The EMBO Journal vol.6 no.1 pp.223–228, 1987

A novel yeast mutant defective in the processing of ras proteins: assessment of the effect of the mutation on processing steps

Asao Fujiyama, Kunihiro Matsumoto[1] and Fuyuhiko Tamanoi

Department of Biochemistry and Molecular Biology, The University of Chicago, 920 East 58th Street, Chicago, IL 60637, and [1]DNAX Research Institute of Molecular and Cellular Biology, 901 California Avenue, Palo Alto, CA 94304, USA

Communicated by G.Tocchini-Valentini

Biosynthesis of RAS1 and RAS2 proteins of *Saccharomyces cerevisiae* involves processing, fatty acid acylation and transport to plasma membranes. We now report the isolation of a mutant, termed *dpr1*, defective in these biosynthetic events. The *dpr1* cells are temperature sensitive for growth and display sterile phenotype specific to *a* cells. The following observations were made using cells overproducing the RAS2 protein. (i) In the *dpr1* cells, the RAS2 proteins remain as precursors and accumulate in the cytoplasm. (ii) The level of the RAS2 proteins in the plasma membrane of the *dpr1* cells is much lower than that in the plasma membrane of wild-type cells. (iii) Fatty acid acylation appears to take place in the *dpr1* cells. These results suggest that the major effect of the *dpr1* mutation is in the processing of the precursor proteins, but not in their fatty acid acylation. Mutants such as *dpr1* should be invaluable for further elucidation of the mechanisms of biosynthesis and transport of the RAS proteins, and presumably also *a* factor.

Key words: a factor/fatty acid acylation/membrane localization/precursor proteins

Introduction

ras genes are present in diverse species which include even the lower eukaryote yeasts and appear to play an essential role in the growth regulation of cells. Products of the *ras* genes exhibit biochemical activities to bind GDP or GTP and hydrolyze GTP (Scolnick *et al.*, 1979; McGrath *et al.*, 1984; Sweet *et al.*, 1984; Gibbs *et al.*, 1984; Tamanoi *et al.*, 1984; Fujiyama *et al.*, 1986; Temeles *et al.*, 1984). In the case of the yeast genes, their products are involved in the stimulation of adenylate cyclase (Toda *et al.*, 1985; Broek *et al.*, 1985).

Mammalian ras proteins are fatty acid acylated and are localized at the inner surface of plasma membranes (Sefton *et al.*, 1982; Shih *et al.*, 1982; Willingham *et al.*, 1980). The membrane localization of the mammalian ras proteins appears to be essential for the activity of the protein, since ras proteins which fail to associate with the plasma membrane are no longer competent in transformation (Willumsen *et al.*, 1985; Weeks *et al.*, 1985). Thus, it is important to elucidate biosynthetic events for the ras proteins leading to their membrane localization. To approach this problem, we have chosen yeast as a model system, since powerful yeast genetics can be employed. We have previously established that the yeast RAS proteins are first synthesized in the cytoplasm as precursors which are rapidly converted to processed forms (Fujiyama and Tamanoi, 1986). The processing can be detected by the difference in mobility on a SDS–polyacrylamide gel and the exact nature of the processing is yet unknown. The processed forms are further modified by fatty acid acylation and only the fatty acid acylated forms are localized at plasma membranes.

Two types of protein fatty acid acylation have been detected in yeast. Wen and Schlesinger (1984) have identified proteins containing palmitic acid covalently bound by an alkali-labile bond. These proteins are also glycosylated. Analyses of secretory mutants have revealed that the palmitic acid acylated proteins accumulate in endoplasmic reticulum when the transport to Golgi is blocked. On the other hand, the fatty acid acylation of these proteins is not detected when the entry into endoplasmic reticulum is blocked. The other type of fatty acid acylation involves myristic acid which is bound to proteins via an alkali-resistant amide linkage to amino-terminal glycine residue (Towler and Glaser, 1986). The fatty acid acylation of the RAS proteins appears to define yet another type of fatty acid acylation. That is, although the fatty acids on the RAS proteins can be released by alkali treatment, they are not glycosylated. Furthermore, an effect of secretory mutations is not observed with the fatty acid acylation of the RAS proteins (Fujiyama and Tamanoi, 1986). This latter observation raises the possibility that the transport of the RAS proteins to the plasma membrane utilizes a novel pathway distinct from the secretory pathway.

S.Powers and M.Wigler have identified a mutation (*supH*), similar to the mutation described in this report (which we call *dpr1*), which reverses heat shock sensitive phenotype of *RAS2*^{val19} cells. This gene has been shown to be allelic to *STE16*, a gene which is necessary for biosynthesis of *a* factor and for mating of *a* cells. It has been suggested that this gene is necessary for a modification that is common to RAS proteins and the *a* factor precursor (I.Herskowitz, S.Michaelis, S.Powers and M.Wigler, personal communication).

To define more clearly the biochemical mechanisms underlying the biosynthesis of the RAS proteins and to gain insight into the novel pathway for the transport of the RAS proteins, we sought to study mutants defective in the biosynthesis of the RAS proteins. Here, we report the isolation and characterization of such a mutant, defective in the *DPR1* gene, and demonstrate that this gene is necessary for modification of RAS2 protein and its proper cellular localization.

Results

Isolation of dpr1 mutants and genetic analyses

Cells of yeast expressing a RAS2^{Val19} protein (a mutant protein in which glycine is replaced by valine at the 19th amino acid residue) are sensitive to heat treatment at 57°C for 5 min. Thus, virtually no viable cells are found after the treatment. This phenotype provided a selection method to isolate the mutants described in this paper. Cells of TK161-R2V (*a leu2 his3 ura3 trp1 ade8 can1 RAS2*^{Val19}) were grown at 25°C, subjected to ethylmethane sulfonate mutagenesis (Lindegren *et al.*, 1965), grown in YPD liquid culture at 25°C and treated at 57°C for 5 min. After collecting the cells, they were plated and grown

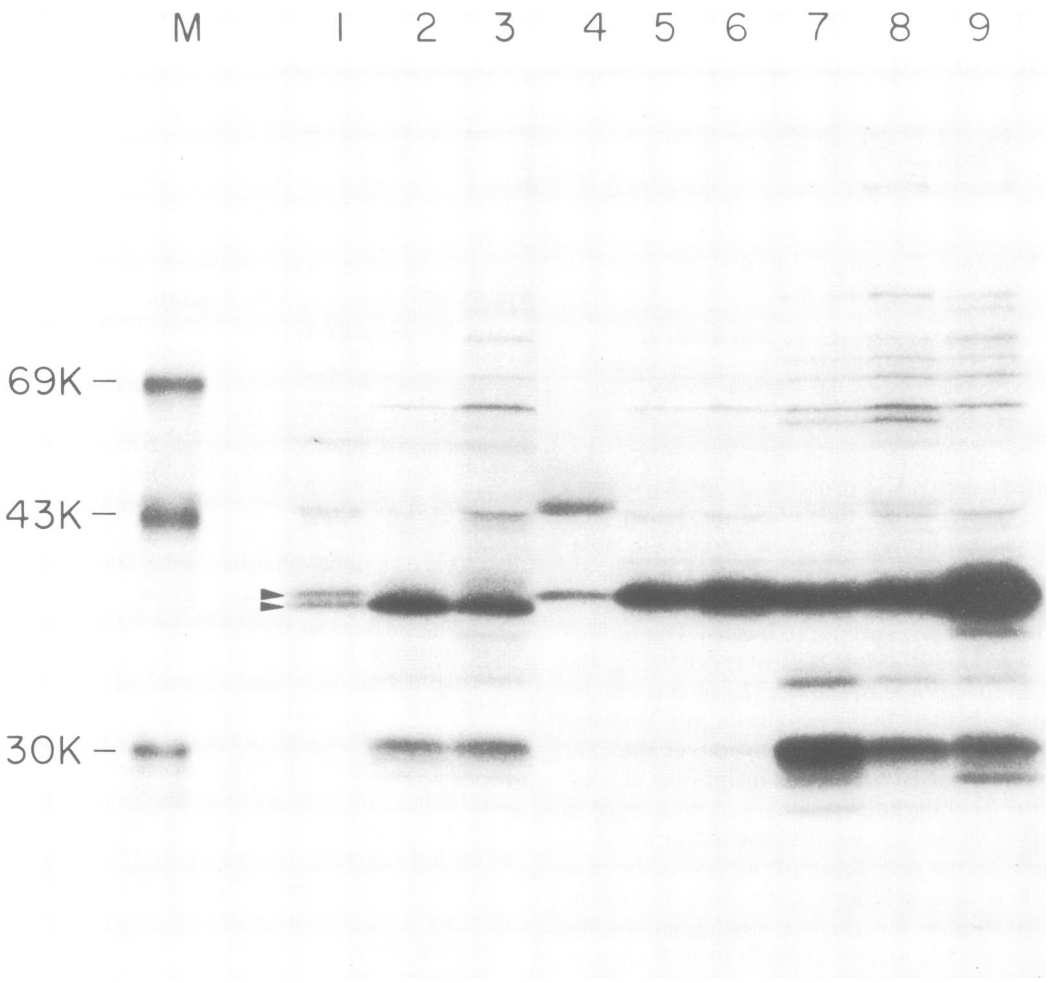

Fig. 1. Immunoprecipitation and SDS−polyacrylamide gel electrophoresis of ^{35}S-labeled RAS2 proteins. The combination of expression plasmids and yeast strains are as follows: **Lane 1**, YEp51-RAS2 in JR25-D. **Lane 2**, YEp51-RAS2 in JR25-2A. **Lane 3**, YEp51-RAS2 in TK161-R2V. **Lane 4**, cell-free translation product. **Lane 5**, YEp52-RAS2 in HR12. **Lane 6**, YEp51-RAS2 in HR13. **Lanes 7** and **8**, YEp51-RAS2 in HR15. **Lane 9**, pYG-RAS2 in HR13. Cells carrying YEp51-RAS2 plasmid were grown in a synthetic medium containing 4% galactose (2% glucose for pYG-RAS2 transformant) and lacking leucine. The *dpr1* strains and TK161-R2V were grown at 25°C and labeled with 10 μCi/ml [^{35}S]methionine (1130 Ci/mmol)for 60 min. (JR25-2A and its derivative JR25-D were grown and labeled at 30°C.) Cell extracts, containing the identical amount of acid-insoluble radiolabeled proteins (3×10^6 c.p.m.) were subjected to immunoprecipitation by monoclonal antibody Y13-259 and SDS−polyacrylamide gel electrophoresis. Poly(A) RNA was isolated from YEp51-RAS2 transformed cells. Mol. wt markers are albumin (Mr 69 000), ovalbumin (Mr 43 000) and carbonic anhydrase (Mr 30 000).

at 25°C. These plates were subjected to one more heat treatment at 57°C for 10 min. The mutants were further selected as temperature sensitive mutants for growth at 35°C. Of the independent 52 mutants isolated, 40 were found to be allelic to *cyr1*. This is expected since alterations of the catalytic subunit of adenylate cyclase may counteract the effect of *RAS2*^{Val19} mutation. Three mutants (HR12, HR13 and HR15), had an extremely low efficiency of mating with α cells and further characterized as follows. The rest, nine isolates, were not analyzed further.

*MAT*α derivatives of three mutants were made by transforming each original mutant with a YCp50-*HO* plasmid. This plasmid contains the wild-type *HO* gene that enables mating-type switching (Jensen *et al.*, 1983). The following two lines of experiments were carried out using these cells. First, *MAT*α mutants which had lost the plasmid were mated to TK161-R2V strain. The resulting diploids (homozygous for *RAS2*^{val19} and heterozygous for *dpr1*) were tested for both heat shock sensitivity at 57°C and temperature sensitivity at 35°C. In each case the diploid was able to grow at 35°C and was sensitive to heat shock treatment at 57°C, indicating that each mutation was recessive. Second, mutant HR12 carrying *MAT*α was crossed to a wild-type strain, and the resulting diploid was sporulated and subjected to tetrad analysis. Temperature sensitivity segregated 2+:2−, indicating that mutant HR12 had a single chromosomal mutation which is designated *dpr1* (defective in the processing of ras proteins). We observed segregations of 4+:0−, 3+:1− and 2+:2− for heat shock sensitivity at 57°C and mating phenotype (negative phenotype is heat shock sensitive and mating deficient). These results indicate that *DPR1* is not linked to the *RAS2* gene and that *dpr1* mutation results in temperature sensitive phenotype in *RAS2*$^+$ cells. No temperature sensitive, heat shock sensitive spore clone was recovered, confirming that temperature sensitive and heat shock resistant phenotypes segregated together. Segregants of the above cross contained *MAT*α *dpr1* cells which were capable of mating with *MATa* wild-type cells. In contrast, all the *MATa dpr1* segregants had an extremely low efficiency of mating with *MAT*α wild-type cells. Thus, *dpr1* mutation results in a sterile phenotype specific to *MATa* cells. Complementation analysis indicated that the three mutants had the same mutation.

Precursor RAS proteins accumulate in dpr1 cells

In order to investigate the fate of RAS proteins in the *dpr1* mutant cells, it was necessary to overproduce the protein. For this pur-

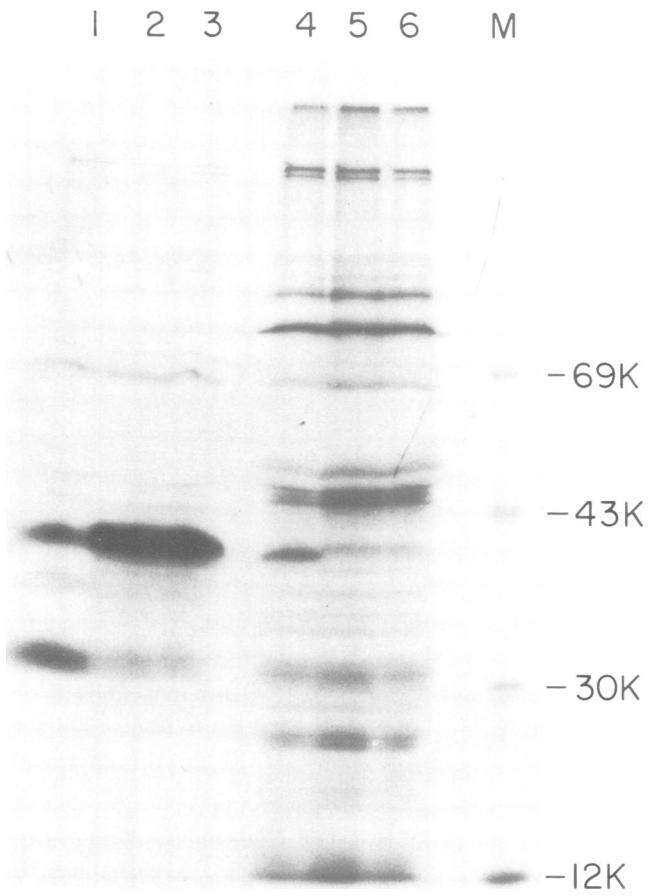

Fig. 2. Distribution of RAS2 proteins. Cells carrying YEp51-RAS2 plasmid were grown in a synthetic medium containing 4% galactose and lacking leucine at 25°C and labeled with 10 μCi/ml [^{35}S]methionine for 1/6−1/2 generation. After breaking the cells, the extracts were separated into soluble and membrane fractions. Fractions containing 2.6 × 10^6 c.p.m. acid-insoluble radioactivity were subjected to immunoprecipitation by Y13-259 and run on a 15% SDS−polyacrylamide gel. **Lane 1**: soluble fraction from TK161-R2V. **Lane 2**: soluble fraction from HR12. **Lane 3**: soluble fraction from HR13. **Lane 4**: membrane fraction from TK161-R2V. **Lane 5**: membrane fraction from HR12. **Lane 6**: membrane fraction from HR13. Mol. wt markers as described in Figure 1.

pose we introduced a plasmid YEp51-RAS2 [a plasmid containing *RAS2* gene under the control of a *GAL10* promoter (Tamanoi *et al.*, 1984)] into the mutant and parental cells. Since the level of endogenous RAS2^{Val19} proteins is negligible, the RAS2 proteins detected in the transformed cells arise solely from the *RAS2* gene on the plasmid. The cells were labelled with [^{35}S]methionine and the labeled proteins were immunoprecipitated. As can be seen in Figure 1, the RAS2 proteins isolated from the *dpr1* cells (HR12, HR13, HR15) migrated slightly more slowly (lanes 5−8) than those isolated from the parental strain TK161-R2V (lane 3) and migrated to the position taken by the RAS2 protein produced in a cell-free translation system (lane 4). Thus, the majority of the RAS2 proteins present in the *dpr1* cells appear to be in the form of a precursor (Fujiyama and Tamanoi, 1986). A small amount of a processed form, however, is present in the *dpr1* cells as described below (Figure 4). We were also able to obtain spontaneously arising cells which accumulate precursor RAS proteins. Even though it is not known whether these cells contain a mutation identical to the *dpr1*, it is clear that the processing of a precursor form is greatly retarded in these cells. Lane 1 of Figure 1 shows the RAS2 proteins obtained from such cells. It is seen that approximately half the proteins are present as a precursor form. The original strain, however, shows only a processed form (lane 2).

Amount of RAS proteins associated with plasma membranes is decreased in dpr1 cells

We next examined whether the transport of the RAS proteins to membranes is affected by the *dpr1* mutation. Cells were labeled with [^{35}S]methionine to achieve steady-state protein labeling, separated into soluble and membrane fractions and the labeled proteins were immunoprecipitated. As shown in Figure 2, the level of the RAS2 protein in the soluble fraction of the *dpr1* cells (HR12 and HR13) was much higher than that observed with the parental strain TK161-R2V. In contrast to this, the RAS2 protein in the membrane fraction of the *dpr1* cells was hardly detected whereas a significant level of the RAS2 protein was observed in the membrane fraction of the parental cells. Thus, the RAS2 protein appears to accumulate in the soluble fraction of the *dpr1* cells. A faint band seen in the membrane fraction of the *dpr1* cells (Figure 2, lanes 5 and 6) migrating slightly slower than a processed RAS2 protein band presumably represents a minor contamination of the soluble RAS2 protein due to its large accumulation in the soluble fraction of the *dpr1* cells. Such a contaminating band was not detected when a similar analysis was carried out (data not shown) with the other mutant JR25-D which accumulated a moderate amount of the soluble protein (see Figure 1). Membrane bound processed RAS2 proteins in the *dpr1* cells, on the other hand, migrate at the same position as the processed RAS2 protein in the parental cells (see Figure 4).

To further confirm the subcellular distribution of the RAS2 protein, sucrose density gradient analyses were carried out with cell extracts. Proteins in the soluble fraction remain at the top of the gradient whereas the proteins associated with membranes sediment. As shown in Figure 3C the RAS2 proteins in the parental strain, as detected by their GDP binding activity, are distributed into two peaks; one at the top of the gradient and the other at the position where plasma membranes sediment. The latter position was identified by assaying vanadate sensitive Mg^{2+}-ATPase activity. The condition used here separates plasma membrane from endoplasmic reticulum membrane (Fujiyama and Tamanoi, 1986). A similar profile was obtained with the other control strain, JR25-2A (α *leu2 ura3 trp1 his3 can1*) (Figure 3B). When extracts from the *dpr1* cells were subjected to the same analysis, however, the vast majority of the RAS2 protein was detected at the top of the gradient, and only a small fraction (~1/50 of the total and <1/3 of the amount detected with the control cells) of the RAS2 protein was found at the position where plasma membranes sediment (Figure 3A). Taken together, these results suggest that the transport of the RAS proteins to membranes is markedly inhibited by the *dpr1* mutation and that the precursor RAS proteins are accumulating in the cytoplasm.

Fatty acid acylation of the RAS proteins takes place in dpr1 cells

Above results demonstrate that the effect of the *dpr1* mutation can be seen in the processing of precursor RAS proteins as well as in their membrane association. We next examined whether the *dpr1* mutation also affected fatty acid acylation of the RAS proteins and whether a drastic decrease in the amount of fatty acid acylated RAS proteins is detected in the *dpr1* cells. The cells were labelled with [^{3}H]palmitic acid, soluble and membrane fractions were separated and immunoprecipitated proteins were analyzed on a SDS−polyacrylamide gel (Figure 4). First, we noted that fatty acid acylated RAS proteins were detected in the *dpr1* cells. A majority of the radioactivity was found in the mem-

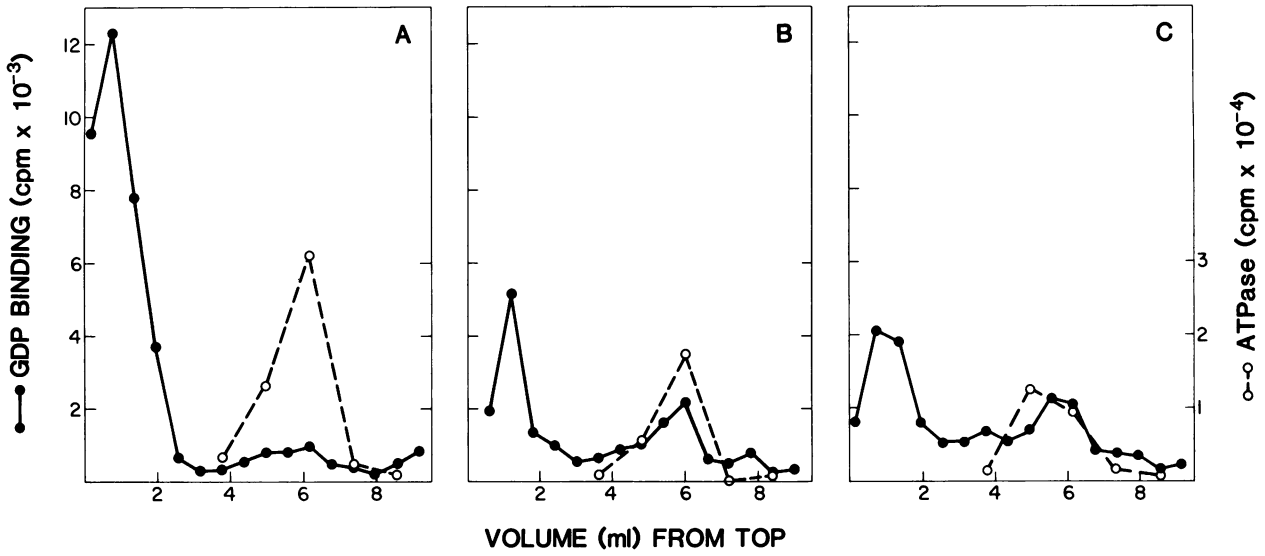

Fig. 3. Fractionation into soluble and membrane fractions by sucrose density gradient sedimentation. Cells carrying YEp51-RAS2 plasmid were grown at 25°C in a 100 ml synthetic medium containing 4% galactose and lacking leucine. After collecting the cells, extracts were fractionated by centrifugation on 9.5 ml gradients of sucrose from 25% (w/w) to 60% (w/w) as described (Fujiyama and Tamanoi, 1986). An aliquot of the fractions collected were treated with 1% Triton X-100 for 1 h on ice and centrifuged for 10 min. The superantant was incubated with [^{3}H]GDP followed by immunoprecipitation with antibody Y13-259 to assay RAS-specific GDP binding activity. (**A**) Sucrose gradient fractions from the extracts of HR12, (**B**) JR25-2A and (**C**) TK161-R2V.

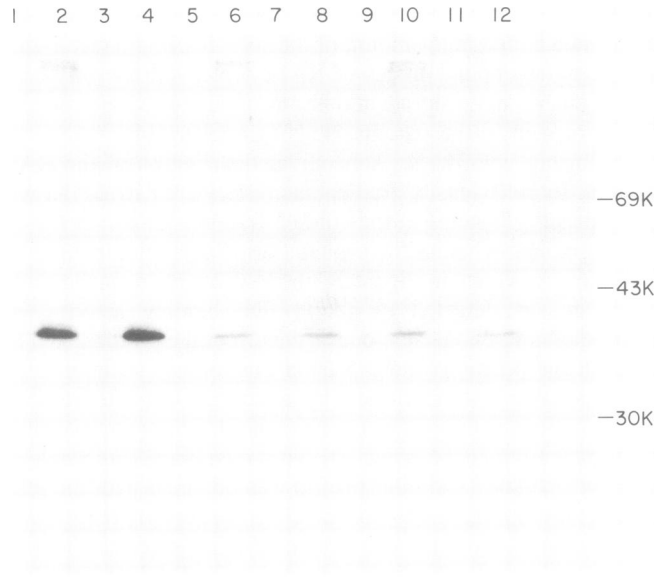

Fig. 4. Immunoprecipitation and SDS–polyacrylamide gel of [^{3}H]palmitic acid-labeled RAS2 proteins. TK161-R2V (**lanes 1–4**), HR12 (**lanes 5–8**), and HR13 (**lanes 9–12**) cells carrying YEp51-RAS2 plasmid were grown in a synthetic galactose medium at 25°C. Half of each cell culture was incubated at 37°C for 30 min prior to labeling. Cells were labeled with 30 μCi/ml [^{3}H]palmitic acid (55 Ci/mmol) at 25°C or 37°C for the period of 30% doubling time. Labeled cells were fractionated into soluble and membrane fractions and were subjected to immunoprecipitation. A fluorogram of the soluble (lanes 1, 3, 5, 7, 9 and 11) and membrane (lanes 2, 4, 6, 8, 10 and 12) fraction samples labeled at 25°C (lanes 1, 2, 5, 6, 9 and 10) or 37°C (lanes 3, 4, 7, 8, 11 and 12) are shown.

brane fraction in both parental and *dpr1* cells, in agreement with our previous results (Fujiyama and Tamanoi, 1986). In contrast to the proteins in the soluble fractions, the proteins from the membranes of the control and the *dpr1* cells migrate with the same mobility. Even though the amount of fatty acid acylated RAS protein is lower in the *dpr1* cells compared to that in the parental cells, this seems to merely reflect the difference in the amount of RAS2 protein in membranes. From densitometric analysis of the autoradiograms obtained after two different exposure times, the amount of the fatty acid acylated RAS2 protein in the *dpr1* cells was found to be ~20% of that detected in the parental cells, which agrees with the actual amount of the protein in the membranes of these strains (see Figure 3). Furthermore, a brief exposure at the non-permissive temperature did not result in a drastic decrease of the amount of fatty acid acylated RAS2 protein. These results suggest that the major effect of the *dpr1* mutation is in the processing of the precursor proteins and not in the fatty acid acylation of the RAS2 protein. In addition, no difference in the profile of total proteins labeled with [^{3}H]palmitic acid was detected between the *dpr1* and the parental cells (data not shown). It is also worth noting that, even though a large amount of the precursor RAS2 protein was present in the soluble fraction of the *dpr1* cells, essentially no palmitic acid radioactivity was observed in the soluble fraction. This is in line with the idea that the precursor forms have to be converted to the processed forms in order to be fatty acid acylated and become membrane associated.

Use of the dpr1 cells for the high level production of RAS proteins

High level production of the wild-type RAS2 protein appears to be deleterious to yeast cells. First, JR25-2A cells overproducing the RAS2 proteins by using *GAL10* promoter do not grow at 37°C in the presence of 4% galactose. Furthermore, our attempts to increase the level of the overproduction by using stronger promoters have been unsuccessful. Above results with the *dpr1* cells, however, have suggested to us that it may be possible to obtain a high level production of the RAS2 protein using these cells, since the overproduced proteins will remain in cytoplasm. To assess this point, we have placed the *RAS2* gene under the control of glyceraldehyde-3-phosphate dehydrogenase promoter. This was accomplished by inserting the *RAS2* gene into *Sal*I site of pYG10, which contains the promoter and *LEU2* gene as a selection marker. When the resulting plasmid pYG-RAS2 was transformed into the *dpr1* cells, LEU$^+$ transformants were obtained. In contrast to this, no transformants were obtained when the same plasmid was transformed into wild-type cells. In these transformants, a large amount of the RAS2 protein was produced as shown in Figure 1 lane 9. The level of the RAS2 produc-

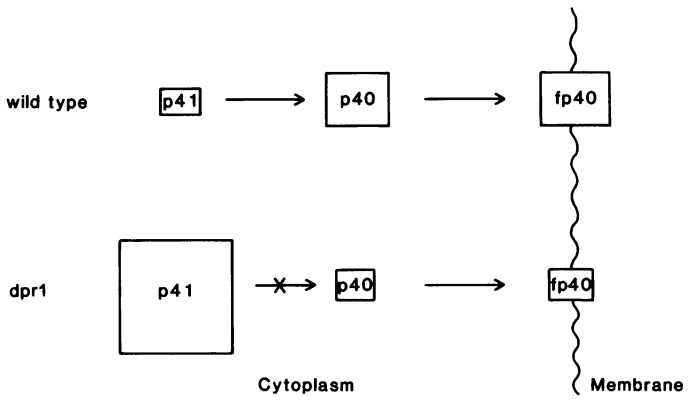

Fig. 5. Effect of the *dpr1* mutation on the pathway of biosynthesis of RAS2 proteins. **X** shows the defective step in *dpr1* cells.

tion was higher than that observed in *dpr1* cells containing YEp51-RAS2 (compare lanes 5 and 9 in Figure 1). Thus, the *dpr1* cells provide a convenient host to accomplish overproduction of the RAS proteins.

Discussion

Here we have described the isolation and characterization of a yeast mutant, *dpr1*, defective in the processing of ras proteins. Mutant ras proteins have been reported (Willumsen *et al.*, 1985; Weeks *et al.*, 1985) which are not fatty acid acylated and do not associate with membranes. However, isolation of a cellular mutant is presented here. The fate of RAS proteins in wild-type and mutant cells is illustrated in Figure 5. First with wild-type cells, precursor forms are quickly converted to processed forms which are then fatty acid acylated and become associated with plasma membranes. Thus, only a minute fraction of the proteins are in the precursor form and a significant fraction of the RAS proteins are found in the plasma membranes. In marked contrast to this, in the *dpr1* cells, processing of precursor forms is defective and the precursor forms accumulate in the cytoplasm. Only a small amount of the RAS proteins are found in plasma membranes. These membrane-bound RAS proteins in the *dpr1* cells are fatty acid acylated, whereas the soluble forms are not. Similar results were obtained when human Ha-ras proteins were expressed in yeast; precursor forms were detected which were processed and then fatty acid acylated. Furthermore, precursor proteins accumulated in the *dpr1* cells (unpublished data). Thus, the *dpr1* mutation affects processing of Ha-ras proteins as well. This also raises the possibility that mammalian cells possess an activity to process precursor ras proteins and a gene closely related to the *DPR1* can be found in mammalian cells.

It has so far been difficult to determine the nature of the processing, mainly because only a small amount of the precursor form is present in wild-type cells. By the use of the *dpr1* cells we can now obtain a large amount of the precursor form which can be compared to the processed form. As a first step toward characterizing the processing, we have isolated the precursor form labeled with radioactive amino acids and determined its amino terminal sequence. Our results (unpublished data) indicate that the first methionine residue is removed in both the precursor and the processed forms and proline is present at the amino terminus in both forms. On the other hand, removal of more than five amino acids at the carboxy terminus is not likely to be taking place. There are only two cysteines located at the fourth and the fifth residues from the carboxy terminus and both forms can be labeled with radioactive cysteine (Fujiyama and Tamanoi, 1986). Taken together, these results suggest that the processing does not involve proteolytic cleavage or involves cleavage of less than four amino acids at the carboxy terminus.

Use of the *dpr1* cells also enabled us to compare biochemical activities of the precursor and the processed forms. We have shown that the precursor forms are active in GDP binding. The increase of the total RAS-specific GDP binding activity observed in the *dpr1* cells corresponds approximately to the increase of the amount of the RAS proteins. Whether the precursor and processed forms have identical specific activity will be clarified by the purification of both forms of the RAS protein. Another useful feature of using the *dpr1* mutant is to overproduce the RAS proteins or their precursors. Since the RAS proteins remain in the cytoplasm, any toxic effect of overproducing the RAS proteins can be minimized. By using the *dpr1* cells, we have been able to boost the production of the RAS proteins approximately 10-fold over the previous level (Tamanoi *et al.*, 1984). We have also been successful in overproducing $RAS2^{Val19}$ or $RAS2^{Thr66}$ proteins as well as mammalian ras proteins.

Yeast cells expressing $RAS2^{Val19}$ proteins are sensitive to heat treatment at 57°C for 5 min, whereas the *dpr1* mutants are resistant to such a treatment. Based on our results, we speculate that the $RAS2^{Val19}$ proteins are accumulated as precursor forms in the cytoplasm of the *dpr1* cells. As a consequence, the *dpr1* mutants may have a decreased amount of the $RAS2^{Val19}$ proteins in membranes and thus the effect of $RAS2^{Val19}$ proteins appears to be minimized. A similar explanation could be made regarding the temperature sensitive growth property of the *dpr1* cells. At the non-permissive temperature, the level of RAS proteins in plasma membranes of the *dpr1* cells may not be sufficient to support the growth of cells. It is possible that cellular localization of other essential gene products is affected as well.

Another phenotype of the *dpr1* mutant is sterile phenotype specific to *a* cells. Since the *dpr1* mutation affects processing of RAS proteins, a similar processing defect may also contribute to this phenotype. One of the possibilities which could account for this phenotype is a processing defect of a mating factor, *a* factor. The *a* factor is produced only in MAT *a* cells and is essential for the mating with MAT α cells. Sequence of the genomic DNA encoding the *a* factor suggests that the *a* factor is first produced as a precursor and is then converted to a mature form (Brake *et al.*, 1985). A sequence cysAA (A is an aliphatic amino acid) which is thought to be required for the processing of mammalian ras proteins (Weeks *et al.*, 1985; Willumsen *et al.*, 1985) is present in the carboxyl terminal region of both the *a* factor precursor and the RAS proteins. Thus, processing of the *a* factor precursor might share characteristics common to the processing of the RAS proteins. Such a proposal has been made using *SupH* mutants (see Introduction) and our results provide strong supporting evidence for this.

What, then, is the biochemical process common to the processing of the two proteins? This does not appear to be fatty acid acylation, since the *dpr1* mutation does not affect this process *per se*. Instead, a modification which precedes the fatty acid acylation is affected by the mutation. While it is not known what this modification involves, the following speculations can be made. Three amino acids at the carboxy terminus might be removed, thus exposing the cysteine residue present in the cysAA sequence. Alternatively, modification of the acceptor residue is required for the attachment of fatty acids to the proteins. Modification of a cysteine residue by farnesyl moiety is detected in peptidal sex hormones of *Tremella mesenterica* (Sakagami *et al.*, 1981)

and the addition of glycerol group to a cysteine is detected in *E. coli* lipoprotein (Hantke and Braun, 1973). Further analyses of the processing of the RAS proteins should clarify this point.

Materials and methods

[^{3}H]GDP (11.3 Ci/mmol; 1 mCi = 37 MBq), [^{3}H]palmitic acid (55 Ci/mmol), [^{32}P]ATP (3000 Ci/mmol) and [^{35}S]methionine (1130 Ci/mmol) were obtained from Amersham.

Plasmids and strains

Genotype of each strain is described in the text. An expression plasmid YEp51-RAS2 has been described (Tamanoi *et al.*, 1984). Plasmids pYG10 and YCp50-*HO* were kindly provided by Kenichi Matsubara and Robert Jensen, respectively. Yeast strain TK161-R2V was provided by Takashi Toda and Michael Wigler.

Detection of radio-labeled RAS proteins

Poly(A) RNA was isolated and subjected to cell-free translation as described previously (Tamanoi *et al.*, 1984). Conditions of immunoprecipitation and SDS–polyacrylamide gel electrophoresis have been described (Tamanoi *et al.*, 1984).

Subcellular fractionation and assay of enzyme activities

Crude extract of yeast cells was fractionated by sucrose density gradient centrifugation and vanadate sensitive Mg^{2+}-ATPase was assayed as a marker enzyme of plasma membrane as described (Fujiyama and Tamanoi, 1986). GDP binding activity was assayed as a marker of RAS protein.

Acknowledgements

We thank Donald Steiner, Howard Tager and Ira Herskowitz for critical reading of the mansucript, Ken-ichi Arai for discussions and advice on this work and R.Jennifer Detrick for stimulating discussions. We also thank William R.Brown for excellent technical assistance and Cheryl Spencer and Anna Wright for typing 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

Brake,A.J., Brenner,C., Najarian,R., Laybourn,P. and Merryweather,J. (1985) In Gething,M.J. (ed.), *Protein Transport and Secretion.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 103–108.

Broek,D., Samiy,N., Fasano,O., Fujiyama,A., Tamanoi,F., Northup,J. and Wigler,M. (1985) *Cell,* **41**, 763–769.

Fujiyama,A. and Tamanoi,F. (1986) *Proc. Natl. Acad. Sci. USA,* **83**, 1266–1270.

Fujiyama,A., Rao,M., Samiy,N. and Tamanoi,F. (1986) In Hicks,J. (ed.), *Yeast Cell Biology.* Alan R.Liss, Inc., NY, pp. 125–149.

Gibbs,J.B., Sigal,I.S., Poe,M. and Scolnick,E.M. (1984) *Proc. Natl. Acad. Sci. USA,* **81**, 5704–5708.

Hantke,K. and Braun,V. (1973) *Eur. J. Biochem.,* **34**, 284–296.

Jensen,R., Sprague,G.F. and Herskowitz,I. (1983) *Proc. Natl. Acad. Sci. USA,* **80**, 3035–3039.

Lindegren,G., Hwang,Y.-L., Oshima,Y. and Lindegren,C.C. (1965) *Can. J. Genet. Cytol.,* **7**, 491–499.

McGrath,J.P., Capon,D.J., Goeddel,D.V. and Levinson,A.D. (1984) *Nature,* **310**, 644–649.

Sakagami,Y., Yoshida,M., Isogai,A. and Suzaki,A. (1981) *Science,* **212**, 1525–1527.

Scolnick,E.M., Papageorge,A.G. and Shih,T.Y. (1979) *Proc. Natl. Acad. Sci. USA,* **76**, 5355–5359.

Sefton,B.M., Trowbridge,I.S., Cooper,J.A. and Scolnick,E.M. (1982) *Cell,* **31**, 465–474.

Shih,T.Y., Weeks,M.O., Gruss,P., Dhar,R., Oroszlan,S. and Scolnick,E.M. (1982) *J. Virol.,* **42**, 253–261.

Sweet,R.W., Yokoyama,S., Kamata,T., Feramisco,J.R., Rosenberg,M. and Gross,M. (1984) *Nature,* **311**, 273–275.

Tamanoi,F., Walsh,M., Kataoka,T. and Wigler,M. (1984) *Proc. Natl. Acad. Sci. USA,* **81**, 6924–6928.

Temeles,G.L., DeFeo-Jones,D., Tatchell,K., Ellinger,M.S. and Scolnick,E.M. (1984) *Mol. Cell. Biol.,* **4**, 2298–2305.

Toda,T., Uno,I., Ishikawa,T., Powers,S., Kataoka,T., Broek,D., Cameron,S., Broach,J., Matsumoto,K. and Wigler,M. (1985) *Cell,* **40**, 27–36.

Towler,D. and Glaser,L. (1986) *Proc. Natl. Acad. Sci. USA,* **83**, 2812–2816.

Weeks,M.O., Hager,G.L., Lowe,R. and Scolnick,E.M. (1985) *J. Virol.,* **54**, 586–597.

Wen,D. and Schlesinger,M.J. (1984) *Mol. Cell. Biol.,* **4**, 688–694.

Willingham,M.C., Pastan,I., Shih,T.Y. and Scolnick,E.M. (1980) *Cell,* **19**, 1005–1014.

Wilumsen,B., Christensen,A., Hubbert,N.L., Papageorge,A.G. and Lowy,D. (1985) *Nature,* **310**, 583–586.

Received on 29 September 1986; revised on 4 November 1986

Note added in proof

S.Michaelis has recently informed us that *supH* (now called *ram*) is allelic to *dpr1*.