Journal of Cellular Biochemistry 36:261–273 (1988)
Molecular Biology of Intracellular Protein
Sorting and Organelle Assembly 57–69

Posttranslational Modification of *ras* Proteins: Detection of a Modification Prior to Fatty Acid Acylation and Cloning of a Gene Responsible for the Modification

F. Tamanoi, E.C. Hsueh, L.E. Goodman, A.R. Cobitz, R.J. Detrick, W.R. Brown, and A. Fujiyama

Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, Illinois 60637

Products of *ras* genes are synthesized as precursors in the cytosol and transported to the plasma membrane by a process which involves posttranslational modification by fatty acid. In this paper, we present evidence for the occurrence in the cytosol of an intermediate modification of *ras* proteins prior to the fatty acid acylation. The modification is detected by a slight shift in the mobility of the protein on SDS polyacrylamide gel. The fatty acid acylation does not contribute to this mobility shift. This modification is affected by the *dpr1* mutation which has recently been shown to affect the processing of yeast *RAS* proteins. To further characterize the nature of the modification event, we have cloned *DPR1* gene from the DNA of *Saccharomyces cerevisiae*. The gene is actively transcribed in yeast cells producing mRNA of approximately 1.6 kb. Genes related to the *DRP1* appear to be present in a distantly related yeast, *Schizosaccharomyces pombe* as well as in guinea pig and human cells.

Key words: ***Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*, subcellular localization, *DPR1* gene, processing of *ras* protein**

Products of *ras* genes are synthesized on free polysomes and are localized at the inner surface of the plasma membrane [1,2]. How *ras* proteins travel to the plasma membrane poses an interesting problem in protein transport, since they appear to be different from typical secretory proteins in that no apparent signallike sequences can be found in these proteins. Instead they are fatty acid acylated and it is possible that this fatty acid serves as an anchor to the plasma membrane by virtue of its hydrophobic properties [3–5].

The association of *ras* proteins to the plasma membrane is essential for their function, since *ras* proteins which are not associated with the plasma membrane

Received February 5, 1987; revised and accepted July 28, 1987.

cannot transform NIH/3T3 cells [4,5]. Studies employing in vitro mutagenesis of *ras* genes have shown that a cysteine located at the fourth position from the carboxy terminus is involved in the fatty acid acylation of *ras* proteins. This cysteine is followed by two hydrophobic amino acid residues, and the sequence CysAAX (where A is an aliphatic amino acid and X is the C-terminal amino acid) is conserved in all *ras* proteins [6–9].

Characterization of the processing of the products of *ras* gene homologues in *Saccharomyces cerevisiae* has provided an insight into these post-translational modification processes. We have shown [10] that the protein products of the homologous genes, *RAS1* and *RAS2*, are synthesized as precursor proteins. In the first modification step, these precursor forms are converted to intermediate forms which migrate slightly faster on SDS polyacrylamide gel electrophoresis than their precursor forms. Fatty acid acylation of these intermediate forms is the second step of modification for *RAS* proteins in yeast and the majority of fatty-acid-acylated *RAS* proteins are localized in the plasma membrane. We have proposed the following scheme for the posttranslational modification: precursor *RAS* → intermediate *RAS* → fatty-acid-acylated *RAS* [10,11]. The implication of this scheme for the processing of *ras* proteins in general is that there should be an identifiable modification of *ras* proteins that necessarily precedes fatty acid acylation. Occurrence of a modification distinct from fatty acid acylation has also been suggested from the characterization of H-*ras* proteins produced in NIH/3T3 cells [12].

In the course of our attempt to express the mammalian H-*ras* gene in yeast, we have noticed that a processing very similar to the one observed with yeast *RAS* proteins takes place with the H-*ras* proteins and that the processing of H-*ras* proteins is slower than that of yeast *RAS* proteins. These observations, the latter in particular, suggested to us that a form of *ras* proteins representing an intermediate in the posttranslational modification can be clearly identified by using this system. In this paper, we describe processing of H-*ras* proteins produced in yeast and show that the processing of *ras* proteins, indeed, occurs in two steps.

The recent isolation of yeast mutants *dpr1* [11] and *ram* [13], which are defective in the processing of *ras* proteins, allows us the opportunity to study this system of modification for *ras* proteins by powerful genetic methods. In the *dpr1* mutant, yeast *RAS2* proteins are accumulated as precursors in the cytosol, suggesting that the *dpr1* mutation primarily affects the conversion of a precursor form to an intermediate form [11]. One interesting phenotype of the *dpr1* and *ram* mutants is a sterility specific of *MATa* cells [11,13]; *MATa* cells having the mutation cannot mate with *MATα* cells. This is apparently due to a defect in the production of mature *a*-factor which is required for the mating with *MATα* cells [13] (R. Sterne and J. Thorner, personal communication). In fact, intracellular accumulation of the unprocessed *a*-factor can be detected in the *dpr1* mutant cells (R. Sterne and J. Thorner, personal communication). Therefore, the intermediate modification that occurs on *ras* proteins prior to their fatty acid acylation also plays a major role in the maturation of the *a*-factor. To gain further insights into this modification event, we have cloned the *DPR1* gene responsible for the modification. It appears to be a single gene in yeast and is actively transcribed. Survey for the presence of genes related to the *DPR1* in diverse organisms suggests that the gene is conserved during evolution.

MATERIALS AND METHODS

Yeast Strains and Materials

The following *S. cerevisiae* strains were used. UC100 (*MATa leu2 trp1 ura3 pep4 prb*) and G441-2D(*MATα leu1*) were obtained from R. Kostriken (Cold Spring Harbor Laboratory) and T. Toda (Cold Spring Harbor Laboratory), respectively. The strains KMY5-2A(*MATα dpr1 his3 leu2 ura3*) and HR13(*MATa dpr1 leu2 his3 ura3 trp1 ade8 can1 RAS2*^{val19}) were obtained from K. Matsumoto (DNAX Research Institute of Molecular and Cellular Biology). [^{35}S]methionine (1,130 Ci/mmol), [^{3}H]palmitic acid (55 Ci/mmol), and [$^{32}\alpha$-P]dCTP (3,000 Ci/mmol) were purchased from Amersham.

Construction of Plasmid DNA

Construction of pYG-H-*ras*, a vector to express H-*ras* gene in yeast, was accomplished by placing the H-*ras* gene under the control of glyceraldehyde 3-phosphate dehydrogenase promoter. For this purpose, the H-*ras* gene was cut out from pOFSP-2 [14] (provided by M. Wigler, Cold Spring Harbor Laboratory) by the digestion with SalI and BanII. After filling the ends with Klenow fragment, the fragment was blunt end ligated to the SalI site of pYG10 (provided by K. Matsubara, Osaka University) which had also been blunt ended by filling the ends. The orientation of the insert was examined by restriction digestions. YEp24-DPR1 was constructed by inserting a BamHI to SalI fragment of *DPR1* into YEp24 DNA. The YEp51-RAS2 plasmid has been described previously [15].

Metabolic Labeling and Immunoprecipitation

Labeling of yeast cells with [^{35}S]methionine or [^{3}H]palmitic acid, fractionation into soluble and membrane fractions, immunoprecipitation of *ras* proteins with monoclonal antibody 259, and SDS polyacrylamide gel electrophoresis were carried out as previously described [10].

Cloning of *DPR1* Gene

For the cloning of *DPR1* gene, a library of *S. cerevisiae* genomic DNA was used. This library (provided by R. Elder, University of Chicago) was constructed by inserting Sau3A partial digests into a BamHI site of YCp19. The DNA was transformed into a temperature-sensitive *dpr1* mutant, HR13, according to Beggs [16] and screened for the ability of transformants to grow at a high temperature (35°C). Plasmid DNAs from the transformants were isolated and recovered by transforming into *E. coli* according to Hanahan [17]. DNAs isolated were used to transform the HR13 cells in order to confirm their complementing ability.

DNA and RNA Analyses

Total yeast RNA was isolated from log-phase cells by breaking cells in the presence of guanidine isothiocyanate. Briefly, cells were suspended in 4 M guanidine isothiocyanate/2 M 2-mercaptoethanol/0.1 M Tris HCl (pH7.5)/5 mM EDTA and broken with glass beads. After removing unbroken cells and glass beads, RNA was purified by CsCl density-gradient centrifugation. Poly(A)$^{+}$ RNA was isolated by using an oligo-dT cellulose column. The RNA samples were size fractionated on an 0.8% agarose/2.2 M formaldehyde gel, transferred to nitrocellulose filter, and hy-

bridized to [^{32}P]*DPR1* probe (specific activity > 10^8 cpm/μg) according to Maniatis et al [18]. Hybridization and washing conditions are described in the figure legends. The *DPR1* probe was prepared by nick translating *DPR1* fragment excised and purified from YCp19-DPR1 plasmid.

S. cervisiae DNA and *Schizosaccharomyces pombe* DNA were isolated as described by Cryer et al. [19]. Guinea pig and human genomic DNAs were kindly provided by K. Agarwal (University of Chicago). Southern hybridization was carried out according to Maniatis et al [18]. Conditions for the hybridization and washing are described in the figure legends.

RESULTS

Detection of an Intermediate Modification Which Precedes Fatty Acid Acylation

Processing of *ras* proteins involves fatty acid acylation and localization at the plasma membrane. Our recent work with yeast *RAS* proteins raised the possibility that a modification distinct from the fatty acid acylation is taking place. However, it was not possible to obtain clear evidence for this point, since the processing of the yeast protein was rapid. In this paper, we have made use of H-*ras* proteins produced in yeast to further investigate this point.

To express H-*ras* gene in yeast, we constructed a plasmid, pYG-H-ras, by placing the H-*ras* gene under the control of a promoter for glyceraldehyde 3-phosphate dehydrogenase (Fig. 1). The level of expression of the H-*ras* gene with this construct was approximately ten times higher than that previously obtained by using *GAL10* promoter [14]. To investigate the processing of H-*ras* proteins, yeast cells carrying pYG-H-ras were labeled with [^{35}S]methionine and the labeled proteins were subjected to immunoprecipitation with monoclonal antibody 259. SDS poly-

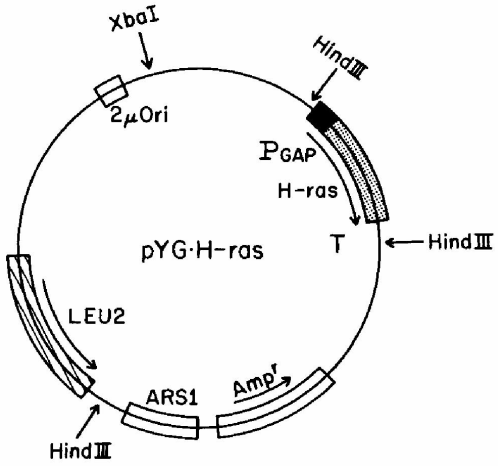

Fig. 1. Construction of pYG-H-ras plasmid. A DNA fragment containing the H-*ras* sequence was inserted downstream of the glyceraldehyde 3-phosphate dehydrogenase promoter (PGAP) of the pYG10 plasmid. In addition, the plasmid contains the *LEU2* gene, *ARS1*, and 2-μm origin as well as a part of pBR322. The direction of transcription taking place on the plasmid is shown by arrows. T indicates a transcription termination sequence from the glyceraldehyde 3-phosphate dehydrogenase gene [25].

acrylamide gel electrophoresis of the precipitates revealed the presence of two bands having apparent molecular weights of 22,000 and 21,000 daltons (Fig. 2A). Because the level of yeast *RAS* proteins is below the detection level, only H-*ras* proteins produced from the gene on the plasmid DNA are detected in this analysis. When the extracts were fractionated into soluble and membrane fractions, the 22,000-dalton form was found exclusively in the soluble fraction whereas the 21,000-dalton form was distributed into both the soluble and membrane fractions (Fig. 2B, lanes 3 and 4).

To investigate which species contain the fatty acid, the culture of cells carrying pYG-H-ras was split into two portions; [^{3}H]palmitic acid was added to the one and [^{35}S]methionine was added to the other. The labeled proteins were immunoprecipitated with the monoclonal antibody 259 and the precipitated proteins were analyzed on SDS polyacrylamide gel. As can be seen in Figure 2, only the 21,000-dalton form in the membrane fraction incorporated ^{3}H fatty acid radioactivity (Fig. 2B, lane 2). The ^{3}H radioactivity was not detected in either the 21,000-dalton form or in the 22,000 form present in the soluble fraction (Fig. 2B, lane 1). Thus, three distinct forms of H-*ras* proteins are detected in yeast cells: the precursor form present in the soluble fraction, the intermediate form present in the soluble fraction, and the fatty acid acylated form present in the membrane fraction. It is worth noting that the apparent molecular weight of the fatty acid acylated form is very similar to that of the

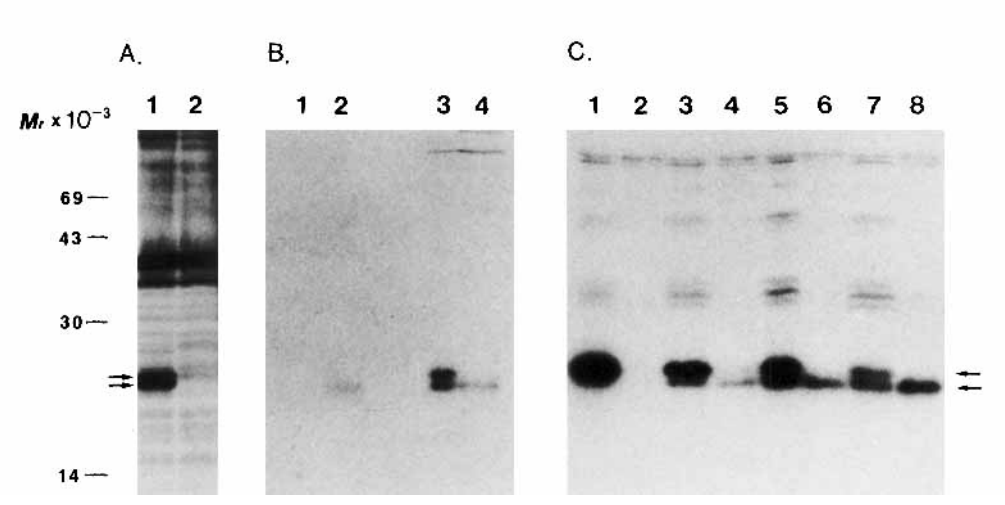

Fig. 2. Analyses of H-*ras* proteins produced in yeast cells. **A:** UC100 cells carrying the pYG-H-ras plasmid were labeled with [^{35}S]methionine. Labeled H-*ras* proteins were immunoprecipitated from total cell extracts with monoclonal antibody 259 and separated on a 15% SDS polyacrylamide gel. Lane 1, with an antibody 259; lane 2, without antibody. Size markers are albumin (*Mr* 69,000), ovalbumin (*Mr* 43,000), carbonic anhydrase (*Mr* 30,000) and lysozyme (*Mr* 14,000). **B:** Fatty acid acylation of the H-*ras* protein is demonstrated. UC100 (pYG-H-ras) cells were labeled either with [^{3}H]palmitic acid (lanes 1 and 2) or with [^{35}S]methionine (lanes 3 and 4) for 2 hr. After separating into soluble (lanes 1 and 3) and membrane (lanes 2 and 4) fractions, H-*ras* proteins were immunoprecipitated and analyzed by SDS/PAGE as described above. **C:** UC100 (pYG-H-ras) cells were pulse labeled with [^{35}S]methionine (40 μCi/ml, 68 Ci/mmol) for 10 min (lanes 1 and 2). After the addition of 550-fold excess of cold methionine, cells were further incubated for 30 min (lanes 3 and 4), 60 min (lanes 5 and 6), and 120 min (lanes 7 and 8). Labeled cells were fractionated into soluble (lanes 1, 3, 5, and 7) and membrane (lanes 2, 4, 6, and 8) fractions. Immunoprecipitation and SDS/PAGE were performed as described above.

intermediate form, suggesting that the fatty acid acylation does not alter the mobility of *ras* proteins on SDS polyacrylamide gel electrophoresis.

The relationship between these three forms of *ras* protein can be deduced from pulse-chase experiments shown in Figure 2C. After a 10-min pulse with [^{35}S]methionine, most of the radioactivity was detected in the precursor form. This is in marked contrast to the processing of yeast *RAS* proteins, in which virtually no precursor forms are detected after a 2-min pulse [10]. When a chase with unlabeled methionine was performed, radioactivity in the intermediate form increased, and eventually there was radioactivity in the fatty-acid-acylated form. These results suggest that the precursor form is converted to the intermediate form, which then becomes fatty acid acylated.

dpr1 Mutation Affects the Intermediate Modification Which Precedes the Fatty Acid Acylation

Recently, a mutant of yeast, *dpr1*, defective in the processing of yeast *RAS* proteins has been isolated. Figure 3 shows the effect of the mutation on the processing of H-*ras* proteins. The plasmid pYG-H-ras was introduced into the *dpr1* mutant cells and the transformants were labeled with [^{35}S]methionine. The *ras* proteins were immunoprecipitated with the monoclonal antibody 259. As can be seen in Figure 3A lane 2, most of the radioactivity in the *dpr1* mutant was detected in the 22,000-dalton

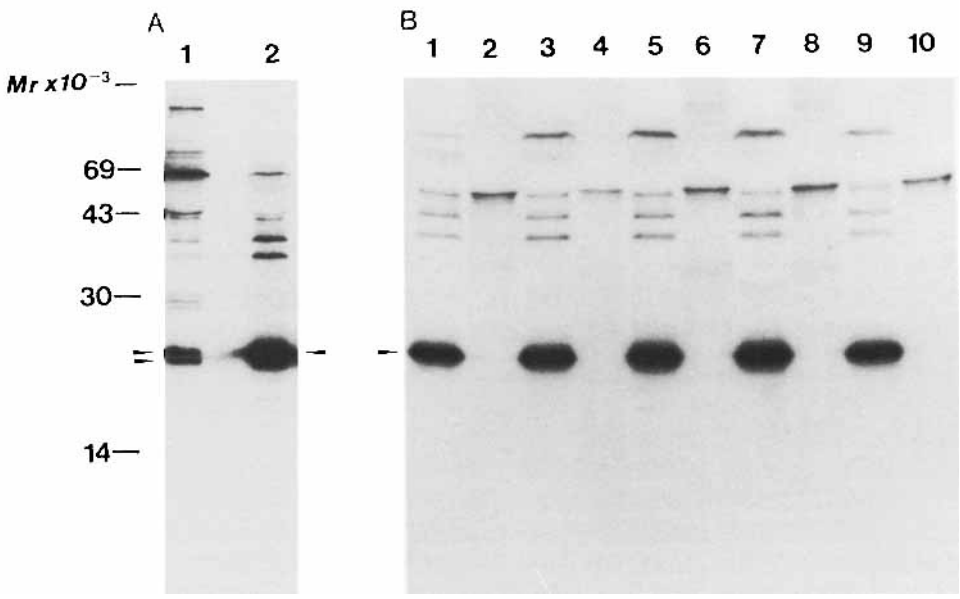

Fig. 3. Effect of the *dpr1* mutation on the processing of H-*ras* proteins produced in yeast cells. **A:** UC100 cells (lane 1) and KMY5-2A cells (lanes 2) were transformed with pYG-H-ras plasmid and labeled with [^{35}S]methionine. Labeled H-*ras* proteins were immunoprecipitated and separated as described in Figure 2. **B:** Pulse-chase analysis of H-*ras* proteins in *dpr1* cells. KMY5-2A cells carrying pYG-H-ras were pulse labeled with [^{35}S]methionine and chased as described in Figure 2C. Pulse labeling for 10 min (lanes 1 and 2), chase for 30 min (lanes 3 and 4), 60 min (lanes 5 and 6), 120 min (lanes 7 and 8), and 240 min (lanes 9 and 10). Labeled cells were separated into soluble (lanes 1, 3, 5, 7, and 9) and membrane fractions (lanes 2, 4, 6, 8, and 10). Immunoprecipitation and SDS/PAGE were performed as described in Figure 2.

precursor *ras* protein. In contrast to this, both the precursor and processed *ras* proteins were detected in parental cells (Fig. 3A, lane 1). The precursor *ras* proteins in the *dpr1* mutant were not processed even after a very lengthy chase with cold methionine (Fig. 2B). Fractionation of extracts of the *dpr1* mutant into soluble and membrane fractions revealed that the H-*ras* proteins were present almost exclusively in the 22,000-dalton form in the soluble fraction. These results suggest that the *dpr1* mutation affects the first modification step; conversion of a precursor form to an intermediate form. In addition, the mutation affects localization of the protein.

Cloning of *DPR1* Gene

Further investigation into the nature of the intermediate modification of *ras* proteins would be greatly facilitated by the identification of a gene responsible for the modification. We cloned the *DPR1* gene by exploiting temperature-sensitive growth of the *dpr1* mutant. Transformation of the *dpr1* mutant HR13 with DNAs from genomic library of *S. cerevisiae* resulted in the isolation of five plasmids (YCp-DPR 12, 21, 30, 41, 311) which complemented the *ts* phenotype. Yeast DNA fragments contained in these plasmids are shown in Figure 4. A common region of 4.5 kb is found in these plasmids.

Another phenotype of the *dpr1* mutant is an *a* sterile phenotype. To demonstrate that the cloned gene also complements this *a* sterile phenotype, each of the five YCp-DPR plasmids described above was introduced into HR13 which is *MATa dpr1*. The transformants of HR13 carrying YCp-DPR were able to mate with G441-2d(*MATα leu1*) cells. On the other hand, HR13 cells not carrying the YCp-DPR plasmid were not able to mate with G441-2D cells. Thus, the plasmid YCp-DPR confers *MATa dpr1* cells the ability to mate with *MATα* cells.

We next examined whether the *DPR1* clones are capable of complementing the deficiency of *ras* protein processing in the *dpr1* cells. A *dpr1* mutant KMY5-2A was transformed with a plasmid YEp51-RAS2 to overproduce yeast *RAS2* protein. The transformants were then transformed with YCp-DPR and selected at 35°C. The two

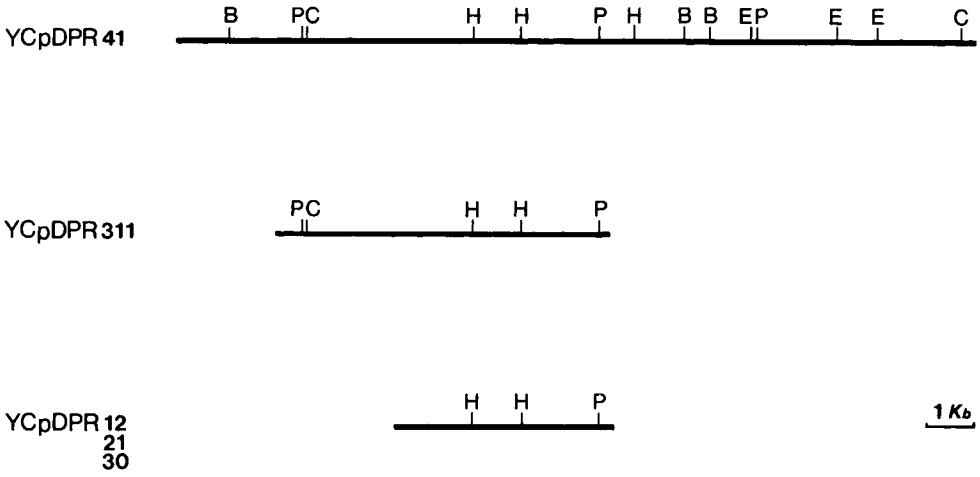

Fig. 4. Structure of cloned *DPR1* fragments. Cleavage sites by restriction endonucleases on cloned DNA fragments are shown. B, BamHI, C, ClaI, E, EcoRI, H, HpaI, P, PstI.

plasmids were maintained on a medium lacking both leucine and uracil. Processing of *RAS2* protein was examined by immunoprecipitating [^{35}S]methionine-labeled *RAS2* protein with the monoclonal antibody 259. As can be seen in Figure 5, processing of precursor *RAS2* proteins was observed in *dpr1* cells transformed with the *DPR1* clones (lanes 2–4). However, in the *dpr1* cells transformed with the vector but lacking the *DPR1* insert, only precursor proteins were detected (Fig. 5, lane1). Thus, the processing of RAS2 protein is complemented by the *DPR1* clones. In an entirely

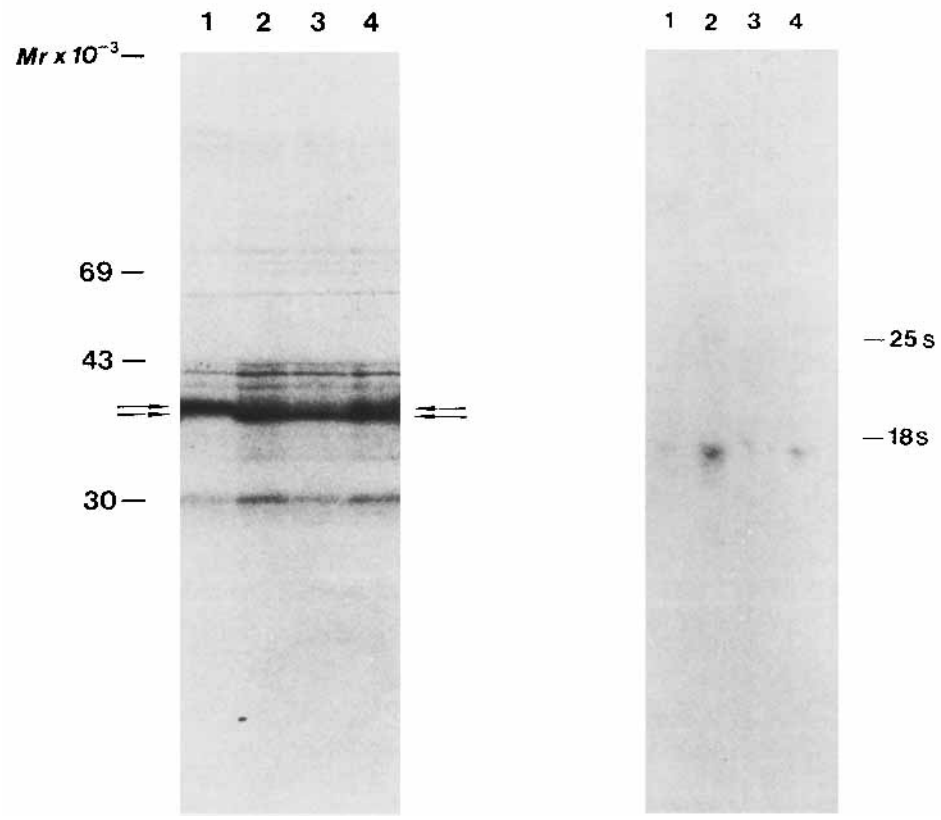

Fig. 5. Complementation of the *dpr1* phenotype by the cloned *DPR1* plasmids. HR13 cells carrying the YEp51-RAS2 plasmid were transformed with YCp19 (lane 1), YCp-DPR12 (lane 2), YCp-DPR41 (lane 3), and YCp-DPR311 (lane 4) DNA. Doubly transformed cells were labeled with [^{35}S]methionine and the processing of yeast *RAS2* proteins was examined as described in Figure 2.

Fig. 6. Blot-hybridization analysis of *DPR1* expression in yeast cells. Total and poly(A)$^+$ RNA were size fractionated on 0.8% agarose/2.2 M formaldehyde gel, transferred to nitrocellulose filter, and hybridized with the *DPR1* probe at 42°C overnight in 5 × SSC/5×Denhardt solution/20 mM sodium phosphate (pH 6.9)/1% SDS/50% formamide. The filter was washed twice for 5 min at room temperature in 2×SSC, followed by a 1-hr wash at 65°C in 0.1×SSC/0.1 M sodium phosphate (pH6.9)/0.05% SDS/1×Denhardt solution, then followed by another 1-hr wash at 65°C in 1×SSC/0.01 M sodium phosphate (pH 6.9)/0.05% × SDS/1×Denhardt solution, and finally washed with 0.1×SSC. Lane 1: 11 μg of poly(A)$^+$ RNA from UC100 cells carrying a plasmid YEp24. Lane 2: 11 μg of poly(A)$^+$ RNA from UC100 cells carrying YEP24-DPR1. Lane 3: 30 μg of total RNA from UC100 cells carrying YEp24. Lane 4: 30 μg of total RNA from UC100 cells carrying YEp24-DPR1. Markers are yeast ribosomal RNAs.

analogous procedure, we have also shown that the *DPR1* clones complement the processing defect of H-*ras* proteins in yeast (data not shown).

Detection of *DPR1* Transcript

Transcription of the *DPR1* gene was assessed by Northern hybridization of yeast RNA with the cloned *DPR1* DNA. As shown in Figure 6, a single band with an approximate size of 1.6 kb was detected. The level of the *DPR1* mRNA is elevated in cells carrying YEp24-DPR1, a multicopy plasmid containing the *DPR1* gene (compare lanes 3 and 4). Similar results were obtained with poly(A)$^+$ RNA, indicating that the *DPR1* mRNAs are polyadenylated. Therefore, the *DPR1* gene is actively transcribed in yeast cells.

DPR1 Gene Appears to Be Conserved During Evolution

The availability of the cloned gene enabled us to search for the presence of genes related to the *DPR1* in various organisms. The cloned gene was first hybridized against genomic DNA from *S. cerevisiae*. As can be seen in Figure 7A, a single band was always found regardless of which endonuclease was used for digestions, suggesting that the *DPR1* gene is a single gene in *S. cerevisiae*, in agreement with other genetic analyses. We next investigated whether genes related to the *DPR1* are present in other organisms. Figure 7B shows the results of hybridization analyses using genomic DNA from *S. pombe*. Clear bands were detected when the hybridization and washing were carried out under nonstringent as well as stringent hybridization conditions. These bands did not correspond to abundant DNA detected by ethidium bromide staining. Figure 8 shows further analyses using guinea pig and human DNAs. In these cases also, we detected bands which did not correspond to repetitive DNA bands seen with ethidium bromide staining. These bands were detected even when the washing was carried out under stringent conditions. These results raise the possibility that genes related to the *DPR1* are present in a distantly related yeast, *S. pombe*, as well as in higher eukaryotes (guinea pig and human cells).

DISCUSSION

The modification of *ras* proteins leading to their acylation by fatty acids involves two distinct modification events: conversion of precursor forms to intermediate forms and fatty acid acylation of the intermediate forms. Our argument that there is an intermediate modification of *ras* proteins prior to their fatty acid acylation rests on the following two pieces of evidence. First, a form distinct from either the precursor or the fatty-acid-acylated form can be detected in the soluble fraction. This molecule displays characteristics expected for an intermediate of processing by pulse-chase experiment. Second, since the intermediate molecule migrates at approximately the same position as the fatty-acid-acylated molecule, it appears that the effect of fatty acid acylation on its mobility on SDS polyacrylamide gel is little, if any. Presence of a modification distinct from fatty acid acylation has also been suggested from the analyses of H-*ras* protein produced in NIH/3T3 cells [12]. However, in two reports that have appeared concerning processing of H-*ras* proteins in yeast [13,20], only precursor and fatty-acid-acylated forms were detected. The reason for the discrepancy is unclear at the moment.

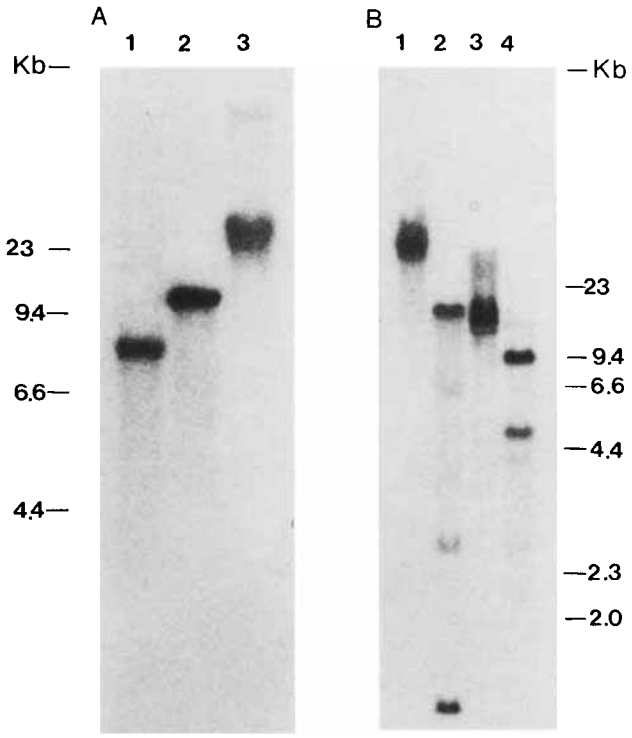

Fig. 7. Southern hybridization analyses of *S. cerevisiae* DNA and *S. pombe* DNA. **A:** DNA from *S. cerevisiae* (3 μg/lane) was digested with either BamHI (lane 1) or EcoRI (lane 2) and hybridized with labeled *DPR1* fragment under stringent conditions, hybridized at 42°C overnight in 50% formamide/5×SSC/5×Denhardt solution/20 mM sodium phosphate (pH 6.5)/0.1% SDS, washed twice at room temperature for 5 min each with 2 × SSC followed by three more washes at 65°C for 1 hr each with 2 × SSC/0.1 × Denhardt solution/0.1 M sodium phosphate, (pH 7.4)/0.1% SDS. Lane 3: No digestion. A Hind III digest of lambda phage DNA was used as a size marker. **B:** DNA from *S. pombe* (20 μg/lane) was digested with EcoRI (lane 2), BamHI (lane 3), and HindIII (lane 4). Hybridization was performed under nonstringent condition; hybridized at 37°C overnight in 5 × SSC/5 × Denhardt solution/20 mM sodium phosphate (pH 6.5)/0.1% SDS, washed twice at room temperature for 5 min each with 2 × SSC followed by three more washes at 56°C for 1 hr each with 2 × SSC/0.1 × Denhardt solution/0.1 M sodium phosphate (pH 7.4)/0.1% SDS. Lane 1: No digestion.

The two modification events seen with *ras* proteins are presumably related. This is suggested from the following two independent observations. First, the *dpr1* mutation blocks the first modification (conversion of a soluble precursor form to a soluble intermediate form), and the amount of fatty-acid-acylated *ras* proteins is greatly reduced in the mutant [11]. This result implies that the first modification is an obligatory step for the second modification (fatty acid acylation). Second, the alteration of cysteine to serine at the CysAAX sequence, a presumed fatty acylation site, also affects the first modification [4], suggesting that the two modifications occur at the same or nearby sites. Our analyses of the amino terminal sequences of the precursor and mature yeast *RAS2* proteins suggest that no modifications occur at the amino terminus. Both proteins were lacking the first methionine predicted from the DNA sequence, but the second amino acid (proline) was present in both cases. Furthermore, formic acid treatment of the two proteins (which cleaves the *RAS2*

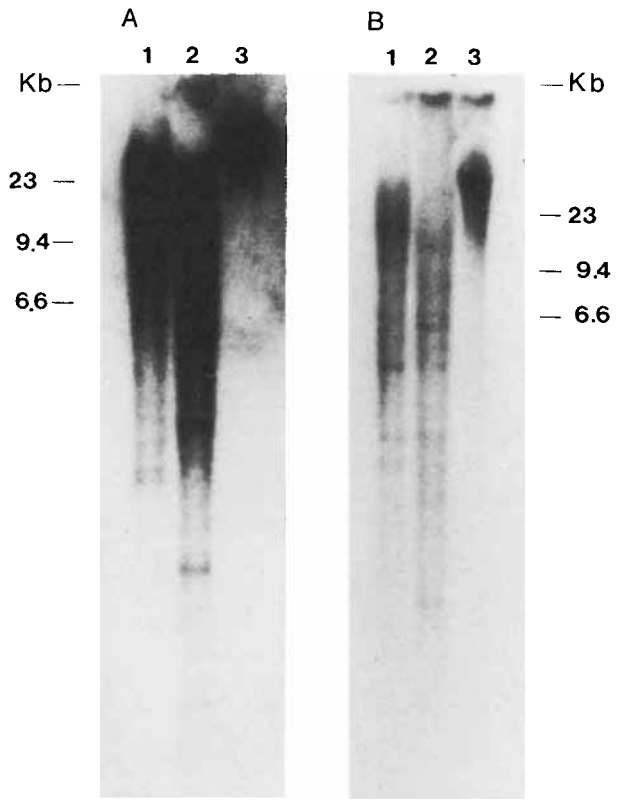

Fig. 8. Southern hybridization analyses of guinea pig and human DNAs; 20 μg of guinea pig DNA (**A**) or human DNA (**B**) was digested with BamHI (lane 1) or EcoRI (lane 2) and run on a 0.8% agarose gel. After transferring DNA onto nitrocellulose filter, the DNA was hybridized with labeled *DPR1* probe. Hybridization was carried out in 5 × SSC/5 × Denhardt solution/20 mM sodium phosphate (pH 6.5)/0.1% SDS at 37°C overnight, washed twice at room temperature for 5 min each with 2 × SSC/0.1 × Denhardt solution/0.1 M sodium phosphate (pH 7.4)/0.1% SDS followed by two more washes at 50°C for 1 hr each in the same buffer, and further washed twice more at 60°C for 1 hr each in the same buffer. Lane 3 shows undigested DNA. A HindIII digest of lambda phage DNA was used as a size marker.

protein between asp39 and pro40 from the amino terminus) produced carboxy terminal fragments which still retained the mobility shift (unpublished).

The exact chemical nature of the modification which precedes fatty acid acylation has not been elucidated. However, recent reports on the structure of mating factors of yeast shed some light on this problem. The cDNA sequence of the *a*-factor of *S. cerevisiae* suggests that the *a*-factor is produced as a precursor of 36 or 38 amino acids which contains a sequence CysValIleAla at the carboxy terminus [21]. The mature *a*-factor, however, contains 12 amino acid residues, and a cysteine which is alkylated is present at the carboxy terminus [22]. The mating pheromone of basidiomycetous yeast, *Rhodosporidium toruloides*, is an undecapeptide having S-farnesylated cysteine at the carboxy terminus [23]. The sequence of the cloned gene revealed the presence of three amino acids following the cysteine [24]. These observations are striking in that the terminal three amino acids are removed in both cases. This result

raises the possibility that the carboxy terminal three amino acids in the *ras* proteins are removed as well before the fatty acid acylation takes place. Direct determination of the carboxy terminal sequence of the *ras* proteins is needed to establish this point.

To facilitate the characterization of the first modification event, we have cloned the *DPR1* gene. The gene cloned by its ability to complement the temperature-sensitive phenotype of the *dpr1* mutant is capable of reversing the *a*-sterile phenotype as well as restoring the ability to process precursor *ras* proteins in the *dpr1* mutant. Thus, all these phenotypes are due to a mutation in a single gene. Powers et al. have cloned a gene, *RAM*, which also affects the processing of *ras* proteins and the *a*-factor precursor [13]. Comparison of the restriction maps of the *RAM* gene and the *DPR1* gene indicates that the two genes are identical. From the Southern analyses, it appears that there is only one *DPR1* gene, although presence of a gene with a limited homology has not been ruled out. The *DPR1* gene is actively transcribed in yeast cells giving rise to a mRNA of approximately 1.6 kb.

Availability of the cloned gene enabled us to investigate whether genes related to the *DPR1* are present in other organisms. Southern hybridization experiments with genomic DNAs suggest that a related gene is present in the distantly related yeast *S. pombe*. By similar hybridization experiments using genomic DNAs from guinea pig and human, we have obtained indications that genes related to the yeast *DPR1* might be present in these higher eukaryotes as well. The *ras* genes are present in diverse species ranging from yeast to human. The genes involved in the modification of the products of the *ras* genes are also conserved during evolution.

ACKNOWLEDGMENTS

We thank Drs. E. Goldwasser, T. Petes, and E. Westbrook for critical reading of the manuscript. This work was supported by NIH grant RO1 CA 41996 and in part by the Louis Block Fund, The University of Chicago.

REFERENCES

1. Shih TY, Weeks MO, Gruss P, Dhar R, Oroszlan S, Scolnick EM: J Virol 42:253, 1982.
2. Willingham MC, Pastan I, Shih TY, Scolnick EM: Cell 19:1005, 1980.
3. Sefton BM, Trowbridge IS, Cooper JA, Scolnick EM: Cell 31:465, 1982.
4. Willumsen BM, Norris K, Papageorge AG, Hubbert NL, Lowy DR: EMBO J 3:2581, 1984.
5. Weeks MO, Hager GL, Lowe R, Scolnick EM: J Virol 54:586, 1985.
6. Taparowsky E, Shimizu K, Goldfarb M, Wigler M: Cell 34:581, 1983.
7. Schejter ED, Shilo BZ: EMBO J 4:407, 1985.
8. Reymond CD, Gomer RH, Mehdy MC, Firtel RA: Cell 39:141, 1984.
9. Powers S, Kataoka T, Fasano O, Goldfarb M, Strathern J, Broach J, Wigler M: Cell 36:607, 1984.
10. Fujiyama A, Tamanoi F: Proc Natl Acad Sci USA 83:1266, 1986.
11. Fujiyama A, Matsumoto K, Tamanoi F: EMBO J 6:223, 1987.
12. Buss JE, Sefton BM: Mol Cell Biol 6:116, 1986.
13. Powers S, Michaelis S, Broek D, Santa Anna-A S, Field J, Herskowitz I, Wigler M: Cell 47:413, 1986.
14. Kataoka T, Powers S, Cameron S, Fasano O, Goldfarb M, Broach J, Wigler M: Cell 40:19, 1985.
15. Tamanoi F, Walsh M, Kataoka T, Wigler M: Proc Natl Acad Sci USA 81:6924, 1984.
16. Beggs JD: Nature 275:104, 1978.
17. Hanahan D: J Mol Biol 166:557, 1983.
18. Maniatis T, Fritsch EF, Sambrook J: "Molecular Cloning: A Laboratory Manual." Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1982.

19. Cryer DR, Eccleshall R, Marmur J: "Methods in Cell Biology," 12. New York: Academic Press, 1975, pp 39–44.
20. Clark SG, McGrath JP, Levinson AD: Mol Cell Biol 5:2746, 1985.
21. Brake AJ, Brenner C, Najarian R, Laybourn P, Merryweather J: "Protein Transport and Secretion." Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1985, pp 103–108.
22. Betz R, Crabb JW, Meyer HE, Wittig R, Duntze W: J Biol Chem 262:546, 1987.
23. Kamiya Y, Sakurai A, Tamura S, Takahashi N, Abe K, Tsuchiya E, Fukui S, Kitada C, Fujino M: Biochem Biophys Res Commun 83:1077, 1978.
24. Akada R, Minomi K, Yamashita I, Miyakawa T, Fukui S: Agric Biol Chem (Jpn) 51:1211, 1987.
25. Holland JP, Holland MJ: J Biol Chem 254:9839, 1979.