but do not bind to collagen type I (ref. 7); LFA-1 ligand binding avidity is modulated through the β-integrin subunit[17]; modulation of platelet integrin GPIIb-IIIa activity[18] and the decrease in integrin function during keratinocyte differentiation[19].

Loss of CAR activity could be a key early step in tumour invasion and metastatis, causing loss of differentiation induction or tumour cell release from cell or extracellular matrix attachments. The findings that SW620, the metastasis-derived line, displays lower binding to extracellular matrix than does SW480, its non-metastasis-derived partner from the same patient, and furthermore, that adhesiveness of SW620 to extracellular matrix is enhanced when transfected with *CAR*, support this notion. In addition, high levels (45–55%) of allelic loss on 16q, in the region of *CAR*, have been demonstrated in breast and prostatic cancers, possibly associated with later stage metastastic tumours[20–22]. *CAR* is therefore a candidate tumour suppressor gene. □

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SEC65 gene product is a subunit of the yeast signal recognition particle required for its integrity

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PROTEIN targeting to the endoplasmic reticulum (ER) in mammalian cells is catalysed by the signal recognition particle (SRP), which consists of six protein subunits and an RNA subunit[1,2]. *Saccharomyces cerevisiae* SRP is a 16S particle, of which only two subunits have been identified: a protein subunit, SRP54p, which is homologous to the mammalian SRP54 subunit, and an RNA subunit, scR1 (ref. 3). The *sec65-1* mutant yeast cells[4] are temperature-sensitive for growth and defective in the translocation of several secreted and membrane-bound proteins. The DNA sequence of the SEC65 gene suggests that its product is related to mammalian SRP19 subunit and may have a similar function[5].

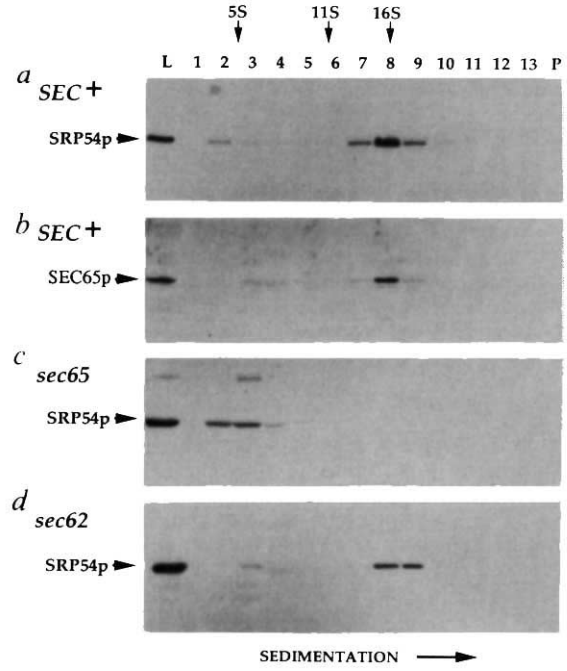

FIG. 1 SEC65p cosediments with *S. cerevisiae* SRP. Crude cell extracts prepared from wild-type (*a* and *b*), *sec65-1* (*c*) or *sec62-1* cells (*d*) were layered over a 5–20% sucrose gradient and centrifuged for 15 h at 40,000 r.p.m. Fractions from the gradient (1–13), load (L) and pellet (P) were analysed, after SDS-PAGE, on western blots developed with either affinity-purified anti-SRP54p (ref. 3; *a, c, d*) or anti-SEC65p (ref. 5; *b*). Western blot analysis of sucrose gradient fractions from a wild-type strain isogenic to *sec65-1* developed with anti-SRP54p were indistinguishable from the blot shown in *b* (data not shown). Sedimentation values of protein standards are indicated.
METHODS. The yeast cell extracts and sucrose gradients were prepared as previously described[3]. The *sec65-1* and *sec62-1* cell extracts were prepared from cells grown at 24 °C. Bound antibodies were visualized on the blots by ^{125}I-labelled second antibody (*a, c* and *d*) or ECL (Amersham; *b*). Anti-SEC65p was raised against a fusion protein[5].

Here we show that SEC65p is a subunit of the *S. cerevisiae* SRP and that it is required for the stable association of another subunit, SRP54p, with SRP. Overexpression of SRP54p suppresses both growth and protein translocation defects in *sec65-1* mutant cells.

To determine whether SEC65p is a component of the *S. cerevisiae* SRP, we first fractionated total cell extracts prepared from wild-type cells by sucrose-density centrifugation. Gradient fractions were analysed on western blots developed with anti-

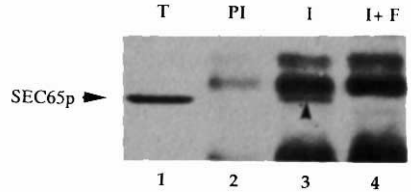

FIG. 2 SEC65p coimmunoprecipitates with SRP54p. Total yeast cell extracts (T, lane 1) and the products of native immunoprecipitations from these extracts, performed with either preimmune serum (PI, lane 2) or anti-SRP54p immune sera in the absence (I, lane 3) or presence of SRP54p-glutathione transferase fusion protein (I+F, lane 4), were examined on western blots developed with anti-SEC65p. Arrowhead indicates SEC65p (lane 3).
METHODS. Native immunoprecipitations were done from 100 μl of crude cell extract (protein concentration ~30 mg ml^{-1}) using Protein-A/Sepharose complexed with anti-SRP54p[3]. The western blot spans a relative molecular mass range of 40,000 to 27,000. Background bands are derived from the heavy and light chains of the first antibody.

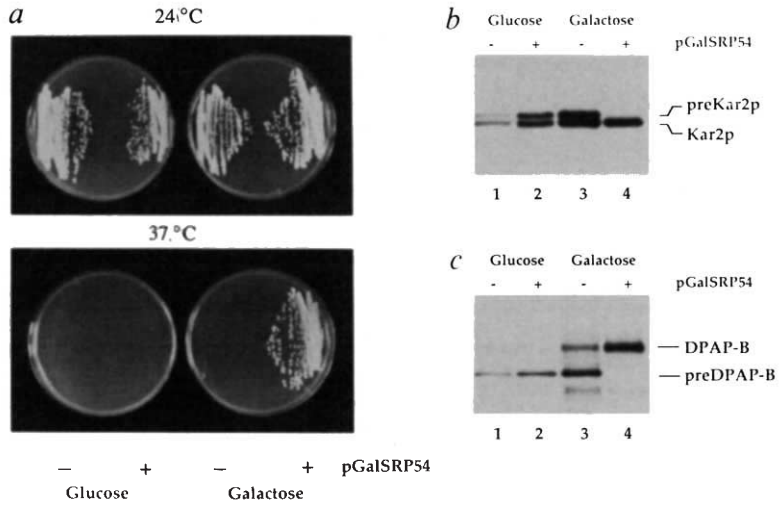

FIG. 3 Overexpression of SRP54p supresses growth and translocation defects of *sec65-1* cells. *a*, Untransformed *sec65-1* cells or cells transformed with pGalSRP54 (ref. 3) were streaked on either YEP-Dex or YEP-Gal plates and grown at either 24 °C or 37 °C. The growth rate of cells containing the plasmid on YEP-Gal was comparable to that of the isogenic wild-type strain (data not shown). *b*, *c*, After growth for 1 h at the nonpermissive temperature (37 °C), untransformed *sec65-1* cells (lanes 1, 3) or cells transformed with pGalSRP54 (lanes 2, 4) were pulse-labelled in either glucose- (lanes 1, 2) or galactose-containing media (lanes 3, 4). Cell extracts were prepared and non-native immunoprecipitations were done using anti-Kar2p (*b*) or anti-DPAP-B (*c*) antibodies. Mature (Kar2p, DPAP-B) and precursor (preKar2p, preDPAP-B) forms of Kar2p and dipeptidyl aminopeptidase-B are indicated.

METHODS. *a*, The sec65-1 cells were transformed using the lithium acetate method[9]. *b*, *c*, The *sec65-1* cell were grown to log phase in YEP-Dex or YEP-Gal liquid medium at 24 °C, shifted to 37 °C for 45 min and pelleted. Cells were resuspended in prewarmed minimal medium plus supplements, and grown for an additional 15 min at 37 °C. Pulse-labelling was done with ^{35}S-methionine (ICN) for 10 min. Cell extraction and non-native immunoprecipitation was performed as described[3].

bodies raised against SRP54p (Fig. 1*a*) and SEC65p (Fig. 1*b*). SEC65p cosediments with SRP54p, which is consistent with the notion that both proteins are components of the same particle. To demonstrate directly that both proteins are SRP subunits, we immunoprecipitated SRP under native conditions with anti-SRP54p. We analysed the precipitated fraction on western blots developed with anti-SEC65p antibodies. SEC65p is immunoprecipitated with anti-SRP45p serum (Fig. 2, lane 3), but not with preimmune serum (lane 2). An excess of SRP54p (added as a fusion protein) prevented precipitation of SEC65p (Fig. 2, lane 4).

Mammalian SRP19 is required for the association of SRP54 with SRP RNA[6–8]. We therefore asked whether SRP is physically altered in *sec65-1* cells. Cell extracts prepared from *sec65-1* cells were fractionated on sucrose-density gradients and the fractions analysed on western blots developed with anti-SRP54p antibodies. The bulk of SRP54p was seen to sediment as an apparently monomeric protein near the top of the gradient (Fig. 1*c*). This effect was not a consequence of presecretory protein accumulation in the mutant cells as SRP54p remained part of a 16S particle in cell extracts prepared from another secretory mutant strain, *sec62-1* (Fig. 1*d*). In western blots of extracts of *sec65-1* cells, the concentration of SEC65p was diminished and after sucrose gradient fractionation the protein could no longer be detected (data not shown). This is consistent with the finding that the concentration of the mutant protein is reduced after a shift to 37 °C (ref. 5). We conclude that in *sec65-1* cells the interaction of SRP54p with the rest of the particle has been disrupted. SEC65p is therefore required for the integrity of SRP.

We next looked for genetic interactions between these two SRP subunits. Overexpression of SRP54p supressed the temperature-sensitive growth phenotype of *sec65-1* cells (Fig. 3*a*). The *sec65-1* cells containing a plasmid-borne copy of the *SRP54* gene under the control of the *GAL1* promoter (pGalSRP54) were able to grow at 37 °C, the nonpermissive temperature, on galactose but not on glucose-containing media. These results are supported by the independent isolation of a multicopy suppressor of the *sec65-1* defect, which has been identified as *SRP54* (ref. 4).

If the overproduction of SRP54p acts directly to rescue *sec65-1* cell growth at 37 °C, then the protein translocation defect in these cells should also be suppressed. To test if this is so, we immunoprecipitated proteins that use of the endoplasmic reticulum translocation system from cell extracts of *sec65-1* cells. Precursor forms of both a soluble protein, Kar2p, and a membrane protein, DPAP-B, were detected in *sec65-1* cells which did not harbour pGalSRP54 (Fig. 3*b*, *c*; lanes 1, 3) as well as in *sec65-1* cells transformed with pGalSRP54 and grown on glucose (Fig. 3*b*, *c*; lane 2). In cells overexpressing SRP54p, however, the untranslocated precursor forms of the proteins were no longer detected (Fig. 3*b*, *c*; lane 4). The overexpression of SRP54p, therefore, efficiently supresses both the growth defect and the protein translocation defect of *sec65-1* cells.

These results agree with biochemical studies of mammalian SRP, which suggest that SRP19 and SRP54 interact during SRP assembly. Because the binding of mammalian SRP54 to SRP RNA requires the presence of SRP19, it has been proposed that SRP19 functions to stabilize a conformation of SRP RNA that forms a high affinity binding site for SRP54 (refs 7, 8). Similarly, yeast SEC65p could function to stabilize a conformation of scR1 that contains a high-affinity binding site for SRP54p. We have shown that the affinity of SRP54p for the rest of SRP is reduced in *sec65-1* cells. High concentrations of SRP54p could drive the formation of a functional SRP by promoting binding of SRP54p to a reduced-affinity binding site and consequently suppress the *sec65-1* mutant phenotype. Two observations[5], however, indicate that the interaction between SEC65p and SRP54p may be more complex. First, the overproduction of SRP54p can stabilize mutant SEC65p in *sec65-1* cells, and second, such overproduction appears insufficient to bypass a requirement for SEC65p in cells in which SEC65 has been deleted. Thus the assembly of SRP54p onto scR1 appears to require SEC65p, and SEC65p and SRP54p could interact directly and/or cooperatively in SRP. □

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