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S.pombe pac1$^+$, whose overexpression inhibits sexual development, encodes a ribonuclease III-like RNase

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The *Schizosaccharomyces pombe pac1* gene is a multicopy suppressor of the *pat1* temperature-sensitive mutation, which directs uncontrolled meiosis at the restrictive temperature. Overexpression of the *pac1* gene had no apparent effect on vegetative growth but inhibited mating and sporulation in wild type *S.pombe* cells. In such cells, expression of certain genes required for mating or meiosis was inhibited. The *pac1* gene is essential for vegetative cell growth. The deduced *pac1* gene product has 363 amino acids. Its C-terminal 230 residues revealed 25% amino acid identity with ribonuclease III, an enzyme that digests double-stranded RNA and is involved in processing ribosomal RNA precursors and certain mRNAs in *Escherichia coli*. The *pac1* gene product could degrade double-stranded RNA *in vitro*. These observations establish the presence of a RNase III homolog in eukaryotic cells. The *pac1* gene product probably inhibits mating and meiosis by degrading a specific mRNA(s) required for sexual development. It is likely that mRNA processing is involved in the regulation of sexual development in fission yeast.

Key words: cell growth/double-stranded ribonuclease/ mating/meiosis/mRNA scarcity

Introduction

Cells of the fission yeast *Schizosaccharomyces pombe* enter meiosis under nitrogen starvation (Egel, 1973; Egel and Egel-Mitani, 1974). Nitrogen starvation triggers expression of genes essential for meiosis apparently through transcriptional activation. These genes include *mei2*, which is required for the transition from the mitotic cell cycle to the meiotic pathway (Shimoda *et al.*, 1985, 1987; Watanabe *et al.*, 1988). Nitrogen starvation also activates transcription of the mating type genes, which are necessary for mating and meiosis (Kelly *et al.*, 1988). The *mei3* gene, another gene essential for meiosis, is inducible by nitrogen starvation only when the cells are heterozygous for the mating type genes (McLeod *et al.*, 1987). That is, induction of *mei3* depends on coexpression of *matP* and *matM*.

A critical post-transcriptional regulation of meiosis has also been elucidated in *S.pombe*. The *pat1* (also called *ran1*) mutation was originally isolated as an inducer of uncontrolled meiosis (Iino and Yamamoto, 1985a; Nurse, 1985). The *pat1* gene product is a negative regulator of meiosis, which must be inactivated for *S.pombe* cells to enter meiosis (Beach *et al.*, 1985; Iino and Yamamoto, 1985b), and has been shown to be a protein kinase (McLeod and Beach, 1988). The *mei3* gene product binds to this kinase and inhibits its activity (McLeod and Beach, 1988). Thus, protein phosphorylation also appears to have a key role in the meiotic control in *S.pombe*.

We have suggested that a combination of two molecular events, that is, inactivation of Pat1 kinase and supply of Mei2 protein, irreversibly commits *S.pombe* cells to meiosis (Watanabe *et al.*, 1988). Even when *pat1* is inactivated, meiosis cannot be initiated unless Mei2 protein is supplied. The *S.pombe pac1* gene is a multicopy suppressor of the uncontrolled meiosis driven by *pat1* and its overexpression inhibits expression of *mei2*, as we showed in our previous publication (Watanabe *et al.*, 1988). Our initial interpretation was that *pac1* might be related to the cAMP cascade in *S.pombe*, because a high level of intracellular cAMP inhibits expression of *mei2* and hence meiosis (Watanabe *et al.*, 1988), whereas loss of intracellular cAMP promotes sexual development (Maeda *et al.*, 1990). We now report a more detailed characterization of *pac1* and show that the *pac1* gene product is, quite unexpectedly, an RNase. Our finding suggests that degradation or processing of a certain RNA(s) may be a novel mechanism regulating sexual development in *S.pombe*.

Results

Isolation and characterization of the pac1 gene

A *pat1*^{ts} (*ran1−3*) mutant (JY628) was transformed with an *S.pombe* genomic library based on an *S.pombe−E.coli* shuttle vector pDB248′ (Beach *et al.*, 1982) to isolate multicopy suppressors of the uncontrolled meiosis driven by *pat1* deficiency. The *ran1−3* allele, which was isolated by Nurse (1985), is leakier than the *pat1−114* allele (Iino and Yamamoto, 1985a) used in most physiological experiments, and was chosen because we thought that we might obtain a larger variety of suppressors with a leakier allele. Transformants of JY628, which were Leu$^+$ due to the *S.cerevisiae LEU2* marker on the vector pDB248′, were replica-plated to examine their growth at 37°C. DNA was prepared from each Leu$^+$ Ts$^+$ transformant and plasmids in the DNA preparation were recovered into an *E.coli* strain. A plasmid named pKF1 retained the suppressor activity when used to retransform an *S.pombe ran1−3* strain, and could also suppress the tighter *pat1−114* allele in JY712.

The pKF1 plasmid carried a 6.0 kb DNA fragment from the *S.pombe* genome. The region responsible for the suppressor activity was limited by subcloning (Figure 1A). A 3.2 kb *Hin*dIII fragment was sufficient to suppress *pat1*. A 2.2 kb *Hin*cII−*Hin*dIII fragment exhibited the suppressor activity when carried on the vector pDB248′ in one orientation, but not in the opposite orientation. This is a phenomenon due to a cryptic promoter activity in pDB248′ and suggests that the *Hin*cII−*Hin*dIII fragment carries all

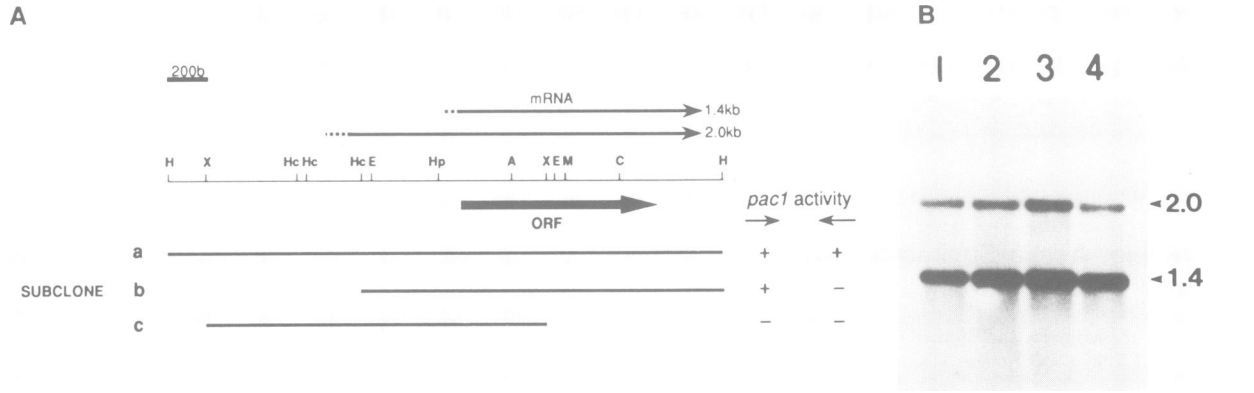

Fig. 1. Structure of the *pac1* gene. **A**. A restriction map of the *pac1* locus is shown together with the position of the open reading frame (ORF) and the two species of *pac1* mRNA. The ability of subclones to confer *pac1* activity is also shown. The subclone b was active only when the direction of insertion matched the direction of transcription from a cryptic promoter on the vector (see text). Restriction enzymes are abbreviated as follows. A, *Acc*I; C, *Cla*I; E, *Eco*RV; H, *Hin*dIII; Hc, *Hin*cII; Hp, *Hpa*II; M, *Mlu*I; and X, *Xba*I. **B**. Northern blot analysis of *pac1* mRNA. Poly(A)$^+$ RNA was prepared from the following strains. 1, a homothallic haploid strain JY450 growing rapidly; 2, JY450 starved for nitrogen for 4 h; 3, a sporogenic diploid strain JY362 growing rapidly; and 4, JY362 starved for nitrogen for 4 h. The 0.4 kb *Xba*I−*Cla*I fragment within the ORF was labeled with ^{32}P and used as the hybridization probe. Note that the loading is not precisely quantitative due to the yield of poly(A)$^+$ RNA in each preparation.

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-1431  TCTAGAGTGGCCCTAAAACATCCATCTTTTCGTAAACATTCTACTCGATAATATTCTTTAACTTGGTTTACGAAATACTAAACTTTTGCTTTAAAATTTG
-1331  TTACTACAATAAACAGTAGCAACGTATTCAACTTTAACTGTAAGCTACGCAGTTTGGTATCTGATTTAAGGATACGTAGAACTCCGGTGAGTTTTCCTTA
-1231  TGGTCTATTTATATTACAATACACAGGTTGTATTAAGTAGCACTGAGTATAGGTATTGTATTAACTGGGTTATAATGTTACCTATCACTAATATAGCTCA
-1131  TAACTGAACTGAGGAACGAGGTTCATTGTATTTATAATATTTCTAAATAGTTGTTATTAACGGGTATCTATGTATATAGACGGATAGTTACCCGTGATTA
-1031  TCAATAAACGGATCTTAGCTAGTATTCCAACATATGACATATAGTGATACGCAACGTAGTTGATCATAGCGTAGTGTAGTATTGCTGACATTAACATACT
 -931  TTGTTGACCGTTGTGCTGCGGTACGCCCTATTCTTTCTCTGCTCATGTTAACCAACGAACGTGGTGAAATAAACCGCTAAGTTGGAAAAAGAGTGTTTCT
 -831  TTCTTCCTTCTCCTTTCTCGTCCCTCACTGCCCAACATAAACAAACTCCCACACATATGAGGTTGAACGAGTCTGCATATTCTTTTTAAAGAAAAGAGTT
 -731  GTTTTCTTATTCATATTGACGGTTTGGTAGTTCCTACGAACTTGCATTTCAGATTGTTTATTTTTCAATCGGGTTTTTGCAAAGTAAGTAGCGTTGTCTT
 -631  GCATCTACGGTGATAGAGCTTTCTTTCAAATAAGGAGGATTGCTGGTCCTTGGTTTGGTCAACATACGGTTTTTAATCACATTTCGATTTTACGCTGCAT
 -531  TTTCGATTTGCTTCAGATATCATTAATTGGTAGATTTGAAGATTATAACATTGAAAAAGACTTTTTTCACTGTAAAAAATATTTATTTTTTTATATTCAG
 -431  CTATTCGAAGAACATTTTTTTTGCATTTTTTTCTTAGCTGGGTAGGTGTTCTTCACATTGGAGAGCTGTTTTTGGTTGATTGTTTTGTTACAAGATTTTT
 -331  TGCATTTGTATATTACACGGAGCTTTTCGTGCTGATCATTTTTTACTTCTTGATTGTTCTTTACTGTTTGTGGTATATTTCTAAAGAAAATACAAAGTAA
 -231  AGTAATTTTGGGTAAATAACTTTTTCGTTGTGGCCCTTAGAACAGTATCCTGGTACTGCACATTTCTTCTACTGACGATATTTTTACCGGAACTCTGTTC
 -131  CGTACTTAATTCTCGGCCATTTTTGACACCGATTTTTTAAGTAGATCCCAATACAGCATTCTTGGATACTTCTGTCTCTTTTAGCACCGTTTTCCTTCTC
  -31  ACAATCTACTTTATATTTCTACCATTTTCCTATGGGACGGTTTAAGAGGCATCATGAAGGGGACTCAGATTCCTCCTCATCCGCTTCCGACTCTTTATCA
                                    M  G  R  F  K  R  H  H  E  G  D  S  D  S  S  S  S  A  S  D  S  L  S
   70  AGAGGAAGAAGGTCTCTTGGTCACAAGCGAAGTAGCCACATAAAAAATCGGCAGTATTATATTTTGGAAAAAAAAATTCGTAAACTCATGTTTGCTATGA
       R  G  R  R  S  L  G  H  K  R  S  S  H  I  K  N  R  Q  Y  Y  I  L  E  K  K  I  R  K  L  M  F  A  M  K
  170  AAGCCTTGCTAGAAGAAACAAAGCATTCAACAAAGGATGATGTGAATTTGGTTATCCCTGGTTCTACTTGGAGCCATATTGAAGGAGTCTACGAAATGTT
         A  L  L  E  E  T  K  H  S  T  K  D  D  V  N  L  V  I  P  G  S  T  W  S  H  I  E  G  V  Y  E  M  L
  270  GAAGTCAAGGCATGATCGCCAAAACGAACCTGTCATTGAAGAACCCTCCTCTCACCCAAAAAATCAAAAAAATCAAGAAAATAATGAACCAACATCTGAG
         K  S  R  H  D  R  Q  N  E  P  V  I  E  E  P  S  S  H  P  K  N  Q  K  N  Q  E  N  N  E  P  T  S  E
  370  GAGTTTGAAGAAGGAGAGTATCCCCCGCCATTACCACCTTTACGGTCTGAGAAACTAAAGGAACAAGTTTTTATGCATATTTCTAGAGCTTATGAAATTT
       E  F  E  E  G  E  Y  P  P  P  L  P  P  L  R  S  E  K  L  K  E  Q  V  F  M  H  I  S  R  A  Y  E  I  Y
  470  ATCCAAATCAGTCGAATCCGAATGAATTGTTGGATATCCATAATGAAAGGCTAGAATTTCTTGGTGACAGCTTTTTTAATTTGTTTACTACGCGTATTAT
         P  N  Q  S  N  P  N  E  L  L  D  I  H  N  E  R  L  E  F  L  G  D  S  F  F  N  L  F  T  T  R  I  I
  570  ATTCAGCAAGTTCCCTCAAATGGATGAAGGAAGTCTTTCCAAACTCCGGGCAAAATTTGTTGGCAACGAAAGTGCTGATAAATTTGCAAGGCTCTATGGG
       F  S  K  F  P  Q  M  D  E  G  S  L  S  K  L  R  A  K  F  V  G  N  E  S  A  D  K  F  A  R  L  Y  G
  670  TTTGATAAAACACTTGTCCTCAGTTATTCTGCCGAAAAAGACCAACTTCGAAAATCTCAAAAGGTCATTGCTGACACTTTCGAAGCATATCTCGGTGCAT
       F  D  K  T  L  V  L  S  Y  S  A  E  K  D  Q  L  R  K  S  Q  K  V  I  A  D  T  F  E  A  Y  L  G  A  L
  770  TGATATTGGATGGACAAGAAGAGACAGCTTTTCAATGGGTTAGCCGACTTCTCCAACCAAAGATTGCCAATATCACTGTTCAACGTCCTATCGATAAGCT
         I  L  D  G  Q  E  E  T  A  F  Q  W  V  S  R  L  L  Q  P  K  I  A  N  I  T  V  Q  R  P  I  D  K  L
  870  GGCTAAATCAAAACTTTTTCACAAGTACAGCACTTTGGGCCACATCGAATATCGCTGGGTCGATGGTGCTGGTGGTTCCGCTGAGGGTTATGTTATTGCC
       A  K  S  K  L  F  H  K  Y  S  T  L  G  H  I  E  Y  R  W  V  D  G  A  G  G  S  A  E  G  Y  V  I  A
  970  TGCATATTTAATGGCAAAGAAGTAGCCCGTGCGTGGGGTGCAAATCAAAAAGACGCGGGCTCTAGGGCAGCTATGCAAGCACTCGAAGTCCTTGCAAAAG
       C  I  F  N  G  K  E  V  A  R  A  W  G  A  N  Q  K  D  A  G  S  R  A  A  M  Q  A  L  E  V  L  A  K  D
 1070  ATTACTCTAAGTTTGCCCGTTAATCCCACATTTTTTATTTTTCTAAAAATTTAAATCCCATTTTATACGATGAATTAATTCGTCACGGTTTCGATCGTTA
         Y  S  K  F  A  R  *
 1170  AAAGGTCTTACTGTCACAGTCAATCTATCTTTTGTTTGGTCCTTATGAAGAATTATTTAAAGTCTTTTTAGCATTTTATATTCTCTTTTAGTTCAAAAAA
 1270  AATATAAAGTTTTTGTTCCTCTGATTATTCCGTATTTCTTTAGATATTTAAGTTAGATAAAACTGGAAACAGCCCATCCATCTATTTGTTTCAAAAAAGG
 1370  AATGTTCACTACTTTACATGATATAATCATGCTGCTTTTAAACACTGTAGAAACTATAAACTGCGGTTTAAGAGGTGTTTCAAGCTT
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Fig. 2. Nucleotide sequence of the *pac1* locus. 2887 nucleotides between the *Xba*I and *Hin*dIII sites flanking the *pac1* ORF are shown. Numbering starts at the first A of the deduced initiation codon. These sequence data are available from EMBL/GenBank/DDBJ under accession number X54998.

or most of the coding region of the gene responsible for suppression, which we named *pac1* (*pat1* compensator), but lacks its authentic promoter.

The nucleotide sequence of a 2.9 kb *Xba*I−*Hin*dIII fragment, which covers the entire *pac1* mRNA (see below), was determined (Figure 2). An uninterrupted open reading frame (ORF) was found, whose direction was consistent with the prediction from the above results. No obvious consensus sequence for *S.pombe* introns was found. The suppressor gene potentially encodes a polypeptide of 363 amino acids, whose calculated molecular weight is ~41 000.

A DNA fragment within the *pac1* ORF hybridized with two transcripts of 2.0 kb and 1.4 kb in length in Northern blot analysis (Figure 1B). The level of these transcripts was hardly affected either by nitrogen starvation or by the mating type of the cell (Figure 1B). Single-stranded oligonucleotide probes revealed that the two species of mRNA are transcribed in the same direction and they could be positioned approximately as shown in Figure 1A by analysis using various restriction fragments as hybridization probes (data not shown). However, it is not yet clear how these two species are related to each other.

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pac1      134 LPPLRSEKIKEQVFMHISRAYEIYPNQSNPNELLDIHNERLEFLGDSFFNLFTTRIIFSK 193
RNaseIII    1 MNPIVINRLQRKLGYTFNHQ-ELLQQALTHRSASSKHNERLEFLGDSILSYVIANALYHR 59

pac1      194 FPQMDEGSLSKLRAKFVGNESADKFARLYGFDKTLVLSYSAERDQLRKSQKVIADTFEAY 253
RNaseIII   60 FPRVDEGDMSRMRATLVRGNTLAELAREFELGECLRLGPGELKSGGFRRESILADTVEAL 119

pac1      254 LGALILDGQEETAFQWVSRLLQPKIANIT-VQRPIDKLAKSKLFHKYSTLGHIEYRWVDG 312
RNaseIII  120 IGGVFLDSDIQTVEKLILNWYQTRLDEISPGDKQKDPKTRLQEYLQGRHLPLPTYLVVQV 179

pac1      313 AGGS-AEGYVIACIFNG-KEVARAWGANQKDAGSRAAMQALEVLAKDYSKFAR 363
RNaseIII 180 RGEAHDQEFTIHCQVSGLSEPVVGTGSSRRKAEQAAAEQALKKLELE 226
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Fig. 3. Comparison of the amino acid sequences of the deduced *pac1* gene product and RNase III (March *et al.*, 1985; Nashimoto and Uchida, 1985). Identical amino acids are shown in white against black.

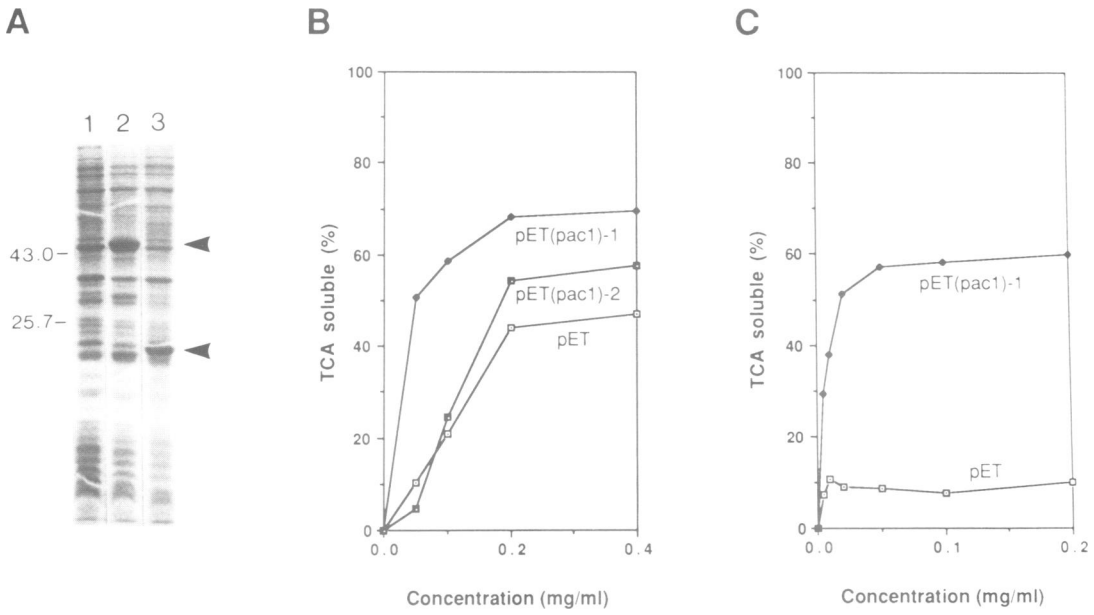

Fig. 4. Double-stranded RNase activity of the *pac1* gene product. **A**. SDS−polyacrylamide gel electrophoresis (SDS−PAGE) patterns of the *E.coli* extracts used for RNase III assay in B. Coomassie Brilliant Blue staining. 1, BL21(DE3)pLysS (*rnc*$^+$) transformed with the pET vector; 2, the same strain transformed with pET(pac1)−1; and 3, the same strain transformed with pET(pac1)−2. Arrows indicate the full-size (lane 2) or a truncated (lane 3) *pac1* product produced. See text for more explanation. **B**. DsRNase activity is expressed by the appearance of TCA-soluble material. Measurements were carried out as in Materials and methods. The abscissa indicates the concentration of proteins in the reaction mix. The reaction proceeds linearly only when the protein concentration is low and comparison of specific activities under such conditions suggests that the pET(pac1)−1 extract is at least five times more active than the others. The host *E.coli* is BL21(DE3)pLysS (*rnc*$^+$). **C**. The same assay as in B but the host is a *rnc*$^-$ derivative of BL21(DE3)pLysS in this case.

Homology between the pac1 gene product and RNase III

The predicted *pac1* gene product was compared with the protein sequences in the NBRF data base and was found to have considerable homology to RNase III of *E.coli*. Although the *pac1* gene product has extra 133 amino acids at its N-terminus, the remaining 230 amino acids share 25% identity with the entire sequence of RNase III (Figure 3). An identical sequence HNERLEFLGDS, is seen at positions 170−180 in *pac1* and at positions 36−46 in RNase III. RNase III loses its activity if glycine in this stretch is replaced by aspartic acid (*rnc−105* mutation) (Nashimoto and Uchida, 1985). The conservation of these residues in the *pac1* gene product suggested that it might have a similar RNase activity to RNase III.

The pac1 gene product is a double-stranded ribonuclease

A standard assay for *E.coli* RNase III is to test its activity in degrading double-stranded RNA (dsRNA) (Robertson *et al.*, 1968). This assay was applied to the *pac1* gene product. The *pac1* gene was expressed in an *E.coli* strain using the T7 promoter-RNA polymerase system (Studier and Moffatt, 1986; Rosenberg *et al.*, 1987). A plasmid, pET(pac1)−1, was designed to produce the complete *pac1* gene product, whereas another plasmid, pET(pac1)−2, could produce only the region homologous to RNase III (amino acids 134−363). Both plasmids directed overproduction of a protein in *E.coli* (Figure 4A). These overproduced proteins could react with antibodies against Pac1 protein, which were prepared using a fusion protein (data not shown). Crude extract prepared from *E.coli* harboring pET(pac1)−1 revealed an activity degrading dsRNA at least five times higher than the basal level of RNase III in *E.coli* (Figure 4B). However, the extract of a pET(pac1)-2 transformant, expressing a truncated *pac1* protein of about the same size as RNase III, did not show an increase in the activity (Figure 4B). To exclude the possibility that RNase III of the *E.coli* host was activated by overproduction of the *pac1* gene product, similar experiments were repeated using an *E.coli* strain deficient in RNase III as the host. The *rnc-105* host was constructed by P1 transduction. This strain showed only

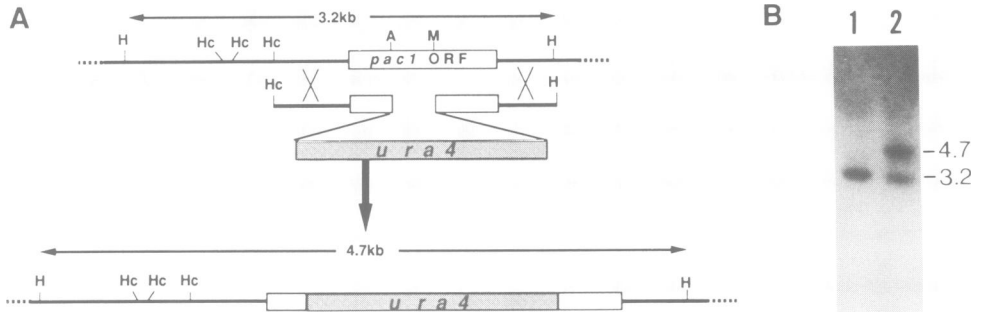

Fig. 5. A scheme for disruption of the genomic *pac1* gene. **A.** A 0.3 kb *Acc*I−*Mlu*I fragment was excised from the *pac1* ORF and a 1.8 kb *ura4*$^+$ cassette (Grimm *et al.*, 1988) was inserted instead. This construct, trimmed at the nearest *Hin*cII and *Hin*dIII sites, was introduced into the genome of a diploid strain JY765 by homologous recombination. **B.** Southern blot analysis which confirms accurate substitution of one wild type allele of *pac1* by the disrupted allele in the resultant diploid JY840. DNA prepared from either JY765 or JY840 was digested with *Hin*dIII, electrophoresed, blotted and probed with a 1.0 kb *Xba*I−*Hin*dIII fragment covering the C-terminal half of the *pac1* ORF. 1, JY765; and 2, JY840.

a limited dsRNA-degrading activity, the identity of which was unclear (Figure 4C), and it was obvious that pET(pac1)−1 could also increase an RNase III-like activity in this strain (Figure 4C). Thus, we conclude that the *pac1* gene product indeed has an RNase III-like activity and that its amino terminal region, which does not exist in RNase III, is necessary for this activity.

Overexpression of pac1 inhibits both mating and sporulation in wild type S.pombe cells

Overexpression of *pac1* suppressed the uncontrolled meiosis of *pat1*^{ts} strains at the restrictive temperature. We examined the effect of *pac1* overexpression in wild type strains. As shown in Table I, a sporogenic diploid strain JY362 sporulated poorly when transformed with pKF1-D2, a *pac1*$^+$ subclone of pKF1 that carried the 3.2 kb *Hin*dIII fragment shown as **a** in Figure 1. Furthermore, mating was almost completely inhibited in the homothallic strain JY450 harboring pKF1-D2 (Table I). Thus, overexpression of *pac1* arrests not only *pat1*-driven uncontrolled meiosis but sexual development in general. To verify that the *pac1* gene product is indeed responsible for the arrest, a derivative of pKF1-D2 with a 4 bp insertion in the *pac1* ORF was constructed as follows. pKF1-D2 was cut with *Mlu*I, the staggered ends produced were filled with DNA polymerase, and the resulting blunt ends were religated. This procedure eliminated the latter half of the *pac1* ORF and added 11 extra amino acids at the C-terminus. The derivative could not cause inhibition either of mating or of sporulation, clearly indicating that it is overproduction of Pac1 RNase that arrests sexual development.

Cells overexpressing *pac1* grew at the same rate as cells carrying only the vector in all media tested, including minimal medium with a reduced amount of either a carbon or a nitrogen source. Thus *pac1* overexpression does not appear to have adverse effects on cell growth.

pac1 is essential for cell growth

A null allele of *pac1* was constructed by gene disruption. The 0.3 kb *Acc*I−*Mlu*I fragment within *pac1* was substituted by a 1.8 kb *ura4*$^+$ gene cassette (Figure 5A). The region deleted from *pac1* corresponds to nearly 28% of the total ORF and includes the conserved HNERLEFLGDS sequence. A *ura4*$^-$ diploid strain JY765 was transformed with a *Hin*cII−*Hin*dIII fragment carrying the disrupted *pac1* allele. Stable Ura$^+$ transformants were isolated and were examined by Southern blot analysis to determine whether a *pac1* allele on the chromosome was correctly substituted by the disrupted allele (Figure 5B). JY840 was thus selected as a putative disruptant.

Table I. Effects of *pac1* overexpression on sexual development

Strain	Plasmid	Mating efficiency (%)	Sporulation efficiency (%)
JY450	pDB248′ (vector)	73	−
	pKF1−D2 (*pac1*$^+$)	0.4	−
JY362	pDB248′	−	87
	pKF1−D2	−	0.5

Tetrad analysis of JY840 revealed that no more than two spores could form colonies in each set of tetrads and that the viable cells were always Ura$^-$. We thus conclude that the *pac1* gene function is essential for vegetative cell growth. The terminal morphology of *pac1*$^-$ spores on germination plates was examined. The spores germinated, formed microcolonies of four to eight cells, and ceased growth. Cell shapes were rather elongated but no hint of uncontrolled meiosis, as seen in *pat1*$^-$ cells, was observed (Figure 6).

Discussion

We previously reported that *S.pombe* cells carrying multiple copies of the *pac1* gene did not express *mei2*, an essential gene for commitment to meiosis, under nitrogen starvation (Watanabe *et al.*, 1988). This explains the inability of such transformants to undergo meiosis, but does not explain their mating deficiency, because *mei2* is dispensable for mating. Why are cells overexpressing *pac1* sterile? We have observed that transcripts of a series of genes required for mating are scarce in these cells (unpublished results). Among them is *ste11*, formerly called *steX* (Watanabe *et al.*, 1988), which is necessary both for mating and meiosis and functions upstream of *mei2* (A.Sugimoto *et al.*, in preparation). The inhibition of *ste11* gene expression would account for the observed sterility.

The *pac1* product is an RNase related to *E.coli* RNase III. This leads to the straightforward interpretation that the scarcity of RNAs essential for sexual development may be due to their degradation by Pac1 RNase. Alternatively, Pac1 RNase may have a key substrate, lack of which inhibits expression of a number of downstream genes. Although the latter possibility appears more likely, a final conclusion

Table II. *S.pombe* strains used

Name	Genotype
JY362	*h⁺/h⁻ leu1/leu1 ade6–M210/ade6–M216*
JY450	*h⁹⁰ leu1 ade6–M216*
JY628	*h⁺ ran1(pat1)–3 leu1*
JY712	*h⁻ pat1–114 leu1*
JY765	*h⁺/h⁻ leu1/leu1 ura4–D18/ura4–D18 ade6–M210/ade6–M216*
JY840	*h⁺/h⁻ leu1/leu1 ura4–D18/ura4–D18 ade6–M210/ade6–M216 pac1⁺/pac1::ura4⁺*

should await identification of the Pac1 RNase substrate(s).

RNase III recognizes stem–loop structures in RNA and cuts them in the stem. RNase III processes the rRNA precursor and certain mRNAs in *E.coli* (Dunn and Studier, 1973). Some mRNAs are translationally activated by RNase III cleavage (Saito and Richardson, 1981), whereas other mRNAs become susceptible to the attack of exonucleases once cleaved by RNase III. The latter class includes the *pnp* mRNA for polynucleotide phosphorylase (Portier *et al.*, 1987; Takata *et al.*, 1987) and the mRNA for RNase III itself (Bardwell *et al.*, 1989). A large number of unidentified messages are also likely to be affected by this enzyme because many *E.coli* proteins change their abundance between *rnc⁺* and *rnc⁻* strains (Gitelman and Apirion, 1980). Computer analysis has shown that several stem–loop structures are possible in the 5′ region of *mei2* or *ste11* mRNA, for instance, although they are somewhat shorter than a typical RNase III cleavage site.

Elevation of intracellular cAMP level causes inhibition of sexual development in *S.pombe* (Calleja *et al.*, 1980; Beach *et al.*, 1985; Watanabe *et al.*, 1988). Overexpression of *pac1* mimics this situation. However, the amount of cAMP in cells overexpressing *pac1* was not higher than in wild type cells (unpublished results). Furthermore, although complete depletion of cAMP from *S.pombe* cells does not arrest cell growth (Maeda *et al.*, 1990), disruption of *pac1* is lethal. These suggest that Pac1 RNase does not cause effects via cAMP.

Pac1 RNase should be active during vegetative cell growth and it is conceivable that this enzyme functions as a 'sweeper' of mRNAs which can potentially induce sexual development and are ectopically expressed in the mitotic cell cycle. We do not know whether its activity changes during the transition from the mitotic cell cycle to the sexual pathway. However, since the Pac1 RNase has an extra N-terminal stretch that RNase III does not have, which could be involved in modulation of Pac1 RNase activity, it is tempting to speculate that Pac1 RNase may be a regulator of sexual development in *S.pombe*.

After RNase III was shown to be involved in processing of rRNA precursors, double-stranded RNase (dsRNase) activity was demonstrated in various animal cells and implicated in processing of rRNA precursors in some instances. Ohtsuki *et al.* (1977) purified a dsRNase from calf thymus, whose molecular weight is ~60 kd, and suggested its involvement in rRNA processing. Hall and Crouch (1977) described a dsRNase named nuclease DII which was subsequently implicated in rRNA processing (Grummt *et al.*, 1979). The nuclease DII appears particularly interesting to us because its molecular weight was estimated to be ~38–40 kd by sucrose gradient sedimentation (Hall and Crouch, 1977) but it migrates as an apparent 46 kd protein in SDS–PAGE (Grummt *et al.*, 1979). The Pac1 RNase behaves similarly: its calculated molecular weight is 41 kd and it migrates as an apparent 45 kd protein in SDS–PAGE (Figure 4A). Thus, possible homology of these two RNases remains an interesting question.

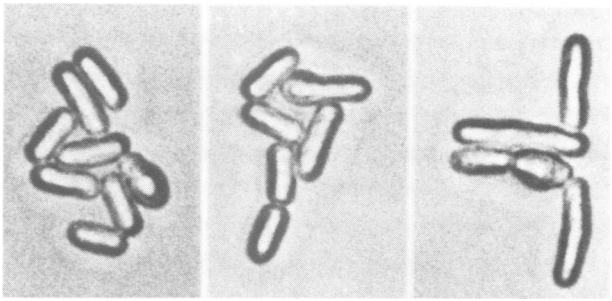

Fig. 6. Terminal morphology of germinated *pac1⁻* spores. Three typical examples are shown. Most deduced *pac1⁻* spores completed germination, divided to give four to eight cells and ceased growth, presumably after consuming the residual *pac1* activity.

It is largely unknown how mRNA undergoes degradation in eukaryotic cells (for review, see Brawerman, 1987). The mRNAs for histone and transferrin receptor are supposed to be cleaved initially by endonucleases which recognize stem–loop structures, and then degraded rapidly (Ross *et al.*, 1986; Müllner and Kühn, 1988), but these endonucleases have not been identified. Further analysis of Pac1 RNase may also provide useful information on the eukaryotic mRNA decay system.

Despite sequence homology and similarity in enzymatic activities, the antibodies we prepared against Pac1 RNase failed to react with RNase III (data not shown). Antibodies against RNase III, provided by D.Court, also failed to react with Pac1 RNase. Reciprocal complementation experiments between the two genes have been unsuccessful so far. This may mean that the two enzymes differ considerably in their gross tertiary structures and substrate specificities.

We would finally like to refer to two studies carried out in *S.cerevisiae*. Mead and Oliver (1983) described the purification of a dsRNase from *S.cerevisiae*, with a molecular weight of 27 kd. This enzyme was more active in nitrogen-starved cells. Pines *et al.* (1988) artificially expressed RNase III in *S. cerevisiae* cells and found that it causes lethality. These results are superficially contradictory to the characteristics of the *S.pombe* Pac1 RNase. A possible explanation of this inconsistency is that there are multiple dsRNases with different substrate specificities. It may be worth trying to identify genes for RNase III-like RNases in *S.cerevisiae* and other organisms by using the conserved region seen in Pac1 RNase as a probe.

Materials and methods

Strains and media

S.pombe strains used in this study are listed in Table II. Media used have been described previously (Egel and Egel-Mitani, 1974; Gutz *et al.*, 1974; Beach *et al.*, 1985; Watanabe *et al.*, 1988). *E.coli* strains for the expression vector system using a T7 promoter were those of Studier and co-workers (Studier and Moffat, 1986; Rosenberg *et al.*, 1987).

Genetic methods and transformation of S.pombe

General genetic methods for *S.pombe* have been described (Gutz *et al.*, 1974). Transformation of *S.pombe* cells was performed as described (Beach *et al.*, 1982; Fukui *et al.*, 1986).

Southern and Northern analyses

Total *S.pombe* DNA was extracted essentially according to Cryer *et al.* (1975). Southern blot analysis was done as described (Southern, 1979). Total cellular RNA was extracted according to Elder *et al.* (1983), either from rapidly growing *S.pombe* cells ($2-5 \times 10^6$ cells/ml) in PM medium or from cells shifted to PM without ammonium chloride and incubated for 4 h. Denaturation of RNA, gel electrophoresis, blotting and hybridization were performed as previously described (Thomas, 1980; Watanabe *et al.*, 1988).

Nucleotide sequence determination

The 3.2 kb *Hin*dIII fragment of pKF1 was cloned in pUC19. Two types of subclones were constructed from it. (i) The *Hin*dIII fragment was divided into various restriction fragments and these were subcloned. (ii) Various deletion plasmids were generated by ExoIII treatment according to Henikoff (1984). The nucleotide sequence of each subclone was determined by the dideoxy-chain termination method (Sanger *et al.*, 1977). The region shown in Figure 2 has been sequenced in both directions at least once.

Assay of RNase III activity

Preparation of *E.coli* crude extracts was according to Dunn (1976). The assay method originates from Dunn (1976) and is described in Nashimoto and Uchida (1985). Instead of the ^{14}C-labeled substrate, tritium-labeled poly(A)·poly(U) was used as a substrate here. RNase III digestion converts TCA-insoluble substrates to a TCA-soluble form (15–20 bp), and RNase III activity is expressed as the percentage of TCA-soluble material produced by the reaction. The reaction mix (100 μl) contained 20 mM Tris–HCl (pH 7.6), 0.1 M KCl, 10 mM $MgCl_2$, 0.1 mM dithiothreitol, 5 μg of [^{3}H]poly(A)·poly(U) (4000 c.p.m.) and a fraction of the *E.coli* extract corresponding to 0.5–20 μg of proteins. The reaction was done at 37°C for 20 min. Protein concentration was estimated with the BioRad Protein Assay Kit according to the protocol of the supplier.

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