Volume 283, number 2, 325–328 FEBS 09787 June 1991

ADONIS 001457939100499I

SRH1 protein, the yeast homologue of the 54 kDa subunit of signal recognition particle, is involved in ER translocation of secretory proteins

Yoshihiro Amaya[1] and Akihiko Nakano[2]

[1]*Department of Biochemistry, Yokohama City University School of Medicine, Yokohama, Kanagawa 236, Japan* and [2]*Department of Biology, Faculty of Science, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*

Received 1 April 1991

The function of the *SRH1* product, the yeast homologue of the 54 kDa subunit of the mammalian signal recognition particle, has been analyzed using a galactose dependent mutant of the gene. *SRH1* has been placed under control of the *GAL1* promoter and introduced into a haploid cell that had its chromosomal *SRH1* copy disrupted. This mutant grows normally on galactose medium but slows down the growth about 10 h after transfer to glucose medium. At the same time, precursor forms of secretory proteins, α-mating factor and invertase, accumulate in the cells. This result indicates that the *SRH1* product is involved in translocation of precursors of secretory proteins across the endoplasmic reticulum membrane in yeast cells.

Signal recognition particle; Translocation; Endoplasmic reticulum; Protein secretion; *SRH1*; *Saccharomyces cerevisiae*

1. INTRODUCTION

Saccharomyces cerevisiae SRH1 [1] (*SRP54*[ac] [2]) encodes a homologue of the 54 kDa subunit of mammalian signal recognition particle (SRP54) [3,4]. Its N-terminal portion is also homologous to the cytosolic domain of the α-subunit of the signal recognition particle receptor [5] including consensus sequence elements for a GTP binding site [6]. The C-terminal portion is an unusual methionine-rich domain containing several repetitive sequences. Gene disruption experiments have shown that the *SRH1* product is essential for cell growth [1,2].

The signal recognition particle (SRP), a complex of six different polypeptide chains and a molecule of 7SL RNA, has been identified as a component involved in protein targeting to and translocation across the endoplasmic reticulum (ER) membrane in a mammalian in vitro reconstitution system, and serves as an adapter between the cytoplasmic protein synthesis machinery and the membrane-bound protein translocation machinery [7]. SRP54 recognizes the signal sequence when it emerges from the ribosome, thereby initiating a series of SRP functions, including peptide elongation arrest and translocation promotion [8,9].

Several genes involved in translocation of precursors of secretory proteins have been identified and characterized in *S. cerevisiae* [10–18]. In contrast to the mammalian in vitro reconstitution systems, translocation of the prepro-α-factor across the ER membrane in yeast system can occur post-translationally depending on a subset of 70 kDa stress proteins and a discrete yeast lysate component(s) [17–23]. On the other hand, preinvertase is translocated efficiently only in a cotranslational reaction [20–23]. In this study, we show that both prepro-α-factor and preinvertase accumulate in galactose-dependent *srh1* mutant cells. We conclude that the *SRH1* product is involved in translocation of precursors of secretory proteins.

Correspondence address: Y. Amaya, Department of Biochemistry, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236, Japan. Fax: (81) (45) 787 2509.

Abbreviations: SRP, signal recognition particle; SRP54, 54 kDa subunit of signal recognition particle; ER, endoplasmic reticulum; GAP, glyceraldehyde-3-phosphate dehydrogenase; YP, 1% yeast extract, 2% polypeptone; MV, Wickerham's minimal medium; PCR, polymerase chain reaction

2. MATERIALS AND METHODS

2.1. *Strains and culture conditions*

Strain YAY101 (*Mata/Matα SRH1/srh1* :: *LEU2 ura3/ura3 leu2/leu2 trp1/trp1 his/his suc2/suc2*) [1] was transformed with *GAL1* promoter-*SRH1* fusion plasmid, pYAS23, using *TRP1* as a marker and subjected to sporulation and tetrad dissection. Spores were scored for auxotroph markers and mating type. The *Leu*$^+$ *Trp*$^+$, α-mating type haploid strain, which contained *GAL1* promoter-*SRH1* fusion gene on the plasmid pYAS23 and the disrupted version of *srh1* in chromosome, was named YAY107 (*Matα srh1* :: *LEU2 ura3 leu2 trp1 his suc2* p[*GAL1-SRH1 TRP1*]). A haploid strain containing wild-type *SRH1*, which showed *Leu*$^-$ *Trp*$^-$ phenotype, was named YAY106 (*Matα SRH1 ura3 leu2 trp1 his suc2*), and used as a control. These strains were transformed with glyceraldehyde-3-phosphate dehydrogenase promoter (GAP) [24] –SUC2 fusion plasmid using *URA3* as a marker to give YAY113 (*Matα srh1* :: *LEU2 ura3 leu2 trp1 his suc2* p[*GAL1-SRH1 TRP1*] p[*GAP-SUC2 URA3*]) and

Published by Elsevier Science Publishers B.V.

YAY112 (*Mata SRH1 ura3 leu2 trp1 his suc2* p[*GAP-SUC2 URA3*]), respectively.

Yeast strains were usually grown at 30°C in YP medium (2% polypeptone and 1% yeast extract) containing 2% glucose (YPD) or in Wickerham's minimal medium (MV) containing 2% glucose and appropriate supplements (MVD). For derepression of the *GAL1* promoter, YP or MV medium was supplemented with 5% galactose and 0.2% sucrose (YPGal and MVGal).

2.2. *Plasmid constructions*

Construction of the *GAL1* promoter-*SRH1* fusion plasmid, pYAS23, is shown in Fig. 1. DNA fragment from 1 to 462 nucleotides of *SRH1* [1] was obtained by polymerase chain reaction (PCR) using primers YAS1 (5'TTCGAATTCATGGTTTTGGCTGATTTGGGGAA3') and YY20(5'GTCAAATGCACCAGCAC3') essentially according to Higuchi et al. [25]. The fragment was inserted into pRS316 [26] by digestion with *Hin*dIII and *Eco*RI. Resulting plasmid was named pYAS1 and subjected to sequence analysis. A 1.7 kb *Hin*dIII fragment of pYYH [1] was inserted into *Hin*dIII site of pYAS1. Orientation of the insert was determined by *Eco*RI digestion. The *GAL1* promoter fragment was excised from pUCG1 [27] by *Hin*dIII/*Eco*RI digestion and blunted by Klenow fragment of DNA polymerase. The fragment was inserted into *Sma*I site of pYAS7. Orientation of the *GAL1* promoter fragment was determined by *Bam*HI digestion. *GAL1* promoter-*SRH1* fusion gene was excised from pYAS18 by digestion with *Nco*I and *Xho*I, located in the multicloning site of pRS316 [26], and inserted into pRS314 containing *TRP1*.

The GAP promoter-*SUC2* fusion plasmid, which directs constitutive expression of preinvertase in yeast cells, was constructed as follows. The GAP promoter fragment was excised from pKTGAP (a gift from A. Toh-e) with *Bam*HI and *Eco*RI digestion, and inserted into pRS316. Resulting plasmid was named pRSGAP. *Hin*dIII-*Pvu*II fragment of pSEY303 (a gift from S.D. Emr), which encodes from A1a-5 to the C-terminus of preinvertase, was inserted into pRSGAP digested with *Sal*I, blunted with T4 polymerase and further digested with *Hin*dIII. Synthetic oligonucleotides encoding N-terminal 4 residues of preinvertase (YA301: 5'AATTCGCATGCTTTTGCA3' and YA302: 5'AGCTTGCAAAAGCATGCG3') were annealed and inserted into the plasmid digested with *Eco*RI and *Hin*dIII. The resulting plasmid was named pGAP-SUC2. DNA manipulations were

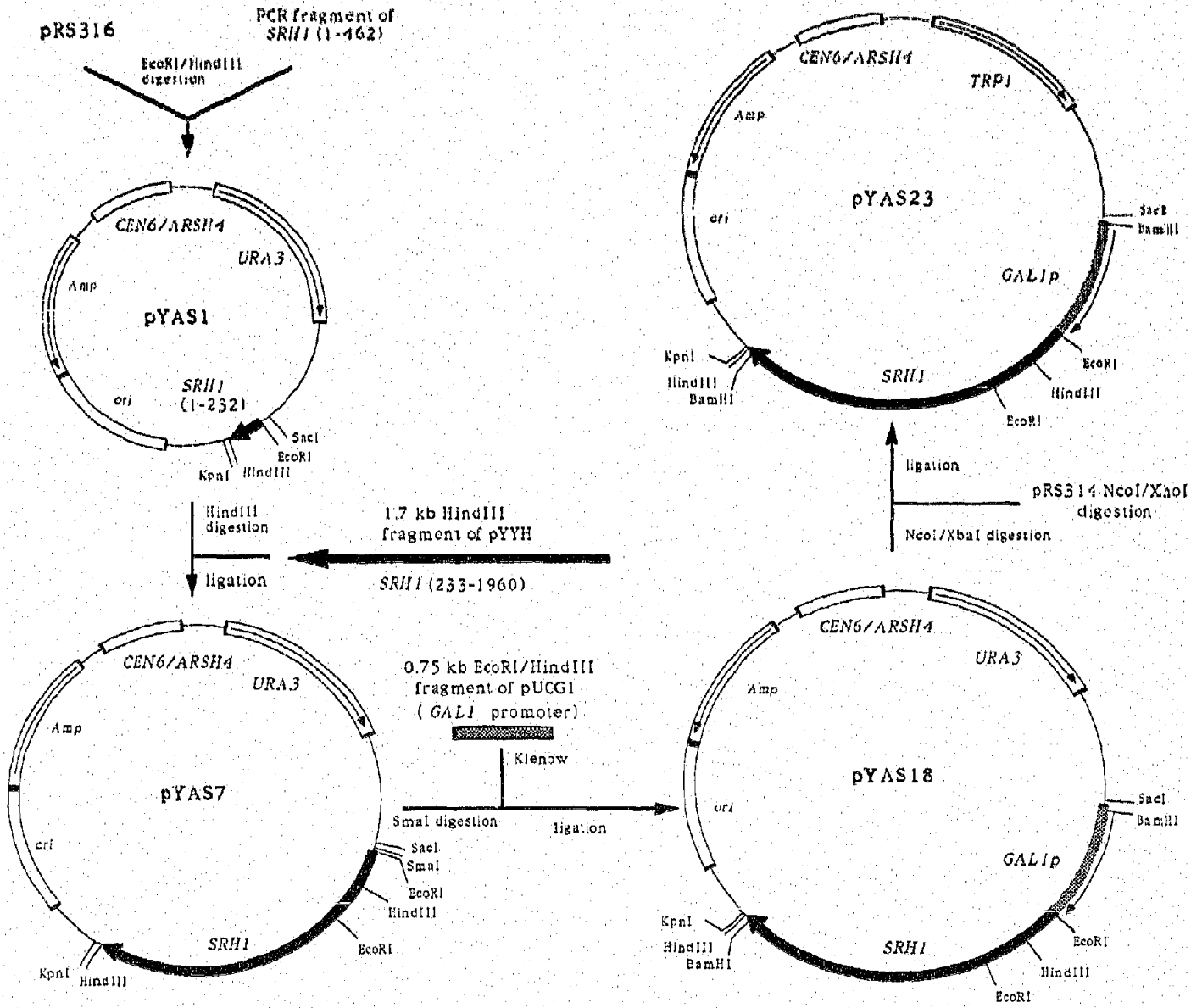

Fig. 1. Construction of the *GAL1* promoter-*SRH1* fusion plasmid. For details see 'Materials and Methods'.

carried out essentially according to Maniatis et al. [28]. Oligonucleotides were synthesized with a DNA synthesizer (Model 381A; Applied Biosystems Japan, Tokyo).

2.3. *Immunoblotting analysis*

Rabbit antibodies against invertase and prepro-α-factor were gifts from R. Schekman and T. Oka, respectively. Preparation of cell lysates and immunoblotting analysis were performed according to previous papers [27,29].

3. RESULTS

To examine the consequences of *SRH1* deficiency, a strain that conditionally expresses *SRH1* gene was generated. This strain, YAY113, harbors chromosomal disruption of the *SRH1* gene, but is rescued by a single-copy of *SRH1* present on a centromeric plasmid. This plasmid contains the coding region of *SRH1* fused to the yeast *GAL1* promoter. Growth of the YAY113 cells on galactose medium (YPGal) was indistinguishable from wild type cells. When these cells were shifted to medium containing glucose (YPD), the *GAL1* promoter was repressed. Growth of the cells was retarded about 10 h after the shift from galactose medium to glucose medium (Fig. 2). At the same time, the repressed mutant cells showed swelling phenotype. The repressed cells were fully viable until 15 h incubation; the cells revived when washed and replenished with galactose, though the viability decreased after a long arrest (more than 20 h) in the presence of glucose (data not shown).

To address whether this depletion of Srh1p interferes with the biogenesis of secretory proteins, extracts prepared from the cells at two stages of Srh1p repression (12 h and 15 h) were immunoblotted with antisera directed against prepro-α-factor and invertase (Fig. 3). In wild type cells, α-factor precursor is known to be rapidly translocated, glycosylated, proteolytically processed, and secreted [27]. In fact, prepro-α-factor did not accumulate either in wild type cells (Fig. 3A, lanes 1, 2, 5, 6) or in the derepressed mutant cells (Fig. 3A lanes 4 and 8). In contrast, there was a remarkable accumulation of prepro-α-factor in the repressed mutant cells (Fig. 3A, lanes 3 and 7). The precursor accumulated in the mutant cells was indistinguishable from that detected in the translocation-defective mutant *sec62* (data not shown).

For the analysis of invertase, we used the GAP promoter-*SUC2* fusion plasmid, pGAP-SUC2, that directs constitutive expression of secretory invertase. Introduction of the plasmid into wild type or the galactose dependent mutant cells had no effect on cell growth or other phenotypes. The highly glycosylated mature form of secretory invertase that was secreted into the periplasm as well as the cytoplasmic form of invertase were observed in either wild type cells or mutant cells (Fig. 3B). In the repressed mutant cells, a considerable amount of the precursor form of secretory in-

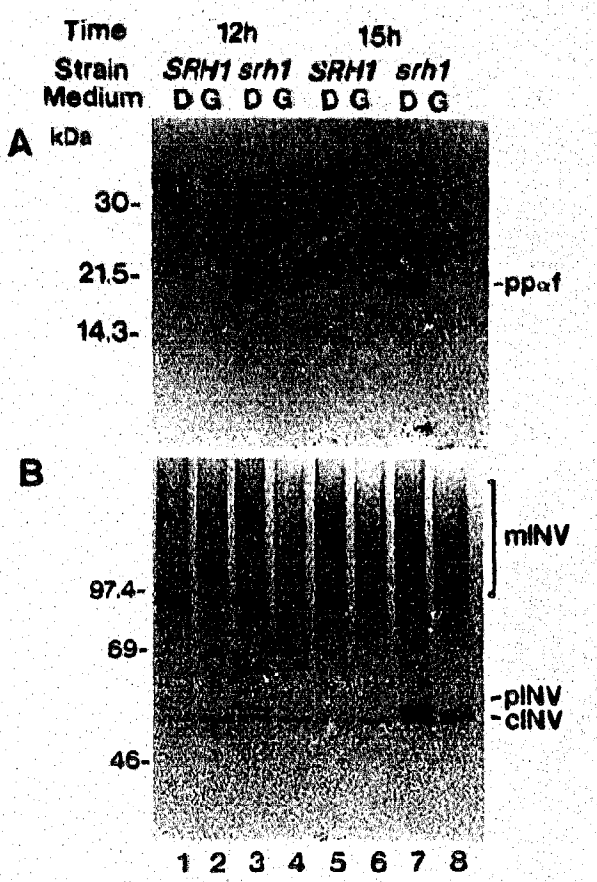

Fig. 3. *srh1* accumulates precursor forms of α-mating factor (A) and invertase (B). Wild type (YAY112) and the galactose-dependent *srh1* mutant (YAY113) was incubated in MVGal (G) and MVD (D) medium. At times indicated (12, 15 h), aliquots were taken and lysates were prepared by glass bead homogenization in SDS. The lysates were electrophoresed in 12.5% (A) and 7.5% (B) SDS-polyacrylamide gels and subjected to immunoblotting analysis. ppα f, prepro-α-factor; mINV, mature form of secretory invertase; pINV, precursor form of secretory invertase; cINV, cytoplasmic form of invertase.

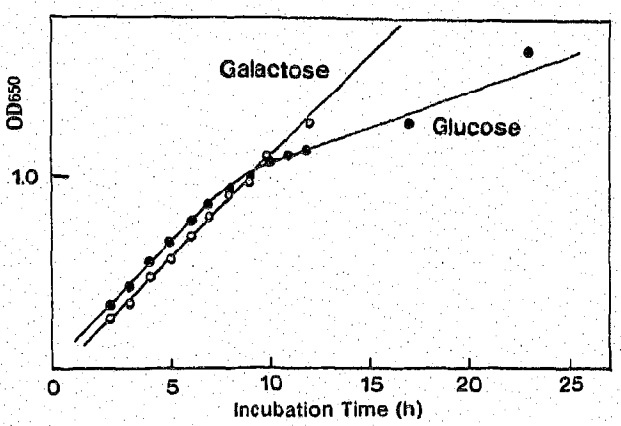

Fig. 2. Growth of the galactose-dependent *srh1* mutant. The galactose-dependent mutant cells (YAY113) that were grown to late late-log phase in YPGal medium, were washed with sterile water, inoculated into either YPGal (galactose) or YPD (glucose), and incubated at 30°C. The increase of cell density was monitored by measuring OD_{650} with a Coleman spectrophotometer.

vertase also accumulated (Fig. 3B, lanes 3 and 7). These results indicate that Srh1p is required for the translocation of secretory proteins.

4. DISCUSSION

Several yeast genes involved in ER translocation of secretory proteins have been identified and characterized [10–18]. *SEC61*, *SEC62* and *SEC63* are assembled with two additional proteins into a multisubunit membrane-associated complex [15]. These membrane proteins may act together to facilitate protein penetration across the ER membrane. Cytosolic [17,18] and ER-lumenal [16] 70 kDa stress proteins are proposed to act as molecular chaperons which unfold the precursor proteins to maintain translocation competence and/or promote assembly-disassembly of translocation machinery components.

In this paper, we have shown that the yeast cells depleted of Srh1p, the homologue of mammalian SRP54, accumulate cytosolic precursor forms of secretory proteins. Two different kinds of precursors are affected by the Srh1 depletion which are known to be translocated either only cotranslationally (pre-invertase) or both co- and post-translationally (prepro-α-factor). This is the first indication that the yeast SRP counterpart is involved in ER translocation of secretory proteins. The mechanism of Srh1p action in co- and post-translational translocation has yet to be elucidated. Considering the good homology to mammalian SRP54, function of Srh1p may well be the recognition of signal sequence and the initiation of the following reactions such as targeting to the ER membrane and translocation promotion. Besides Srh1p, homologues of 7SL RNA [30] and α-subunit of SRP receptor [31] are found in *S. cerevisiae*, although genetic and biochemical interactions with *SRH1* are not precisely revealed. *S. pombe* homologue of the SRP54 is shown to be associated with the homologue of 7SL RNA in vivo [32]. *E. coli* homologues of 7SL RNA and SRP54 also bind together and play some roles in translocation of precursor of β-lactamase [31,32]. Taken together, it seems that the SRP homologues have conserved its role in membrane translocation throughout evolution. More detailed analysis of the Srh1p function by both genetic and biochemical approaches is now under way.

Acknowledgments: We thank Philip Hieter, Yoshikazu Ohya, Akio Toh-e and Scott Emr for plasmids; Toshihiko Oka and Randy Schekman for antibodies; Raymond Deshaies for a *sec62* strain; Shuh-ichi Nishikawa for discussions; and Yasuhiro Anraku, Masataka Mori and Takeshi Nishino for encouragement. This work was supported by Grants-in-Aid (02304063 and 02780152) from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

[1] Amaya, Y., Nakano, A., Ito, K. and Mori, M. (1990) J. Biochem. 107, 457–463.
[2] Hann, B.C., Poritz, M.A. and Walter, P. (1989) J. Cell Biol. 109, 3223–3230.
[3] Römisch, K., Webb, J., Herz, J., Prehn, S., Frank, R., Vingron, M. and Dobberstein, B. (1989) Nature 340, 478–482.
[4] Bernstein, H.D., Poritz, M.A., Strub, K., Hoben, P.J., Brenner, S. and Walter, P. (1989) Nature 340, 482–486.
[5] Lauffer, L., Garcia, P.D., Harkins, R.N., Coussens, L., Ullrich, A. and Walter, P. (1985) Nature 318, 334–338.
[6] Connoly, T. and Gilmore, R. (1989) Cell 57, 599–610.
[7] Walter, P., Gilmore, R. and Blobel, G. (1984) Cell 38, 5–8.
[8] Wiedmann, M., Kurzchalia, T.V., Bielka, H. and Rapoport, T.A. (1987) J. Cell Biol. 104, 201–208.
[9] Siegel, V. and Walter, P. (1988) Cell 52, 39–49.
[10] Deshaies, R.J. and Schekman, R. (1987) J. Cell Biol. 105, 633–645.
[11] Rothblatt, J.A., Deshaies, R.J., Sanders, S.L., Daum, G. and Schekman, R. (1989) J. Cell Biol. 109, 2641–2652.
[12] Deshaies, R.J. and Schekman, R. (1989) J. Cell Biol. 109, 2653–2664.
[13] Sadler, I., Chaing, T., Kurihara, T., Rothblatt, J., Way, J. and Silver, P. (1989) J. Cell Biol. 109, 2665–2675.
[14] Deshaies, R.J. and Schekman, R.J. (1990) Mol. Cell Biol. 10, 6024–6035.
[15] Deshaies, R.J., Sanders, S.L., Feldman, D.A. and Schekman, R. (1991) Nature 349, 806–808.
[16] Vogel, J.P., Misra, L.M. and Rose, M.D. (1990) J. Cell. Biol. 110, 1885–1896.
[17] Deshaies, R.J., Koch, B.D., Werner-Washburne, M., Craig, E.A. and Schekman, R. (1988) Nature 332, 800–805.
[18] Chirico, W.J., Waters, M.G. and Blobel, G. (1988) Nature 332, 805–810.
[19] Hansen, W., Garcia, P.D. and Walter, P. (1986) Cell 45, 397–406.
[20] Rothblatt, J.A. and Meyer, D.I. (1986) Cell 44, 619–628.
[21] Waters, M.G. and Blobel, G. (1986) J. Cell Biol. 102, 1543–1550.
[22] Rothblatt, J.A. and Meyer, D.I. (1986) EMBO J. 5, 1031–1036.
[23] Rothblatt, J.A., Webb, J.R., Ammerer, G. and Meyer, D.I. (1987) EMBO J. 6, 3455–3463.
[24] Holland, J.P. and Holland, M.J. (1979) J. Biol. Chem. 254, 9839–9845.
[25] Higuchi, R., Krummel, B. and Saiki, R. (1988) Nucleic Acids Res. 15, 7351–7363.
[26] Sikorski, R.S. and Hieter, P. (1989) Genetics 122, 19–27.
[27] Nakano, A. and Muramatsu, M. (1989) J. Cell Biol. 109, 2677–2691.
[28] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[29] Nakano, A., Brada, D. and Schekman, R. (1988) J. Cell Biol. 107, 851–863.
[30] Felic, F., Cesareni, G. and Hughes, J.M.X. (1989) Mol. Cell. Biol. 3260–3268.
[31] Poritz, M.A., Bernstein, H.D., Strub, K., Zopf, D., Wilhelm, H. and Walter, P. (1990) Science 250, 1111–1117.
[32] Ribes, V., Römisch, K., Giner, A., Dobberstein, B. and Tollervey, D. (1990) Cell 63, 591–600.