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Sce3, a suppressor of the *Schizosaccharomyces pombe* septation mutant *cdc11*, encodes a putative RNA-binding protein

Susanne Schmidt, Kay Hofmann[1] and Viesturs Simanis*

Cell Cycle Control Laboratory and [1]Biocomputing Unit, Swiss Institute for Experimental Cancer Research (ISREC), Chemin des Boveresses 155, 1066 Epalinges, Switzerland

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ABSTRACT

In the fission yeast *Schizosaccharomyces pombe*, the *cdc11* gene is required for the initiation of septum formation at the end of mitosis. The *sce3* gene was cloned as a multi-copy suppressor of the heat-sensitive mutant *cdc11-136*. When over-expressed, it rescues all mutants of *cdc11* and also a heat-sensitive allele of *cdc14*, but not the *cdc14* null mutant. Deletion shows that *sce3* is not essential for cell proliferation. It encodes a putative RNA-binding protein which shows homology to human eIF4B. Immunolocalisation indicates that Sce3p is located predominantly in the cytoplasm. Elevated expression of *sce3* increases the steady-state level of *cdc14* mRNA. Possible mechanisms of its action are discussed.

INTRODUCTION

The fission yeast *Schizosaccharomyces pombe* provides a simple eukaryotic system to study the control of cytokinesis and its co-ordination with mitosis (1). Fission yeast cells grow mainly by elongation at their tips, and divide by binary fission after forming a centrally placed septum. As in higher eukaryotes, initiation of cytokinesis is dependent upon the onset of mitosis (2). In *S.pombe*, F-actin is seen as patches or dots at sites of cell growth or division. During interphase, it is found at the growing ends of the cell, and, after the onset of mitosis, it relocates to form an equatorial ring whose position anticipates the site of septum formation (3,4). The septum grows inwards from the cell cortex at the end of mitosis, when the spindle breaks down. Secondary septa are formed on either side of the primary septum, which is then dissolved to bring about cell separation. F-actin is then relocated to the old (pre-existing) end of the cell, from where growth resumes.

Some of the proteins which control the onset of septum formation and cytokinesis in fission yeast have been identified. The products of the *arp3*, *sop2*, *cdc3*, *cdc4*, *cdc8*, *cdc12*, *cdc15* and *rng2* genes are required for actin rearrangement and/or to stabilise the actin ring (5–13). The *dmf1* protein is implicated in assuring correct positioning of the medial ring: mutants of the *dmf1*/*mid1* gene misplace the division septum at high frequency (12,14). Immunofluorescence studies have shown that the products of the *dmf1*, *cdc3*, *cdc4*, *cdc8*, *cdc12* and *cdc15* genes are associated with the medial ring (6,7,9,10,12–14).

At the end of mitosis, the Cdc7p kinase, the Spg1p GTPase and the activities of Cdc11p and Cdc14p are required for septation (5,15–17). A conditional, heat-sensitive mutation in any of these genes results in the failure to make a division septum, though F-actin ring formation is not impaired (9,17). Cells become highly elongated and multinucleate, as growth and nuclear division continue in the absence of cell division. Spg1p and Cdc7p interact directly (17), and genetic evidence suggests that *cdc11* and *cdc14* are part of the same complex responsible for regulating septum formation (18). The *plo1* kinase appears to be required for both actin ring formation and septation, and increased expression can induce septum formation from G_1 and G_2 (19). Once septation has been initiated, the products of the *cdc16* and *byr4* genes are required to limit the cell to making a single septum per cell cycle (2,20,21). Cloning and characterisation of the *cdc11* gene would further our understanding of the mechanisms responsible for the induction of septum formation, and its co-ordination with mitosis. To date, we have not been successful in cloning *cdc11*; however, we isolated several multi-copy suppressors. The characterisation of one of these, *sce3*, is presented here.

MATERIALS AND METHODS

Yeast physiological and genetic techniques

Standard techniques were used for growth, manipulation and synchronisation of fission yeast (22). Cells were grown in yeast extract (YE), malt extract (ME) or EMM2 minimal medium, supplemented as required with 50 mg/ml of adenine, uracil, leucine, histidine or lysine. The vitality stain Phloxin B (Sigma) was added to YE or EMM2 medium at a concentration of 5 mg/ml, to identify diploid or mutant cells on plates. Selection synchrony was performed as described (17), using a Beckman JS 5.0 elutriation system. Cell number was measured with a Coulter counter and the percentage of septated cells was determined by staining with Calcofluor. Other techniques have been referred to previously (9). The yeast strains used in this study are listed in Table 1. All mutants were outcrossed to wild-type at least twice before use. Crosses were performed on ME plates at 25°C, according to (23), and the resulting tetrads were dissected using a Leitz micromanipulator.

All transformations of *S.pombe* cells were performed by the lithium acetate method, unless stated otherwise (24). To induce expression of genes under the control of the thiamine repressible

*To whom correspondence should be addressed. Tel: +41 21 692 5888; Fax: +41 21 652 6933; Email: viesturs.simanis@isrec.unil.ch

nmt1 promoter, in strains carrying either a pREP vector (pREP3, pREP41, pREP81) (25,26) or the pINT5 integrated vector (as described in 16), cells were grown to mid-exponential phase in EMM2 medium containing 2 mM thiamine. They were then collected by centrifugation, washed twice in sterile water and reinoculated into EMM2 medium lacking thiamine. At 25°C, under these conditions, the promoter is induced after 12–13 h.

Table 1. List of strains used in this study

Strain	Genotype	Reference
SP0007	*leu1-32 h*$^{-}$	(5)
SP0560	*ade6M210/ade6M216 leu1-32/leu1-32 ura4D18/ura4D18 h*$^{+}$*/h*$^{-}$	This study
SP0699	*cdc11-136 leu1-32 h*$^{-}$	This study
SP0701	*cdc11-136 ura4D18 h*$^{-}$	This study
SP0763	*cdc14-118 leu1-32 h*$^{-}$	(15)
SP1387	*sce3::ura4*$^{+}$*/sce3*$^{+}$ *ade6M216/ade6M216 h*^{90} */h*$^{+}$	This study
SP1397	*sce3::ura4*$^{+}$ *h*$^{-}$	This study
SP1680	*leu1::pint5(ura4*$^{+}$*)-sce3 ura4D18 h*$^{-}$	This study

The source of each original mutant is given. Auxotrophic markers (*leu1-32*, *ura4D18*, *ade6M210* or *ade6M216*) were crossed into the backgrounds as required. Expression cassettes based upon pINT5 were introduced into backgrounds either by transformation of DNA fragments, or by appropriate genetic crosses.

Molecular biology techniques

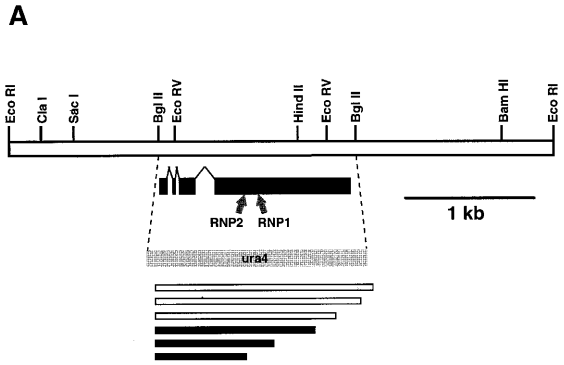
Standard methods were used throughout for DNA and RNA manipulations (27). Sequencing was performed by the dideoxy chain termination method on both strands, using the Sequenase 2.0 Kit (United States Biochemical Corp.), and Exonuclease III deletions were done with the 'Erase-a-base' Kit (Promega), according to the manufacturer's instructions. Techniques for *S.pombe* plasmid recovery and DNA and RNA extractions have been described (22). Northern and Southern blots were performed by capillary transfer onto GeneScreen Plus membranes (NEN), hybridisation and washing of the filters were done according to the manufacturer's protocol. Probes were made by random hexamer priming (27).

Cloning of the *sce3* gene

Schizosaccharomybes pombe strain SP0701, *cdc11-136 ura4D18 h*$^{-}$, was transformed to uracil prototrophy by the protoplast method (28), using a wild-type *S.pombe* genomic DNA library, based on the pUR18 vector (29). Transformants were replica-plated onto minimal medium containing Phloxin B, at 36°C. Six plasmids were rescued from colonies able to grow at the restrictive temperature. The *sce3* cDNA was isolated from a wild-type *S.pombe* cDNA library (30), by hybridisation with the *Cla*I–*Hin*dII fragment, and sequencing confirmed the presence of the three introns.

Deletion of the *sce3* gene

To create a *sce3* null allele, the *Bgl*II–*Bgl*II fragment of the genomic clone, encompassing the whole *sce3* ORF, was replaced by the *S.pombe ura4*$^{+}$ gene (Fig. 1A). This plasmid was then cut with *Eco*RI and the resulting 4.4 kb fragment was gel-purified and used to transform the diploid strain SP0560 to uracil prototrophy,



B

MGPKKSTKMSLNAFLGDESFGSTNWADDIDDLPALPQDRTTSTYRATPSS
ADAGYNAPSSTFESVRSPPESRREGGMGSGYQRDAIPIPSEPPFTAHVGN
LSFDLTENDLGDFFGEGVTSIRLVIDPLTERSRGFGYVEFETADTLSAAL
ALSGEDLMGRPVRITVAEPRRSFAREERSTGDWVRRGPLPPAEPAESPFG
KRRTNSGRFRDPARDPSDRVREEPREWVRRGPLPPRESSERPRLNLKPRS
SSNVNTEATPSATTTTSSKPKRDPFGGAKPVDNTSVLQRVEEKLAKRTQS
FRREDNANRERSTSRKPSADKAEKTDKTDAIAEKVSDIRLGDGEKKSSET
DSEVAATKTPATEDAPATNAGEAEEEEGWTKIGKGRKH*

Figure 1. Structure of *sce3* locus and sequence of Sce3p. **(A)** Representation of the *sce3* locus. Some relevant restriction sites are shown. The black bars represent the positions of the four exons of the *sce3* gene. The *Bgl*II–*Bgl*II fragment replaced by the *ura4*$^{+}$ gene to make the *sce3* null allele is also shown. The bars below the gene structure indicate subclones of *sce3* tested for their ability to complement *cdc11-136*. Open bars indicate that a clone is able to rescue, solid bars indicate that it cannot. **(B)** The predicted protein sequence of Sce3p is shown, using the one-letter code for amino acids. The residues conserved in the RNP domain are underlined. The asterisk represents a STOP codon.

creating the diploid (SP1387). Chromosomal DNA was extracted from wild-type diploid cells (SP0560), from the diploid heterozygous for the *sce3* deletion (SP1387), and from the four resulting spores, and was digested with *Eco*RI. The disruption was verified by Southern blotting, using the *ura4*$^{+}$ gene and the *Bgl*II fragment of *sce3* as probes.

Schizosaccharomyces pombe protein extracts

Protein extracts were performed using a slightly modified version of the previously described method (16). *Schizosaccharomyces pombe* cells were collected and washed in ice-cold STOP buffer (0.9% NaCl, 1 mM NaN_3, 10 mM EDTA, 50 mM NaF). Cells were lysed with 1 ml of acid washed beads (Sigma), by vortexing 2 min at 4°C, in the presence of a modified extraction buffer (60 mM β-glycerolphosphate, 25 mM MOPS pH 7.2, 15 mM $MgCl_2$, 15 mM EGTA, 1 mM DTT, 0.1 mM sodium orthovanadate, 1 mM PMSF, 20 µg/ml leupeptin, 40 µg/ml aprotinin). Concentration of proteins was determined using the BioRad Protein Assay Kit.

Production of antisera and Western blotting

The *Hin*cII–*Bgl*II C-terminal fragment of *sce3* was cloned into a *Sma*I-digested pGEX-3X plasmid to produce a GST fusion protein. This part of the protein lacks the two putative RNA-binding motifs. *Escherichia coli* DH5α cells were transformed with this construct, and induced to produce the fusion protein by addition

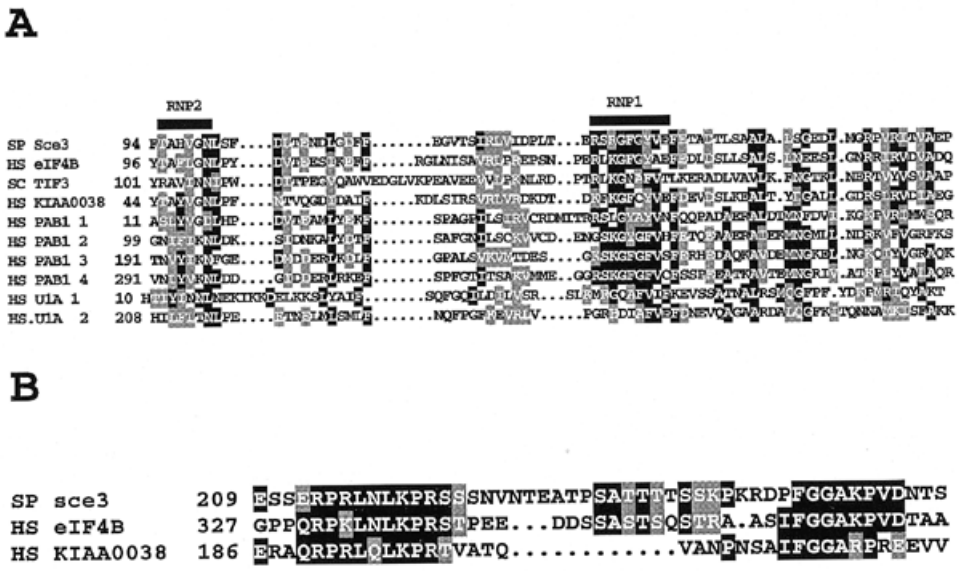


Figure 2. Alignment of Sce3p with related proteins from the sequence databases. **(A)** The single RNP domain of Sce3p is aligned with the single domain in human eIF4B, *S.cerevisiae* TIF3, human KIAA0038, the four domains of human PAB1 and the two domains of human U1A. Conserved residues are shown on a black background, while conservative substitutions are shaded in grey. Gaps were introduced as required to maximise homology. Abbreviations: SP, *S.pombe*; HS, human; SC, *S.cerevisiae*. **(B)** Homology between Sce3p, human eIF4B and KIAA0038 in the domain C-terminal to the RNP motif. Abbreviations and homologies are coded as in (A).

of IPTG to 0.1 mM. After an additional 3 h growth at 37°C, cells were collected by centrifugation. The bacterial pellet was resuspended in ice-cold PBS (phosphate-buffered saline) pH 7.2, containing 50 µg/ml leupeptin, 1% (v/v) aprotinin, 1 mM PMSF (phenylmethylsulfonyl fluoride) and 1% (v/v) Triton X-100, sonicated, and then centrifuged 10 min at 10 000 *g*. The supernatant was then passed through a glutathione–agarose column equilibrated in the same buffer, containing protease inhibitors as described. The protein was eluted from the column with 5 mM reduced glutathione. As degradation products were also eluted by this method, the proteins were finally separated on a 10% sodium dodecyl sulfate (SDS) polyacrylamide gel. Proteins were visualised by incubating the gel in ice-cold 0.25 M KCl. The fusion protein band was excised, and the protein was electroeluted in 15 mM Tris base, 72 mM glycine, 0.05% (w/v) SDS, pH 8.3.

A rabbit (New Zealand White) was injected intramuscularly at monthly intervals with 150–200 µg of protein emulsified in incomplete Freund's adjuvent. The serum was affinity purified according to standard procedures (31). SDS–PAGE and Western blots were performed onto nitrocellulose as described (31). The affinity purified *sce3* antibodies were used at 1/10 000 dilution. Bound antibodies were detected using peroxidase-coupled secondary antibodies and revealed with ECL (Amersham), or with alkaline phosphatase-coupled secondary antibodies, at a 1/7500 dilution.

Microscopy

Staining with DAPI (4′-6′ diamidino-2-phenylindol, Sigma), Calcofluor (Fluorescent Brightener N°28, Sigma) and rhodamine-conjugated phalloidin was performed as described (3). Tubulin was detected as described (32) using TAT-1 (33), diluted 1/50. The *sce3* affinity purified polyclonal antibody was used at a 1/100 dilution, after fixation of cells with 4% PFA. The secondary antibody was goat anti-mouse or goat anti-rabbit IgG, CY3 conjugated, used at a dilution of 1/800 and 1/400, respectively. Photographs were taken using a Zeiss Axiophot microscope with ×100 objective onto Kodak Tri-X Pan film and processed according to the manufacturer's protocol.

RESULTS

Cloning of the *sce3* gene

To try to clone the *cdc11* gene by complementation, the strain *cdc11-136 ura4D18* was transformed to uracil prototrophy with a wild-type *S.pombe* genomic DNA library (Materials and Methods). Colonies were allowed to form at 25°C on selective medium, replica plated to 36°C, and plasmids were recovered from colonies able to grow at the restrictive temperature. Three different genes were isolated from this screen, one of which, *sce3*, is discussed here. Hybridisation against an ordered cosmid library (34) mapped *sce3* to cosmid 16C3, close to the centromere of Chromosome II. The *cdc11* gene is known to be located near the rDNA cluster on Chromosome III, at the opposite end to *ura4*. Thus, *sce3* is a multicopy suppressor of *cdc11-136*.

Three structurally related plasmids were recovered, and one with a 5.2 kb insert was used for restriction mapping and further analysis (Fig. 1A). A combination of unidirectional exonuclease III deletions and subcloning of convenient restriction fragments was used to define a 2.1 kb region responsible for the rescue of *cdc11-136*. The gene found in this region was named *sce3* (suppressor of cdc eleven). Sequencing of the *sce3* genomic DNA and cDNA indicated the presence of three introns at the 5′ end of the coding sequence. The accession number for the DNA sequence in the EMBL database is AJ000318. *Sce3* rescues all three tested alleles of *cdc11* (*cdc11-119*, *cdc11-123*, *cdc11-136*), and also rescues the *cdc14-118* mutant but not a *cdc14* null allele. However, increased expression of *sce3* does not rescue *cdc7-24*, *cdc15-140*, *cdc16-116* or mutants of *cdc2*.

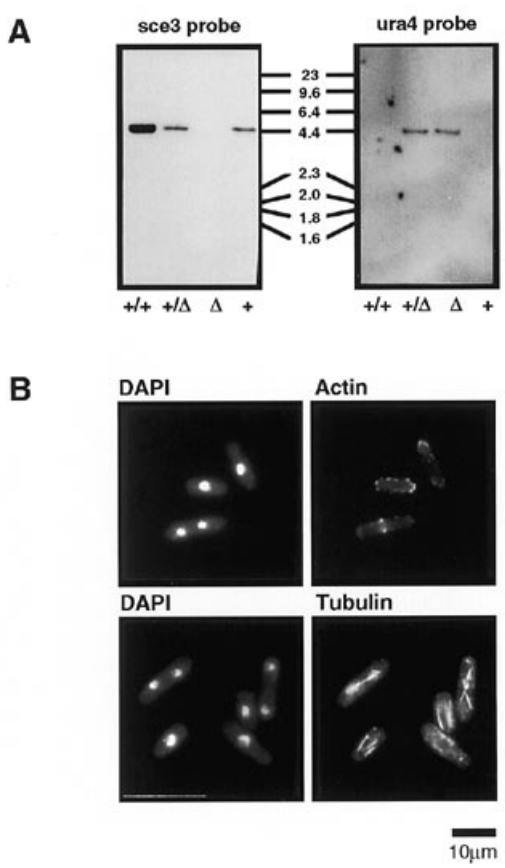


Figure 3. Deletion of *sce3* indicates that it is not an essential gene. **(A)** DNA was extracted from SP0560 (+/+), SP1387 (+/Δ), SP1397 (Δ) and SP0328 (wild-type) strains. The DNA was digested and probed with either a *sce3* probe or a *ura4* probe (as described in Materials and Methods) on a Southern blot. Note that there is no *sce3* signal in the haploid null mutant. In the blot probed with *ura4*, only the two strains carrying the deletion show a band. **(B)** Cells from strain SP1397, a haploid strain carrying the *sce3* null allele, were fixed and stained with DAPI, rhodamine conjugated phalloidin or the TAT-1 antibody.

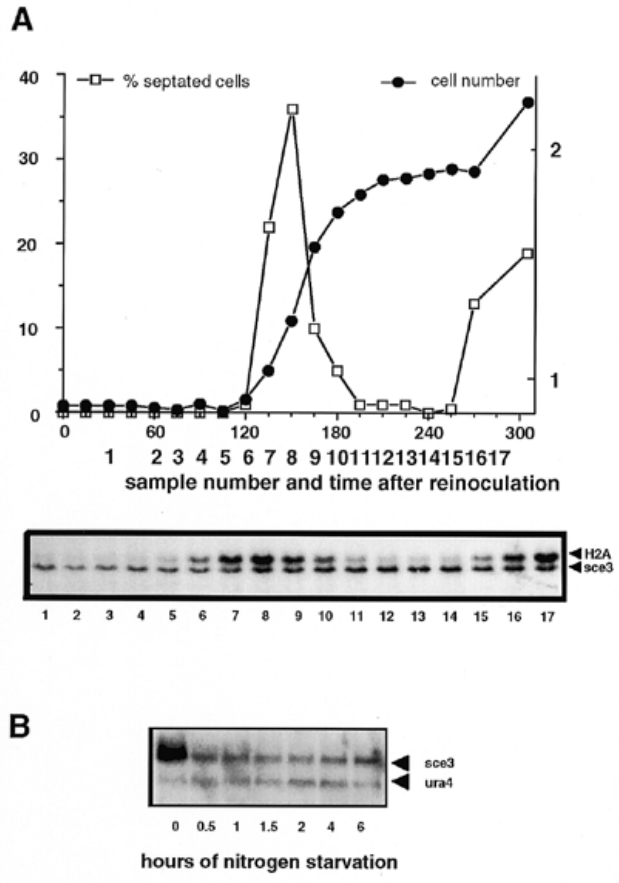


Figure 4. The *Sce3* mRNA level is constant in exponential growth, but decays rapidly in response to starvation. **(A)** Cells were synchronised by elutriation and small G_2 cells were reinoculated into fresh medium at 36°C. Samples were taken every 15 min. Cell number was determined using a Coulter Counter and the percentage of septated cells by dark field microscopy. RNA extracts were prepared at each timepoint and Northern blotted. The blot was probed either with a *sce3 Bgl*II–*Bgl*II fragment or with a fragment of H2A, as control. **(B)** Cells were grown to mid-exponential phase in minimal medium and then washed twice and reinoculated into a medium lacking nitrogen. Samples were taken every 30 min or every hour following the medium-shift. RNA was extracted and Northern blotted, and probed with a *sce3* probe [same fragment then in (A)] or with a *ura4 Hin*dIII–*Hin*dIII fragment.

The *sce3* gene encodes a protein of 388 amino acids, with a calculated molecular weight of 42680, whose sequence is shown in Figure 1B. Database comparisons revealed the presence of an RNP domain in the N-terminal region of the protein. The RNP domain is known to be involved in binding RNA. An alignment of the Sce3p RNP domain with those most similar to it is shown in Figure 2A. Overall, Sce3p is most homologous to human Initiation Factor 4b (eIF4b). In addition to the RNP domain the homology extends into the C-terminal region (Fig. 2B), which was shown, in the case of eIF4b, to be implicated in binding RNA (35,36). Interestingly, C-terminal truncations of *sce3* tested for their ability to complement a *cdc11-136* mutant (Fig. 1A) implicate this C-terminal region as important for *sce3* function, since fragments lacking it but still containing both RNP motifs are not able to rescue a *cdc11-136* strain.

Sce3 is not essential for cell proliferation

To determine whether the *sce3* gene is essential for cell proliferation, one copy of the entire coding sequence was replaced by the *ura4*$^+$ gene in a diploid strain (Fig. 1A; Materials and Methods). Correct replacement was verified by Southern blot analysis (Fig. 3A). After sporulation and tetrad dissection, all four spores in each tetrad were able to form a colony, and the *ura4*$^+$ gene segregated 2:2, indicating that *sce3* is not essential for cell division. The *sce3*::*ura4*$^+$ null mutant showed no heat or cold sensitivity when compared to an otherwise isogenic wild-type strain, and divided at a similar rate to wild-type (not shown). Staining of *sce3::ura4*$^+$ cells with rhodamine-conjugated phalloidin, to reveal the F-actin, and TAT-1, to reveal microtubules, showed that they are indistinguishable from wild-type cells. F-actin rings were clearly observed prior to septum formation, and microtubules were seen in either the typical interphase array, or as short mitotic spindles (Fig. 3B). Thus, loss of *sce3* function interferes neither with cell division, nor with the normal cytoskeletal rearrangements that occur during the cell cycle.

Double mutants between *sce3::ura4*$^+$ and other mutants implicated in septum formation were created by tetrad dissection. Genetic interactions were observed only in the case of

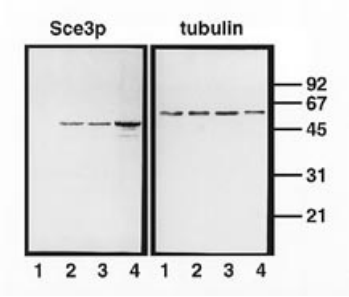


Figure 5. An antiserum to Sce3p recognises a 43 kDa protein. Proteins were extracted from either SP1397 cells (lane 1), SP0007 cells (lane 2), SP0573 cells (lane 3) or SP1680 cells (lane 4). The Western blot was probed with either affinity purified antibody to Sce3p, or with TAT-1, as loading control. Note the missing Sce3p band in the *sce3* null allele (lane 1), and the strong band in cells overexpressing (lane 4).

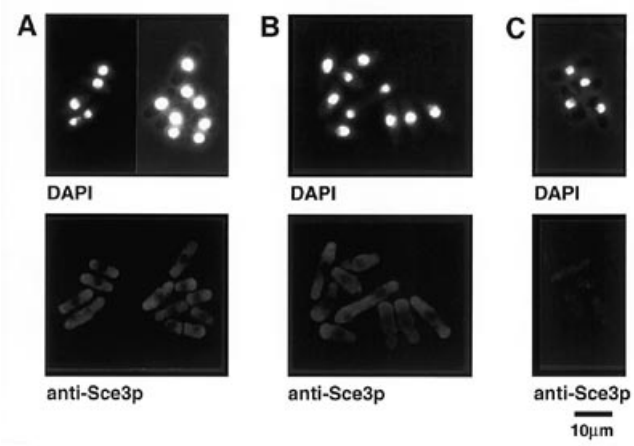


Figure 6. Sce3p is predominantly a cytoplasmic protein. **(A)** Wild-type cells were grown to mid-exponential phase in EMM2 medium, fixed and stained with DAPI and CY3 coupled anti-Sce3p antibody (Materials and Methods). Note that the localisation of Sce3p is predominantly cytoplasmic. **(B)** Cells SP1680 were grown in thiamine-free medium for 18 h at 25°C to induce Sce3p expression. Cells were treated as described in (A). **(C)** Cells deleted for *sce3* (SP1397) were processed as in (A). The absence of any signal confirms the specificity of the anti-Sce3p antibody.

sce3::ura4$^+$ *cdc11-136* and *sce3::ura4*$^+$ *cdc14-118*, where the restrictive temperature was lowered by 2–3°C as compared to each single mutant. No additive effects were observed with all other mutants tested (*cdc7-24*, *cdc15-140*, *cdc16-116*).

Sce3 mRNA level is constant in exponential growth but decays rapidly in response to nitrogen starvation

We tested if the *sce3* transcript varied in its level during the cell cycle. A culture of wild-type cells was synchronised by elutriation and small, G_2 cells were reinoculated into fresh medium. Samples were taken at the indicated times after reinoculation, RNA extracts were prepared and analysed on a Northern blot by hybridisation with a *sce3* probe. As shown in Figure 4A, the steady state level of the *sce3* mRNA does not change significantly through the cell division cycle during exponential growth, while the H2A control transcript fluctuates, as expected. In contrast, when RNA is extracted from cells entering stationary phase after shift to nitrogen-free medium, the steady-state level of *sce3* mRNA decreased very rapidly (within 30 min after shift), while the *ura4* control transcript remained constant (Fig. 4B). This abrupt change in *sce3* mRNA level suggests that its function is not required during exit from the cell cycle.

Immunolocalisation of Sce3p

To localise Sce3p in the cell, a polyclonal antibody directed against the C-terminus of the protein was produced by immunising a rabbit (Materials and Methods). After affinity purification, the antibody recognised a protein of the expected size (43 kDa) on a Western blot of protein extracts made from wild-type cells. This band was not detected in extracts from cells deleted for the *sce3* gene (Fig. 5) and was much stronger in extracts from cells containing a multicopy plasmid expressing high levels of the *sce3* gene. This confirms that the protein recognised is Sce3p.

Immunolocalisation studies were performed on wild-type cells (SP0007), cells deleted for *sce3* (SP1397) and cells overexpressing *sce3* (SP1680). This showed that Sce3p is predominantly cytoplasmic at all stages of the cell cycle both in wild-type cells (Fig. 6A) and in cells overexpressing *sce3* (Fig. 6B). No signal was seen in *sce3::ura4*$^+$ cells (Fig. 6C), confirming the specificity of the staining.

Overexpression of *sce3* in wild-type cells does not impede cell cycle progression

Wild-type cells expressing the *sce3* gene from a single integrated copy under the control of the inducible *nmt1* promoter were grown to mid-exponential phase, and shifted to thiamine-free medium for 18 h at 25°C. Cells were fixed and stained for actin and tubulin, using rhodamine-conjugated phalloidin and TAT-1 antibody. All the expected cytoskeletal rearrangements occurred normally: normal spindle and interphase microtubule configurations were observed. F-actin formed a ring at the onset of mitosis and was otherwise located at the tips of interphase cells (data not shown). Thus, cells expressing high levels of Sce3p are indistinguishable from wild-type, with respect to cytoskeletal rearrangements.

To test the effect of altering the gene dosage of *sce3* upon *cdc14* mRNA level, we prepared RNA extracts from wild-type cells, *cdc11-136* cells and *cdc14-118* cells, all of them either overexpressing *sce3*, or not, and from cells deleted for *sce3*, and analysed them on a Northern blot with a probe against *cdc14* (Fig. 7). This demonstrated that in all tested backgrounds, the level of *cdc14* mRNA was always higher in the presence of high amounts of Sce3p than in cells with wild-type levels of Sce3p. Quantitation of the bands using a Phospho-Imager revealed a 2–3-fold increase in *cdc14* mRNA level (data not shown).

DISCUSSION

The *sce3* gene was cloned as a multicopy suppressor of a *cdc11-136* mutant. It can also rescue two other alleles of *cdc11* and *cdc14-118*, but none of the other cytokinesis mutants tested. It does not rescue a *cdc14* null allele, so it does not bypass the requirement for *cdc14*. We showed that deletion of *sce3* renders cells more sensitive to the *cdc11-136* and *cdc14-118* mutations, but has no other effect by the criteria we have tested.

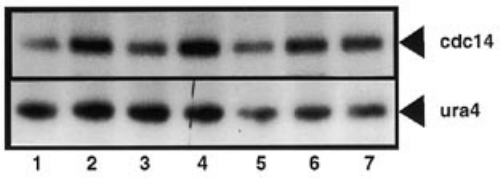


Figure 7. *Cdc14* mRNA level is increased in cells overexpressing *sce3*. RNA extracts were prepared and blotted (Materials and Methods) from the following strains (grown at 36°C): wild-type cells (SP0007) (lane 1), *cdc11-136* cells (SP0699) (lane 3) and *cdc14-118* cells (SP0763) (lane 5). Immediately adjacent to each of these lanes is the corresponding strain overexpressing *sce3* (lanes 2, 4 and 6). Lane 7 is an RNA extract from cells deleted for *sce3* (SP1397). The Northern blot was probed with a probe recognising *cdc14*, and a *ura4* probe as loading control. Note the increase in the amount of cdc14 mRNA in each lane overexpressing *sce3* (lanes 2, 4 and 6).

Database comparisons indicate that *sce3* contains one RNP motif in its N-terminal region. RNA-binding proteins often contain one or more copies of this domain (37–39). Within the RNP motif, the RNP1 and RNP2 sub-motifs play a crucial role in RNA-binding, as shown by *in vitro* mutagenesis (40,41) and the crystal structure of the domain (42). Based on this, it is likely that Sce3p will be an RNA-binding protein.

What is the biological function of Sce3p?

RNP motif containing proteins have been implicated in a wide range of biological processes. Since loss of Sce3p has no overt phenotype in an otherwise wild-type background, it is not clear in which event(s) it participates. However, the protein most homologous to Sce3p, in searches of the sequence databases, is human eIF4B, which is an RNA-binding protein that binds to, and stimulates, the RNA helicase eIF4A, thereby promoting the association of mRNA to the 40S subunit of the ribosome. The RNA-binding specificity of human eIF4B is conferred both by the residues within the RNP domain, and in a second RNA-binding domain located in the C-terminal region of the protein (35,36). The homology between eIF4B and Sce3p also extends to this second domain. Interestingly, C-terminal deletions of Sce3p that still contained both RNP1 and RNP2, but lacked this second domain, were unable to rescue the *cdc11-136* mutant, consistent with this region being important for Sce3p function. The *TIF3* gene is thought to be the *Saccharomyces cerevisiae* homologue of eIF4B (43). However, Sce3p is more closely related to eIF4B in the RNP domain, and Tif3p does not contain the second, C-terminal RNA-binding region found in *sce3* and eIF4B. In this context, it is interesting to note that *S.pombe* eIF4E also shares some features with its human counterpart that are not conserved in the equivalent gene in *S.cerevisiae* (44). Both the *TIF3* and *sce3* null mutants are viable, though the former is cold sensitive (43). It is therefore possible that Sce3p plays an eIF4B-like role in the initiation of translation. The lack of observable phenotype for the *sce3* null in a wild-type background, suggests that it is redundant with another protein for eIF4B function.

Regulation of *sce3* at the level of transcription

Exit from the cell cycle and entry into the quiescent G_0 state is an active process that is not yet well understood at the molecular level (45). During the transition from G_1 to G_0, cells down-regulate some genes required for cell cycle progression, and some metabolic genes; for example, DNA polymerase and DNA primase (46), and a subunit of ribonucleotide reductase (47), are constitutively expressed in cycling cells, but are lost upon exit to G_0. The fact that the steady-state level of the *sce3* mRNA decreases rapidly upon nitrogen starvation suggests that *sce3* function is not required upon entry into stationary phase. Examination of the 3′ untranslated region of the mRNA shows that it contains a number of U-rich tracts, which have been implicated as a feature contributing to mRNA instability (48). Whether this is essential for the rapid degradation of *sce3* mRNA in response to starvation is unknown.

How does increased expression of Sce3p rescue mutants in *cdc11* and *cdc14*?

It has been proposed that under optimal conditions for translation, mRNAs are longer-lived (49). If Sce3p functions in the initiation of translation, its role may be to facilitate the association of a subset of mRNAs, perhaps including *cdc14*, with the ribosome. It is possible that an increased level of Sce3p facilitates association of the *cdc14* mRNA with the ribosome, and thereby increases the stability of the mRNA. In the absence of any feedback regulation of the *cdc14* mRNA, a decrease in the rate of degradation will result in an increase in its steady state level. Alternatively, Sce3p may bind the *cdc14* mRNA directly, and prevent its degradation in a manner independent of translation. In both cases, the net result will be an increase in the amount of Cdc14p. Since it is known that *cdc14-118* has considerable residual activity at the non-permissive temperature (5), it is possible that a small increase in the level of mutant protein provides sufficient activity to permit cell division. In the absence of Sce3p the level of *cdc14* mRNA does not change, but *cdc14-118* mutant cells have a reduced restrictive temperature: it is possible that the *cdc14* mRNA is translated less efficiently and so the level of the mutant protein decreases, reducing the total amount of *cdc14* activity, and rendering the cells more heat sensitive. Whether Sce3p also affects the level of *cdc11* mRNA awaits cloning of the *cdc11* gene.

It is clear that there is some specificity in the suppression of cell cycle mutants by *sce3*, since it has no effect upon other leaky mutants, such as *cdc7*, or upon *cdc2* mutants. In the latter case, two human RNP motif containing proteins (Scr2 and Scr3) were isolated as suppressors of *S.pombe cdc2* and *cdc13* mutations (50). Interestingly, these were also proposed to function by facilitating the translation of the mutant protein.

Studies of *S.cerevisiae* have shown that another factor implicated in translation initiation can be a suppressor of conditional mutants. *DED1* encodes an RNA helicase implicated in the initiation of translation (51). A mutant allele of DED1 has been identified as a suppressor of a splicing mutant (52), and increased expression of DED1 can suppress the growth defect of an RNA polymerase III mutant (53).

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