Mol Gen Genet (1994) 244:183–188

ORIGINAL PAPER

Christine Lang-Hinrichs · Ingo Queck · Georg Büldt
Ulf Stahl · Volker Hildebrandt

The archaebacterial membrane protein bacterio-opsin is expressed and N-terminally processed in the yeast *Saccharomyces cerevisiae*

Received: 18 August 1993 / Accepted: 31 January 1994

Abstract The *bop* gene codes for the membrane protein bacterio-opsin (BO), which on binding all-*trans*-retinal, constitutes the light-driven proton pump bacteriorhodopsin (BR) in the archaebacterium *Halobacterium salinarium*[3]. This gene was cloned in a yeast multi-copy vector and expressed in *Saccharomyces cerevisiae* under the control of the constitutive *ADH*1 promoter. Both the authentic gene and a modified form lacking the precursor sequence were expressed in yeast. Both proteins are incorporated into the membrane in *S. cerevisiae*. The presequence is thus not required for membrane targeting and insertion of the archaebacterial protein in budding yeast, or in the fission yeast *Schizosaccharomyces pombe*, as has been shown previously. However, in contrast to *S. pombe* transformants, which take on a reddish colour when all-*trans*-retinal is added to the culture medium as a result of the in vivo regeneration of the pigment, *S. cerevisiae* cells expressing BO do not take on a red colour. The precursor of BO is processed to a protein identical in size to the mature BO found in the purple membrane of *Halobacterium*. The efficiency of processing in *S. cerevisiae* is dependent on growth phase, as well as on the composition of the medium and on the strain used. The efficiency of processing of BR is reduced in *S. pombe* and in a retinal-deficient strain of *H. salinarium*, when retinal is present in the medium.

Key words Bacterio-opsin · Expression · Yeast *Saccharomyces cerevisiae* · Membranes

Communicated by C.P. Hollenberg

C. Lang-Hinrichs (✉) · I. Queck · U. Stahl
Technische Universität Berlin, FG Mikrobiologie und Genetik, Gustav-Meyer-Allee 25, D-13355 Berlin, Germany

G. Büldt[1] · V. Hildebrandt[2]
Freie Universität Berlin, FB Physik, Abt. Biophysik, Arnimallee 14, D-14195 Berlin, Germany

Present addresses:
[1] Forschungszentrum Jülich, IBI-2 Institut für Biologische Strukturforschung, D-52425 Jülich, Germany
[2] Max-Planck-Institut für Biochemie, Abt. Membranbiochemie, D-82152 Martinsried bei München, Germany

[3] The designation *H. salinarium* instead of the former designation *H. halobium* is used throughout this paper following the classification of Tindall (1992)

Introduction

Bacteriorhodopsin (BR) is a membrane protein of the archaebacterium *Halobacterium salinarium* and functions as a light-driven proton pump (Oesterhelt and Stoeckenius 1971). It is present in the purple membrane and contains all-*trans*-retinal as a chromophore covalently bound to lysine 216. BR is a well-characterized integral membrane protein with seven α-helices spanning the lipid bilayer. With respect to structure, BR is similar to G-protein coupled receptors that form part of membrane-associated signal transduction systems (Pardo et al. 1992).

The *bop* gene, which encodes the apo-protein bacterio-opsin (BO), has been isolated from *H. salinarium* and sequenced (Dunn et al. 1981). Heterologous expression of the gene has, to date, only been possible in *Escherichia coli* (Dunn et al. 1987) and in the fission yeast *Schizosaccharomyces pombe* (Hildebrandt et al. 1989). The gene has also been reintroduced into *Halobacterium* (Ni et al. 1990; Ferrando et al. 1993). The N-terminal sequence of the precursor of BO consists of 13 amino acids and its composition has no similarities with known leader sequences in eubacteria or eukaryotes. The *bop* gene has been modified in such a way as to allow its integration into eukaryotic expression cassettes, either as the mature form (without the precursor sequence) or as the precursor form (with the N-terminal sequence) (Hildebrandt et al. 1991). Both forms of the gene can be expressed in *S. pombe* and lead to formation of functional, membrane-integrated bacteriorhodopsin after in vivo incorporation of retinal. The apoprotein is highly unstable in *E. coli* and *E. coli* is not able to process BO even when it is expressed as a hybrid protein

fused to various *E. coli* leader sequences (Karnik et al. 1990). The apoprotein precursor is processed in several steps in fission yeast and in halobacteria, resulting in at least two intermediate forms on Western blots (Wölfer et al. 1988; Miercke et al. 1989).

The expression of BO in budding yeast is interesting for several reasons. Firstly, it is still unclear how BO is translocated into the membrane in *Halobacterium* (Gropp et al. 1992) and in fission yeast. If BO can be expressed in *S. cerevisiae*, specific mutant strains for studying the translocation pathway are available among the so-called *sec* mutants (Schwencke 1991). Secondly, BR is one of the seven-helix receptors and can serve as a model for studying and comparing signal transduction pathways in *S. pombe* and *S. cerevisiae*. This paper describes our studies of the expression of BO in *S. cerevisiae* as a basis for further analyses. The intracellular location of the protein has been determined and we show that *S. cerevisiae* is capable of processing the apoprotein precursor, with an efficiency which is dependent on culture conditions and the strain used.

Materials and methods

Strains and plasmids

The *E. coli* strain used was K12 SF8 (Chan and Tye 1980). The *S. cerevisiae* strains used were AH22 (*MAT***a** *leu2-3 leu2-112 his* 4-519 *can1*; Hinnen et al. 1978) and cl3-ABYS86 (*MAT*α *pra1-1 prb1-1 cps1-3 ura* 3 *Δ5 leu2-3 leu2-112 his*$^-$; kindly provided by Professor D. Wolf, Stuttgart). The *S. pombe* strain used had the genotype *h*$^-$ *leu1-32* (Hildebrandt et al. 1989). *E. coli* was grown in LB medium (0.5% Bacto-tryptone, 0.5% yeast extract, 1% NaCl, pH 7.2); ampicillin was added at 50 µg/ml when needed. *S. cerevisiae* was grown in either complete medium (YE: 0.5% yeast extract, 2% glucose, pH 6.3) or minimal medium [YNB: 0.67% yeast nitrogen base (Difco), 2% glucose, amino acids at 40 mg/l as required]. The antibiotic G418 (Geneticin, Sigma, Munich FRG) was added for selection at 250 µg/ml in solid medium and at 100 µg/ml in liquid medium. *S. pombe* cells transformed with the plasmid pEVBOp (containing the *bop* gene) were grown in minimal medium with 6% glucose as described previously (Hildebrandt et al. 1991). *H. salinarium* strains JW5 and ET1001 (an overproducer of purple membrane) were grown in complex medium (Oesterhelt and Stoeckenius 1974). All-*trans*-retinal was purchased from Sigma; retinal was added to the cultures at 6-h intervals to a concentration of 2 µM.

The bacterio-opsin gene was cloned either as the precursor (*bop*) or as the mature form (*bom*); the construction of the inserts was described previously (Hildebrandt et al. 1989). The plasmid pPT2a (Lang-Hinrichs et al. 1989) is a 2 µm-based vector that confers resistance to G418. A derivative of pPT2a containing the multiple cloning site of pUC19 inserted downstream of the *ADH1* promoter in pPT2a was used for constructing the expression vectors (pPT2b). Cleavage of pPT2b with *Bam*HI and *Hin*dIII and insertion of the *bop* gene from plasmid pBSBOp (Hildebrandt et al. 1989), followed by ligation of the Tn903 kanamycin resistance gene (Lang-Hinrichs et al. 1989) as a selection marker at the *Pvu*II site (as a *Hin*dII fragment; kanamycin resistance Genblock; Pharmacia, Freiburg FRG) yielded pPBOpK. As an alternative selection marker, the yeast (*S. cerevisiae*) *LEU2* gene, on a 2.8 kb *Hpa*I fragment from plasmid pDFM11, was inserted at the *Pvu*II site of pPT2b, giving plasmid pPBOpL. [pDFM11 is a derivative of pDam1 (Beach et al. 1980) bearing a 2.2 kb *Hin*dIII fragment of pFL1 (Chevallier et al. 1980) carrying the 2 µm DNA origin of replication.] Plasmid pPBOmK was constructed in the same way as pPBOpK; the *bom* gene was derived from plasmid pBSBOm (Hildebrandt et al. 1989). Plasmid pKXL34 (pKX34; Lang-Hinrichs et al. 1989), containing the *LEU2* gene but no BO gene sequences, was used as a control.

Transformations

Transformation of *E. coli* and yeast was carried out according to standard procedures (Himeno et al. 1984; Ito et al. 1983). Regeneration of yeast cells for expression of G418 resistance was for 4–20 h before plating on selective medium (Lang-Hinrichs et al. 1989).

RNA preparation

RNA was extracted from yeast cells in the early log phase of growth and analysed on agarose gels as previously described (Lang-Hinrichs et al. 1990).

Protein extraction and immunoblotting

Whole-cell proteins were isolated from yeast cells after disruption with glass beads (0.45 mm diameter) in TN buffer [150 mM NaCl, 50 mM TRIS-HCl, pH 7.5, 1% sodium dodecyl sulfate (SDS), 2 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF)]. Proteins were separated in 12.5% SDS/polyacrylamide gel electrophoresis (PAGE) and electrophoretically transferred to nitrocellulose filters (Sartorius, Göttingen FRG). A polyclonal rabbit antiserum was raised against BR by immunization with purple membrane; 200 µg of purple membranes in 1 ml phosphate-buffered saline (PBS) were mixed with 1 ml complete Freund's adjuvant in a bath sonifier and subcutaneously injected into a rabbit. Booster injections with a suspension of incomplete Freund's adjuvant and purple membranes were administered four times at intervals of 6 weeks. Two weeks later serum was prepared in Corvac™ serum separator tubes (style HR 88831-302015; Sherwood Medical, St. Louis, Mo., USA). The secondary antibody used for detection of immune complexes was pig anti-rabbit antibody conjugated to horseradish peroxidase (Dako, Hamburg FRG). The secondary antibody was detected using diaminobenzidine as a substrate or by using the ECL Western blotting system (Amersham, Braunschweig, FRG) according to the recommendations of the manufacturer.

Isolation and fractionation of membranes

Crude membranes of *S. cerevisiae* were isolated from spheroplasts as follows. Spheroplasts were carefully homogenized, and the suspension was then centrifuged (5 min, 3000 × g) to remove cell debris. Membranes were pelleted from the supernatant by spinning for 1 h at 38000 rpm in a Beckman SW28 rotor. Cytosolic proteins were precipitated from the supernatant with 10% trichloroacetic acid (TCA). The crude membranes were resuspended in 50 mM TRIS-HCl, pH 7.5, 300 mM NaCl, 20 mM $MgCl_2$, 1 mM PMSF, washed twice in the same buffer and finally resuspended in 50 mM TRIS-HCl, pH 7.5, 150 mM NaCl.

Membrane fractions were obtained from cells grown to mid-log phase ($OD_{600\ nm}$ 8.0). Cells were washed with sterile water, resuspended in 4% glucose, 100 mM TRIS-HCl, pH 8.0, 10 mM EDTA, 0.2 mM PMSF and disrupted with glass beads (0.45 mm diameter). The suspension was diluted with 20 volumes of STED-10 (10% sucrose, 10 mM TRIS-HCl, pH 7.6, 1 mM EDTA, 1 mM DTT) and the cell debris was removed by centrifugation at 1000 × *g* for 10 min. The membranes were recovered by centrifugation at 30000 × *g* for 30 min, resuspended in STED-10 and carefully homogenized. Fractionation was carried out following zonal centrifugation in sucrose gradients. The membrane fraction was first loaded onto a gradient of 20–53% sucrose, and centrifugation was performed for 6 h at 13000 rpm in a Sorvall HB4 rotor. The gradient was divided into two fractions at 35% sucrose; the mem-

branes in each fraction were recovered by centrifugation and loaded onto 20–40% and 40–60% sucrose gradients, respectively. Fractions of 500 µl were collected and assayed for the following marker enzymes: H^+ ATPase for plasma membrane; UDPase for Golgi membranes; α-mannosidase for vacuolar membranes; cytochrome *c* oxidase for mitochondria, and NADPH cytochrome *c* oxidoreductase for endoplasmic reticulum (ER). The assays were performed according to the protocol of Fasano et al. (1991). Protein concentration was determined using the micro-protein assay of Bio-Rad (Munich FRG). Purple membranes of *H. salinarium* were isolated essentially as described (Oesterhelt and Stoeckenius 1974). Plasma membranes were obtained from *S. pombe* cells according to the protocol of Hildebrandt et al. (1991).

Results

Expression of bacterio-opsin in *S. cerevisiae*

The bop gene from *H. salinarium* was expressed as the mature form (*bom*) and the precursor form (*bop*) from the *ADH*1 promoter on 2 µm-based plasmids in *S. cerevisiae*. The plasmids contained as a selection marker either the Tn903 kanamycin resistance gene coding for G418 resistance in yeast (plasmids pPBOmK and pPBOpK) or the *S. cerevisiae LEU*2 gene (pPBOpL). Both the mature form and the precursor form of the *bop* gene were shown to result in transcripts (Fig. 1) and translation products (Fig. 2) in the yeast.

The size of the major transcript was approximately 1100 nucleotides, corresponding well to the expected length (780 bp of gene sequence + 50 bp of 5′ untranslated sequences + 200 bp of the terminator region of the yeast *TRP*1 gene + polyA tail). Larger transcripts, which probably start at sequences further upstream in the promoter sequence (Ruohonen et al. 1991), were also detectable. Transcription of the archaebacterial *bop* gene in *S. cerevisiae* was also achieved using the *ADH* promoter from *S. pombe* on a 2 µm-based plasmid and the inducible *GAL*1 promoter of *S. cerevisiae* on an

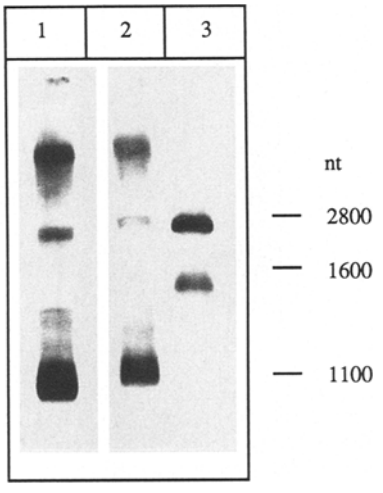

Fig. 1 Northern blot analysis of RNA from *Saccharomyces cerevisiae* AH22/pPBOmK (lane 1) and AH22/pPBOpK (lane 2). The markers (lane 3; sizes given in nucleotides) are length standard I from Boehringer Mannheim. Cells were harvested in early log phase. The *bop* gene was used as a probe

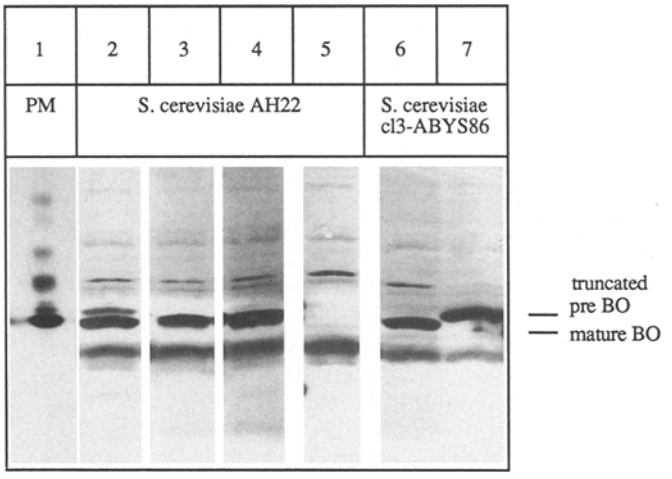

Fig. 2 Western blot analysis of whole cell proteins of *S. cerevisiae* detected with polyclonal antibodies against *H. salinarium* purple membrane. Lane 1, 20 ng of purple membrane as a positive control; lane 2, AH22/pPBOpL; lane 3, AH22/pPBOmK; lane 4, AH22/pPBOpK; lane 5, AH22/pKXL34 as a negative control showing the cross reaction of the antiserum with yeast proteins; lane 6, cl3-ABYS86/pPBOmK; lane 7; cl3-ABYS86/pPBOpK. Yeast cells were harvested in late-log phase (OD_{600} of 8–10); 100 µg of yeast protein was loaded in each lane. Note that AH22 expresses two BO species from pPBOpL but only the mature form from pPBOmK. In strain cl3-ABYS86/pPBOpK, the mature form is not seen

ars-based vector (data not shown). The best expression, however, was achieved using the *S. cerevisiae ADH*1 promoter on the 2 µm vector pPT2b.

Translation products of the *bop* gene were detected by immunoblotting analysis using a polyclonal antibody directed against purple membranes. The proteins detected correspond in size to BR forms present in the purple membrane of *Halobacterium* (Fig. 2). BO could be detected both in strain AH22 and in strain cl3-ABYS86. The latter strain was used as a host because it is deficient in three major vacuolar proteinases and has frequently been used to circumvent degradation of heterologous proteins (Kopetzki et al. 1989).

Localization of bacterio-opsin in *S. cerevisiae*

Cells of strains AH22/pPBOpK and AH22/pPBOmK were fractionated in order to determine the subcellular location of BO. Crude membranes were prepared and analysed for BO by immunological methods. BO was clearly enriched in the membrane fraction of both strains and was not present in the cytosolic fraction (Fig. 3). BO was localized more precisely by density-gradient fractionation of the cellular membranes. Membranes were identified in the gradient fractions by using antibodies directed against the mitochondrial Rieske iron-sulfur protein, and by assays for marker enzymes as described in the Materials and methods. BO was detected at a sucrose concentration of 39–41% in fractions that exhibited H^+-ATPase activity and thus contained plasma membranes (data not shown).

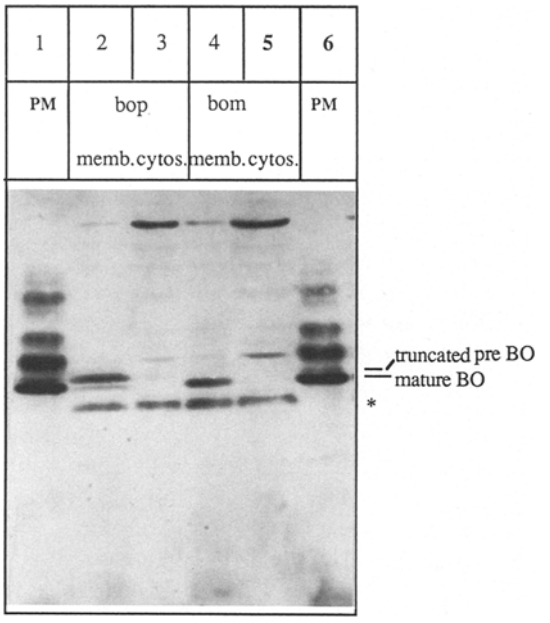

Fig. 3 Western blot analysis of crude membrane preparations of strains *S. cerevisiae* AH22/pPBOpK and AH22/pPBOmK grown in complete medium. Lanes 1 and 6, 20 ng of purple membrane protein; lane 2, crude membrane fraction of AH22/pPBOpK (100 µg proteins); lane 3, cytosolic fraction of AH22/pPBOpK (100 µg proteins); lane 4, crude membrane fraction of AH22/pPBOmK (100 µg proteins); lane 5, cytosolic fraction of AH22/pPBOmK (100 µg proteins). The *asterisk* marks the position of a band that results from cross reaction with yeast protein (see also Fig. 2). Note that BO is present only in the membrane fractions

Processing of bacterio-opsin in *S. cerevisiae*

Only one major translation product was detected in strains expressing the *bom* gene, which encodes the mature form of BO, whereas two protein bands were observed in strain AH22 expressing the precursor form (*bop*; see Fig. 2, lane 2). These bands correspond in size to the fully processed and the intermediate forms of BR present in the purple membrane (Fig. 2). The unprocessed form representing the primary translation product can be detected in *Halobacterium* and in *S. pombe* transformed with the BO gene when retinal is present. In *S. cerevisiae* AH22/pPBOpK the unprocessed form was not detected under these conditions (see Fig. 4A).

The partially processed BO intermediate was detected both in strain AH22 and in the protease-deficient strain cl3-ABYS86 (Fig. 2, lanes 2, 7). In strain AH22 the processing efficiency is dependent on the composi-

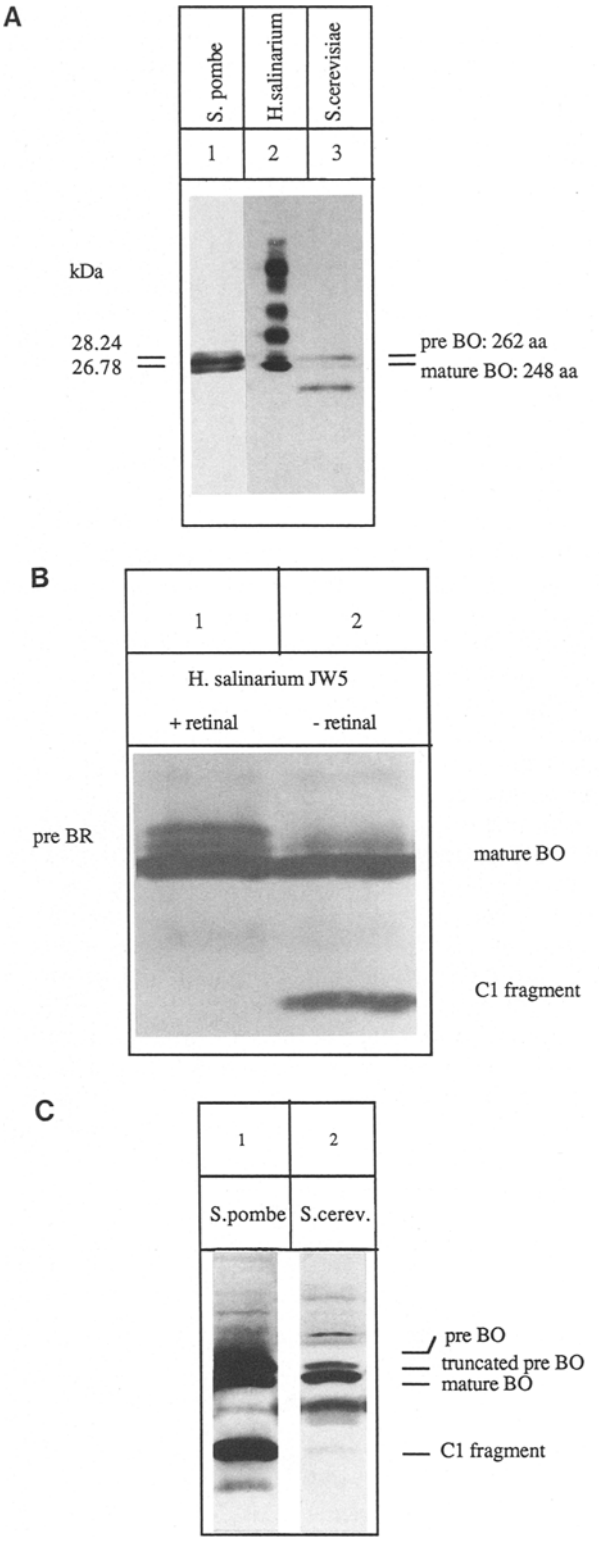

Fig. 4A Western blot analysis of plasma membranes of *Schizosaccharomyces pombe* cells transformed with pEVBOp (lane 1; 5 µg protein), purple membranes of *Halobacterium salinarium* (lane 2; 20 ng protein) and plasma membranes of *S. cerevisiae* AH22 cells transformed with pPBOpK (lane 3; 100 µg protein). Cells were grown in the presence of retinal. In *S. cerevisiae*, only the partially processed intermediate is observed; the unprocessed form is still not detectable even after prolonged exposure of the blot. **B** Western blot analysis of plasma membrane proteins from *H. salinarium* JW5 cells grown in the presence (lane 1) or absence (lane 2) of all-*trans*-retinal. (*BR* Bacteriorhodopsin, *BO* bacterio-opsin). Note the appearance of the proteolytic C1 fragment in the cells grown in the absence of retinal. **C** Western blot analysis of whole cell proteins of *S. pombe*/pEVBOp cells (lane 1) and *S. cerevisiae* AH22/pPBOpL cells (lane 2). Both strains were grown in minimal medium to late-log phase without the addition of retinal. Note that the C1 fragment is only seen in S. pombe and that pre BO is not observed in *S. cerevisiae*. 100 µg of protein was loaded in each lane.

tion of the medium and on growth phase. Processing is efficient in cells grown in minimal medium, but is incomplete in cells grown in complete medium (data not shown). Processing is also more efficient in the late log phase than in the early log phase; this is in agreement with observations made for strain ET1001 of *H. salinarium* (Wölfer et al. 1988) and for *S. pombe* (Hildebrandt et al. 1991). The proteinase-deficient strain cl3-ABYS86 is, however, unable to process BO completely. Expression of the precursor form of the BO gene in complete medium results in only one protein band (Fig. 2, lane 7), which corresponds in size to an intermediate, partially processed form of bacterio-opsin.

Stability of bacterio-opsin in *S. cerevisiae* and retinal incorporation

Retinal is necessary to allow BO to function as a proton pump and also for the stability of the protein, both in *Halobacterium* and in recombinant *S. pombe* cells expressing BO (Hildebrandt et al. 1993). In the *Halobacterium* mutant strain JW5, which does not synthesize retinal, degradation products of BO, such as the C1 fragment resulting from endopeptidase cleavage at amino acid 71 (Fig. 4B), are observed. The same degradation product is observed when recombinant *S. pombe* cells are grown without the addition of exogenous all-*trans*-retinal (Fig. 4C, lane 1). No such correlation is observed in *S. cerevisiae*; the C1 fragment is seen only as a very weak band in strain AH22/pPBOpL grown without retinal (Fig. 4C).

Retinal must be incorporated into the protein in order for functional bacteriorhodopsin to be formed. This in turn leads to a reddish coloration of the cells. With *S. pombe* transformants and retinal-deficient *Halobacterium* mutants this colour change can be achieved by adding all-*trans*-retinal to the culture medium. The *S. cerevisiae* AH22 transformants were also cultivated in the presence of the chromophore; however, the cells did not take on a red colour under the growth conditions that resulted in red *S. pombe* cells. Crude membrane preparations of *S. cerevisiae* transformants (AH22/pPBOpK) also did not show a change of colour when retinal was added. The lack of colour change might result from the lower level of BO expressed in the *S. cerevisiae* cells in comparison with *S. pombe*. Quantification of BO was performed by Western blot analyses using BR in purple membrane as a reference. The amount of BO relative to whole cell proteins was calculated to be 0.01% in *S. cerevisiae* transformants and 0.1% in *S. pombe* transformants.

Discussion

In this paper we have shown that archaebacterial bacterio-opsin can be expressed as a membrane protein in the budding yeast *S. cerevisiae*. This yeast is one of the best characterized eukaryotic hosts for heterologous genes; the successful expression of bacterio-opsin provides the basis for further studies on those elements and processes necessary for the expression and function of membrane proteins in this host.

Bacterio-opsin is found in the membrane fraction in *S. cerevisiae* regardless of whether it is expressed from the gene with the presequence (*bop*) or without the presequence (*bom*). This finding indicates that the presequence is not required for translocation or membrane insertion, and raises the question of how bacterio-opsin is inserted into the membrane. It has not been established whether membrane insertion occurs post-translationally or co-translationally in *H. salinarium* (Frevert et al. 1989). It is assumed that two translocation mechanisms exist in *Halobacterium*, as bacterio-opsin and bacterio-opsin-like proteins have a presequence different from that of other membrane proteins, such as the halobacterial glycoprotein CGS (Gropp et al. 1992). Whether a translocation mechanism differing from the normal pathway is used for the membrane insertion of BO in the yeast *S. cerevisiae* will have to be examined in further experiments by using *S. cerevisiae* translocation mutants.

The 13-amino acid presequence encoded by plasmids pPBOpL and pPBOpK has no influence on the stability of the protein in *S. cerevisiae*, in contrast to the situation in *E. coli* cells expressing BO (Karnik et al. 1990). Processing of the 13-amino acid leader does occur in *S. cerevisiae*, as proteins corresponding in size to mature BO are present. Processing in *S. cerevisiae* is dependent both on growth phase and growth medium and on the strain used. Processing is more efficient in later growth phases and when cells are grown in minimal medium. An increase in proteolytic activity is generally observed in yeast cells grown under conditions of nitrogen limitation, or in cultures proceeding from logarithmic to stationary phase growth (Rendueles and Wolf 1988). Similarly, BO can be detected in the precursor form in early log phase in *Halobacterium*; the intermediate and mature forms are detected in late log phase and the mature form is enriched in the stationary growth phase (Wölfer et al. 1988).

BO is detected in *S. cerevisiae* in two forms, one corresponding in size to the intermediate, partially processed form, the other to the mature form. The first processing step, which leads to the intermediate, is probably very efficient and rapid, so that the largest precursor has not been detected in *S. cerevisiae* to date. This step also occurs in the proteinase-deficient strain cl3-ABYS86, which is, however, unable to process BO completely. The reason for the incomplete processing is not clear; the *PEP4* gene product (one of the proteases missing in cl3-ABYS86) has pleiotropic effects and is also involved in proteolytic processing of the vacuolar carboxypeptidase Y (Rendueles and Wolf 1988). It is noteworthy that the same size classes of BO have previously been detected in *S. pombe* transformants grown in the presence of sub-optimal amounts of retinal (retinal was added to the culture at irregular intervals and not after every second cell division; Hildebrandt et al. 1991).

The addition of retinal to the cultures did not result in red *S. cerevisiae* cells. In contrast to recombinant *S. pombe* cells, which become reddish when grown in the presence of retinal, *S. cerevisiae* cells remain colourless. We assume that the amount of BO capable of incorporating the chromophore is too low in *S. cerevisiae* transformants to result in a visible change in colour. Further experiments are necessary to determine whether the BO formed in *S. cerevisiae* is present in the correct conformation.

As shown in this paper, BO is one of the seven-helix membrane proteins that can be expressed in yeast. Expression of membrane proteins of the same class has previously been achieved in yeast for the human M1 muscarinic receptor (Payette et al. 1990), the human β_2 adrenergic receptor (King et al. 1990), the rat $5HT_2$ serotonin receptor, and the human D_2 dopamine receptor (Sander 1992). Thus, *S. cerevisiae* seems to be well-suited as host for the expression of seven-helix receptors, especially as several of these heterologous proteins have been shown to be functional in this organism.

Acknowledgements We thank Wolfram Koch for help with some of the experiments and Roslin Bensmann for correcting the English. The protease-deficient strain has been kindly provided by Dieter Wolf, Stuttgart.

References

Beach D, Piper M, Shall S (1980) Isolation of chromosomal origins of replication in yeast. Nature 284:185–187

Chan CSM, Tye B (1980) Autonomously replicating sequences in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 77:6329–6333

Chevallier MR, Bloch JC, Lacroute F (1980) Transcriptional and translational expression of a chimeric bacterial-yeast plasmid in yeasts. Gene 11:11–19

Dunn R, McCoy J, Simsek M, Majumdar A, Chang SH, RajBhandary UL, Khorana HG (1981) The bacteriorhodopsin gene. Proc Natl Acad Sci USA 78:6744–6748

Dunn R, Hackett NR, McCoy J, Kimura K, Khorana HG (1987) Structure-function studies on bacteriorhodopsin. I. Expression of the bacterio-opsin gene in *Escherichia coli*. J Biol Chem 262:9246–9254

Fasano O, Hurt E, Serrano R, Tollervey D (1991) EMBO Practical Course, Handbook: Cellular and molecular biology of yeast, EMBL, Heidelberg FRG

Ferrando E, Schweiger U, Oesterhelt D (1993) Homologous bacterio-opsin-encoding gene expression via site-specific vector integration. Gene 125:41–47

Frevert U, Lange M, Wrede P (1989) Immunoelectron microscopic study on the biogenesis of bacteriorhodopsin. Endocytobiosis Cell Res 6:171–182

Gropp R, Gropp F, Betlach MC (1992) Association of the halobacterial 7S RNA to the polysome correlates with expression of the membrane protein bacterioopsin. Proc Natl Acad Sci USA 89:1204–1208

Hildebrandt V, Ramezani-Rad M, Swida U, Wrede P, Grzesiek S, Primke M, Büldt G (1989) Genetic transfer of the pigment bacteriorhodopsin in the eukaryote *Schizosaccharomyces pombe*. FEBS Lett 243:137–140

Hildebrandt V, Polakowski F, Büldt G (1991) Purple fission yeast: overexpression and processing of the pigment bacteriorhodopsin. Photochem Photobiol 54:1009–1016

Hildebrandt V, Fendler K, Heberle J, Hoffmann A, Bamberg E, Büldt G (1993) Bacteriorhodopsin expressed in *Schizosaccharomyces pombe* pumps protons through the plasma membrane. Proc Natl Acad Sci USA 90:3578–3582

Himeno M, Shibata T, Kawahara Y, Hanaoka Y, Komano T (1984) Effect of polyethylene glycol in plasmid DNA solution on transformation of $CaCl_2$-treated *Escherichia coli* cells. Agric Biol Chem 48:657–662

Hinnen A, Hicks JB, Fink GR (1978) Transformation of yeast. Proc Natl Acad Sci USA 75:1929–1933

Ito H, Fukuda Y, Murata K, Kimura A (1983) Transformation of intact yeast cells treated with alkali ions. J Bacteriol 153:163–168

Karnik S, Doi T, Molday R, Khorana HG (1990) Expression of the archaebacterial bacterio-opsin gene with and without signal sequences in *Escherichia coli*: the expressed proteins are located in the membrane but bind retinal poorly. Proc Natl Acad Sci USA 87:8955-8959

King K, Dohlman HG, Thorner J, Caron MG, Lefkowitz RJ (1990) Control of yeast mating signal transduction by a mammalian β_2-adrenergic receptor and G_s α subunit. Science 250:121–123

Kopetzki E, Buckel P, Schumacher G (1989) Cloning and characterization of baker's yeast α-glucosidase: over-expression in a yeast strain devoid of vacuolar proteinases. Yeast 5:1–24

Lang-Hinrichs C, Berndorff D, Seefeldt C, Stahl U (1989) G418 resistance in the yeast *Saccharomyces cerevisiae*: comparison of the neomycin resistance genes from Tn5 and Tn903. Appl Microbiol Biotechnol 30:388–394

Lang-Hinrichs C, Dössereck C, Fath I, Stahl U (1990) Use of the Tn903 neomycin resistance gene for promoter analysis in the fission yeast *Schizosaccharomyces pombe*. Curr Genet 18:511–516

Miercke LJW, Ross PE, Stroud RM, Dratz EA (1989) Purification of bacteriorhodopsin and characterization of mature and partially processed forms. J Biol Chem 264:7531–7535

Ni B, Chang M, Duschl A, Lanyi J, Needleman R (1990) An efficient system for the synthesis of bacteriorhodopsin in *Halobacterium halobium*. Gene 90:169–172

Oesterhelt D, Stoeckenius W (1971) Rhodopsin-like protein from the purple membrane of *Halobacterium halobium*. Nature 233:149–152

Oesterhelt D, Stoeckenius W (1974) Isolation of the cell membrane of *Halobacterium halobium* and its fractionation into red and purple membrane. Methods Enzymol 31:667–678

Pardo L, Ballesteros JA, Osman R, Weinstein H (1992) On the use of the transmembrane domain of bacteriorhodopsin as a template for modeling the three-dimensional structure of guanine nucleotide-binding regulatory protein-coupled receptors. Proc Natl Acad Sci USA 89:4009–4012

Payette P, Gossard F, Whiteway M, Dennis M (1990) Expression and pharmacological characterization of the human M1 muscarinic receptor in *Saccharomyces cerevisiae*. FEBS Lett 266:21–25

Rendueles PS, Wolf DH (1988) Proteinase function in yeast: biochemical and genetic approaches to a central mechanism of post-translational control in the eukaryote cell. FEMS Microbiol Rev 54:17–46

Ruohonen L, Penttilä M, Keränen S (1991) Optimization of *Bacillus* α-amylase production by *Saccharomyces cerevisiae*. Yeast 7:337–346

Sander P (1992) Expression of membrane-localized receptors in the yeast *Saccharomyces cerevisiae*. PhD Thesis, Johann-Wolfgang-Goethe-Universität, Frankfurt

Schwencke J (1991) Vacuoles, internal membraneous systems and vesicles. In: Rose AH, Harrison JS (eds) The yeasts, vol 4. Academic press, London, pp 347–432

Tindall BJ (1992) The family *Halobacteriaceae*. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The prokaryotes. Springer, New York, pp 769–808

Wölfer U, Dencher NA, Büldt G, Wrede P (1988) Bacterioopsin precursor is processed in two steps. Eur J Biochem 174:51–57