Mol Gen Genet (1990) 224:72–80

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A yeast mutant, *PRP20*, altered in mRNA metabolism and maintenance of the nuclear structure, is defective in a gene homologous to the human gene *RCC1* which is involved in the control of chromosome condensation

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Received April 23, 1990

Summary. We report on the characterization of the yeast *prp20-1* mutant. In this temperature-sensitive mutant, multiple steps of mRNA metabolism are affected. The *prp20-1* mutant strain showed alterations in mRNA steady-state levels, defective mRNA splicing and changes in transcription initiation or termination when shifted from the permissive to the non-permissive temperature. In addition, a change in the structure of the nucleus in these cells became apparent. Electron microscopy revealed an altered structure of the nucleoplasm of *prp20-1* mutant cells when grown at the non-permissive temperature that was not observed in cells grown at the permissive temperature or in wild-type cells. The wild-type *PRP20* gene was isolated and sequenced. The putative PRP20 protein has a molecular weight of 52 kDa. We found that the *PRP20* gene is identical to the yeast *SRM1* gene (Clark and Sprague 1989). In addition, the PRP20 protein sequence shows significant sequence similarity to the human RCC1 protein (Ohtsubo et al. 1987). This protein has been implicated in the control of chromosome condensation. Based on the phenotype of the *prp20-1* mutant and the observed sequence similarity to the human RCC1 protein, we postulate that the yeast PRP20 protein is involved in the control of nuclear organization.

Key words: *Saccharomyces cerevisiae* – Splicing – Nuclear structure

Introduction

The splicing of intron-containing pre-mRNA is one step in the maturation of mRNA in eukaryotic cells. The removal of introns takes place in the nucleus and occurs on a large complex, the spliceosome (for reviews, see Green 1986; Padgett et al. 1986). The identification of temperature-sensitive mutants of *Saccharomyces cerevisiae* defective in mRNA splicing has allowed the identification of several protein components involved in the formation of the spliceosome (Vijayraghavan and Abelson 1989). In addition, genetic studies have established the role of several small nuclear RNA species in the splicing process (Guthrie and Patterson 1988). However, recent results have indicated that the different steps in mRNA maturation may not be independent of each other: it seems likely that the formation of the spliceosome occurs while the pre-mRNA is still being transcribed (Aebi et al. 1986; Beyer and Osheim 1988). In addition, the formation of the spliceosome may prevent the export of unspliced pre-mRNA from the nucleus to the cytoplasm (Legrain and Rosbash 1989). The observation that a given mRNA species follows a "track" from the place of synthesis to the nuclear envelope indicates that at least in higher eukaryotic cells the different maturation steps of mRNA are linked to a nuclear structure (Lawrence et al. 1989).

The yeast system should prove very useful in the study of the influence of the nuclear structure on RNA metabolism. In a mutant defective in this nuclear organization, several different steps in mRNA metabolism may be affected. Indeed, in a collection of temperature-sensitive splicing mutants, we may have found such a mutant (*prp20;* Vijayraghavan et al. 1989). This mutant was identified as a conditional splicing mutant using the accumulation of pre-mRNA under non-permissive conditions as an assay (Vijayraghavan et al. 1989). The *prp20* mutant was distinct from other *prp* mutants in that accumulation of oversized pre-mRNA was also detected. This observation led us to the hypothesis that *prp20* may represent a mutant in which several steps of mRNA metabolism are affected.

Here, we describe additional phenotypic features caused by the *prp20-1* mutation. A mutation in the *PRP20* gene not only leads to a defect in mRNA splicing

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but also alters the steady state levels of different mRNA species. We also observed new RNA species in a *prp20-1* mutant strain when grown under non-permissive conditions. In addition, an alteration in the ultrastructure of the nucleus in this mutant was observed. We isolated the *PRP20* gene by functional complementation. It encodes a protein of 52 kDa which is identical to the SRM1 protein described by Clark and Sprague (1989). The 52 kDa protein sequence shows a high degree of similarity to that of the human RCC1 protein. This human protein is thought to be involved in the control of chromosome condensation.

Materials and methods

Strains and media. The original isolate of the *prp20-1* strain has been described (Vijayraghavan et al. 1989). The strains prp20/2A (*MATα prp20-1 ade2-101 his3Δ200 ura3-52*) and prp20/2C (*MATa prp20-1 ade2-101 his3Δ200 ura3-52 lys2-801*) are segregants of the first backcross to the wild-type strain SS328 (*MATα ade2-101 his3Δ200 lys2-801 ura3-52*). The tester strain 29-4A (*MATa leu2 his3Δ200 ura3-52*) was used for backcrosses to strains containing stable integrates of the plasmid pYIp50/*PRP20*. The strains RL92 (*MATa prp(rna)2 leu2-3 leu2-112 ura3-52*; Lustig et al. 1986) and NOY237 (*MATa rpaΔ190::URA3 ura3-52 leu2-3, 112 his4-Δ401 trp1-Δ1 CAN*^{r}pGAL-*RPA190;* Wittekind et al. 1990) were used as reference strains for microscopy. Strain 20B-12-1 (*MATα pep4-3 ppc1 prb1 his1*) (obtained from E. Jones, Carnegie-Mellon University, Pittsburgh, PA) served as a wild-type reference. Yeast media were prepared according to Sherman et al. (1983).

RNA extraction and Northern blot analysis. Total RNA was extracted from exponentially growing cells using the glass beads method described by Sherman et al. (1983). Poly(A)$^+$ RNA was selected using oligo(dT)-cellulose chromatography. The RNA was analyzed on a 1.2% agarose gel in 50 mM Hepes, 1 mM EDTA, 6% formaldehyde. In each lane was added 10 μg total or 2 μg poly(A)$^+$ RNA. Blots were prepared according to Vijayraghavan et al. (1989). As probes were used the 0.8 kb *Sal*I-*Hpa*I fragment of the *TCM1* gene (Schultz and Friesen 1983), and 3.4 kb *Sal*I-*Eco*RI fragment of the *PAB* gene (Sachs et al. 1986), and the 0.8 kb *Bam*HI fragment and the 0.2 kb *Pst*I-*Xba*I fragment of the *PRP20* gene (see Fig. 4C), labeled according to the random priming method of Feinberg and Vogelstein (1983). Reprobing of the blots was done after removal of the probe by boiling in 0.1 × SSC, 0.1% SDS for 10 min.

Yeast transformation, isolation and sequence determination of the PRP20 *gene.* Yeast strains were transformed according to the lithium acetate method of Ito et al. (1983). For the isolation of the *PRP20* gene, a plasmid pool (Rose et al. 1987, obtained from Scott Emr) consisting of yeast DNA partially digested with *Sau*3A cloned into the vector YCp50 was transformed into the strain prp20/2C and transformants were selected on minimal medium lacking uracil. The transformants were tested for temperature resistance by replica-plating on YEPD medium and incubation at 37° C. Positive clones were tested for simultaneous loss of the Ura$^+$ and Ts$^+$ phenotype by selecting Ura$^-$ colonies on 5-fluoro-orotic acid plates (Boeke et al. 1984). After isolation of the corresponding plasmid DNA, one plasmid, which contained an insert of approx. 13 kb, was analyzed further. Subfragments were cloned in the vector pPHYC18, a YCp50 derivative containing the polylinker sequence of the Bluescript vectors (Stratagene) (Paul Hermann, personal communication). These subfragments were tested for the ability to complement the *prp20-1* mutation in the strain prp20/2C. A complementing 6.1 kb *Eco*RI-*Xba*I fragment was analyzed further. Nested deletions entering the fragment from either site were created using the Stratagene protocol (Stratagene, San Diego) and cloned into the pPHYC18 vector to test their complementing ability. The appropriate region was sequenced by creating nested deletions as described above and the sequence of both strands was determined using Sequenase (USB).

Integration of the PRP20 *gene at the* prp20 *locus.* The 5.9 kb *Sal*I–*Xba*I fragment (Fig. 4C) was cloned as a *Sal*I-*Eco*RI fragment into the YIp5 vector cleaved with *Sal*I and *Eco*RI. The *Eco*RI site of the *PRP20* fragment derives from polylinker sequences directly following the *Xba*I site. The resulting plasmid was linearized with *Mlu*I and used for transformation of the strain prp20/2A to the Ura$^+$ phenotype. Four independently transformed strains were selected and crossed to the strain 29-4A. Tetrad analysis of these crosses were performed as described by Sherman et al. (1983).

Microscopy. The strains prp20/2C, RL92 and 20B-12-1 were grown to mid log phase at 23° C and then shifted to 37° C. Aliquots were taken at different time intervals and prepared for examination by microscopy. Strain NOY237 was grown to mid log phase in YPGal (1% yeast extract, 2% peptone, 2% galactose) to allow expression of the *RPA190* gene under the control of the *GAL1* promoter. The cells were collected and kept for 6 h in YEPD medium to stop *RPA190* expression effectively (Wittekind et al. 1990). Fixing and staining of the cells has been described (Clark 1990a; Clark and Abelson 1987). For electron microscopy, the cells were prepared as described by Clark (1990b).

Results

As described previously (Vijayraghavan et al. 1989), the *prp20-1* mutant was isolated from a collection of about 1000 temperature-sensitive mutants based on the phenotype of pre-mRNA accumulation upon a shift from permissive (23° C) to non-permissive temperature (37° C). In order to verify that the ts-phenotype and pre-mRNA accumulation co-segregated, the original isolate (ts319)

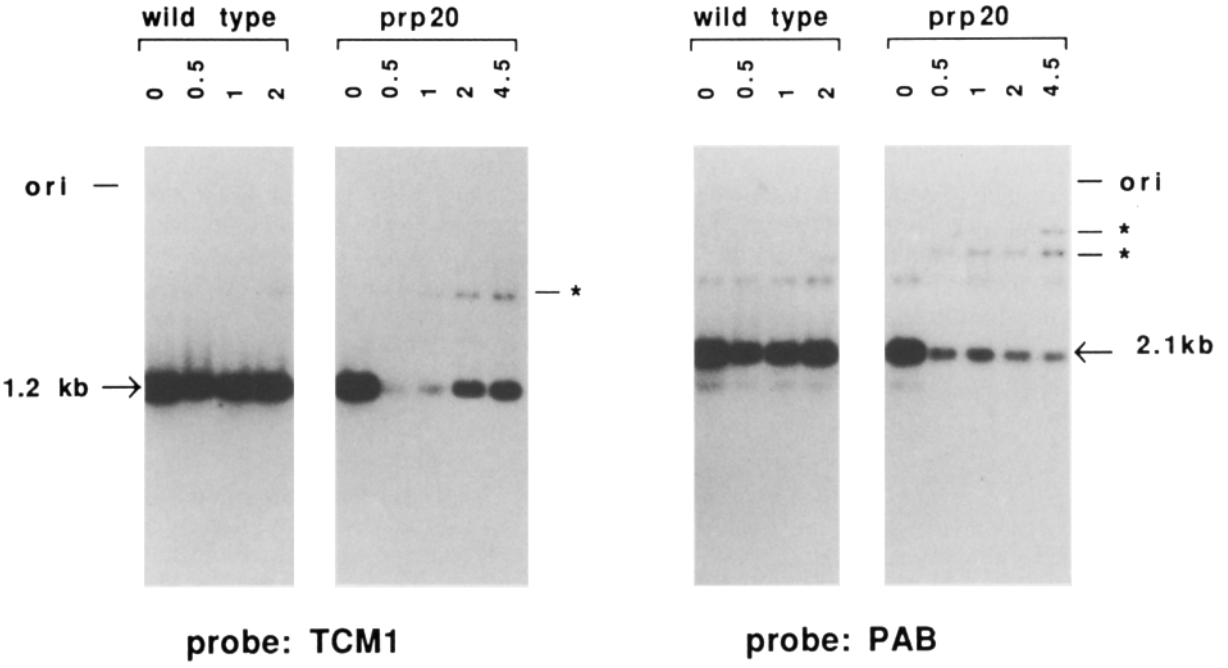

Fig. 1. Expression of the *TCM1* and *PAB* genes in the wild type and the *prp20-1* mutant at 23° and 37° C. The two yeast strains SS328 and prp20/2C were grown at 23° C in YEPD medium to mid log phase ($OD_{546}=2$). At time 0, the cultures were shifted to 37° C and RNA was extracted at the indicated times (hours). Poly(A)$^+$-RNA was selected by oligo(dT)-cellulose chromatography and 2 μg RNA was applied per lane, subjected to electrophoresis and transferred to nitrocellulose membrane. The blot was probed first with a probe specific for *TCM1*. After removal of the *TCM1* signals, the blot was reprobed with a *PAB*-specific probe. The *asterisks* mark bands which appear in the *prp20-1* mutant strain upon the shift to non-permissive conditions

(Vijayraghavan et al. 1989) was crossed to the strain SS328 and the resulting tetrads were analyzed. In five tetrads tested, the ts-phenotype and pre-mRNA accumulation co-segregated, indicating that the two phenotypes were caused by the same mutation (data not shown).

The prp20-1 *mutation affects mRNA metabolism*

The *prp20-1* mutant differed from all other mRNA splicing-deficient mutants in the initial screen. In Northern blot analysis using intron sequences of actin as a probe, new actin RNA species which were longer than the actin pre-mRNA were detected in addition to unspliced pre-mRNA (Vijayraghavan et al. 1989). In order to determine the effect of the *prp20-1* mutation on the steady-state level of non-spliced mRNA species, we performed Northern blot analysis of RNA from a *prp20-1* strain grown for different time periods at the non-permissive temperature (37° C). As probes, we used DNA sequences specific for either the gene encoding the poly(A)-binding protein (*PAB*) (Sachs et al. 1986) or the ribosomal protein gene *TCM1* (Schultz and Friesen 1983). For the Northern blots, total RNA as well as oligo(dT)-selected poly(A)$^+$ RNA was used. In Fig. 1, only the results obtained using the poly(A)$^+$ RNA are shown; they did not differ significantly from those seen with total RNA. In both cases, we observed a dramatic drop in the steady-state level of the transcripts in the *prp20-1* mutant after 30 min incubation at the non-permissive temperature. Whereas the steady-state level of *PAB* transcripts decreased continuously in the *prp20-1* mutant, the *TCM1* RNA level, after an initial drop, increased upon longer incubation at 37° C (Fig. 1).

In addition to the normal-sized transcripts, both probes detected oversized transcripts in the *prp20-1* mutant (Fig. 1, marked by asterisks). These transcripts appeared 30 min after the temperature shift and their levels increased with time. Since these transcripts were present in equivalent ratios in total and in oligo(dT)-selected RNA, we concluded that they were polyadenylated.

The prp20-1 *mutation leads to a morphological change in the structure of the nucleus*

As described above, the *prp20-1* mutation affects the steady-state level of non-spliced mRNA and the splicing of actin pre-mRNA and leads to the appearance of new, oversized RNA species. We speculated that this pleiotropic effect was correlated with an altered structure of the nucleus. In order to test this hypothesis, a *prp20-1* mutant strain was analyzed microscopically after a shift from permissive to non-permissive temperature. First, we examined the effect of the *prp20-1* mutation on the nuclear structure by light microscopy. The nucleolus-specific silver staining of whole mount cells (Clark 1990a) revealed that upon temperature shift, the structure of the nucleolus was affected quite dramatically (Fig. 2). Only 30 min after the shift, the clearly defined cap-like structure of the nucleolus (Fig. 2A, arrowhead)

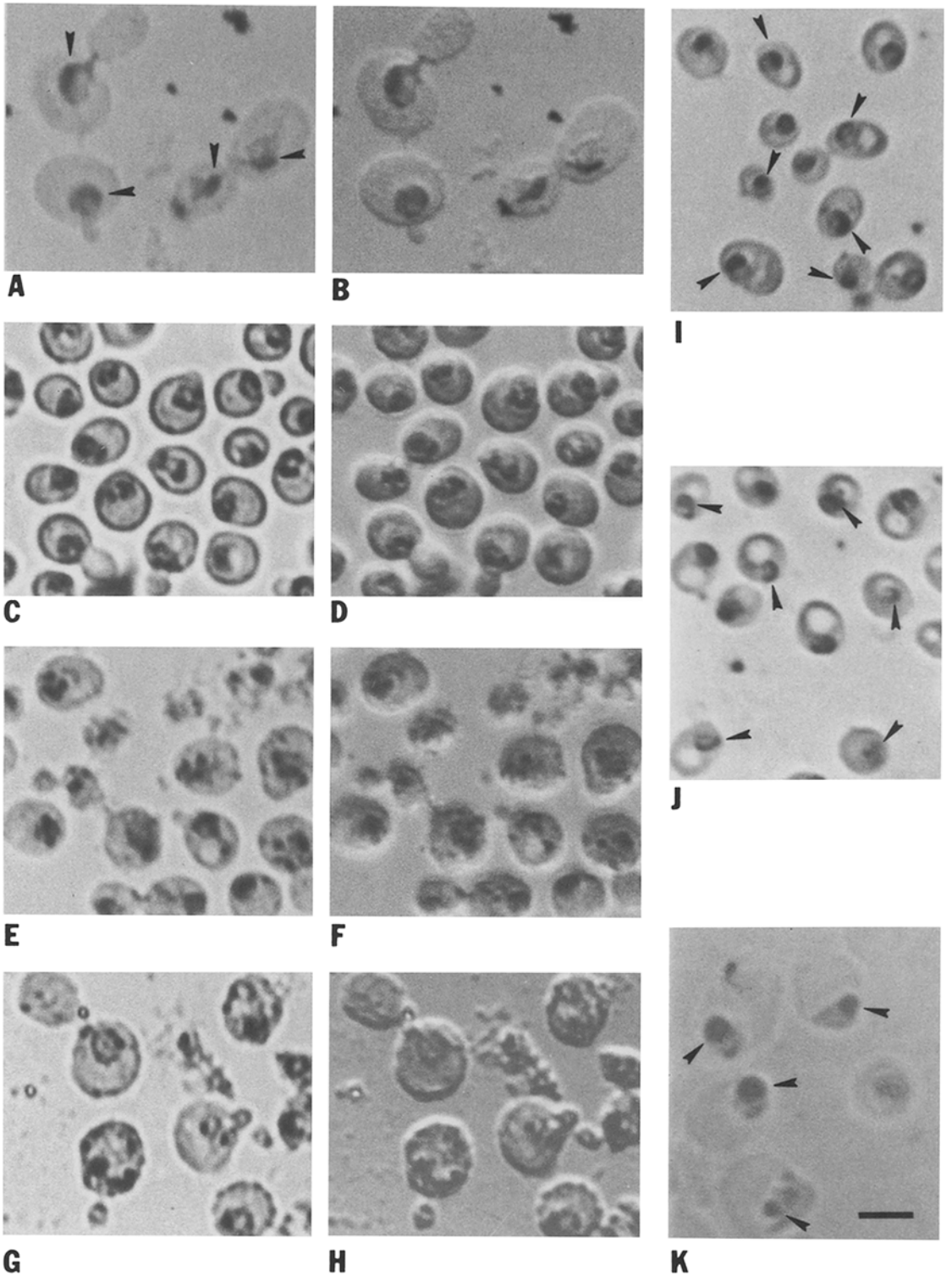

Fig. 2A–K. Nucleolus-specific silver staining of *prp20* cells grown for various time at 37° C. The strain prp20/2C was grown to mid log phase at permissive temperature (23° C) and then shifted to 37° C. Samples were removed at different times (**A, B** grown at 23° C; **C, D** 0.5 h at 37° C; **E, F** 1 h at 37° C; **G, H** 2 h at 37° C) and the cells were processed for light microscopy. As controls, a wild-type strain, grown at 37° C (**I**), a temperature-sensitive *prp2* strain, grown at 37° C for 1 h (**J**) and the strain NOY237, grown for 6 h in YEPD (**K**) are shown. **A, C, E, G, I, J** and **K** Bright field illumination; **B, D, F** and **H** Nomarski optics. The *arrowheads* point to intact nucleolar structures. Bar represents 5 μm

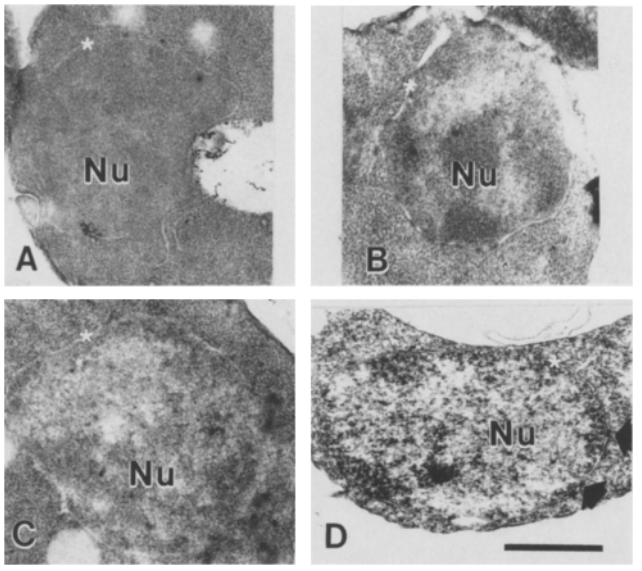

Fig. 3A–D. The ultrastructural changes in the nucleoplasm caused by the *prp20-1* mutation. **A** An ultrathin section of the nucleus of a *prp20-1* mutant cell grown at 23° C. **B, C** and **D** Thin sections of the nuclei of *prp20-1* mutant cells grown at 37° C for 0.5, 1 and 2 h, respectively. *Nu* nucleus. The *white asterisks* indicate the lumen of the nuclear envelope. The *black arrowheads* in **D** point to nuclear pore structures. Bar represents 5 μm

was altered (Fig. 2C and D). After 2 h, no defined nucleolus could be detected in the mutant cells. This effect was specific for the *prp20-1* mutation, since wild-type cells (Fig. 2I) and the temperature-sensitive *prp2* mutant grown at 37° C (Fig. 2J) as well as the RNA polymerase I-deficient strain NOY237 (Fig. 2K) did not show this nucleolar disruption. Changes in the appearance of the cytoplasm, for example, the presence of large vacuolar structures, were also detected in the *prp20-1* mutant strain. However, these changes were not specific for the *prp20-1* mutant, since such structures were also visible in the other temperature-sensitive strains.

A similar change in the ultrastructure of the nucleoplasm was revealed by electron microscopy. Figure 3 shows the ultrastructural appearance of the yeast nucleus resulting from the *prp20-1* defect. Grown at 23° C, the nucleoplasm of *prp20-1* mutant cells stained at a uniform density throughout the nucleus (Fig. 3A); it was separated from a very similarly stained cytoplasm by the nuclear envelope (Fig. 3A, white asterisk). Upon growth at non-permissive temperature, the nucleoplasm lost this uniform staining characteristic and clumps of stained material appeared (Fig. 3B–D). The disruption of the nucleus by the *prp20-1* mutation seemed to affect primarily the nucleoplasm. Even after 2 h at non-permissive temperature (Fig. 3D) the nuclear envelope (white asterisks) and the nuclear pores (black arrows) remained structurally intact.

Isolation of the PRP20 *locus*

In order to isolate the *PRP20* gene, a plasmid pool (Rose et al. 1987, obtained from Scott Emr) containing yeast chromosomal DNA partially digested with *Sau*3A and ligated into the vector YCp50 was used to transform yeast strain prp20/2C to uracil prototrophy. Resulting transformants were then tested for growth at the restrictive temperature (37° C). From approximately 30000 uracil auxotrophs, around 50 temperature-resistant strains were obtained. Several of these strains were tested for simultaneous loss of the Ura^+ and the Ts^+ phenotype, using the 5-fluorouracil selection method (Boeke et al. 1984). From 4 of these strains, the plasmid DNA was isolated, amplified in *Escherichia coli* and tested for the ability to transform strain prp20/2C to Ura^+ and Ts^+. All 4 plasmids, which proved to have identical yeast DNA inserts, complemented the *ura3-52* as well as the *prp20-1* mutation. One plasmid was chosen for further analysis. Different fragments of the insert were subcloned and tested for complementation of the *prp20-1* mutation. A complementing 6.1 kb *Eco*RI-*Xba*I fragment (Fig. 4C) was analyzed in more detail by creating two sets of nested deletions from either side of the fragment and testing individual deletions for the ability to complement the *prp20-1* mutation. This mapping reduced the size of the DNA required for complementation to 2.5 kb. The DNA sequence of this region was determined.

The DNA fragment sequenced showed a single, long open reading frame (ORF), encoding a potential polypeptide of 482 amino acid residues (see below). In order to confirm that this region was the *PRP20* gene, the 5.9 kb *Sal*I-*Xba*I fragment (Fig. 4C) was subcloned on the vector YIp5 and the linear DNA, cleaved at the single *Mlu*I site (see Fig. 4C) was used to transform yeast strain prp20/2A to uracil prototrophy. Four different Ura^+ clones were selected and crossed to the tester strain 29-4A. Resulting tetrads were analyzed. In three of these crosses, no temperature-sensitive segregants (in a total of 18 tetrads) were observed, indicating integration of the plasmid DNA in or very close to the *prp20* locus. One cross yielded a 2:2 segregation of the ts-phenotype. However, in this cross, the YIp5 plasmid marker *URA3* and the *prp20* locus were closely linked, since all 7 tetrads were of parental ditype with regard to these two markers. This linkage was not observed in crosses including chromosomal *prp20* and *ura3* mutations (data not shown) and indicated integration of the plasmid DNA at the chromosomal *prp20* locus in this transformant. We have not analyzed the exact structure of the *prp20* gene in this strain; however, gene conversion may account for the Ura^+, Ts^- phenotype of this transformant.

Since in all transformed strains tested, the linearized plasmid integrated at the *prp20* locus, we conclude that the cloned DNA fragment contained the *PRP20* gene.

Analysis of the transcription of the PRP20 *gene*

We analyzed the expression of the *PRP20* gene in wild-type and *prp20-1* mutant cells in a manner similar to that described above for *PAB* and *TCM1* transcription. First, we used a uniformly labeled, 0.8 kb *Bam*HI frag-

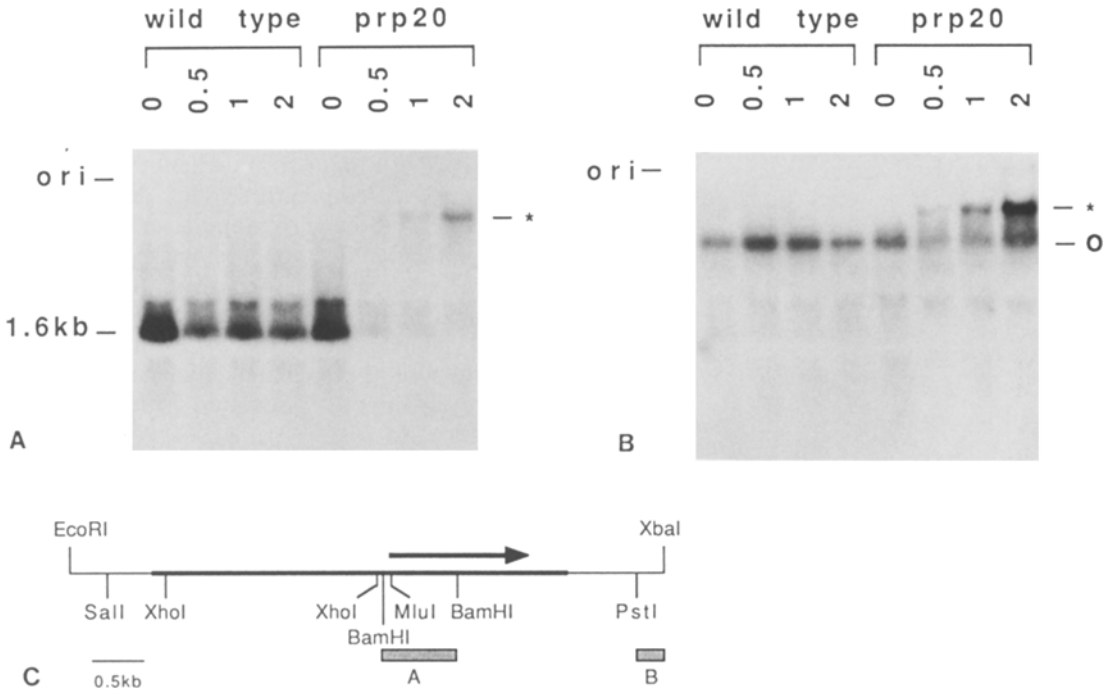

Fig. 4A–C. Expression of the *PRP20* gene in wild-type and *prp20-1* mutant cells. The two yeast strains SS328 and prp20/2C were grown at 23° C in YEPD medium to mid log phase ($OD_{546}=2$). At time 0, the cultures were shifted to 37° C and RNA was extracted at the times indicated (hours). 10 μg of total RNA was loaded per lane. The blot in **A** was probed with the 0.8 kb *Bam*HI fragment from the *PRP20* coding region uniformly labeled with ^{32}P. The blot in **B** was probed with the uniformly labeled 0.25 kb *Pst*I-*Xba*I fragment located downstream of the *PRP20* coding region. The blot in **A** was exposed for 16 h to X-ray film, whereas that in **B** was exposed for 48 h. The *asterisks* mark the position of the oversized transcript of the *PRP20* locus found in the *prp20-1* mutant strain grown at 37° C. In **B**, the *circle* indicates a transcript of unknown origin which is detected by the *Pst*I-*Xba*I probe only. This RNA species was detected in RNA from wild-type as well as from mutant cells. In **C**, a partial restriction map of the *PRP20* locus is shown. The sites of cleavage by selected restriction enzymes are given. The *thick line* indicates the sequenced region. The *arrow* shows the location of the open reading frame of the *PRP20* gene. The *shaded bars* indicate the DNA fragments used as probes for Northern blot analysis

ment (Fig. 4C) as probe. This fragment covers the 5′ terminal part of the *PRP20* ORF. Wild-type RNA yielded a major band at around 1.6 kb and a minor band of slightly lower mobility (Fig. 4A). As seen for the *TCM1* and *PAB* transcripts (Fig. 1), shifting the *prp20-1* mutant strain to non-permissive conditions led to a drastic decrease in the steady-state level of *prp20* transcripts and the appearance of a new, oversized transcript which could not be detected in wild-type RNA. The levels of these transcripts increased with time. Furthermore, these oversized transcripts also hybridized to a probe derived from sequences located downstream of the *PRP20* gene. The uniformly labeled *Pst*I-*Xba*I fragment (located downstream of the *PRP20* ORF, Fig. 4C) detected the same oversized transcripts as seen with the probe derived from the *PRP20* coding region (Fig. 4B). In addition, this probe hybridized to a transcript of approximately 3 kb present in wild-type as well as in *prp20-1* cells, indicating an undefined transcription unit located downstream of the *PRP20* gene. We concluded that the oversized transcripts detected in this experiment contained sequences derived from the *PRP20* coding region as well as sequences which are encoded downstream of the *PRP20* gene. In order to define the polarity of these oversized transcripts, we used an oligonucleotide probe (positions 1020–1003 of the *PRP20* gene; Clark and Sprague 1989) complementary to the *PRP20* mRNA. This probe hybridized only to the wild-type and mutant *PRP20* transcripts and not to the oversized RNA detected with the two randomly primed probes (data not shown). We therefore concluded that the oversized transcript contained sequences complementary to the *PRP20* mRNA. Transcription of these RNA species must have initiated downstream of the *PRP20* gene and extended into the *PRP20* coding region.

The PRP20 *gene is identical to the* SRM1 *gene*

The *PRP20* gene contains an ORF encoding a polypeptide of 482 amino acid residues with a calculated molecular weight of 52 kDa. We found that the *PRP20* sequence is identical to the previously reported sequence of the *SRM1* gene of *S. cerevisiae* (Clark and Sprague 1989). The *SRM1* gene was identified by a temperature sensitive, recessive mutation which restored mating competence to a *MATα* cell lacking the *STE3* receptor.

The PRP20 protein sequence is similar to that of RCC1, *a human protein involved in chromosome condensation*

We used the PIR database (release 21) to look for sequence similarities between PRP20 and known proteins.

Fig. 5. Alignment of the amino acid sequences of the RCC1 (top) and the PRP20 (bottom) proteins to show the seven repeats. The N- and C-terminal parts of each repeat are shown together with the number of amino acids which separate them. Gaps (−) are included to maximize the degree of similarity. Identical (|) or similar (.) amino acids in both sequences are marked. At the bottom, conserved positions in which 10 or more of the 14 repeats show identical or similar amino acid residues are indicated by *asterisks*

Only the human RCC1 showed significant similarity to the PRP20 protein sequence. The two proteins had 30% identity over a length of 474 amino acid residues. The *RCC1* gene was defined in the temperature-sensitive hamster cell line tsBN2, which at non-permissive temperature shows premature chromosome condensation (Nishimoto et al. 1978, 1981). Subsequently, the human cDNA was isolated by functional complementation (Kai et al. 1986; Ohtsubo et al. 1987). The amino acid sequence of RCC1 reveals the presence of seven repeated units of about 60 amino acid residues (Ohtsubo et al. 1987). These seven repeats share identical or chemically similar amino acids at several positions. We observed a similar repeated unit in the PRP20 sequence (Fig. 5). The sequence similarity to the human protein was most pronounced at the termini of these repeated units, whereas the middle portion of the repeats showed a lesser degree of conservation. As in the human protein, these repeated sequences had a high glycine content and four of the glycine residues were highly conserved in the human as well as in the yeast protein (Fig. 5).

Discussion

We have studied the effect of the *prp20-1* mutation on mRNA metabolism. Initially, this mutant was isolated because it accumulated intron-containing pre-mRNA upon a shift to the non-permissive temperature and no correctly spliced actin mRNA was detected (Vijayraghavan et al. 1989). In addition to this splicing deficiency, we found that the steady-state levels of the unspliced *PAB, TCM1* (Fig. 1) and *PRP20* transcripts (Fig. 4) decreased in the *prp20-1* mutant strain when it was shifted to the non-permissive temperature. This decrease has not been observed for other *prp* mutants: a *prp2* mutation has no significant effect on the steady-state level of unspliced mRNA species (Rosbash et al. 1981, M. Aebi, unpublished results). We postulate that the decrease in unspliced mRNA in the *prp20* strain is not a secondary effect of the splicing deficiency but may reflect a phenotype directly associated with the mutation.

The steady-state level of *TCM1* transcripts in the *prp20-1* mutant increased upon longer incubation at the non-permissive temperature even though shifting the *prp20-1* mutant from permissive to non-permissive temperature led to a rapid cessation of cell growth at every step of the cell cycle (M. Aebi, unpublished results). In this respect, *TCM1* transcription differed from that of the other genes tested. Since the *TCM1* gene lacks the transcriptional control HOMOL1 and RPG boxes (Mager 1988) found in most other ribosomal protein genes, we have analyzed the expression of the unspliced ribosomal protein gene *S33* (Teem et al. 1984), which also lacks these control regions. Though the steady-state level of *S33* rapidly decreased upon shift to the non-permissive temperature, no recovery was observed (data not shown). At present, we have no explanation for the aberrant behavior of *TCM1* transcription in the *prp20-1* mutant.

In addition to the rapid depletion of unspliced mRNA species, we observed aberrant transcripts induced by a shift to the non-permissive temperature. We analyzed these new transcripts in the case of the *PRP20* transcription unit. These oversized transcripts initated downstream of the *PRP20* locus and extended into the *PRP20* coding region, leading to RNA which was complementary to the normal *PRP20* mRNA. We did not analyze the transcription initation sites for these RNAs and therefore, incorrect transcription initation or altered transcription termination and/or polyadenylation may have accounted for the appearance of such transcripts in the *prp20-1* strain.

The observation that the PRP20 protein is similar to the human *RCC1* gene product may explain the pleiotropic phenotype observed in the *prp20-1* mutant. The human *RCC1* gene and its corresponding cDNA have been isolated by functional complementation of a temperature-sensitive mutation in the hamster cell line tsBN2 (Kai et al. 1986; Ohtsubo et al. 1987). This cell line showed premature chromosome condensation upon a shift to non-permissive temperature (Nishimoto et al. 1978, 1981) in the S- and G2-phases and inhibition of RNA and protein synthesis in the G1-phase. We have not analyzed the effect of the *prp20-1* mutation at the mRNA level at different phases of the cell cycle, however, as seen with tsBN2 cells, we observed a structural change in the organization of the nucleoplasm, a rapid

decrease in mRNA steady-state levels and altered transcription initiation and/or termination upon shift to the non-permissive temperature. Based on the similarity of the PRP20 and RCC1 protein sequences as well as the similarity of the phenotypes observed in the *prp20-1* and tsBN2 mutants we speculate that the *PRP20* gene product could be involved in the control of a yeast nuclear process analogous to chromosome condensation in higher eukaryotic cells. A PRP20-dependent alteration in yeast chromatin structure could explain the observed changes in mRNA production. The rapid accumulation of unspliced pre-mRNA could be explained by the altered nuclear structure induced at the non-permissive temperature. This hypothesis is supported by the finding that the PRP20 protein is localized in the nucleus (M. Clark and M. Aebi, unpublished experiments).

The *PRP20* and the recently reported *SRM1* gene are identical. Clark and Sprague (1989) have identified the *SRM1* gene in a search for mutations that restored mating competence to a *MATα* strain lacking the *STE3* receptor. The *srm1-1* mutation conferred a temperature-sensitive phenotype. When grown at 33° C, *srm1-1* mutants were able to mate in a receptorless background, whereas at 37° C, no mating at all could be observed and the growth of the cells was completely arrested. Clark and Sprague (1989) concluded that the SRM1 protein is a component of the pheromone response pathway; however, an additional role in the vegetative life cycle was reported as well. We have no direct explanation for the fact that two different selection procedures led to the identification of the same gene. However, since we observed a drastic change in the steady-state levels of different mRNA species in *prp20-1* cells when grown at the non-permissive temperature, we can speculate that these altered mRNA levels may deregulate the pheromone response pathway and therefore allow receptorless strains to mate. Alternatively, the SRM1/PRP20 protein could have a function in the pheromone response pathway as well as in the control of nuclear structure in vegetatively growing cells.

Acknowledgments. We thank Scott Emr for the plasmid bank, Paul Hermann for the vector pPHYC18, M. Nomura for strain NOY237, Tim Mager for the ribosomal protein probes, Michel Dalrymple and Jean Beggs for bringing the *RCC1* similarity to our attention and Charles Weissmann for critical reading of the manuscript. M.A. was supported by the Swiss National Science Foundation, grant 83.483.0.87. M.W.C. was supported by National Institutes of Health postdoctoral fellowship No. 5732GM1008-02 and National Institutes of Health Grant GM 32637 to J.A.

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Communicated by C.P. Hollenberg