Three proteins required for early steps in the protein secretory pathway also affect nuclear envelope structure and cell cycle progression in fission yeast

Anna Matynia[1,3,*], Sandra S. Salus[2,3,*] and Shelley Sazer[1,2,3,‡]

[1]Department of Molecular and Cellular Biology, [2]Graduate Program in Cell and Molecular Biology, [3]Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas 77030

*These authors contributed equally to this work

‡Author for correspondence (e-mail: ssazer@bcm.tmc.edu)

Accepted 18 October 2001
Journal of Cell Science 115, 421-431 (2002) © The Company of Biologists Ltd

Summary

The Ran GTPase is an essential protein that has multiple functions in eukaryotic cells. Fission yeast cells in which Ran is misregulated arrest after mitosis with condensed, unreplicated chromosomes and abnormal nuclear envelopes. The fission yeast sns mutants arrest with a similar cell cycle block and interact genetically with the Ran system. sns-A10, sns-B2 and sns-B9 have mutations in the fission yeast homologues of *S. cerevisiae* Sar1p, Sec31p and Sec53p, respectively, which are required for the early steps of the protein secretory pathway. The three sns mutants accumulate a normally secreted protein in the endoplasmic reticulum (ER), have an increased amount of ER membrane, and the ER/nuclear envelope lumen is dilated. Neither a post-ER block in the secretory pathway, nor ER proliferation caused by overexpression of an integral ER membrane protein, results in a cell cycle-specific defect. Therefore, the arrest seen in sns-A10, sns-B2 and sns-B9 is most likely due to nuclear envelope defects that render the cells unable to re-establish the interphase organization of the nucleus after mitosis. As a consequence, these mutants are unable to decondense their chromosomes or to initiate of the next round of DNA replication.

Key words: Protein secretion, Endoplasmic reticulum, sar1, sec31, pmm1, Phosphomannomutase, *SEC53*, Ran, Nuclear envelope, Cell cycle, pim1-d1, Fission yeast, *S. pombe*

Introduction

The endoplasmic reticulum (ER) is the cytoplasmic organelle where the early steps of the protein secretory pathway are localized. Protein secretion begins with the co-translational insertion of polypeptide chains into the ER (Walter et al., 1984). Proteins are glycosylated and folded in the lumen of the ER and packaged into COPII coated vesicles that bud off of the ER and deliver their cargo to the Golgi apparatus (Orlean, 1992; Springer et al., 1999). After proteins transit through the Golgi apparatus they are targeted to either the vacuole or the plasma membrane (Pfeffer, 1999).

Although the ER and the nucleus differ in structure, their membranes and lumens are continuous and both are the sites of protein translation (Gant and Wilson, 1997). The nuclear envelope (NE) separates the contents of the nucleus from the cytoplasm but is perforated with nuclear pore complexes (NPC), through which small molecules and ions freely diffuse and large molecules are selectively transported (Gant and Wilson, 1997).

Higher eukaryotes undergo an open mitosis in which the nuclear envelope breaks down before and is re-assembled after mitosis (Gant and Wilson, 1997). Lamins are critical for nuclear structure, forming a lattice that anchors the nuclear pores and the chromatin to the NE (Gant and Wilson, 1997). Lamins are also required for the assembly of DNA replication complexes (Ellis et al., 1997; Newport and Forbes, 1987), indicating the importance of nuclear structure for nuclear-specific functions.

Yeasts undergo a closed mitosis in which spindle assembly and chromosome segregation take place within the confines of the NE. The shape of the nucleus changes from round to oblong as the spindle elongates during mitosis and eventually pinches off into two discrete organelles (Hurt et al., 1992; McCully and Robinow, 1971). Several yeast NPC components essential for pore distribution in the NE (Belgareh and Doye, 1997) and an inner NE protein required for proper NE structure (Kops and Guthrie, 2001) have been characterized. However, neither lamins nor other structural elements that affect nuclear organization or the changes in nuclear structure associated with the entry and exit of cells from mitosis have been identified in yeast.

Several *S. pombe* mutants, including pim1-d1 (Sazer and Nurse, 1994) and the sns mutants (Matynia et al., 1998), are unable to re-establish the interphase state of the nucleus after mitosis. pim1-d1 is a temperature-sensitive lethal mutant that arrests after mitosis with condensed, unreplicated chromosomes and fragmented NEs (Demeter et al., 1995; Sazer and Nurse, 1994). The *pim1* gene encodes the guanine nucleotide exchange factor (GEF) for the Spi1p GTPase, a member of the evolutionarily conserved family of Ran GTPases. The RanGTPase is essential for three aspects of nuclear structure and function: nucleocytoplasmic transport, mitotic spindle formation, and the structure and post-mitotic re-assembly of the NE (Sazer and Dasso, 2000). No significant defects in nucleocytoplasmic transport have been seen when the Ran system is perturbed in *S. pombe* (S.S.S., J. Demeter

and S.S., unpublished) (Fleig et al., 2000), making it unlikely that NE fragmentation is a secondary consequence of transport defects. A nucleocytoplasmic transport-independent role for the Ran GTPase in NE structure and function has also been observed in vitro (Sazer and Dasso, 2000).

The temperature-sensitive sns mutants (septated not in S-phase) phenotypically resemble pim1-d1 (Matynia et al., 1998). Amongst this collection are ten complementation groups that are not mutated in known components of the Ran GTPase system but interact genetically with Ran or its regulators (Matynia et al., 1998). We report here that three of the sns strains, sns-A10, sns-B2 and sns-B9, are mutated in *S. pombe* genes encoding proteins whose *S. cerevisiae* homologues, Sar1p, Sec31p and Sec53p, respectively, are required for protein modification and secretion from the ER. At the restrictive temperature sns-A10, sns-B2 and sns-B9 have abnormally dilated NE lumens and increased amounts of ER, characteristics they share with comparable budding yeast mutants. However, the fission yeast secretory mutants are unique because they are unable to progress normally through the cell cycle. This cell cycle block is not due simply to a proliferation of ER membrane or to an inability to secrete proteins from the cell. We propose that it is the defect in the structure of the NE that interferes with the reestablishment of the interphase organization of the nucleus.

Materials and Methods

Yeast strains and cell culture

All strains were derived from the wild-type haploid strain 972 *h*$^-$ (Leupold, 1970); mutants sns-A10, sns-B2 and sns-B9 (Matynia et al., 1998), and ypt1-VN and ypt2-VN (Armstrong et al., 1994; Craighead et al., 1993) have been previously characterized. The wild-type strain SS767 *leu1-32, ura4-D18, ade6-M210, h*$^-$ containing an integrated pREP42-*GFP-pap1-LEU2* plasmid (Toone et al., 1998) was crossed to sns-A10, sns-B2 and sns-B9 by standard methods. The nda3-311 cold-sensitive strain (Hiraoka et al., 1984) and Δpuc1 deletion strain (Forsburg and Nurse, 1994) were used to genetically link the *sar1* locus with the sns-A10 temperature-sensitive allele. The HMG CoA reductase gene was overproduced in wild-type *S. pombe* cells harboring plasmid pPL238, which contains the *S. cerevisiae HMG1* gene under the regulation of the high-strength *S. pombe nmt1* promoter (Lum and Wright, 1995); gift of R. Wright, University of Washington. Temperature-sensitive phenotypes were characterized in cells grown to mid-log phase at 25°C (permissive temperature) and then shifted to 36°C (restrictive temperature) for 4 hours, unless otherwise stated. Cells were grown in Edinburgh Minimal Medium (EMM) or Yeast Extract (YE) (Moreno et al., 1991) supplemented with amino acids and/or 1 M sorbitol where stated. Ectopic expression of genes from the high strength (3×), medium strength (42×) or low strength (81×) thiamine-regulatable *nmt1* promoter was repressed by addition of 5 μg/ml thiamine and de-repressed by washing three times in media without thiamine (Forsburg, 1993; Maundrell, 1990). Standard methods were used for random spore or tetrad analysis and to isolate haploid double mutant strains (Moreno et al., 1991). Temperature-sensitive colonies were identified by replica plating to YE containing the vital dye, phloxine B (Sigma) (Moreno et al., 1991).

Library screens and DNA manipulations

The genes mutated in sns-A10, sns-B2 and sns-B9 were cloned by complementation of the temperature sensitive lethality. Either a genomic library in the pUR19 vector (Barbet et al., 1992) (gift of Tony Carr, University of Sussex, UK), or a cDNA library (gift of Bruce Edgar, Fred Hutchinson Cancer Research Center and Chris Norbury, Imperial Cancer Research Fund, UK), in which transcription is controlled by the 3X-*nmt1* promoter, was introduced by electroporation or lithium acetate transformation (Moreno et al., 1991; Okazaki et al., 1990). Plasmids that restored growth at the restrictive temperature were recovered from yeast, amplified in bacteria and retransformed into yeast to confirm rescue of the temperature-sensitive lethality. Upon subcloning into pBluescript II KS (pBS) (Stratagene), inserts were sequenced using Sequenase version 2.0 (United States Biochemical). A BLAST search of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/cgi-bin/BLAST/) and of the Sanger Center (www.sanger.ac.uk/Projects/S_pombe/blast_server.html) databases was used to identify the genes contained in the plasmid inserts. Protein alignments were performed using ClustalW and displayed using Boxshade (www.ch.embnet.org/software/BOX_form.html). Southern blot (Sambrook et al., 1989) analysis was performed as previously described (Patterson et al., 1995) using [^{32}P]dATP labeled DNA probes prepared using Prime-It (Stratagene).

One 2.8 kb genomic plasmid, pUR19-GAM1, and one 1.0 kb cDNA plasmid, pREP3X-*sar1*, which cross-hybridized, fully rescued the temperature sensitivity of sns-A10. The 1.3 kb genomic *Hin*DIII fragment that conferred rescue of the temperature sensitivity lies between *nda3* and *puc1* on chromosome 2 (Hoheisel et al., 1993) and random spore analysis was performed to show linkage of the temperature-sensitive mutation in sns-A10 to both *nda3* and *puc1*.

The genomic plasmid XIX/pUR rescued the temperature sensitivity of sns-B2 and mutant strains in which this plasmid was homologously integrated showed linkage to the temperature-sensitive sns-B2 locus via random spore analysis.

A homologous integrant of one genomic clone, isolated 25 times from the genomic library and 90 times from the cDNA library, was shown to be linked to the temperature-sensitive mutation in sns-B9 by random spore analysis.

Deletion strains and expression constructs

For use in the generation of deletion constructs, the *ura4* gene was subcloned into the *Sma*I site in pBS to create the plasmid pBS-*ura4*. To generate *sar1* null strains, a PCR fragment was synthesized from this template using 100 nt primers corresponding to 80 nt immediately 5′ or 3′ of the *sar1* coding sequence and 20 bases of the pBS polylinker sequence flanking the *ura4* gene (Bahler et al., 1998). The PCR product was gel purified and transformed into wild-type *leu1-32/leu1-32 ura4-D18/ura4-D18 ade6-M210/ade6-M216* h$^+$/h$^-$ diploid cells. Gene replacement was verified by PCR using an internal *ura4* primer and a primer flanking the deletion construct. The heterozygous null strain Δsar1-6 was transformed with the pREP3X-*sar1* plasmid and haploid Δsar1-6 cells expressing *sar1* from the *nmt1* promoter were isolated.

To delete the *sec31* gene, the 5′ flanking sequence (–18 to –824 relative to the start codon) and the 3′ flanking sequence (+67 to +941 relative to the stop codon) were amplified by PCR and subcloned into the *Pst*I/*Cla*I and *BamH*I/*Sac*I sites of pBS-*ura4*. The resulting *sec31*-knockout fragment was gel purified and transformed into a wild-type *leu1-32/leu1-32 ura4-D18/ura4-D18 ade6-M210/ade6-M216* h$^+$/h$^-$ diploid strain containing pSLF172-81X-*sec31*. Diploid *ura4*$^+$ colonies were isolated and Southern analysis was performed to confirm the replacement of the *sec31* gene with *ura4*. To construct the pSLF172-81X-*sec31* expression plasmid, the *sec31* ORF was first PCR amplified and cloned into the *ura4* based plasmid pSLF173 (Forsburg and Sherman, 1997) to create pSLF173-*sec31*. A *LEU2* marked, low-strength *nmt1*-controlled expression plasmid was constructed by cleaving pSLF172 (Forsburg and Sherman, 1997) and subcloning the multiple cloning site and terminator into pRep81X, creating pSLF172-81X. Finally, the *sec31* ORF from pSLF173-*sec31*was subcloned into pSLF172-81X to create pSLF172-81X-*sec31*.

A deletion construct of *pmm1*, pBS-*ura4-pmm1*, containing the *ura4* gene between 3 kb of 3′ *pmm1*-flanking sequence and 80nt of 5′ *pmm1*-flanking sequence was constructed. The 3.7 kb *Sma*I-*Bst*EII fragment from pBS-*ura4-pmm1* was gel purified and used to transform diploid cells as above. Gene replacement was verified by Southern blot analysis. For promoter shutoff experiments in haploid Δ*pmm1* deletion strains, the rescuing *pmm1* cDNA in the pREP3X vector was subcloned into pREP81X and transformed into heterozygous *pmm1/pmm1::ura4* diploid strains. Two independent *LEU2* transformants were sporulated and haploid Δpmm1 cells containing pREP81X-*pmm1* were isolated.

DNA sequence analysis

To identify mutant residues, the open reading frames of the *sar1* locus in sns-A10 and of the *sec31* locus in sns-B2 were amplified by PCR and sequenced using either the CYCLIST exo⁻ PFU kit (Stratagene) or ThermoSequenase Cycle Sequencing Kit (Amersham Life Science Inc.).

Acid phosphatase glycosylation analysis

The glycosylation profile of the secreted protein acid phosphatase was analyzed by western blot. Wild-type, sar1-1, sec31-1 (in EMM), ypt1-VN and ypt2-VN cells (in YE) were grown to mid-log phase and shifted to 36°C for 4 hours. pmm1-1 cells (in YE) were grown to mid-log phase at 25°C, incubated in 500 μg/mL cycloheximide (Sigma) for 1 hour at 25°C, followed by 1 hour at 36°C to inhibit protein synthesis. These cells were collected by centrifugation, washed, re-inoculated into 36°C media without cycloheximide, and harvested after an additional 3 hour incubation at 36°C. Extracts were prepared in HB buffer (Moreno et al., 1991). Proteins were separated by 10 or 12% SDS-PAGE and transferred to an Immobilon-P nylon membrane (Millipore). Acid phosphatase was detected by western blot analysis using the monoclonal anti-acid phosphatase antibody Ab 7B4 (a generous gift of J. Armstrong, University of Sussex, UK) as previously described (Schweingruber et al., 1986) and anti-HRP secondary antibodies using the ECL detection system (Amersham Life Science).

Microscopy

Cells were observed with a Zeiss Axioskop fluorescence microscope and photographed on color slide film or with a DVC 1300 Black and White CCD camera with QED software. To determine cell cycle stage distribution, wild-type, sec31-1 and wild-type cells overexpressing *HMG1* (grown in EMM), and wild-type, sar1-1, pmm1-1, ypt1-VN and ypt2-VN (grown in EMM supplemented with sorbitol) were ethanol fixed and stained with DAPI as previously described (Moreno et al., 1991). Wild-type, sar1-1, and sec31-1 cells grown in EMM, and pmm1-1 cells grown in EMM supplemented with sorbitol were prepared for electron microscopy by high-pressure freeze substitution as previously described (Demeter et al., 1995), except that samples were substituted in 1% osmium tetroxide containing 0.1% uranyl acetate instead of tannic acid. Samples were post-fixation stained with 1% aqueous uranyl acetate and Reynold's lead citrate and observed on a Hitachi H7000 electron microscope. A minimum of 130 cells was scored for each sample. Live Δsar1 and Δpmm1 cells were stained with 3,3′-dihexyloxacarbocyanine iodide ($DiOC_6$; Molecular Probes) to visualize cellular membranes and with Hoechst 33342 (Sigma) to visualize the DNA (Demeter et al., 1995).

Nuclear protein transport assays

Wild-type or mutant sns strains containing the integrated pREP42-*GFP-pap1-LEU2* plasmid were grown to mid-log phase in EMM at 25°C, with (wild-type, sar1-1 and pmm1-1) or without (wild-type and sec31-1) 1 M sorbitol in the absence of thiamine to induce GFP-Pap1p expression from the *nmt1* promoter. Cells were shifted to 36°C for 4 hours and viewed both live and after fixation in methanol/formaldehyde (Demeter et al., 1995) either before or after the addition of 0.8 M hydrogen peroxide to test for import and export, respectively.

Results

sns-A10, sns-B2 and sns-B9 are mutated in genes that encode proteins whose homologues are required for early stages of the protein secretory pathway

In the previously described sns screen (Matynia et al., 1998), three temperature-sensitive lethal mutants, sns-A10, sns-B2 and sns-B9, were isolated whose terminal phenotypes closely resembled that of cells in which the Ran GTPase is misregulated. The genes mutated in these strains were identified by complementation of their temperature sensitive lethality (see Materials and Methods).

One cDNA that rescued the temperature sensitivity of sns-A10 corresponded to the complete ORF of the *sar1* gene, which had previously been cloned in *S. pombe* by its ability to complement a temperature sensitive *S. cerevisiae* Sar1p GEF mutant, *sec12-1* (d'Enfert et al., 1992). The *S. pombe* Sar1 protein is 71% identical to *S. cerevisiae* Sar1p (Nakano and Muramatsu, 1989) and 63% identical to *M. musculus* SAR1A (Shen et al., 1993) (Fig. 1A). The *S. cerevisiae* Sar1p is a small GTPase required for vesicular transport of proteins from the ER to the Golgi apparatus (Nakano and Muramatsu, 1989). Two nucleotide changes were identified in the genomic *sar1* locus of the sns-A10 mutant strain: G79A, which results in a glycine to serine change at amino acid position 27, and G99A, a silent mutation (Fig. 1A). Because *sar1* was linked genetically to the temperature-sensitive locus in the sns-A10 strain (see Materials and Methods), the sns-A10 allele of *sar1* contained a mutation, and the *sar1* null mutant was rescued by expression of a plasmid borne copy of *sar1* (see below) sns-A10 was renamed sar1-1.

One genomic plasmid that rescued the temperature sensitivity of sns-B2 was sequenced and found to contain a single ORF (*S. pombe* cosmid SPBC8D2) encoding a protein 28% identical to *S. cerevisiae* Sec31p (Goffeau et al., 1996) and 29% identical to the Sec31 protein homologue in *H. sapiens* (Tang et al., 2000) (Fig. 1B). The *S. cerevisiae* Sec31 protein is required for budding of COPII vesicles from the ER during the process of protein secretion (Salama et al., 1997). The sns-B2 *sec31* locus has three G to A nucleotide mutations that would result in amino acid changes S7N, E72K and G80D in the N-terminus of the protein (Fig. 1B). The sns-B2 strain will be referred to as sec31-1 because the temperature sensitivity of the sns-B2 mutant was linked to the *sec31* locus and the *sec31* locus in sns-B2 contained three mutations.

One cDNA that rescued the temperature sensitivity of sns-B9 was sequenced and found to contain a single ORF, *pmm1*, which encodes phosphomannomutase. *S. pombe* Pmm1p is 72% identical to *S. cerevisiae* Sec53p (Goffeau et al., 1996) and is 56% identical to *H. sapiens* phosphomannomutase 1 (Hansen et al., 1997) (Fig. 1C). The enzyme phosphomannomutase is required for the synthesis of mannose-1-GDP, which is required for synthesis of the carbohydrate used for glycosylation of proteins destined for secretion (Feldman et al., 1987; Orlean, 1992). Based on the

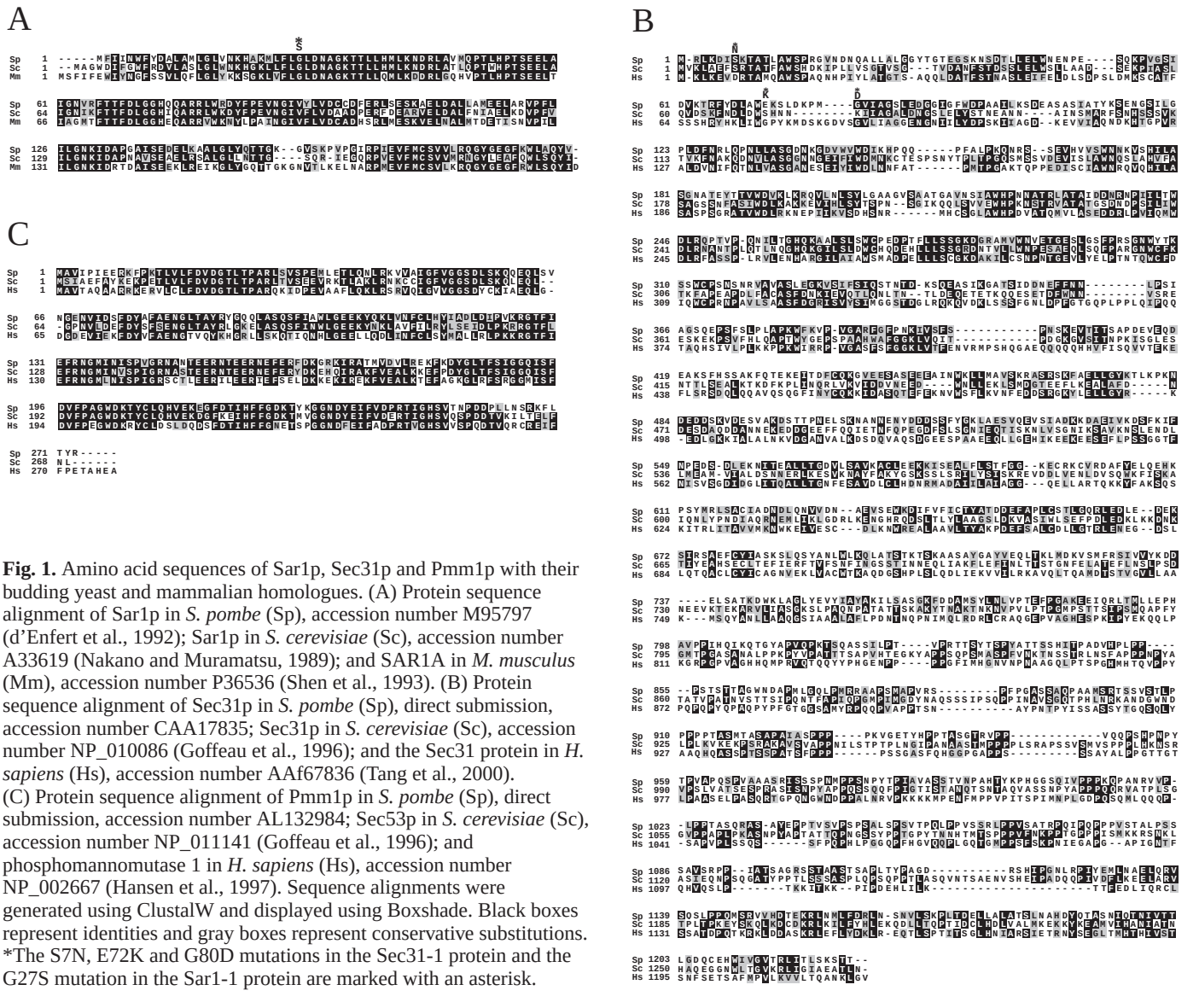

Fig. 1. Amino acid sequences of Sar1p, Sec31p and Pmm1p with their budding yeast and mammalian homologues. (A) Protein sequence alignment of Sar1p in *S. pombe* (Sp), accession number M95797 (d'Enfert et al., 1992); Sar1p in *S. cerevisiae* (Sc), accession number A33619 (Nakano and Muramatsu, 1989); and SAR1A in *M. musculus* (Mm), accession number P36536 (Shen et al., 1993). (B) Protein sequence alignment of Sec31p in *S. pombe* (Sp), direct submission, accession number CAA17835; Sec31p in *S. cerevisiae* (Sc), accession number NP_010086 (Goffeau et al., 1996); and the Sec31 protein in *H. sapiens* (Hs), accession number AAf67836 (Tang et al., 2000). (C) Protein sequence alignment of Pmm1p in *S. pombe* (Sp), direct submission, accession number AL132984; Sec53p in *S. cerevisiae* (Sc), accession number NP_011141 (Goffeau et al., 1996); and phosphomannomutase 1 in *H. sapiens* (Hs), accession number NP_002667 (Hansen et al., 1997). Sequence alignments were generated using ClustalW and displayed using Boxshade. Black boxes represent identities and gray boxes represent conservative substitutions. *The S7N, E72K and G80D mutations in the Sec31-1 protein and the G27S mutation in the Sar1-1 protein are marked with an asterisk.

linkage between the temperature-sensitive locus in sns-B9 and the *pmm1* locus (see Materials and Methods), and the rescue of the *pmm1* null strain by expression of a plasmid borne copy of *pmm1* (see below), the sns-B9 strain will be referred to as pmm1-1.

sar1-1, sec31-1 and pmm1-1 accumulate a normally secreted protein in the ER

To determine whether there are defects in the secretion of proteins from the ER in the sar1-1, sec31-1, and pmm1-1 mutant strains, the glycosylation profile of a secreted protein, acid phosphatase, was analyzed. In wild-type cells incubated at 25°C (Fig. 2A, lane 1; Fig. 2B, lane 1) or at 36°C for up to 4 hours (Fig. 2A, lanes 2,3,4; Fig. 2B, lane 2), acid phosphatase was fully processed with regard to the addition of carbohydrate groups as previously reported (Ayscough and Warren, 1994; Schweingruber et al., 1986). When core N-linked glycosyl groups are added to acid phosphatase in the ER it is converted to its 72 kDa form, and after further modification in the Golgi apparatus the protein migrates as a diffuse band of greater than 95 kDa (Schweingruber et al., 1986).

In sar1-1 cells, acid phosphatase accumulated in its 72 kDa core glycosylated form (arrow) even at 25°C (Fig. 2A, lane 5), and this form increased in abundance upon incubation at 36°C (Fig. 2A, lanes 6-8), indicating a block in its secretion from the ER (Schweingruber et al., 1986).

The glycosylation profile of acid phosphatase in sec31-1 cells also revealed a block in secretion from the ER (Fig. 2B). In sec31-1 cells at 25°C (Fig. 2B, lane 3), a low level of the 72 kDa form of acid phosphatase (arrow) and high molecular weight smears indicated normal protein secretion. However, sec31-1 cells incubated at 36°C for 4 hours, accumulated a high level of the 72 kDa ER form of acid phosphatase (Fig. 2B, lane 4), indicating that protein secretion from the ER is inhibited.

pmm1-1 cells accumulated low levels of underglycosylated ER forms of acid phosphatase at 36°C (data not shown). To characterize the glycosylation defect more clearly, a pulse experiment was performed using cycloheximide-treated cells to visualize only the newly synthesized forms of acid

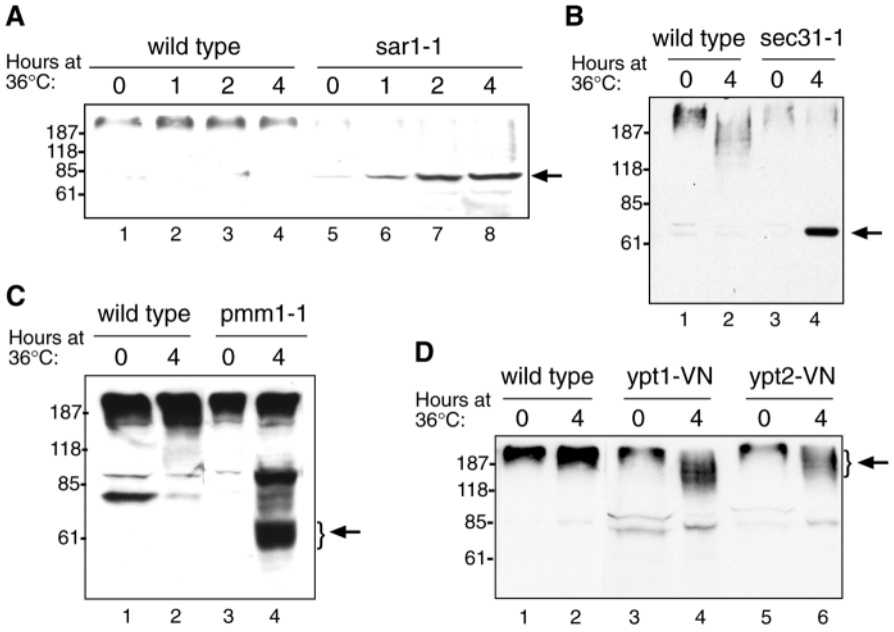

Fig. 2. sar1-1, sec31-1 and pmm1-1 accumulate ER forms of acid phosphatase. Equal quantities of cell extracts were resolved by SDS-PAGE, transferred to a nylon membrane and probed with the anti-acid phosphatase monoclonal antibody 7B4 (Schweingruber et al., 1986) unless otherwise stated. (A) Wild-type (lanes 1-4) and sar1-1 (lanes 5-8) cells were harvested after 0, 1, 2 or 4 hours at 36°C. (B) Wild-type (lanes 1,2) and sec31-1 (lanes 3,4) cells were harvested after incubation for 0 or 4 hours at 36°C. (C) Wild-type (lanes 1,2) and pmm1-1 (lanes 3,4) cells were treated with cycloheximide for 1 hour at the permissive temperature, washed, and released into pre-warmed media without cycloheximide at 36°C. Untreated cells and cells incubated for 4 hours at 36°C were harvested. (D) Wild-type (lanes 1,2), ypt1-VN (lanes 3,4) and ypt2-VN (lanes 5,6) cells were harvested after 0 and 4 hours at 36°C. Three times as much protein was loaded for the ypt mutants at 25°C to allow better visualization of the acid phosphatase. The arrow indicates the 72 kDa core glycosylated ER form (A,B), the 45-75 kDa incompletely glycosylated ER forms (C) or the high molecular weight Golgi modified forms of acid phosphatase (D).

phosphatase (see Materials and Methods). The migration of acid phosphatase as a high molecular weight smear indicated that it is fully processed in wild-type cells at 25°C or 36°C and in pmm1-1 cells at 25°C (Fig. 2C, lanes 1-3). By contrast, pmm1-1 cells at 36°C accumulated unglycosylated or partially glycosylated acid phosphatase, which migrate between 54 and 72 kDa (Fig. 2C, lane 4, arrow) (Schweingruber et al., 1986).

Previously characterized strains defective at post-ER stages of the protein secretory pathway were used as negative controls for these experiments (Armstrong et al., 1994; Craighead et al., 1993). Mutants defective in the fusion of secretory vesicles to either the Golgi apparatus (ypt1-VN) (Armstrong et al., 1994) or the plasma membrane (ypt2-VN) (Craighead et al., 1993) processed acid phosphatase similarly to wild-type cells at 25°C (Fig. 2D, lanes 1-3,5). At 36°C, ypt1-VN and ypt2-VN accumulated the high molecular weight species indicative of Golgi-modified forms of acid phosphatase (Fig. 2D, lanes 4,6, arrow) (Ayscough and Warren, 1994).

sar1, sec31 and pmm1 are essential genes

To determine the consequences of loss of *sar1*, *sec31* and *pmm1*, null alleles of each were generated by replacing the ORF with the selectable *ura4* gene (see Materials and Methods). When *sar1*$^+$/*sar1::ura4* heterozygous null diploids were sporulated no *ura4*-positive haploid colonies grew, indicating that *sar1* is an essential gene. Analysis of 15 complete tetrads from the *sar1*$^+$/*sar::ura4* strain identified 14 tetrads in which viable to non-viable spores segregated 2:2 and one tetrad with one viable and three non-viable spores. None of the viable colonies were *ura4*-positive. These data confirmed that *sar1* is an essential gene in *S. pombe*, as previously reported (d'Enfert et al., 1992). Microscopic examination of 30 *sar1* null spores indicated that 83% were unable to germinate while 13% formed small colonies of 1-3 cells, the majority of which were septated. The viability of *sar1* null cells was rescued by the *sar1* cDNA when expressed from the high level *nmt1* promoter but not when expression was repressed by the presence of thiamine (see Materials and Methods). The terminal phenotype of haploid Δsar1 cells was similar to that of the sar1-1 mutant: 48 hours after promoter shutoff, 27% of Δsar1 cells were septated and binucleate with condensed chromosomes, as compared with only 9% septated, binucleate cells in control cultures in which the promoter was not repressed.

Numerous attempts to delete one copy of *sec31* in a wild-type diploid strain were unsuccessful, perhaps due to haplo-insufficiency. To facilitate the generation of a heterozygous null strain, the wild-type diploid was transformed with pSLF172-81X-*sec31*, a LEU2-containing plasmid in which wild-type *sec31* expression was driven by the lowest strength *nmt1* promoter (see Materials and Methods) due to the toxicity of higher levels of *sec31* expression (data not shown). One heterozygous diploid *sec31*$^+$/*sec31::ura4* strain was obtained and random spore analysis of >2000 germinated spores produced no *ura4*-positive colonies, indicating that *sec31* is an essential gene. Analysis of 28 tetrads revealed that spore viability was low: two weeks after tetrad dissection only 16% of the spores grew into colonies, while 63% remained ungerminated. The remaining 21% of the spores did germinate but either divided less than twice (17%) or formed microcolonies (4%). None of the colonies or microcolonies was *ura4*-positive, providing further evidence that *sec31* is an essential gene. A Δ*sec31* strain kept alive by expression of *sec31* from the lowest strength *nmt1* promoter could not be isolated. Therefore, promoter shut-off experiments to study the terminal phenotype of Sec31p-depleted cells in more detail could not be performed. It is clear, however, that *sec31* is an essential gene that is required for normal sporulation.

The sporulation and germination of *pmm1*$^+$/*pmm1::ura4* heterozygous null diploids yielded 8100 colonies, only 16 of which were *ura4*-positive haploids, suggesting that *pmm1* is an

essential gene**.** Analysis of 26 complete tetrads from two different heterozygous null strains (*pmm1*$^+$/*pmm1::ura4*-2 and *pmm1*$^+$/*pmm1::ura4*-5) identified 25 tetrads in which viable to non-viable colonies segregated 2:2, and one tetrad with no viable colonies. None of the viable colonies were *ura4*-positive. These data confirm that *pmm1* is an essential gene. Microscopic examination of the tetrad dissection plates revealed that, of 54 *pmm1* null spores examined, 98% germinated. Of these, 52% formed single, rounded cells and 47% arrested as single, septated cells.

The viability of Δpmm1 cells was rescued by expression of the *pmm1* cDNA from the lowest strength *nmt1* promoter, but not when expression was repressed. 48 hours after promoter repression, Δpmm1 cells stopped dividing and arrested with a terminal phenotype similar to that of the pmm1-1 temperature-sensitive mutant: 19% were septated, binucleated cells with condensed chromosomes.

sar1-1, sec31-1 and pmm1-1 accumulate cytoplasmic membranes

Defects in the membranes of sar1-1, sec31-1 and pmm1-1 were previously characterized using the vital lipophilic fluorescent dye, $DiOC_6$, which binds to all cellular membranes (Matynia et al., 1998). Compared with wild-type cells, there appeared to be an increase in cytoplasmic membranes in sar1-1, sec31-1 and pmm1-1, and the nuclear envelopes could not be clearly visualized in sar1-1 or pmm1-1. Promoter shut off experiments (described above) showed that 27% of Δsar1 and 19% of Δpmm1 cells accumulated abnormal membranes that could be visualized with $DiOC_6$ (Fig. 3; compare E with A, and G with C). By contrast, the nuclear envelopes of two previously characterized *S. pombe* secretory mutants, ypt1-VN and ypt2-VN, which are defective in post-ER stages of the pathway, could be clearly delineated (data not shown) (Craighead et al., 1993). These data indicate that the membrane accumulation observed in sar1-1, Δsar1, sec31-1, pmm1-1 and Δpmm1 is not a general characteristic of fission yeast protein secretory mutants.

To more clearly delineate their membrane abnormalities, sar1-1, sec31-1 and pmm1-1 were examined by electron microscopy (see Materials and Methods). In wild-type cells grown at 36°C, the nuclear envelope was a circular structure containing nuclear pores (Fig. 4A). In sar1-1, sec31-1 and pmm1-1, the nuclear envelopes were intact at the restrictive temperature, but the nuclear envelope lumens were dramatically dilated and easier to visualize than in wild-type cells (Fig. 4B,C,D, arrowheads). This defect is more clearly seen at higher magnification (compare Fig. 4E with Fig. 4F,G,H). sar1-1, sec31-1 and pmm1-1 mutant cells also displayed a dramatic increase in cytoplasmic membranes that were dilated and easier to visualize than in wild-type cells (Fig. 4B,C,D, arrows). In wild-type *S. pombe* cells the ER surrounds and is continuous with the nuclear envelope and extends through the cytoplasm to the cell periphery where it lies immediately adjacent to the plasma membrane (Pidoux and Armstrong, 1992; Pidoux and Armstrong, 1993). Since the excess cytoplasmic membranes seen in the mutants emanated from the nuclear envelope and cell periphery and lacked nuclear pores, they are most likely derived from the ER. These membranes were uniformly distributed throughout the cytoplasm in sar1-1 (Fig. 4B), appeared to 'bubble' off from both the nucleus and the cell periphery in sec31-1 (Fig. 4C), and accumulated around the nucleus in pmm1-1 (Fig. 4D). 56% of sar1-1, 68% of sec31-1, and 50% of pmm1-1 cells had accumulated ER membranes and dilated nuclear and ER lumens (Table 1).

Nucleocytoplasmic transport is normal in sar1-1, sec31-1 and pmm1-1

To test whether the structurally abnormal nuclear envelopes affect nuclear function, nuclear protein import and export in sar1-1, sec31-1 and pmm1-1 was assayed by monitoring the localization of the GFP-Pap1p reporter protein. Pap1p is a stress response transcription factor that shuttles between the nucleus and cytoplasm (Toone et al., 1998). At steady state, GFP-Pap1p is actively exported from the nucleus and its localization appears to be exclusively cytoplasmic. Upon oxidative stress, such as exposure to hydrogen peroxide, Pap1p rapidly accumulates in the nucleus (Toone et al., 1998). GFP-

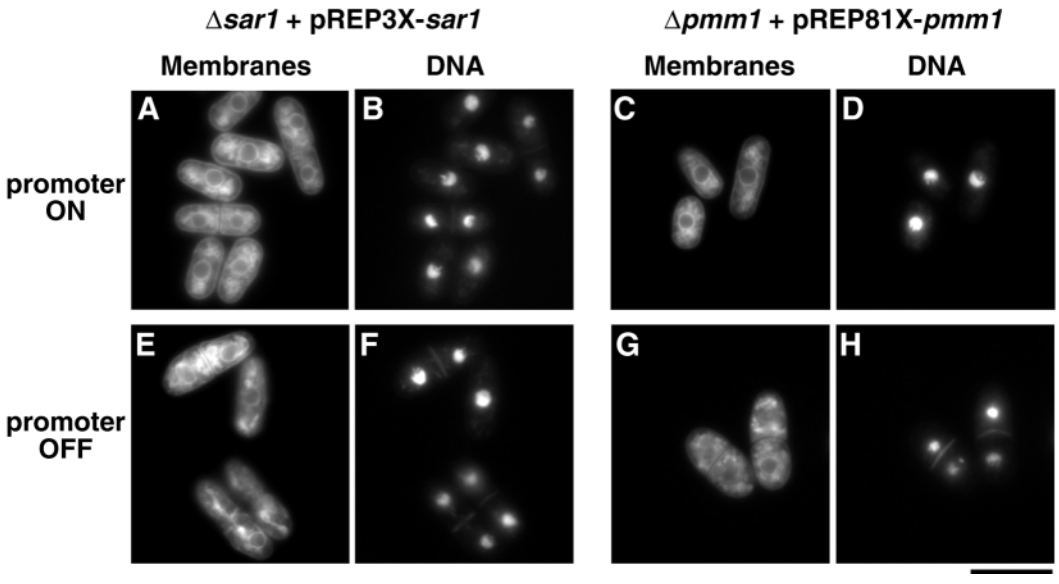

Fig. 3. Δsar1 and Δpmm1 null strains accumulate cytoplasmic membranes. Δsar1, containing pREP3X-*sar1*, and Δpmm1, containing pREP81X-*pmm1*, were grown in media either without thiamine, to allow expression (promoter ON; A-D) or with thiamine, to repress expression (promoter OFF; E-H). After 48 hours, live cells were stained with both $DiOC_6$, to visualize cellular membranes (A,C,E,G), or Hoechst, to visualize DNA (B,D,F,H). Cells in which the promoter was off contain an increase in cellular membranes compared with cells in which the promoter remained on. Bar, 10 μm.

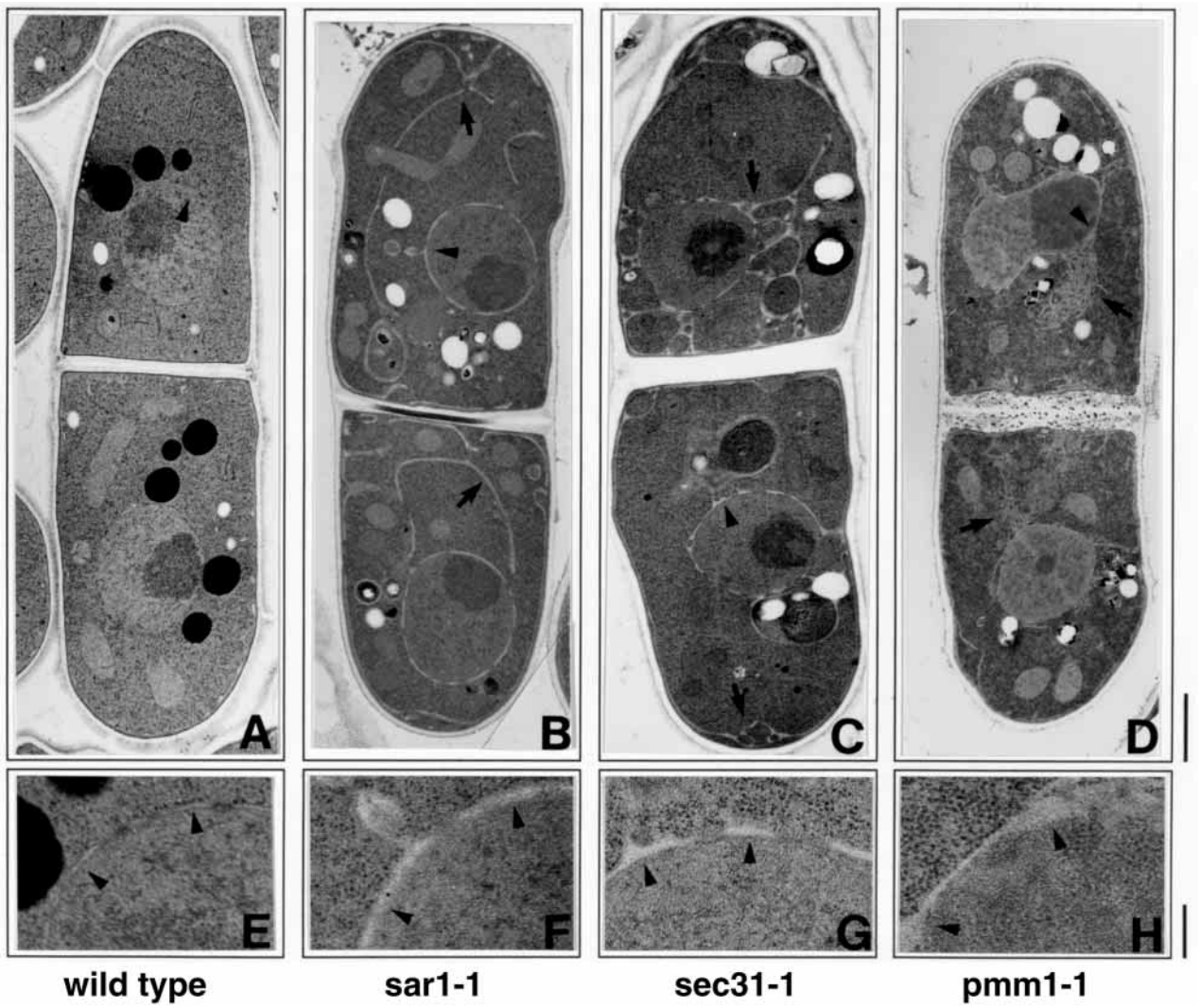

Fig. 4. sar1-1, sec31-1 and pmm1-1 have accumulated ER membranes and dilated nuclear envelope lumens. Electron micrographs of wild-type (A,E), sar1-1 (B,F), sec31-1 (C,G) and pmm1-1 (D,H) cells grown for 4 hours at 36°C. Wild-type cells contain nuclei with normal morphology and few cytoplasmic membranes. sar1-1, sec31-1 and pmm1-1 have dilated nuclear envelope lumens and accumulated ER membranes (B,C,D, arrows). The nuclear envelopes, indicated by arrowheads, are shown at higher magnification to compare the width of the lumen of wild-type with sar1-1, sec31-1 and pmm1-1 strains (compare E with F,G,H). Bar, 1 μm (A-D); 0.2 μm (E-H).

Pap1p was localized predominantly to the cytoplasm in wild-type cells (Fig. 5A) as well as in the sar1-1, sec31-1 and pmm1-1 mutants grown at the restrictive temperature, suggesting that nuclear protein export was not adversely affected in these cells (Fig. 5C,E,G). When hydrogen peroxide was added for 15 minutes, GFP-Pap1p relocalized to the nucleus in wild-type (Fig. 5B) and mutant cells (Fig. 5D,F,H) at 36°C. These data indicate that the nuclear envelopes of sar1-1, sec31-1 and pmm1-1 are intact and competent for nucleocytoplasmic transport.

The cell cycle arrest of sar1-1, sec31-1 and pmm1-1 is not due to a general defect in protein secretion

sar1-1, sec31-1 and pmm1-1 mutants arrest after mitosis but before S-phase (Matynia et al., 1998). To ask if the protein secretion defect is responsible for this cell cycle arrest, the terminal phenotypes of these three strains were compared with those of ypt1-VN and ypt2-VN (Armstrong et al., 1994; Craighead et al., 1993), which are defective in post-ER stages of secretion. sar1-1, sec31-1 and pmm1-1 arrested at the restrictive temperature with a high percentage of septated, binucleated cells (Table 2). However, the cell cycle distribution of ypt1-VN and ypt2-VN was comparable with that of wild-type cells (Table 2). These data indicate that a block in protein secretion alone is not sufficient to cause the cell cycle arrest observed in pmm1-1, sec31-1 and sar1-1.

Proliferation of ER membranes alone does not result in a cell cycle arrest in *S. pombe* cells

We next wanted to ask whether ER proliferation in the absence of nuclear envelope dilation could cause a cell cycle arrest. It has been shown previously in *S. pombe* and *S. cerevisiae* that increasing the levels of the *S. cerevisiae* Hmg1p causes a proliferation of ER membranes called karmellae that surround

Table 1. sar1-1, sec31-1 and pmm1-1 cells accumulate ER membranes and dilated nuclear envelopes

Strain	Normal membranes (%)	Abnormal membranes (%)		
		Excess*	Dilated NEs‡	Both
Wildtype	97	1	2	0
sar1-1	33	3	8	56
sec31-1	24	1	7	68
pmm1-1	23	5	22	50

Membrane morphology was determined by examination of electron micrographs of at least 130 cells per sample.

*Cells containing excess ER membranes were qualitatively defined as those containing at least twice as much ER as found in a typical wild-type cell.

‡Cells containing dilated NEs were qualitatively defined as those containing NEs that were at least twice as wide as a typical wild-type NE.

Table 2. Neither ER membrane proliferation nor defects in post-ER stages of the secretory pathway cause cell cycle arrest

Strain	Septated (%)		Unseptated (%)	
	Mononucleate	Binucleate	Mononucleate	Binucleate
Wildtype	0	9	87	4
ypt1-VN	0	10	87	3
ypt2-VN	0	12	86	2
sar1-1	0	41	55	4
sec31-1	0	45	50	5
pmm1-1	0	38	59	3
Wildtype overexpressing *HMG1*	0	12	82	6

The percentage of cells that were septated, binucleate or mononucleate was determined by microscopic examination of greater than 200 DAPI-stained cells.

the nucleus but do not perturb the nuclear envelope (Lum and Wright, 1995; Wright et al., 1988). Wild-type *S. pombe* cells expressing *HMG1* from the highest strength *nmt1* gene promoter (Wright et al., 1988) for 24 hours had a cell cycle distribution comparable with that of wild-type cells (Table 2) and with wild-type cells in which *HMG1* expression was repressed (data not shown). The distribution in all three samples remained similar after 48 hours of *HMG1* expression (data not shown). These data indicate that ER proliferation alone, in the absence of nuclear envelope abnormalities, is not sufficient to cause a cell cycle arrest in *S. pombe* cells.

Discussion

sns-A10, sns-B2, and sns-B9 are mutated in genes required for protein secretion and cell cycle progression

The sns (septated not in S-phase) mutant screen identified temperature sensitive *S. pombe* mutants unable to complete the mitosis to interphase transition (Matynia et al., 1998). Like the previously characterized RanGEF mutant pim1-d1, the sns mutants arrest after mitosis but before S-phase with condensed unreplicated chromosomes, a medial septum and abnormal NEs. Among the ten strains not mutated in *pim1*, the terminal phenotypes of sns-A10, sns-B2 and sns-B9 most closely resemble that of pim1-d1 and these three mutants also have genetic interactions with components of the Ran GTPase system. The genes mutated in sns-A10, sns-B2, and sns-B9 were identified by complementation of their temperature-sensitive lethality. All three were found to encode proteins required for protein secretion: sns-B9 is mutated in *pmm1*, sns-A10 is mutated in *sar1*, and sns-B2 is mutated in *sec31*.

Phosphomannomutase (Pmm) is an evolutionarily conserved enzyme that converts mannose-6-phosphate to mannose-1-phosphate, the precursor for the synthesis of cellular carbohydrate moieties required for N-linked and O-linked glycosylation and GPI anchor synthesis (Orlean, 1992). Phosphomannomutase is essential for secretion because proteins must be properly glycosylated and folded to be recognized as substrates for secretion from the ER. In the *S. cerevisiae* sec53-6 temperature sensitive phosphomannomutase mutant, proteins that are normally secreted are improperly glycosylated and accumulate in the ER lumen (Ferro-Novick et al., 1984). We have shown that the fission yeast pmm1-1 phosphomannomutase mutant also accumulates partially glycosylated acid phosphatase in the ER when incubated at the restrictive temperature.

In contrast to the secretory defect caused by defective phosphomannomutase, *S. cerevisiae* strains with defects in Sar1p or Sec31p are competent for glycosylation but have a

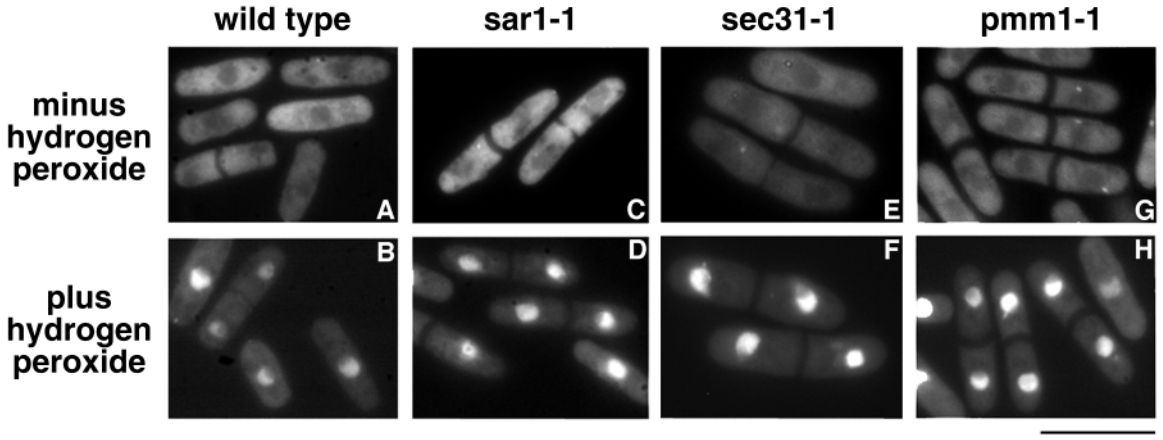

Fig. 5. Nuclear protein import and export are normal in sar1-1, sec31-1 and pmm1-1. Wild-type, sar1-1, sec31-1 and pmm1-1 cells with integrated GFP-Pap1p under the medium strength *nmt1* promoter were incubated at 25°C until mid-log phase, then shifted to 36°C for four hours. Cells were photographed either without addition of hydrogen peroxide, showing that GFP-Pap1p is exported from the nucleus (A,C,E,G) or after exposure to 0.8 mM hydrogen peroxide for 15 minutes, showing that GFP-Pap1p is imported into the nucleus (B,D,F,H). Bar, 10 μm.

block in secretion because cells are unable to form the COP II coat on ER secretory vesicles. This accumulation of proteins in the ER is accompanied by an accumulation of ER membrane (Salama et al., 1997). Sar1p is a soluble GTPase and its interaction on the cytoplasmic face of the ER with its GEF (Sec12p) initiates COP II coat formation (Springer et al., 1999). Once Sar1p is converted to its active GTP-bound state, two cytoplasmic complexes, Sec23p/Sec24p and Sec13p/Sec31p, are recruited to the ER membrane and incorporated into the COP II vesicle coat. Sec23p, the Sar1p GAP, then stimulates Sar1p-GTP hydrolysis rendering the COPII vesicle competent for fusion to the cis-Golgi.

Structural homologues of Sar1p have been identified in a variety of organisms (Goffeau et al., 1996; Shen et al., 1993). Their functional conservation was demonstrated in *S. cerevisiae* by showing that a mutation in the Sar1p GEF (*sec12-1*) can be rescued by expression of either the fission yeast or budding yeast *sar1* gene (d'Enfert et al., 1992). The *sar1-1* mutant allele identified in the sns screen contains a nucleotide mutation that results in the amino acid substitution G27S in the G1 region of the GTPase, a highly conserved motif required for nucleotide binding (Bourne et al., 1991). Mutations in this region that cause temperature-sensitive lethality and are predicted to inhibit nucleotide binding in *S. cerevisiae* Sar1p are defective in vesicle budding from the ER (Yamanushi et al., 1996). The *S. pombe* sar1-1 G27S mutation renders the protein temperature sensitive and acid phosphatase accumulates in its 72 kDa ER glycosylated form in mutant cells incubated at the restrictive temperature.

S. cerevisiae Sec31p is a component of a cytoplasmic complex, which is recruited to the ER membrane after Sar1p activation and is required for COP II coat formation and vesicle budding from the ER (Salama et al., 1997). The *S. pombe* Sec31 protein is similar to Sec31p in *S. cerevisiae* (Goffeau et al., 1996) and *H. sapiens* (Tang et al., 2000). There are three nucleotide changes in the *sec31-1* allele resulting in amino acid changes S7N, E72K and G80D. At comparable positions, the Sec31 proteins of *S. cerevisiae* and *H. sapiens* also have a serine and a glycine, respectively. All three *sec31-1* mutations are located in the N-terminal portion of the protein which has been shown to be required for binding of *S. cerevisiae* Sec31p to Sec13p, an interaction which is necessary for COP II coat formation (Shaywitz et al., 1997).

pmm1-1, sar1-1, sec31-1 and mutants in their *S. cerevisiae* homologues (Ferro-Novick et al., 1984; Nakano and Muramatsu, 1989; Salama et al., 1997; Yamanushi et al., 1996) accumulate normally secreted proteins in the ER, although they interfere with secretion by different mechanisms. All three of these *S. pombe* mutants exhibit a similar cell cycle arrest at the mitosis to interphase transition. Therefore, this defect is most likely the direct or indirect result of the block in protein secretion from the ER, a characteristic of all three mutants, rather than a glycosylation defect, which is unique to the pmm1-1 mutant.

In *S. cerevisiae*, several secretory mutants are transiently blocked in nuclear protein import, suggesting a possible connection between protein secretion and NE function (Nanduri et al., 1999). However, sar1-1, sec31-1 and pmm1-1 are competent for nucleocytoplasmic import and export.

S. pombe pmm1-1, sar1-1 and sec31-1 exhibit a proliferation of ER membranes and a swelling of the ER/NE lumen. Because the membranes and lumens of the nuclear envelope and the ER are continuous, the inability to secrete proteins from the ER may directly result in the observed dilation of the nuclear envelope and ER lumen. Mutations in *S. cerevisiae* Sar1p, Sec31p and Sec53p also block protein secretion from the ER and cause proliferation of ER membranes (Ferro-Novick et al., 1984; Salama et al., 1997; Yamanushi et al., 1996). What distinguishes the *S. pombe* secretory mutants from their *S. cerevisiae* counterparts is their cell cycle arrest after mitosis but before S phase with condensed, unreplicated chromosomes.

A general defect in protein secretion is not sufficient to cause a cell cycle arrest in fission yeast

If the cell cycle arrest seen in sar1-1, sec31-1 and pmm1-1 in *S. pombe* is due to defects in the secretion of a specific protein(s) or lipid(s) from the cell, then mutants defective in protein secretion at later steps of the secretory pathway should also exhibit a similar cell cycle arrest. This hypothesis was tested by examining cell cycle progression in the ypt1-VN and ypt2-VN mutants, which can secrete proteins from the ER but are unable to fuse secretory vesicles to the Golgi apparatus or plasma membranes, respectively (Armstrong et al., 1994; Craighead et al., 1993). We found that neither ypt1-VN nor ypt2-VN undergoes a cell cycle block at the restrictive temperature. A block in protein secretion is therefore not sufficient to cause a cell cycle stage specific arrest. These data suggest that the cell cycle block in sar1-1, sec31-1, and pmm1-1 is caused specifically by the accumulation of proteins in the ER, or the consequences thereof, rather than from an inability to deliver proteins to the Golgi apparatus or the plasma membrane.

Cell cycle progression in budding yeast does not depend on protein glycosylation or secretion

Among the more than 40 secretion defective strains that have been characterized in *S. cerevisiae*, none has been reported to exhibit a classical cell division cycle (cdc) arrest (Ferro-Novick et al., 1984; Jedd et al., 1995; Newman and Ferro-Novick, 1987; Novick et al., 1980; Segev and Botstein, 1987). This suggests that protein secretion per se is not essential for cell cycle progression in budding yeast.

Many secreted proteins require glycosylation for proper folding in and subsequent secretion from the ER. Several previously published studies in *S. cerevisiae* suggest that protein glycosylation affects cell cycle progression and a number of genes required for protein glycosylation have cell cycle regulatory elements in their promoters (Kukuruzinska and Lennon-Hopkins, 1999). However, most *S. cerevisiae* mutants deficient in glycosylation do not exhibit classical cell cycle regulatory defects (Kukuruzinska and Lennon-Hopkins, 1999) indicating that protein glycosylation is not required for cell cycle progression.

ER proliferation is not sufficient to cause a cell cycle arrest in fission yeast

To determine whether the ER membrane accumulation seen in pmm1-1, sar1-1 and sec31-1 interferes with cell cycle progression, we asked whether cells that have excess ER membranes but do not have nuclear defects undergo a cell cycle arrest. In fission yeast, overexpression of *HMG1*, the gene that

encodes the budding yeast ER resident protein HMG-CoA reductase, causes the proliferation of ER into stacked paired membranes around the nucleus (called karmellae) and peripheral membrane stacks near the plasma membrane (Lum and Wright, 1995). These cells are viable and have ultrastructurally normal NEs that divide at mitosis (Lum and Wright, 1995). We found that wild-type cells overproducing Hmg1p progress normally through the cell cycle suggesting that the accumulation or proliferation of ER membranes in the absence of NE abnormalities is not sufficient to cause a cell cycle arrest in fission yeast.

Improper nuclear structure may result in a cell cycle arrest

We propose that the cell cycle arrest seen in pmm1-1, sec31-1 and sar1-1 is a result of abnormalities in the NE, rather than defects in glycosylation, ER proliferation or protein secretion. There are several examples in *S. cerevisiae* of structural and functional links between the nucleus and the ER: (1) A recent screen identified *S. cerevisiae* mutants with irregularly shaped or multilobed nuclei that are mutated in genes whose products are required for ER to Golgi transport (Kimata et al., 1999); (2) Deletion of genes that encode two ER transmembrane proteins results in morphological defects in the nucleus and defects in meiosis (Siniossoglou et al., 1998); and (3) A *S. cerevisiae* strain mutated in the ER lumenal chaperone BiP (encoded by *KAR2*) is defective in fusion of the ER and nuclear membranes during mating (Brizzio et al., 1999). Taken together, these results support the hypothesis that nuclear structure and/or function can be impaired by ER defects.

The genetic interactions observed between components of the RanGTPase system and *sar1-1*, *sec31-1*, and *pmm1-1*, which cause impaired protein secretion from the ER, can be explained by the fact that all result in nuclear envelope structural defects. While the swollen nuclear envelopes of sar1-1, sec31-1 and pmm1-1 remain intact and competent for nuclear protein import and export, the nuclear envelope of pim1-d1 mutant cells, mutated in the RanGEF, undergoes fragmentation at the restrictive temperature. This is presumably due to defects in a process(es) other than general protein secretion as this strain has no apparent protein secretory defect (data not shown). All four strains may have nuclear defects in interphase that are exacerbated at mitosis when the nuclear envelope undergoes dramatic structural changes.

Although the NE of yeasts does not undergo the cycles of breakdown and reformation that occur in higher eukaryotes, there may be a similar requirement for the re-establishment of proper nuclear architecture prior to the initiation of DNA replication (Gant and Wilson, 1997). In the fission yeast *S. pombe*, the dilation of the NE lumen caused by the failure to secrete proteins from the ER may interfere with this process. The condensed chromatin in sar1-1, sec31-1 and pmm1-1 arrested cells and their failure to enter S phase after mitosis indicates that chromosome decondensation and perhaps other structural re-arrangements required for the correct establishment of the interphase nucleus, do not occur in cells with abnormal NEs.

We thank Ngoctuyen Ong for sequencing the *sar1* locus in the sar1-1 strain. We gratefully acknowledge John Armstrong for supplying ypt1-VN, ypt2-VN as well as the anti-acid phosphatase antibodies, and Robin Wright for providing the *HMG1* overexpression plasmid. We also thank Mary Morphew for preparation of EM samples and Hank Adams and Frank Herbert in the Baylor Integrated Microscopy Core Laboratory for sectioning and staining of EM samples. This work was supported by the National Institutes of Health (GM49119).

References

Armstrong, J., Pidoux, A., Bowden, S., Craighead, M., Bone, N. and Robinson, E. (1994). The ypt proteins of *Schizosaccharomyces pombe*. *Biochem. Soc. Trans.* **22**, 460-463.

Ayscough, K. and Warren, G. (1994). Inhibition of protein synthesis disrupts the golgi apparatus in the fission yeast, *Schizosaccharomyces pombe*. *Yeast* **10**, 1-11.

Bahler, J., Wu, J. Q., Longtine, M. S., Shah, N. G., McKenzie, A., III, Steever, A. B., Wach, A., Philippsen, P. and Pringle, J. R. (1998). Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. *Yeast* **14**, 943-951.

Barbet, N., Muriel, W. J. and Carr, A. M. (1992). Versatile shuttle vectors and genomic libraries for use with *Schizosaccharomyces pombe*. *Gene* **114**, 59-66.

Belgareh, N. and Doye, V. (1997). Dynamics of nuclear pore distribution in nucleoporin mutant yeast cells. *J. Cell Biol.* **136**, 747-759.

Bourne, H. R., Sanders, D. A. and McCormick, F. (1991). The GTPase superfamily: conserved structure and molecular mechanism. *Nature* **349**, 117-127.

Brizzio, V., Khalfan, W., Huddler, D., Neh, C. T., Andersen, S. S. L., Latterich, M. and Rose, M. D. (1999). Genetic interactions between *KAR7/SEC71*, *KAR8/JEM1*, *KAR5* and *KAR2* during nuclear fusion in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* **10**, 609-626.

Craighead, M. W., Bowden, S. l., Watson, R. and Armstrong, J. (1993). Function of the *ypt2* gene in the exocytic pathway of *Schizosaccharomyces pombe*. *Mol. Biol. Cell* **4**, 1069-1076.

d'Enfert, C., Gensse, M. and Gaillardin, C. (1992). Fission yeast and a plant have functional homologues of the Sar1 and Sec12 proteins involved in ER to Golgi traffic in budding yeast. *EMBO J.* **11**, 4205-4211.

Demeter, J., Morphew, M. and Sazer, S. (1995). A mutation in the RCC1-related protein Pim1 results in nuclear envelope fragmentation in fission yeast. *Proc. Natl. Acad. Sci. USA* **92**, 1436-1440.

Ellis, D. J., Jenkins, H., Whitfield, W. G. F. and Hutchison, C. J. (1997). GST-lamin fusion proteins act as dominant negative mutants in *Xenopus* egg extracts and reveal the function for the lamina in DNA replication. *J. Cell Sci.* **110**, 2507-2518.

Feldman, R. I., Bernstein, M. and Schekman, R. (1987). Product of SEC53 is required for folding and glycosylation of secretory proteins in the lumen of the yeast endoplasmic reticulum. *J. Cell Biol.* **262**, 9332-9339.

Ferro-Novick, S., Novick, P., Field, C. and Schekman, R. (1984). Yeast secretory mutants that block the formation of active cell surface enzymes. *J. Cell Biol.* **98**, 35-43.

Fleig, U., Salus, S. S., Karig, I. and Sazer, S. (2000). The fission yeast Ran GTPase is required for microtubule integrity. *J. Cell Biol.* **151**, 1101-1112.

Forsburg, S. L. (1993). Comparison of *Schizosaccharomyces pombe* expression systems. *Nucleic Acids Res.* **21**, 2955-2966.

Forsburg, S. L. and Nurse, P. (1994). Analysis of the *Schizosaccharomyces pombe* cyclin puc1: evidence for a role in cell cycle exit. *J. Cell Sci.* **107**, 601-613.

Forsburg, S. L. and Sherman, D. A. (1997). General purpose tagging vectors for fission yeast. *Gene* **191**, 191-195.

Gant, T. M. and Wilson, K. L. (1997). Nuclear assembly. *Annu. Rev. Cell Dev. Biol.* **13**, 669-695.

Goffeau, A., Barrell, B. G., Bussey, H., Davis, R. W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J. D., Jacq, C., Johnston, M. et al. (1996). Life with 6000 genes. *Science* **274**, 563-567.

Hansen, S. H., Frank, S. R. and Casanova, J. E. (1997). Cloning and characterization of human phosphomannomutase, a mammalian homologue of yeast SEC53. *Glycobiol.* **7**, 829-834.

Hiraoka, Y., Toda, T. and Yanagida, M. (1984). The NDA3 gene of fission yeast encodes beta-tubulin: a cold-sensitive nda3 mutation reversibly blocks spindle formation and chromosome movement in mitosis. *Cell* **39**, 349-358.

Hoheisel, J. D., Maier, E., Mott, R., McCarthy, L., Grigoriev, A. V., Schalkwyk, L. C., Nizetic, D., Francis, F. and Lehrach, H. (1993). High

resolution cosmid and P1 maps spanning the 14 Mb genome of the fission yeast *S. pombe*. *Cell* **73**, 109-120.

Hurt, E. C., Mutvei, A. and Carmo-Fonseca, M. (1992). The nuclear envelope of the yeast *Saccharomyces cerevisiae*. *Int. Rev. Cytol.* **136**, 145-184.

Jedd, G., Richardson, C., Litt, R. and Segev, N. (1995). The Ypt1 GTPase is essential for the first two steps of the yeast secretory pathway. *J. Cell Biol.* **131**, 583-590.

Kimata, Y., Lim, C. R., Kiriyama, T., Nara, A., Hirata, A. and Kohno, K. (1999). Mutation of the yeast ε-COP gene *ANU2* causes abnormal nuclear morphology and defects in intracellular vesicular transport. *Cell Struct. Funct.* **24**, 197-208.

Kops, A. and Guthrie, C. (2001). An essential nuclear envelope integral membrane protein, Brr6p, required for nuclear transport. *EMBO J.* **20**, 4183-4193.

Kukuruzinska, M. A. and Lennon-Hopkins, K. (1999). *ALG* gene expression and cell cycle progression. *Biochim. Biophys. Acta* **1426**, 359-372.

Leupold, U. (1970). Genetic methods for *Schizosaccharomyces pombe*. *Methods Cell. Physiol.* **4**, 169-177.

Lum, P. Y. and Wright, R. (1995). Degradation of HMG-CoA reductase-induced membranes in the fission yeast, *Schizosaccharomyces pombe*. *J. Cell Biol.* **131**, 81-95.

Matynia, A., Mueller, U., Ong, N., Demeter, J., Granger, A. L., Hinata, K. and Sazer, S. (1998). Isolation and characterization of fission yeast sns mutants defective at the mitosis-to-interphase transition. *Genetics* **148**, 1799-1811.

Maundrell, K. (1990). nmt1 of fission yeast. *J. Biol. Chem.* **265**, 10857-10864.

McCully, E. K. and Robinow, C. F. (1971). Mitosis in the fission yeast *Schizosaccharomyces pombe*: a comparative study with light and electron microscopy. *J. Cell Sci.* **9**, 475-507.

Moreno, S., Klar, A. and Nurse, P. (1991). Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol.* **194**, 795-823.

Nakano, A. and Muramatsu, M. (1989). A novel GTP-binding protein, Sar1p, is involved in transport from the endoplasmic reticulum to the Golgi apparatus. *J. Cell Biol.* **109**, 2677-2691.

Nanduri, J., Mitra, S., Andrei, C., Liu, Y., Yu, Y., Hitomi, M. and Tartakoff, A. M. (1999). An unexpected link between the secretory pathway and the organization of the nucleus. *J. Biol. Chem.* **274**, 33785-33789.

Newman, A. P. and Ferro-Novick, S. (1987). Characterization of new mutants in the early part of the yeast secretory pathway isolated by a [^{3}H]-mannose suicide selection. *J. Cell Biol.* **105**, 1587-1594.

Newport, J. W. and Forbes, D. J. (1987). The nucleus: structure, function, and dynamics. *Annu. Rev. Biochem.* **56**, 535-565.

Novick, P., Field, C. and Schekman, R. (1980). Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. *Cell* **21**, 205-215.

Okazaki, K., Okazaki, N., Kume, K., Jinno, S., Tanaka, K. and Okayama, H. (1990). High-frequency transformation method and library transducing vectors for cloning mammalian cDNAs by trans-complementation of *Schizosaccharomyces pombe*. *Nucleic Acids Res.* **18**, 6485-6489.

Orlean, P. (1992). Enzymes that recognize dolichols participate in three glycosylation pathways and are required for protein secretion. *Biochem. Cell Biol.* **70**, 438-447.

Patterson, T. E., Stark, G. R. and Sazer, S. (1995). A strategy for quickly identifying all unique two-hybrid or library plasmids within a pool of yeast transformants. *Nucleic. Acids Res.* **23**, 4222-4223.

Pfeffer, S. R. (1999). Transport-vesicle targeting: tethers before SNAREs. *Nat. Cell Biol.* **1**, E17-E22.

Pidoux, A. L. and Armstrong, J. (1992). Analysis of the BiP gene and identification of an ER retention signal in *Schizosaccharomyces pombe*. *EMBO J.* **11**, 1583-1591.

Pidoux, A. L. and Armstrong, J. (1993). The BiP protein and the endoplasmic reticulum of *Schizosaccharomyces pombe*: fate of the nuclear envelope during cell division. *J. Cell Sci.* **105**, 1115-1120.

Salama, N. R., Chuang, J. S. and Schekman, R. W. (1997). *SEC31* encodes an essential component of the COPII coat required for transport vesicle budding from the endoplasmic reticulum. *Mol. Biol. Cell* **8**, 205-217.

Sambrook, J., Fritsch, E. R. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. New York: Cold Spring Harbor Laborarory Press.

Sazer, S. and Dasso, M. (2000). The Ran decathlon: multiple roles of Ran. *J. Cell Sci.* **113**, 1111-1118.

Sazer, S. and Nurse, P. (1994). A fission yeast RCC1-related protein is required for the mitosis to interphase transition. *EMBO J.* **13**, 606-615.

Schweingruber, A. M., Schoenholzer, F., Keller, L., Schwaniger, R., Traschel, H. and Schweingruber, M. E. (1986). Glycosylation and secretion of acid phosphatase in *Schizosaccharomyces pombe*. *Eur. J. Biochem.* **158**, 133-140.

Segev, N. and Botstein, D. (1987). The *ras*-like yeast *YPT1* gene is itself essential for growth, sporulation, and starvation response. *Mol. Cell. Biol.* **7**, 2367-2377.

Shaywitz, D. A., Espenshade, P. J., Gimeno, R. E. and Kaiser, C. A. (1997). COPII subunit interactions in the assembly of the vesicle coat. *J. Biol. Chem.* **272**, 25413-25416.

Shen, K. A., Hammond, C. M. and Moore, H. P. (1993). Molecular analysis of SAR1-related cDNAs from a mouse pituitary cell line. *FEBS Lett.* **335**, 380-385.

Siniossoglou, S., Santos-Rosa, H., Rappsilber, J., Mann, M. and Hurt, E. (1998). A novel complex of membrane proteins required for formation of a spherical nucleus. *EMBO J.* **17**, 6449-6464.

Springer, S., Spang, A. and Schekman, R. (1999). A primer on vesicle budding. *Cell* **97**, 145-148.

Tang, B. L., Zhang, T., Low, D. Y., Wong, E. T., Horstmann, H. and Hong, W. (2000). Mammalian homologues of yeast sec31p: an ubiquitously expressed form is localized to endoplasmic reticulum (ER) exit sites and is essential for ER-Golgi transport. *J. Biol. Chem.* **275**, 13597-13604.

Toone, W. M., Kuge, S., Samuels, M., Morgan, B. A., Toda, T. and Jones, N. (1998). Regulation of the fission yeast transcription factor Pap1 by oxidative stress: requirement for the nuclear export factor Crm1 (exportin) and the stress-activated MAP kinase Sty1/Spc1. *Genes Dev.* **12**, 1453-1463.

Walter, P., Gilmore, R. and Blobel, G. (1984). Protein translocation across the endoplasmic reticulum. *Cell* **38**, 5-8.

Wright, R., Basson, M., D'Ari, L. and Rine, J. (1988). Increased amounts of HMG-CoA reductase induce 'karmellae': a proliferation of stacked membrane pairs surrounding the yeast nucleus. *J. Cell Biol.* **107**, 101-114.

Yamanushi, T., Hirata, A., Oka, T. and Nakano, A. (1996). Characterization of yeast *sar1* temperature-sensitive mutants, which are defective in protein transport from the endoplasmic reticulum. *J. Biochem.* **120**, 452-458.