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Sequence Analysis of the *Schizosaccharomycs pombe* Homologue of the *CDC3* Gene in *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae **has a highly ordered ring of filaments that lies just inside the cytoplasmic membrane in the region of the mother-bud neck. Mutants defective in any one of the four cell-division cycle genes (*CDC3, CDC10, CDC11, CDC12*) fail to form these filaments and exhibit a pleiotropic phenotype that includes failure to complete cytokinesis and abnomal bud growth. However, the role of the filament is not clear. In order to find out the role of filament, the similar gene in *S. pombe* (called *cdc103*$^+$) to the *CDC3* was cloned and sequenced. Here I report the sequence analysis of the *cdc103*$^+$. Comparison of the predicted amino acid sequences of *cdc103*$^+$ and *CDC3* revealed that they share significant similarity (43% identity and 56% identity or similarity) to each other.**

Key words: ***Schizosaccharomyces pombe, Saccharomyces cerevisiae, CDC3***

One of the fundamental problems of cell biology is understanding the mechanism by which cells elicit changes in shape and intracellular spatial organization. The budding yeast *Saccharomyces cerevisae* provides a very useful system to study morphogenetic processes in cell division. The cell-division cycle of *S. cerevisiae* involves several morphogenetic events including (i) selection of a nonrandom budding site; (ii) deposition of a ring of chitin at the site of bud emergence; (iii) localization of new cell wall growth to the region bounded by the chitin ring, resulting in selective growth of the bud; (iv) nuclear migration to the region of the mother-bud neck; and (v) cytokinesis and formation of a septal cell wall. As in other eukaryotes, cytoskeletal components, including both actin (1, 14, 21) and microtubules (1, 4, 12, 13, 14), are believed to play important roles in these processes.

S. cerevisiae also contains another cytoskeletal element, of unknown biochemical nature, which may be involved in cellular morphogenesis. This is a highly ordered array of filaments, ~10-nm in diameter, which lies just inside of the cytoplasmic membrane in the region of the mother-bud neck (4, 5). EM studies suggest that these filaments appear at about the time of bud emergence and disappear just before cytokinesis. Temperature-sensitive mutants defective in any of four distinct cell-division cycle genes (*CDC3, CDC10, CDC11,* and *CDC12*) lack these filaments and display a pleiotropic phenotype when shifted to the restrictive temperature (1, 5, 9, 22). These mutants fail to complete cytokinesis, fail to properly localize chitin to the bases of buds formed at the restrictive temperature, and display hyperpolarized bud growth; DNA synthesis, nuclear division, and budding continues, resulting in the formation of multinucleate, multibudded cells.

CDC3, CDC10, CDC11, and *CDC12* have been cloned and sequenced, revealing that they encode a family of related proteins that are 25~37% identical in amino acid sequence (8). In addition, antibodies specific for the products of *CDC3, CDC10, CDC11,* and *CDC12* have been used to show by immunofluorescence that these proteins localize to the mother-bud neck in the vicinity of the filaments (16, 17, 18). Filament staining disappeared in *cdc3, cdc10, cdc11,* and *cdc12* mutants shifted to the restrictive temperature. These results suggest strongly that *CDC3, CDC10, CDC11,* and *CDC12* encode constituents of the 10-nm filaments; however, the predicted amino acid sequences of these genes lack similarity to other proteins, inculding known filament-forming proteins. Thus, *CDC3, CDC10, CDC11,* and *CDC12* may encode a novel class of filaments-forming proteins.

Despite the accumulated information about the filament proteins, the role of these proteins in morphogenetic processes remains unclear. In order to better understand the function of the neck-filament proteins and to determine if they are specific for the budding lifestyle of *S. cervisiae,* we have undertaken a search for homologues of *CDC3, CDC10, CDC11,* and *CDC12* in the fission yeast *Schizosaccharomyces pombe. S. pombe* was selected because it is evolutionarily distant (19) and morphologically distinct from *S. cerevisiae. S. pombe* cells are hemispherical-capped cylinders which grow by length extension alone. *S. pombe* divides by binary fission in which a septum is formed across the middle of the cell, resulting in two nearly equal-sized cells; this is very different from the asymmetrical-division process of budding used by *S. cerevisiae.* Moreover, *S. pombe* does not form any structures analogous to the mother-bud neck during the course of its cell-division cycle, nor have any filamentous structures resembling the filaments been described. Thus, identification of homologues of the neck-filament proteins and characterization of their function in *S. pombe* may provide important clues about their possible role in cellular morphogenesis in both yeasts as well as their possible general distribution in other eukaryotes. Here I report the DNA sequence of an *S. pombe* homologue (called *cdc103*$^{+}$) of the *S. cerevisiae CDC3* gene.

Materials and Methods

DNA was sequenced by the dideoxy chain-termination method (25) using the Sequenase DNA sequencing kit (United States Biochemical). Phages M13mp18 and M13 mp19 (27) and plasmid pBluescript were used to generate single-strand DNA templates. Nested deletions were generated using exonuclease III (3, 10). Oligonucleotides used as sequencing primers were synthesized. Oligonucleotides used as primers to the sequence of two exon/intron junctions of the *cdc103*$^{+}$ cDNA clone were 5′-ATCCGTTCAACTGACGC-3′ (complementary to nucleotides +244 to +260 of the sense strand of the genomic clone), 5′-CATGCTATAAGTGCTATG-3′ (identical to nucleotides +692 to +709 of the sense strand of the genomic clone), and 5′-GGTATTCTACTGATC-3′ (complementary to nucleotides +952 to +970 of the sense strand of the genomic clone). The oligonucleotide used as a primer to the sequence of the 3′-end of the *cdc103*$^{+}$ cDNA clone was 5′-TTCTCAGAAGGTTCAAGA-3′ (identical to nucleotides +1306 to +1323 of the sense strand of the genomic clone).

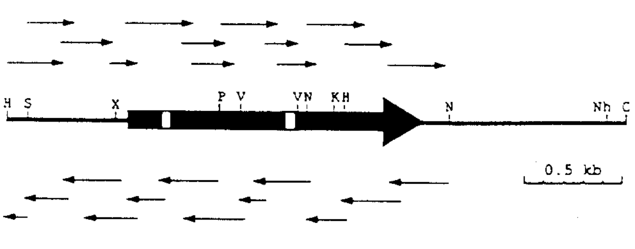

Fig. 1. Restriction map and sequencing strategy for the *cdc103*$^{+}$ region. The directions and extents of sequencing are shown by arrows above and below the restriction map. The open reading frames are indicated by the heavy arrow; introns are indicated as open boxes on this arrow. Restriction endonuclease cleavage sites are indicated as follows; C, *Cla*I; H, *Hin*dIII; K, *Kpn*I; N, *Nde*I; Nh, *Nhe*I; P, *Pst*I; S, *Sal*I; V, *Eco*RV; and X, *Xho*I.

Result

The DNA sequence of both strands of a 2.3 kb region surrounding the *Pst*I site were determined (Fig. 1) ; this revealed three long open reading frames of 180, 597, and 630 bp, interrupted by two apparent short introns of 37 and 45 bp (Fig. 2). Both of the putative introns contained the consensus sequences apparently re-

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AAGCTTCTAA TGTTTTAGTT TGATCTTACT TTTTCGTAGG AAAAGAAATA TAGATATTTG TTTCTGAATC ATCTATTAGC   -641
ATGAAAATGT TGGCCAGGCA ATAATTTCTA ACTGTGTTTT ATGTCTAATA ATTAAACTTT TAAAATCGTT GAGGGTCACA   -561
TTGTCGACGT ACAAGTTCAA TTGAATAAAT AATCAGTTGT GCAATAAATT GCATTTGCAT TAATGCTCAA TTCAACGACT   -481
ACTGCCCAAG GTTCTATATT AATAGAAAAA TAAATATTTT AAATTAAATT CAAAAGATTT AGATGATTGC ATGCTGAAAG   -401
GCTATTCAAA CAAATAATTT AGGTATAAAT AAAAGGTCAA AAAATGTTTT TAAACAAATA CAGATAATGG ATAATTTTAA   -321
TTCATTAGAT TTATCCAATT GTTAAAAAAA ATAATTGTAA AATTAACAAG ACAACTAAAA CAATGGAATA ACAATGTTTA   -241
GTATCTTTTT ATTTAATGCA TCCCAGCCGT GTATATTTCG AGGTTAAGGA CCTAAATTCG ACGCGACAGT GAATTAAGAA   -161
ATTTCAAAAA AGCTCTATAA TTTCGTGAAA CATAATTAAG AAAAAAATTG GTTAAGTTTA ATTTCCTACT TGAGCAAATA    -81
GATGTTCATG CTCGAGCTCC TAGCTACACT CTTCATCACA TACATAACCA CCAACACGGG GGTTTTCGAT AATTTCTGAC     -1

ATG GCG TCA ATG GTA CTC GCG GAT GGT ATG CCT ACA GTT AAA GAT GAT TCC ACT AGA AGC AGG GGT    +66
 M   A   S   M   V   L   A   D   G   M   P   T   V   K   D   D   S   T   R   S   R   G

TCC GAC GTT GAT TCT TTC ACA TCT ACA GAT AAT GTA ACG CAA ATA AAT GTT GAG GCA GCC ATT TCA   +132
 S   D   V   D   S   F   T   S   T   D   N   V   T   Q   I   N   V   E   A   A   I   S

GAA AAC AAG AAT GAA GAA AAA CCC ATT CAG GAT AAT TCT GAA CAA GAG GTAATCAATTATTTAAAATTCCT   +203
 E   N   K   N   E   E   K   P   I   Q   D   N   S   E   Q   E

TTACTAATATTTAG  TTC AAT CCG CAC GTT AGT ATA ATC CAG CGT CAG TTG AAC GGA TAC GTT GGA TTC   +271
                 F   N   P   H   V   S   I   I   Q   R   Q   L   N   G   Y   V   G   F

GCT AGT CTT CCT AAT CAA TGG CAT CGT CGT TGT GTT CGT CAA GGT TTT AAT TTC AAC GTA TTA GTA   +337
 A   S   L   P   N   Q   W   H   R   R   C   V   R   Q   G   F   N   F   N   V   L   V

TTA GGG GAA AGC GGT TCA GGG AAA TCT ACA CTT GTG AAT ACG CTA TTG AAT AGA GAT GTT TAC CCA   +403
 L   G   E   S   G   S   G   K   S   T   L   V   N   T   L   L   N   R   D   V   Y   P

CCG ACC CAG AAA TCT TTA ACT GGG GAT TTT GGA GTG AAC CCA GAA CCC ACT GTT ATG ATC AAC TCT   +469
 P   T   Q   K   S   L   T   G   D   F   G   V   N   P   E   P   T   V   M   I   N   S

TCT GCA GTT GAA ATA GTG GAA AAT GGT ATC AGT CTT CAA TTA AAT GTA ATT GAT ACA CCC GGT TTT   +535
 S   A   V   E   I   V   E   N   G   I   S   L   Q   L   N   V   I   D   T   P   G   F

GGC GAT TTT ATT GAC AAC ACC GAT TGT TGG CAA CCA GTT TTG ACA GAT ATC GAG GGT CGC TAT GAT   +601
 G   D   F   I   D   N   T   D   C   W   Q   P   V   L   T   D   I   E   G   R   Y   D

CAA TAT CTT GAG CTT GAA AAG CAC AAT CCT CGA TCT ACT ATT CAA GAT CCA AGA GTT CAT GCT TGT   +667
 Q   Y   L   E   L   E   K   H   N   P   R   S   T   I   Q   D   P   R   V   H   A   C

ATA TTT TTT ATT CAG CCT ACC GGT CAT GCT ATA AGT GCT ATG GAG CTT CGA GTT ATG TTG GCT TTG   +733
 I   F   F   I   Q   P   T   G   H   A   I   S   A   M   E   L   R   V   M   L   A   L

CAC GAG AAA GTA AAT ATT ATA CCC ATC ATT GCG AAA GCC GAT ACA CTA ACG GAT GAT GAA CTT AAC   +799
 H   E   K   V   N   I   I   P   I   I   A   K   A   D   T   L   T   D   D   E   L   N

TTT ACG AAG GAA ATG  GTAAGCCCTAATCTTGATTTAAAGTTGCTAATGAAAAAATGATAG  ATT TTG AGA GAT ATC   +874
 F   T   K   E   M                                                   I   L   R   D   I

CAA TAC CAC AAT ATC AGA ATC TTC TTC CCT CCG ACA TAT GAG ACC GAT GAT CCT GAA TCA GTG GCA   +940
 Q   Y   H   N   I   R   I   F   F   P   P   T   Y   E   T   D   D   P   E   S   V   A

GAA AAT GCA GAC ATC ATG AGT AGA ATA CCT TTT GCT ATA ATT GCT TCT AAT ACA TTC GTG GTC AAC  +1006
 E   N   A   D   I   M   S   R   I   P   F   A   I   I   A   S   N   T   F   V   V   N

AAT GAA GGA AAG CGC GTC CGC GGG AGG CGG TAC CCA TGG GGC GTT GTT GAA GTC GAT AAT GAA GAG  +1072
 N   E   G   K   R   V   R   G   R   R   Y   P   W   G   V   V   E   V   D   N   E   E

CAT TCT GAT TTC CCT AAG CTT CGT GAA ATG CTT ATT CGA ACA CAC TTA GAA GAA CTC AAA GAA CAG  +1138
 H   S   D   F   P   K   L   R   E   M   L   I   R   T   H   L   E   E   L   K   E   Q

ACA AAT AAG CTG TAT GAA GCG TAT CGT ACT GAA CGG TTG CTT AGC AGC GGA ATA TCA CAA GAT CAC  +1204
 T   N   K   L   Y   E   A   Y   R   T   E   R   L   L   S   S   G   I   S   Q   D   H

TCC GTT TTT CGT GAA GTC AAC CCT AGT GCT AAA CTC GAA GAG GAG CGT GCC TTA CAC GAA GAG AAA  +1270
 S   V   F   R   E   V   N   P   S   A   K   L   E   E   E   R   A   L   H   E   E   K

TTG ATG AAA ATG GAA GCA GAA ATG AAA ACC ATT TTT TCT CAG AAG GTT CAA GAA AAA GAA GAT CGT  +1336
 L   M   K   M   E   A   E   M   K   T   I   F   S   Q   K   V   Q   E   K   E   D   R

CTT AAA CAA TCT GAA AAC GAG TTA CGT ACC CGT CAT CGC GAA ATG AAG GCA GCA TTG GAG AAG CAA  +1402
 L   K   Q   S   E   N   E   L   R   T   R   H   R   E   M   K   A   A   L   E   K   Q

AAA GCT GAC TTA ATT GAT CAT AAA AAT CGG TTA ATG CAA GCT AAA GCT GCG GCC GAA AAT GAG AAG  +1468
 K   A   D   L   I   D   H   K   N   R   L   M   Q   A   K   A   A   A   E   N   E   K

AGT AAA AGG AAG TTT TTT AAA TAG TCT CCT TAC TAG CTG ATA TTT ATT TGT ATG ACA CCG CAG TTC  +1534
 S   K   R   K   F   F   K   -               -

TACTGCTATC TCCTTCTTTG TTATTTAATT ACTACTTAAA GTCCAATATT GGGCTGTATA TCTTTCCTTG ATTATTCTAC  +1614
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Fig. 2. DNA sequence of the *cdc103*$^{+}$ region and predicted amino acid sequence of cdc103 protein. Numbering of the DNA sequence is such that +1 is the first base of the first ATG-initiated open reading frame. Consensus intron splice sequences are indicated by a underline.

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cdc103+  MASMVLADGMPTVKDDSTRSRGSDVDSFTSTDNVTQINVEAAISENKNEE  50
CDC3     MSL-------------------------------------------KEEQ  7

cdc103+  KPIQDNSEQE------FN--------------------------------  62
CDC3     VSIKQDPEQEERQHDQFNDVQIKQESQDHDGVDSQYTNGTQNDDSERFEA  57

cdc103+  --------------------------PHVSIIQRQLNGYVGFASLPN  83
CDC3     AESDVKVEPGLGMGITSSQSEKGQVLPDQPEIKFIRRQINGYVGFANLPK  107

cdc103+  QWHRRCVRQGFNFNVLVLGESGSGKSTLVNTLLNRDVYPPTQKSLTGDFG  133
CDC3     QWHRRSIKNGFSFNLLCVGPDGIGKTTLMKTLFNND-----------DIE  146

cdc103+  VNPE----------------------------PTVMINSSAVEIVENGI  154
CDC3     ANLVKDYEEELANDQEEEEGQGEGHENQSQEQRHKVKIKSYESVIEENGV  196

cdc103+  SLQLNVIDTPGFGDFIDNTD-CWQPVLTDIEGRYDQYLELEKHNPRSTIQ  203
CDC3     KLNLNVIDTEGFGDFLNNDQKSWDPIIKEIDSRFDQYLDAENKINRHSIN  246

cdc103+  DPRVHACIFFIQPTGHAISAMELRVMLALHEKVNIIPIIAKADTLTDDEL  253
CDC3     DKRIHACLYFIEPTGHYLKPLDLKFMQSVYEKCNLIPVIAKSDILTDEEI  296

cdc103+  NFTKEMILRDIQYHNIRIFFPPTYETDDPESVAENADIMSRIPFAIIASN  303
CDC3     LSFKKTIMNQLIQSNIELFKPPIYSNDDAENSHLSERLFSSLPYAVIGSN  346

cdc103+  TFVVNNEGKRVRGRRYPWGVVEVDNEEHSDFPKLREMLIRTHLEELKEQT  353
CDC3     DIVENYSGNQVRGRSYPWGVIEVDNDNHSDFNLLKNLLIKQFMEELKERT  396

cdc103+  NK-LYEAYRTERLLSSGISQDHSVFREVNPSAKLEEERALHEEKLMKMEA  402
CDC3     SKILYENYRSSKLAKLGIKQDNSVFKEFDPISKQQEEKTLHEAKLAKLEI  446

cdc103+  EMKTIFSQKVQEKEDRLKQSENELRTRHREMKAALEKQKADLIDHKNRLM  452
CDC3     EMKTVFQQKVSEKEKKLQKSETELFARHKEMKEKLTKQLKAL--------  488

cdc103+  QAKAAAENEKS----------------------KRKFFK  469
CDC3     -----EDKKKQLELSINSASPNVNHSPVPTKKKGFLR  520

Identity  : 202 ( 43.1%)
Similarity: 61 ( 13%)
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Fig. 3. Alignment of the predicted amino acid sequences of cdc103 protein and CDC3 protein. The single-amino acid code is used. The character to show that two aligned residues are identical is ':'. The character to show that two aligned residues are similar is '·'. Amino acids said to be 'similar' are: A, S, T; D, E; N, Q; R, K; I, L, M, V; F, Y, W.

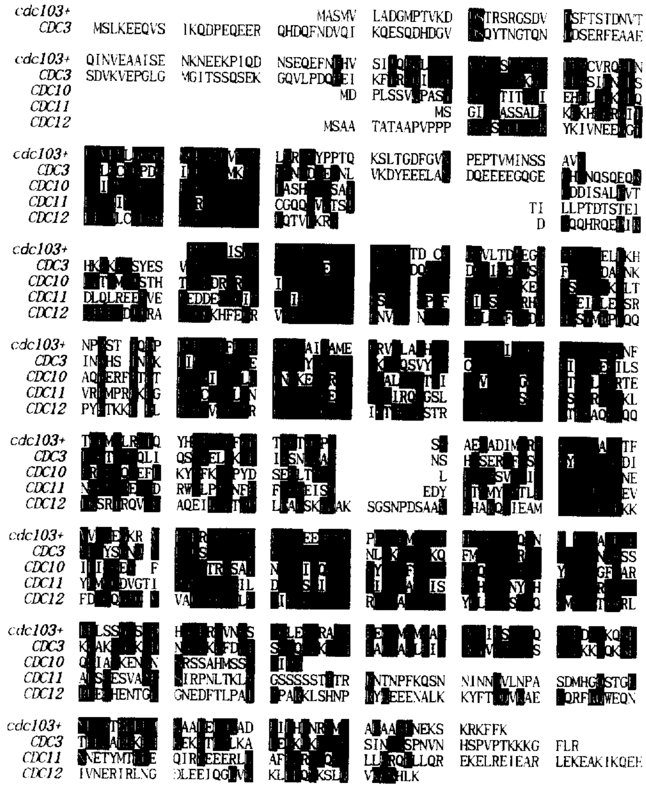

Fig. 4. Alignment of the predicted amino acid sequences of cdc103 protein and CDC10, CDC11, and CDC12 protein. The single-amino acid code is used.

quired for splicing in *S. pombe* (5′-GTANG... 21 to 101 bp... CT(G/A)A...5 to 18 bp...AG-3′; 24), except that the 37 nucleotide intron has a T rather than a G in the fifth position of the 5′-splice site. That these putative introns are actually spliced out *in vivo* was demonstrated by sequencing the appropriate junctions in a cDNA clone of *cdc103*$^{+}$ (see Materials and Methods). Sequencing of the cDNA clone also helped to verify the identification of the initiatior ATG codon as shown in Fig. 2: the cDNA sequence matched that of the genomic clone between nucleotides −20 (the end of the cDNA clone) and +180, and no consensus splicing signals are present in the genomic sequence between nucleotide −20 and the in-frame stop codons at positions −36 to −34 and −60 to −58. However, it is not ruled out that the ATG codons at nucleotides +10 to +12 and +28 to +30 might serve as initiators. The putative primary stop codon is followed by two additional in-frame stop codons within the next 80 nucleotides. The identity of the primary stop codon was also confirmed by sequencing the appropriate region of the cDNA clone.

The putative spliced *cdc103*$^{+}$ open reading frame should encode a protein of 469 amino acids with a molecular weight of 53,745 Daltons and a net charge of −3 at pH 7.0 (Fig. 2). Comparison of the predicted amino acid sequence of cdc103 protein with that of CDC3 protein shows that the two proteins are closely related: 43% of the amino acids are identical and an additional 13% are similar (Fig. 3). The similarity is particularly striking in the central portions of the proteins: 50% identity (69% identity or similarity) from amino acids 149 to 257 with a single amino acid gap; and 60% identity (69% identity or similarity) from amino acids 314 to 440 with a single amino acid gap. The predicted amino acids sequence of cdc103 protein is also similar to those of CDC10, CDC11, and CDC12 protein (27~35% identity and 10~15% additional similarity) (Fig. 4).

Discussion

We have identified a *S. pombe* homologue (called *cdc 103*$^+$) of the *S. cerevisiae CDC3* gene. The gene encoding this homologue was cloned from an expression library of *S. pombe* genomic DNA using antibodies specific for CDC3 protein as a probe (15). DNA sequence analysis revealed an open reading frame of 469 codons which was interrupted by two short introns. The predicted amino acid sequence of *cdc103*$^+$ displayed significant similarity, 43% identity (56% identity or similarity), to that of *CDC3*; the level of similarity was much more pronounced, 50~60% identity (69% identity or similarity), when central portions of the predicted amino acid sequences of the two proteins were compared. The predicted amino acid sequence of *cdc103*$^+$ was also similar, although to a lesser extent (27~35%), to that of *CDC10, CDC11,* and *CDC12,* as might be expected since *CDC3, CDC10, CDC11,* and *CDC12* encoded a family of related proteins.

The level of similarity between the products of *CDC3* and *cdc103*$^+$ is significant considering the evolutionary divergence of these yeasts (19). Other proteins that are involved in similar processes in these yeast share comparable levels of amino acid sequence variation. For example, the p34 protein kinase encoded by *CDC28* in *S. cerevisiae* and *cdc2*$^+$ in *S. pombe* share 62% amino acid sequence identity (2, 11, 20), calmodulin encoded by *CMD1* in *S. cerevisiae* and *cam1*$^+$ in *S. pombe* share 56% amino acid sequence identity (6, 26); and orotidine-5'-phosphate decarboxylase encoded by *URA3* in *S. cerevisiae* and *ura4*$^+$ in *S. pombe* share 51% amino acid identity (7, 23). Thus, the products of *CDC3* and *cdc103*$^+$ may perform similar functions at the molecular level. Moreover the presence of such similar proteins in these distantly related yeasts suggests that *CDC3* (and *CDC10, CDC11, CDC12*) homologues may be present in other eukaryotes.

To find the function and intracellular localizaton of cdc103 protein in the *S. pombe* cell division cycle, β-galactosidase-cdc103 protein and anthranilate synthase-cdc 103 protein fusion proteins are under construction and will be used to generate the antibodies specific for cdc 103 protein. Immunofluorescence study using these antibodies will show the intracellular localization and the possible function of the cdc103 protein.

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