolated by complementation and can be transferred from one yeast to the other. From these experiments we establish that *S. cerevisiae cdc* 28 can complement *cdc* 2 mutations of *S. pombe*, suggesting that the start events have a similar molecular basis in the two organisms.

Isolation of the *S. pombe cdc* 2 gene

A sequence containing a *cdc* start gene of *S. cerevisiae* has been selected from a bank of budding yeast DNA by its ability to complement the appropriate *cdc* mutation[8]. The development of techniques for high frequency transformation of *S. pombe* and the construction of a gene bank in a yeast–bacterial shuttle vector[9,10], makes the same approach feasible in *S. pombe*.

A *Hin*dIII partial *S. pombe* gene bank was made in vector pDB262, which contains the *S. cerevisiae leu* 2 gene and can complement the *leu* 1.32 mutation of *S. pombe*[9,10]. The gene bank was used to isolate the *cdc* 2 start gene by transforming a *cdc* 2.33 *leu* 1.32 strain to Leu^+ prototrophy and replica-plating the resultant 30,000 clones to 35 °C, at which temperature *cdc* 2.33 cells elongate and die (Fig. 2*b*; ref. 11). Clones that continued to divide at 35 °C were isolated and the plasmids which they contained were recovered in *Escherichia coli*[9,10]. One of these plasmids was found to complement the *cdc* 2.33 and *cdc* 2.M26 mutations when retransformed back into strains containing these mutations. The dividing cells in these transformant clones were elongated (Fig. 2*c*) compared with wild-type dividing cells (Fig. 2*a*), indicating incomplete suppression of the mutant phenotype by the insert on the plasmid. The plasmid was termed pcdc2.3(Sp) and contained a 7-kilobase (kb) insert. A 1.4-kb *Hin*dIII fragment from this insert was recloned into vector pDAM6 which contains the *S. cerevisiae leu* 2 and pBR322 but no sequence capable of supporting replication in *S. pombe*. This plasmid, pcdc2.32(Sp), was transformed into a *S. pombe leu* 1.32h^- strain and some of the Leu^+ prototrophic transformants formed were found to be mitotically stable and were presumed to have arisen by plasmid integration into the chromosomal *cdc* 2 gene. This was confirmed for two of the integrants by preparing DNA from cells of the *leu* 1.32h^- strain before integration and after integration of pcdc2.32(Sp). The DNA was digested with *Bam*HI, an enzyme which does not cut within the 1.4-kb *Hin*dIII insert but cuts once in the vector pDAM6 (Fig. 3). After Southern transfer the digested DNAs were probed with ^{32}P-labelled pcdc2.32(Sp) (Fig. 3). The 13-kb *Bam*HI fragment of DNA from cells before integration was altered to two fragments of 17 and 8 kb after integration for both integrants (see Fig. 3 for explanation). This result demonstrates that pcdc2.32 (Sp) integrated at the chromosomal site homologous to the 1.4-kb *Hin*dIII insert. We demonstrated that this site was the *cdc* 2 locus by showing that the Leu 1^+ marker phenotype of the plasmid was closely linked to *his* 3, a marker localized within 1 centimorgan of the *cdc* 2 locus[12]. The two pcdc2.32(Sp) integrants in strain *leu* 1.32 h^- were crossed to a *his* 3^- *leu* 1.32 h^+ strain. The resultant zygotes were sporulated and the phenotypes of 400 spores of both crosses examined. In both cases less than seven recombinant spores (*leu* 1^+ *his* 3^- or *leu* 1^- *his* 3^+) were observed, indicating that the *S. cerevisiae leu* 2 gene must have integrated within 1.7 centimorgans of *his* 3. Therefore, pcdc2.3(Sp) contains the *S. pombe cdc* 2 start gene.

Isolation of a *S. cerevisiae* sequence complementing *cdc* 2

Having shown that a sequence containing the *cdc* 2 gene could be isolated from *S. pombe*, we next tried to establish whether

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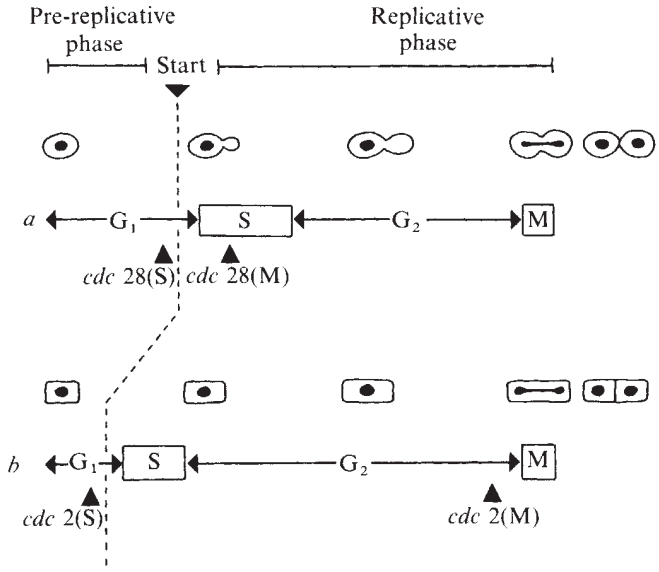

Fig. 1 Schematic representation of cell cycle control in *S. cerevisiae* (*a*) and *S. pombe* (*b*). Start divides the two cycles operationally into an uncommitted pre-replicative phase and a committed replicative phase. The pre-replicative phase occupies most of G_1. It is the major expandable part of the cycle, becoming much longer at slow growth rates. The figure represents rapidly growing cells in which the pre-replicative phase is longer in *S. cerevisiae* than in *S. pombe*. The transition or execution points of *cdc* 28 and *cdc* 2 are shown as filled triangles, the ones appropriate for start are labelled *cdc* 28(S) and *cdc* 2(S) and those appropriate for mitosis are *cdc* 28(M) and *cdc* 2(M).

there are any sequences in *S. cerevisiae* which have the same function as the *cdc* 2 gene. This can be done by introducing a *S. cerevisiae* gene bank into *S. pombe* and testing for complementation of the *cdc* 2.33 mutation. Previously, such complementation has only been observed for genes involved in intermediary metabolism such as *leu* 2 (refs 9, 10). A *S. cerevisiae* gene bank has been constructed in YEp13 (provided by K. Nasmyth), a vector which contains the *leu* 2 gene and can autonomously replicate in *S. pombe*. This gene bank was used to transform the *S. pombe* strain *cdc* 2.33 *leu* 1.32 in a manner identical to that described above for the isolation of pcdc2.3(Sp). A plasmid, pcdc2(Sc), was isolated which complemented the *cdc* 2.33 and *cdc* 2.M26 mutations. This plasmid contained *S. cerevisiae* sequences as its insert (data not given) and generated *cdc* 2^+ transformants having the same elongated appearance at the restrictive temperature as the *S. pombe* pcdc2.3(Sp) (Fig. 2*c*). Thus, *S. cerevisiae* contains DNA sequences which can functionally complement mutations in the *cdc* 2 gene.

S. cerevisiae cdc 28 can complement *cdc* 2

As *cdc* 2 is a start gene in *S. pombe*, pcdc2(Sc) might contain one of the four known *S. cerevisiae* start genes[5,6]. All of these have been isolated in the plasmids pcdc28, 36, 37 and 39 (ref. 8 and S. Reed, unpublished results) using vector YRp7 or its derivative YRp7′. The four plasmids were digested with *Hin*dIII and after Southern transfer were probed with ^{32}P-pcdc2(Sc). The hybridization observed in this experiment indicated that the inserts of pcdc2(Sc) and pcdc28 (containing the *cdc* 28 gene) contained common sequences. In particular, both plasmids had a 2.3-kb *Hin*dIII fragment in common. To test this was the same sequence, the 2.3-kb *Hin*dIII fragment was recovered from a digest of pcdc28, then nick-translated and hybridized to the similarly sized fragment from pcdc2(Sc) (Fig. 4). As the pcdc28 sequences are unique in the *S. cerevisiae* genome (S. Reed, unpublished results) this hybridization establishes that pcdc2(Sc) contains *cdc* 28 sequences. To confirm that pcdc2(Sc) contains a functional *cdc* 28 gene, the plasmid was transformed into a *cdc* 28.4 *leu* 2.3 *S. cerevisiae* strain (provided by I. Johnson). The resultant Leu^+ prototrophs grew at 36 °C, the restrictive temperature of *cdc* 28.4, demonstrating that pcdc.2(Sc) can complement a *cdc* 28 mutation and thus contains a functional *cdc* 28 gene. These experiments show that the *cdc* 28 gene contained within pcdc2(Sc) can complement mutations of *cdc* 2. To test whether the *cdc* 28 gene contained within pcdc28 will also complement mutations of *cdc* 2, the pcdc28 sequences first had to be transferred to a different vector because YRp7 cannot be selected for in *S. pombe*; pcdc28 was partially digested with *Sau*3A and ligated with *Bam*HI-digested pDB248. The crude ligation mix was used to transform the *cdc* 2.33 *leu* 1.32 *S. pombe* strain to Leu^+ prototrophy. About 30% of the transformants were able to form colonies at the restrictive temperature of 35 °C. The cells of these transformants were smaller (Fig. 2*c*) than wild-type cells (Fig. 2*a*) and were very similar to certain *wee* mutant alleles of *cdc* 2 (Fig. 2*d*). These *wee* mutant alleles have been interpreted as having an abnormally high *cdc* 2 gene product activity. Note that the behaviour of pcdc28 in *S. pombe cdc* 2.33 strains differs from that of pcdc2(Sc), which resulted in weaker complementation and the formation of elongated

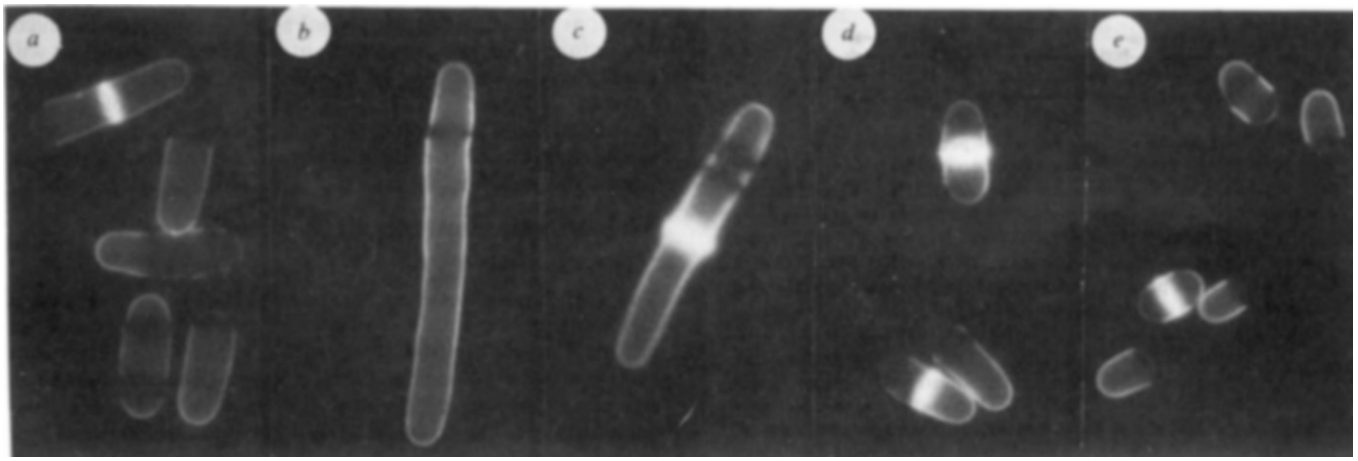

Fig. 2 Cells of *S. pombe* strains stained with Calcofluor and photographed by fluorescence microscopy. In these conditions the septa of dividing cells fluoresce intensely. *a*, Wild-type 972; *b*, *cdc* 2.33 *leu* 1.32; *c*, *cdc* 2.33 *leu* 1.32 containing pcdc2.3(Sp); *d*, *cdc* 2.1w (previously called *wee* 2.1); *e*, *cdc* 2.33 *leu* 1.32 containing pcdc28 in pDB248. Cells were grown in minimal medium at 25 °C overnight and were transferred to 35 °C in yeast extract medium for 7 h before staining as described in ref. 11. The cells in *e* include some dividing at a small size, similar to *cdc* 2.1w, and others which are highly elongated such as *cdc* 2.33 which have lost the plasmid and are unable to divide. pcdc2.3(Sp) is based on vector pDB262 which contains part of the yeast 2 μm circle, *leu* 2 and the bacterial plasmid pTR262. pDB248 is similar but with pTR262 replaced by pBR322[9,10].

cells (Fig. 2c). It is unknown why these two plasmids differ in their behaviour but as they were located in different vectors and were isolated from different gene banks the plasmid copy number and the flanking chromosomal and vector sequences could be different. Either of these two factors could influence the final activity of the *cdc* 28 gene product in *S. pombe*.

S. pombe cdc 2 cannot complement *cdc* 28

As *cdc* 28 can complement *cdc* 2 mutations of *S. pombe*, it was of interest to determine whether *cdc* 2 could complement *cdc* 28 mutations of *S. cerevisiae*. This was tested by transforming the *S. cerevisiae* strain *cdc* 28.4 *leu* 2.3 to Leu^+ prototrophy using pcdc2.3(Sp). The Leu^+ transformants did not grow at 36 °C, forming enlarged cells that were both budded and unbudded. This failure to complement indicates that the *S. pombe cdc* 2 gene cannot substitute for a defective *cdc* 28 gene in *S. cerevisiae*, which could be due to inadequate *cdc* 2 expression or inability of the *cdc* 2 gene product to provide the entire function of *cdc* 28. It has been shown that the 5′ regulatory sequences of the cytochrome *c* and alcohol dehydrogenase genes differ in *S. pombe* and *S. cerevisiae* (ref. 13 and P. Russell, personal communication). Thus the 5′ sequences of *cdc* 2 may be unable to initiate transcription at a sufficient level in *S. cerevisiae* to complement mutations of *cdc* 28.

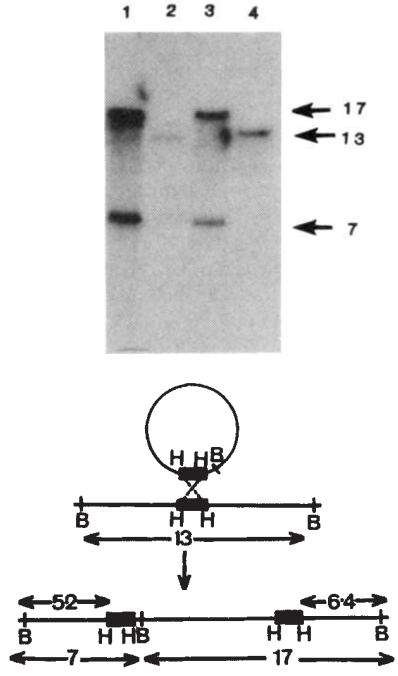

Fig. 3 A model of the integration of pcdc2.32(Sp) into the *cdc* 2 site on chromosome II (bottom) and an insert showing Southern blot transfers of *S. pombe* chromosomal DNA digested with chromosomal DNA (top) prepared from: tracks (1) and (3), pcdc2.32(Sp) integrants; tracks (2) and (4), the *leu* 1.32 strain before integration. B, *Bam*HI sites; H, *Hin*dIII sites. All procedures for DNA preparation, nick-translation with ^{32}P-CTP and Southern blot transfers are described in refs 9, 10. The fragments produced by integration can be explained as follows. The 1.4-kb *Hin*dIII fragment (shown as a thick line) derived from pcdc2.3(Sp) is contained within a 13-kb *Bam*HI chromosomal fragment. When pcdc2.32(Sp) of total length 11 kb integrates via homologous recombination at the *cdc* 2 site, a tandem duplication of the 1.4-kb *Hin*dIII fragment is formed which flanks the vector sequences of pDAM6. As pcdc2.32(Sp) contains only one *Bam*HI site situated in the vector next to the chromosomal insert, two new *Bam*HI fragments are generated with a combined length of 24 kb made up of the original *Bam*HI chromosomal fragment of 13 kb and pcdc2.32(Sp) (11 kb). The precise sizes of the two *Bam*HI fragments generated can be explained if it is assumed that the 1.4-kb *Hin*dIII fragment is located 5.2 and 6.4 kb from the chromosomal *Bam*HI sites.

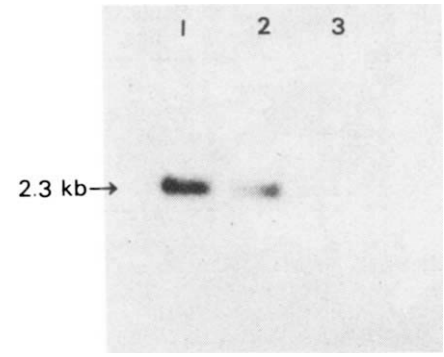

Fig. 4 Southern blot transfers of DNAs digested with *Hin*dIII and probed with the 2.3-kb *Hin*dIII fragment derived from pcdc28 (see text). Track (1), pcdc28; (2), pcdc2(Sc); (3), YEp 13. Note that a single 2.3-kb fragment hybridizes in pcdc28 and pcdc2(Sc) but there is no hybridization with YEp 13. All procedures for DNA preparation, nick-translation with ^{32}P-CTP and Southern blot transfers have been described elsewhere[9,10].

cdc 2 and *cdc* 28 genes perform similar functions in both G_1 and G_2

The requirement for the *S. pombe cdc* 2 gene in G_1 at start has already been described; *cdc* 2 is also required in G_2 before mitosis (Fig. 1). Whether cells become blocked in G_1 or G_2 depends on their position in the cell cycle at the time of temperature shift[7]. It has recently been shown that certain *cdc* 28 mutants of *S. cerevisiae* also arrest in G_2 just before mitosis as well as in G_1 at start[14]. Thus *cdc* 28 also has a dual role in the cell cycle at start and at mitosis, and is presumably providing both these functions when complementing *cdc* 2 mutations of *S. pombe*. This strongly suggests that the molecular function of the *cdc* 2 and *cdc* 28 genes is the same or very similar. To determine whether the *cdc* 2 and *cdc* 28 sequences are also closely related, pcdc2.3(Sp) was digested with *Hin*dIII and after Southern transfer was hybridized with ^{32}P-pcdc2(Sc). No hybridization of the inserts was detected in normal stringency conditions (0.75 M NaCl, 65 °C). This lack of sequence homology is not surprising as the two yeasts are not closely related and no hybridization was observed between *S. pombe leu* 1 and *S. cerevisiae leu* 2 genes, both of which encode the same enzyme, β-isopropylmalate dehydrogenase[9,10]. DNA sequencing will be required to establish the precise relationship between *cdc* 2 and *cdc* 28.

The molecular mechanism of the *cdc* 2 and *cdc* 28 gene functions at start and mitosis is unknown. As the gene products are required for two very different cell cycle events, they will possibly have some sort of triggering coordinating role. We envisage a molecular function such as that of protein modifications in the control of many other cellular processes[15], modulating different molecular activities at start and mitosis, but via the same mechanism.

The mitotic controls in *S. pombe* and *S. cerevisiae*

The *cdc* 2 gene function has been shown to be the major rate-limiting step in *S. pombe*, determining the timing of mitosis[16,17]. *wee* mutant alleles at the *cdc* 2 locus result in a cell cycle having a shorter G_2 than normal and a reduced cell size at mitosis and cell division. Before mitosis can occur, the cell must grow to a critical size and this requirement is reduced in the *wee* mutants[25,26].

In *S. cerevisiae* there is no mitotic control analogous to that found in *S. pombe*. Mitosis is thought to occur when a certain time has elapsed after 'start'[18,19]. The difference in control between the two yeasts may be related to the fact that mitosis is apparently initiated very early in the cell cycle in *S. cerevisiae*. A short intranuclear spindle forms by the end of G_1 (ref. 20). This structure elongates later in the cycle to form the full mitotic

spindle during mitosis. The requirement of *cdc* 28 for mitosis is also completed early, about one-tenth of a cell cycle after start (Fig. 1; ref. 14). These observations suggest that the initiation of mitosis occurs early in the cell cycle in *S. cerevisiae*, a situation which is different from most eukaryotic cells such as *S. pombe*. When *cdc* 28 is introduced into *S. pombe* as pcdc28, mitosis is initiated prematurely so that cell division occurs at a small size (Fig. 2*e*). One explanation for this could be that the *cdc* 28 gene lacks some of the regulatory functions of *cdc* 2 which ensure that mitosis is delayed until the *S. pombe* cell has grown to normal size. *cdc* 28 may not have these functions because this control is not normally operative in *S. cerevisiae*.

Cell cycle control in other organisms

The *cdc* 2 and *cdc* 28 genes have major roles in the cell cycle control of *S. pombe* and *S. cerevisiae* and must perform identical or similar molecular functions. But the two organisms are not closely related as judged by both taxonomic criteria[21] and the comparison of 5S RNA[22] and cytochrome *c*[23] sequences, which show <70% homology. Despite this considerable evolutionary divergence, the molecular mechanisms of start and of the control initiating mitosis appear to have been highly conserved, thus we believe that these control mechanisms may also be applicable to other eukaryotes. In mammalian cells, G_1 restriction or transition points analogous to start in the yeasts have been proposed as the major point of cell cycle control[24]. Thus it is possible that the control in mammalian cells may also involve a functional equivalent of the *cdc* 2 and *cdc* 28 gene products.

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Arrangement of human immunoglobulin heavy chain constant region genes implies evolutionary duplication of a segment containing γ, ε and α genes

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Cosmid clones containing the human γ, ε and α heavy chain constant region genes and an ε pseudogene have been isolated. All these genes have a switch sequence detectable by hybridization. We have studied overlapping cosmids covering two separate regions of the genome, and the gene order in each of these regions was found to be γ–γ–ε–α. This implies an evolutionary duplication in this multigene family involving γ, ε and α genes.

MOST known mammalian genes are organized in multigene families whose members share sequence homology. Such families include the globin genes[1,2], interferon genes[3], growth hormone genes[4], genes of the major histocompatibility complex[5] and immunoglobulin genes. The immunoglobulin heavy (H) chain genes consist of four linked families of gene segments: the variable (V_H), diversity (D), joining (J_H) and constant (C_H) gene segments. The C_H gene segments determine the class of the H-chain polypeptide. Both man and mouse have five classes of H chain, known as μ, δ, γ, ε and α, which differ in their biological activities. In both species the γ chains are further divided into four subclasses, and in man there are two subclasses of α chain. When the active H-chain gene is formed by $V_H/D/J_H$ integration[6–8] the C_H gene closest to the J_H locus, the μ gene, is the first to be expressed while the other C_H genes can subsequently be expressed by H-chain switching mechanisms. One of these mechanisms, which operates for each C_H gene except δ, involves deletion of all C_H genes between the V gene and the newly expressed C_H gene[9–12]. This switch deletion has been proposed to involve recombination, perhaps by unequal sister chromatid exchange[13–15], between homologous, internally repetitive sequences (called switch or S regions) which were found upstream of each mouse C_H gene except δ[14,16–18]. It was similarly found in man that the μ gene S region has a repetitive sequence and is homologous with a region upstream of $\gamma 2$ but not δ[19]. Comparison of S regions of mouse μ, γ and α genes with their human counterparts has shown strong evolutionary conservation [19–22].

The order of the C_H genes in the mouse has been determined as 5′-μ-δ-$\gamma 3$-$\gamma 1$-$\gamma 2b$-$\gamma 2a$-ε-α-3′, and no pseudogenes have been reported[18]. The existence of two α-chain subclasses in man and the finding that human DNA contains a pseudo γ gene ($\psi\gamma$)[22,23] and three ε-like genes[24–26] implied that the arrangement of human C_H genes was different from that in the mouse. Some aspects of the arrangement of human C_H genes have already been published. The J_H–μ–δ region was mapped and found to be organized similarly to the mouse[19,27], the $\gamma 2$ and $\gamma 4$ genes were shown to be about 19 kilobases (kb)

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