

FIG. 4 PAC1 inhibits a MAP kinase signal transduction pathway leading to increased gene expression. The effect of PAC1 on the SRE-mediated expression of a luciferase reporter gene was examined using a transient transfection assay. The reporter plasmid pfos-Tk-Luc contains *c-fos* enhancer sequences including the SRE element (nucleotides -357 to -276) cloned upstream of a fragment of the thymidine kinase promoter (nucleotides -200 to 70) and the firefly luciferase gene.

**METHODS.** Transient transfections of CV-1 cells were done as described<sup>23</sup>. Cells were transfected with 2 µg of a luciferase reporter plasmid (pfos-Tk-Luc), 1 µg of a control plasmid (pCH110) expressing  $\beta$ -galactosidase activity, 1 µg of the ERK2 expression plasmid pCMV-p41<sup>mapk</sup>, and 1 µg of the PAC1 expression plasmid pMT2-PAC1 (ref. 8). Total DNA in the transfection was maintained at 10 µg using pUC13 as carrier DNA. Some cells (black bars) were stimulated with 10 ng ml<sup>-1</sup> PMA. The cells were collected 48 h after transfection and the extracts obtained were assayed for  $\beta$ -galactosidase and luciferase activity<sup>23</sup>. The data are presented as the activity ratio of luciferase (light units per mg protein) and  $\beta$ -galactosidase (absorbance units per min per mg protein) (mean  $\pm$  s.d.,  $n=3$ ).

tion of endogenous PAC1 protein in Jurkat, and that ERK2 phosphorylation and activity in G6 cells are modulated by levels of PAC1 that are substantially lower than those of transiently transfected COS cells.

PAC1 is a nuclear phosphatase that is likely to act upon MAP kinases that are translocated into the nucleus following activation<sup>6,8</sup>. To examine the effect of PAC1 expression on a nuclear event linked *in vivo* to MAP kinase activation, we used a transient transfection assay that measures the transcriptional activity of the *c-Fos* serum response element (SRE). In transiently transfected CV-1 cells, SRE-regulated reporter gene expression increased ~2-fold following PMA treatment. This PMA-stimulated expression was eliminated in the presence of constitutive PAC1 (Fig. 4). Transfection with ERK2 increased the basal SRE-mediated reporter gene expression, and this was augmented by PMA treatment. PAC1 coexpression eliminated the increased basal and PMA-stimulated SRE activity resulting from ERK2 expression. These results show that increases in SRE activity resulting from PMA treatment or from increased ERK2 expression and activation are inhibited *in vivo* by PAC1. Although the specific nuclear events that act upon the SRE are not directly addressed by these experiments, a PMA-stimulated, MAP kinase-mediated pathway involving the SRF/Elk-1 ternary complex has been described<sup>12,15</sup>.

A mitogen-induced phosphatase in fibroblasts, 3CH134/CL100, has been described<sup>11,16</sup>. 3CH134 has ~80% similarity with the carboxy-terminal 15K catalytic domain of PAC1, whereas the amino-terminal region of each protein (corresponding to potential regulatory domains) share ~30% similarity<sup>11,16</sup>. PAC1 is maximally expressed in haematopoietic tissues, whereas 3CH134 is expressed predominantly in several other tissues<sup>8,16</sup>. 3CH134 has been shown to dephosphorylate ERK1 and ERK2 *in vitro*<sup>10,17,18</sup> and to dephosphorylate ERK1 and ERK2 in transiently transfected COS cells<sup>19</sup>. The presence of more than one ERK phos-

phatase that can be distinctly regulated represents a means by which pleiotropic kinase activation could be differentially modulated. □

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- Ruderman, J. V. *Curr. Opin. Cell Biol.* **5**, 207-213 (1993).
- Nishida, E. & Gotoh, Y. *Trends biochem. Sci.* **18**, 128-131 (1993).
- Ahn, N. G., Seger, R. & Krebs, E. G. *Curr. Opin. Cell Biol.* **4**, 992-999 (1992).
- Gonzalez, F. A. et al. *J. Cell Biol.* **122**, 1089-1101 (1993).
- Alvarez, E. et al. *J. Biol. Chem.* **266**, 15277-15285 (1991).
- Chen, R., Sarnecki, C. & Blenis, J. *Molec. cell. Biol.* **12**, 915-927 (1992).
- Davis, R. J. *J. Biol. Chem.* **268**, 14553-14556 (1993).
- Rohan, P. J. et al. *Science* **259**, 1763-1766 (1993).
- Payne, D. M. et al. *EMBO J.* **10**, 885-892 (1991).
- Zheng, C.-F. & Guan, K.-L. *J. Biol. Chem.* **268**, 16116-16119 (1993).
- Keyse, S. M. & Emslie, E. A. *Nature* **359**, 644-647 (1992).
- Marais, R., Wynne, J. & Treisman, R. *Cell* **73**, 381-393 (1993).
- Hill, C. S. et al. *Cell* **73**, 395-406 (1993).
- Graham, R. & Gilman, M. *Science* **251**, 189-192 (1991).
- Gille, H., Sharrocks, A. D. & Shaw, P. E. *Nature* **358**, 414-417 (1992).
- Charles, C. H., Abler, A. S. & Lau, L. F. *Oncogene* **7**, 187-190 (1992).
- Alessi, D. R., Smythe, C. & Keyse, S. M. *Oncogene* **8**, 2015-2020 (1993).
- Charles, C. H., Sun, H., Lau, L. F. & Tonks, N. K. *Proc. natn. Acad. Sci. U.S.A.* **90**, 5292-5296 (1993).
- Sun, H., Charles, C. H., Lau, L. F. & Tonks, N. K. *Cell* **75**, 487-493 (1993).
- Laemmli, U. K. *Nature* **227**, 680-685 (1970).
- Siegel, J. N. in *Current Protocols in Immunology* (eds Coligan, J. E., Kruisbeek, A. M., Margulies, D. H., Shevach, E. M. & Strober, W.) 11.2.4-11.2.17 (Wiley, New York, 1991).
- Kameshita, I. & Fujisawa, H. *Analyt. Biochem.* **183**, 139-143 (1989).
- Seth, A., Gonzalez, F. A., Gupta, S., Raden, D. L. & Davis, R. J. *J. Biol. Chem.* **267**, 24796-24804 (1992).
- Northwood, I. C., Gonzalez, F. A., Wartmann, M., Raden, D. L. & Davis, R. J. *J. Biol. Chem.* **266**, 15266-15276 (1991).
- Harlow, E. & Lane, D. *Antibodies. A Laboratory Manual* 1-726 (Cold Spring Harbor Laboratory Press, New York, 1988).

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## Evolutionary conservation of components of the protein translocation complex

Enno Hartmann, Thomas Sommer, Siegfried Prehn\*, Dirk Görlich†, Stefan Jentsch‡ & Tom A. Rapoport§

Max-Delbrück Centre for Molecular Medicine, Robert-Rössle Strasse 10, 12135 Berlin-Buch, Germany

\* Institute for Biochemistry, Humboldt-University Berlin, Hessische Strasse 3-4, 10115 Berlin, Germany

‡ ZMBH, Center for Molecular Biology, University Heidelberg, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany

**PROTEIN translocation into the mammalian endoplasmic reticulum requires the Sec61p complex, which consists of three membrane proteins<sup>1</sup>. The  $\alpha$ -subunit, the homologue of Sec61p of yeast<sup>2-4</sup>, shows some similarity to SecYp<sup>5</sup>, a key component of the protein export apparatus of bacteria<sup>6,7</sup>. In *Escherichia coli*, SecYp is also associated with two other proteins (SecEp and band-1 protein)<sup>8,9</sup>. We have now determined the sequences of the  $\beta$ - and  $\gamma$ -subunits of the mammalian Sec61p complex. Sec61- $\gamma$  is homologous to SSS1p, a suppressor of *sec61* mutants in *Saccharomyces cerevisiae*, and can functionally replace it in yeast cells. Moreover, Sec61- $\gamma$  and SSS1p are structurally related to SecEp of *E. coli* and to putative homologues in various other bacteria. At least two subunits of the Sec61/SecYp complex therefore seem to be key components of the protein translocation apparatus in all classes of organisms.**

The Sec61p complex was purified from canine pancreatic microsomes<sup>1</sup>. Partial amino-acid sequences were determined for

† Present address: Wellcome/CRC Institute, Tennis Court Road, Cambridge CB2 1QR, UK.

§ To whom correspondence should be addressed.

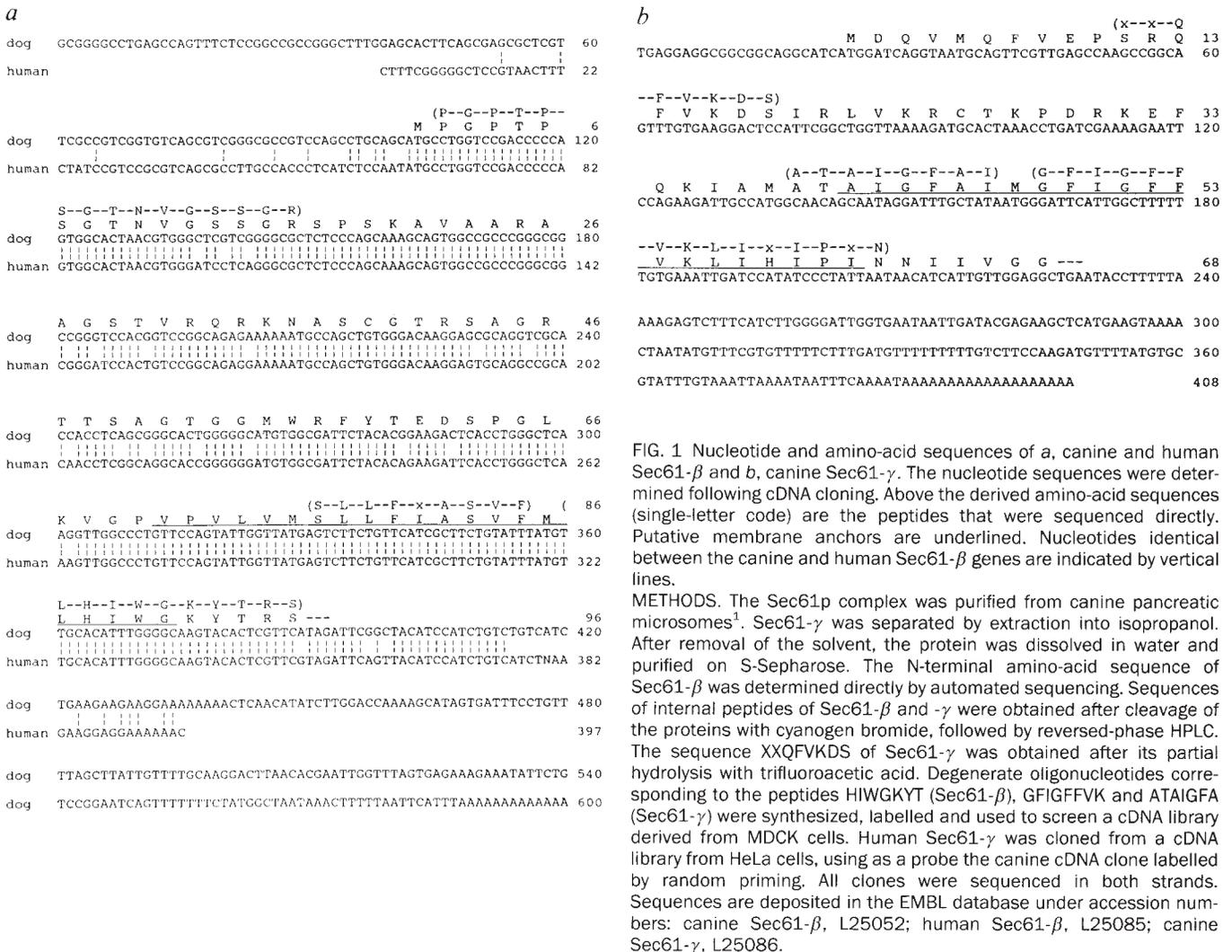


FIG. 1 Nucleotide and amino-acid sequences of *a*, canine and human Sec61- $\beta$  and *b*, canine Sec61- $\gamma$ . The nucleotide sequences were determined following cDNA cloning. Above the derived amino-acid sequences (single-letter code) are the peptides that were sequenced directly. Putative membrane anchors are underlined. Nucleotides identical between the canine and human Sec61- $\beta$  genes are indicated by vertical lines.

**METHODS.** The Sec61p complex was purified from canine pancreatic microsomes<sup>1</sup>. Sec61- $\gamma$  was separated by extraction into isopropanol. After removal of the solvent, the protein was dissolved in water and purified on S-Sepharose. The N-terminal amino-acid sequence of Sec61- $\beta$  was determined directly by automated sequencing. Sequences of internal peptides of Sec61- $\beta$  and - $\gamma$  were obtained after cleavage of the proteins with cyanogen bromide, followed by reversed-phase HPLC. The sequence XXQFVKDS of Sec61- $\gamma$  was obtained after its partial hydrolysis with trifluoroacetic acid. Degenerate oligonucleotides corresponding to the peptides HIWGWKYT (Sec61- $\beta$ ), GFIFFFVK and ATAIGFA (Sec61- $\gamma$ ) were synthesized, labelled and used to screen a cDNA library derived from MDCK cells. Human Sec61- $\gamma$  was cloned from a cDNA library from HeLa cells, using as a probe the canine cDNA clone labelled by random priming. All clones were sequenced in both strands. Sequences are deposited in the EMBL database under accession numbers: canine Sec61- $\beta$ , L25052; human Sec61- $\beta$ , L25085; canine Sec61- $\gamma$ , L25086.

the  $\beta$ - and  $\gamma$ -subunits and the corresponding complementary DNAs were cloned. The cDNAs of canine and human Sec61- $\beta$  and of canine Sec61- $\gamma$  code for amino-acid sequences that contain the expected peptides (Fig. 1*a, b*). The predicted sizes of the proteins ( $M_r$ s 10,000 and 7,500) agree reasonably well with those determined by SDS-PAGE electrophoresis (14,000 and 8,000  $M_r$ , respectively)<sup>1</sup>. Both proteins are predicted to span the membrane once, with a hydrophobic segment close to the C terminus, and have their N terminus located in the cytoplasm. They seem to belong to a class of 'tail-anchored' membrane proteins<sup>10</sup> which includes the v- and t-SNAREs implicated in vesicular transport<sup>11</sup>.

A homologue of the mammalian Sec61- $\beta$  was found in the GenBank database in the plant *Arabidopsis thaliana* (35% identical amino acids; Fig. 2*a*). Sec61- $\gamma$  is similar to a protein of rice (72% identical amino acids; Fig. 2*b*) and to partial sequences of cDNAs of mice and *Caenorhabditis elegans* (data not shown). Of particular interest is the identity of 45% amino acids between Sec61- $\gamma$  and SSS1p (Fig. 2*b*), a suppressor of *sec61* temperature-sensitive (*ts*) mutants in *S. cerevisiae* (ref. 12, and T.S. and S.J., unpublished results) which is essential for protein translocation and for the viability of yeast cells<sup>12</sup>. Thus, yeast also seems to have a Sec61p complex that includes homologues to the mammalian Sec61- $\alpha$  and Sec61- $\gamma$ .

Homologues of Sec61- $\gamma$  were also found in prokaryotes. A sequence in the archaeobacterium *Sulfolobus solfataricus* contains 20% identical amino acids (Fig. 2*b*), and all Sec61- $\gamma$  sequences are related to SecEp of *E. coli* and SecEp-like sequences of various other bacteria (Fig. 2*c*). The homologies among the

members of the Sec61- $\gamma$ /SSS1/SecEp family are statistically significant as analysed with a multiple alignment computer program (Fig. 2 legend). The gene for the Sec61- $\gamma$  homologue of *S. solfataricus* is found in a similar arrangement on the genome as the *secE* genes of eubacteria, preceding sequences homologous to the transcription factor nusG and to the ribosomal proteins L11 and L1 of *E. coli*. The conserved region of *E. coli* SecEp is limited to the third putative membrane-spanning segment and the region immediately preceding it, exactly the domain that is essential for its function in protein translocation<sup>13</sup>. The putative SecEp homologues of other bacteria do not contain the preceding, non-essential sequences.

We have determined whether the mammalian Sec61- $\gamma$  can functionally replace the yeast protein SSS1p *in vivo*. A diploid, heterozygous null mutant of *S. cerevisiae* was constructed in which one copy of *SSS1* was deleted ( $\Delta$ *sssl/SSS1*). A multicopy plasmid was then introduced which carries the gene for the mammalian Sec61- $\gamma$  under the control of the GAL promoter which can be induced by galactose and repressed by glucose. Tetrad analysis of this strain on glucose medium produced only two viable spores, confirming that *SSS1* is an essential gene<sup>12</sup>. Haploid cells carrying the deletion ( $\Delta$ *sssl*) but containing the plasmid were viable on galactose (Fig. 3*a*, left) but not on glucose (Fig. 3*a*, right), indicating that lack of SSS1p can be compensated for by expression of Sec61- $\gamma$ . The generation time on

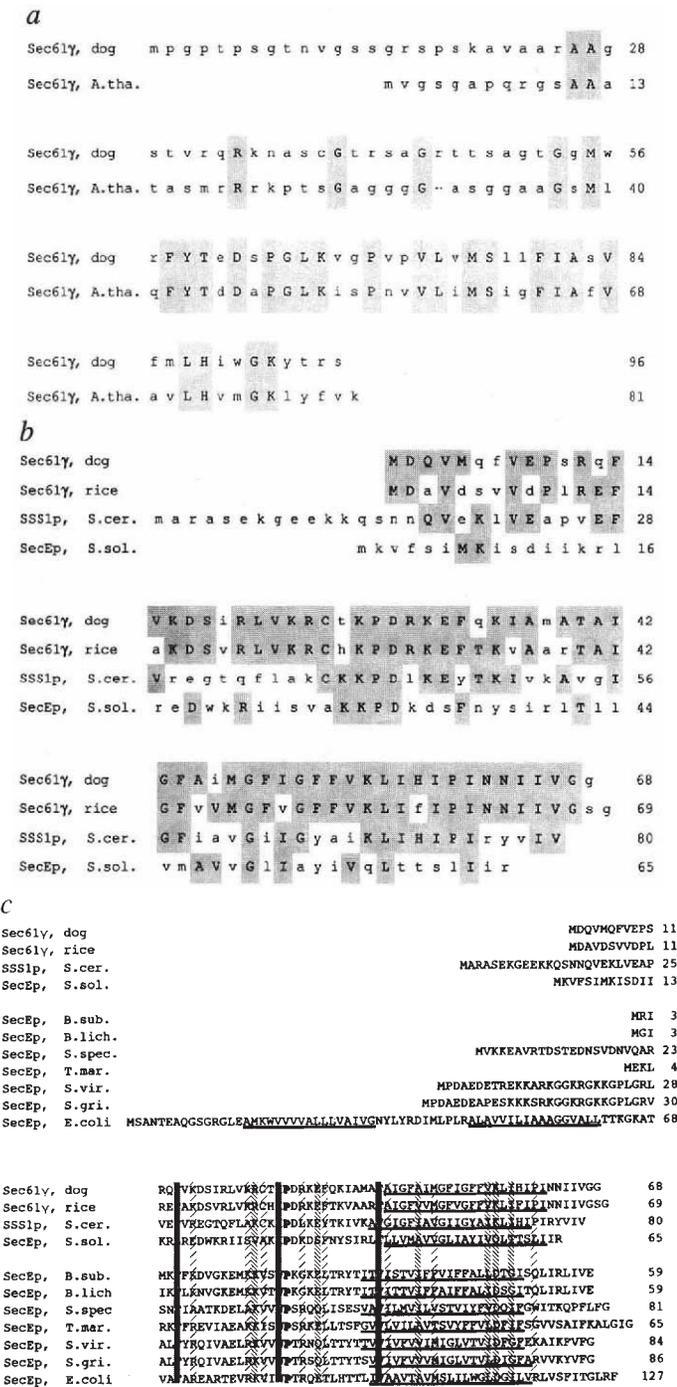


FIG. 2 Homologues of Sec61-β and Sec61-γ detected in the database. **a**, Comparison of the sequence of canine Sec61-β with that of its homologue in the plant *A. thaliana*. Identical residues are indicated in grey. **b**, Comparison of the sequence of canine Sec61-γ with that of its eukaryotic and archaeobacterial homologues. **c**, Multiple alignment of the eukaryotic/archaeobacterial Sec61-γ sequences with the eubacterial SecEp sequences. Positions of conservation are indicated by a hatched background, with the dark shading corresponding to those at which the fewest amino-acid exchanges occur. The conserved proline is indicated in bold. **METHODS.** The search for homologous sequences was made using TBLASTN<sup>21</sup> and TFASTA<sup>22</sup>. The following entries in GenBank were found: Sec61-β, *A. thal.*, ATTS1834 (*A. thaliana*) (the sequence of the clone in the database was redetermined and corrected); Sec61-γ, rice, RIC1124A; SSS1p, *S. cer.*, YSCAFR (*S. cerevisiae*); SecEp, *S. sol.*, SSDOCK (*S. solfataricus*); SecEp, *B. sub.*, BACSHRBPNS (*Bacillus subtilis*); SecEp, *B. lich.*, BACSIGH (*Bacillus licheniformis*); SecEp, *S. spec.*, SPMULTG (*Synechocystis spec.*); SecEp, *T. mar.*, TMNUSG (*Thermogata maritima*); SecEp, *S. vir.*, STMVBRA (*Staphylococcus virsini*); SecEp,

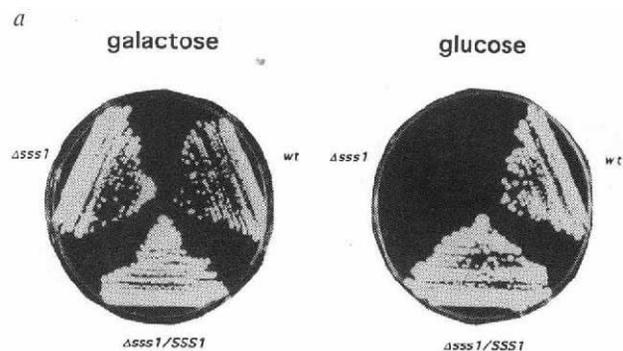
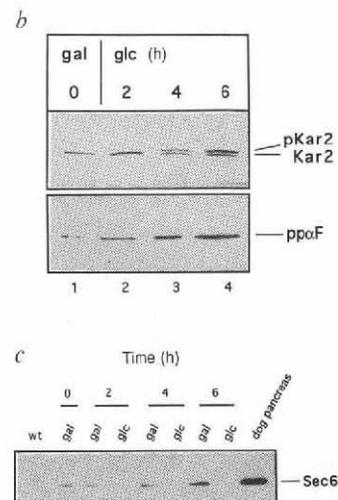


FIG. 3 Sec61-γ can functionally replace SSS1p in *S. cerevisiae*. **a**, A diploid, heterozygous deletion mutant ( $\Delta$ sss1/SSS1), a haploid wild type (wt) and a haploid deletion mutant ( $\Delta$ sss1), all carrying a multicopy plasmid with the mammalian SEC61-γ-gene under control of the GAL10 promoter, were tested for growth at 30 °C for 3 days on either galactose or glucose. **b**, A culture of  $\Delta$ sss1 cells was grown at 30 °C on galactose (gal) minimal medium to express Sec61-γ from a plasmid, and then shifted to glucose (glc). At the times indicated, the cells were labelled with <sup>35</sup>S-methionine for 10 min, proteins were extracted and immunoprecipitated with antibodies against KAR2 or  $\alpha$ -factor. The labelled proteins were separated by SDS-PAGE (12 and 18% acrylamide, respectively) and visualized by fluorography. pKAR2, ppaF, precursors to KAR2 and pro- $\alpha$ -factor, respectively. **c**, To follow the level of Sec61-γ, an experiment parallel to that in **b** was carried out, except that one portion of the cells continued growth on galactose. At different time points, cells were broken and crude membrane fractions were prepared, analysed by SDS-PAGE (7.5–17% acrylamide) and immunoblotted with antibodies specific for Sec61-γ. Positive and negative controls were carried out with membrane preparations from canine pancreas and wild-type yeast cells.



METHODS. The SSS1 gene was isolated by searching for multicopy suppressors of the sec61-2 ts-mutation (T.S. & S.J. unpublished data). The gene was disrupted by deleting the coding region between nucleotides -20-109 and replacing it by a 2.4-kb fragment carrying the ADE2 marker gene. A linearized plasmid containing  $\Delta$ sss1::ADE2 was transformed into a diploid wild-type strain (YTX69 *mat a/a*, homozygous *his3-11, -15, leu2-3, -112, trp1-1, ura3-1, ade2-1, can1-100*), resulting in a heterozygous mutant strain (YTX77,  $\Delta$ sss1::ADE2/SSS1). The complete coding region of Sec61-γ was amplified by the polymerase chain reaction at nucleotides 1-354 using specific oligonucleotide primers. The fragment was cloned behind the GAL10 promoter and the entire expression cassette was moved into the plasmid pRS424 (*TRP1*, 2  $\mu$ m)<sup>25</sup>. Tetrad analysis, immunoprecipitation and immunoblot analysis were done<sup>5,26</sup>. Antibodies against Sec61-γ were raised<sup>5</sup> against a synthetic peptide corresponding to the first ten amino acids.

*S. gri.*, SGNUSG (*Staphylococcus griseus*); SecEp, *E. coli*, ECOSECE (*E. coli*). A putative homologue of Sec61-β was also found in *S. solfataricus* (GenBank entry: SSORNPB). Partial sequences homologous to Sec61-γ found in the database: Sec61-γ mouse, MUSTUMSEQD; Sec61-γ, *C. elegans*, T01535; SecEp, *Thermus thermophilus*, S49804S1. Multiple alignment of the Sec61-γ/SecEp sequences was carried out using CLUSTAL<sup>23</sup>. The statistical significance of the homologies was analysed with MACAW<sup>24</sup> using only one representative of highly related sequences and choosing as search lengths those of the individual proteins. The probability that the homology occurs by chance was estimated to be  $3.3 \times 10^{-16}$ .

galactose of the complemented  $\Delta$ sss1 strain was approximately equal to that of the wild type.

We also tested whether the translocation defect, which occurs upon depletion of SSS1p from yeast cells and is indicated by the accumulation of precursors of exported proteins<sup>12</sup>, can be reversed by expression of Sec61- $\gamma$ . Little accumulation of precursors to  $\alpha$ -factor or Kar2p (BiP) was observed if the expression of Sec61- $\gamma$  was induced in the presence of galactose (Fig. 3b, lane 1), indicating that the export of these proteins from the cytoplasm was close to normal. After shift to glucose, a severe defect of translocation developed (Fig. 3b, lanes 2–4), concomitant with the depletion of Sec61- $\gamma$  from the cells (Fig. 3c, lanes 4, 6, 8). After 6 h, the cells ceased to grow (data not shown).

Signal sequences are similarly structured and exchangeable<sup>14</sup> and one mechanism by which they are recognized—that involving the signal recognition particle—exists in all organisms<sup>15–19</sup>. It is now clear that the component mediating the actual membrane passage of a polypeptide, the Sec61/SecYp complex, is also ubiquitous: two of its subunits, Sec61- $\alpha$ /SecYp and Sec61- $\gamma$ /SSS1/SecEp, have been found in mammals, yeast and bacteria and, where tested, they are associated with each other. It remains to be seen whether Sec61- $\beta$ , which so far has been found only in plants, also exists in yeast and whether it is related to the band-1 protein of the *E. coli* SecYp complex.

It is likely that Sec61- $\alpha$ /SecYp forms a channel that guides polypeptides across the membrane<sup>5,20</sup>. Such a channel would be gated in two dimensions: perpendicular to the plane of the membrane to let polypeptides across, and horizontally within the membrane to release membrane-spanning regions of membrane proteins into the phospholipid bilayer and perhaps to let signal sequences in. Although the function of Sec61- $\gamma$ /SSS1/SecEp is unknown, it may be involved in the second gating mechanism. Such a function would be consistent with the fact that this essential polypeptide, containing little more than a membrane-spanning region and a few adjacent residues, has remained separate from its multi-spanning partner during evolution. □

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- Görllich, D. & Rapoport, T. A. *Cell* **75**, 615–630 (1993).
- Deshaies, R. J. & Schekman, R. *J. Cell Biol.* **105**, 633–645 (1987).
- Rothblatt, J. A., Deshaies, R. J., Sanders, S. L., Daum, G. & Schekman, R. *J. Cell Biol.* **109**, 2641–2652 (1989).
- Stirling, C. J., Rothblatt, J., Hosobuchi, M., Deshaies, R. & Schekman, R. *Molec. Biol. Cell* **3**, 129–142 (1992).
- Görllich, D., Prehn, S., Hartmann, E., Kalies, K. U. & Rapoport, T. A. *Cell* **71**, 489–503 (1992).
- Ito, K. et al. *Cell* **32**, 789–797 (1983).
- Schatz, P. J. & Beckwith, J. A. *Rev. Genet.* **24**, 215–248 (1990).
- Brundage, L., Hendrick, J. P., Schiebel, E., Driessen, A. J. M. & Wickner, W. *Cell* **62**, 649–657 (1990).
- Akimaru, J., Matsuyama, S. I., Tokuda, H. & Mizushima, S. *Proc. natn. Acad. Sci. U.S.A.* **88**, 6545–6549 (1991).
- Kutay, U., Hartmann, E. & Rapoport, T. A. *Trends Cell Biol.* **3**, 72–75 (1993).
- Söllner, T. et al. *Nature* **362**, 318–324 (1993).
- Esnault, Y., Blondel, M.-O., Deshaies, R. J., Schekman, R. & Kepes, F. *EMBO J.* **12**, 4083–4093 (1993).
- Schatz, P. J., Bieker, K. L., Ottemann, K. M., Silhavy, T. J. & Beckwith, J. *EMBO J.* **10**, 1749–1757 (1991).
- von Heijne, G. *J. molec. Biol.* **184**, 99–105 (1985).
- Poritz, M. A. et al. *Science* **250**, 1111–1117 (1990).
- Ribes, V., Romisch, K., Giner, A., Dobberstein, B. & Tollervy, D. *Cell* **63**, 591–600 (1990).
- Phillips, G. J. & Silhavy, T. J. *Nature* **359**, 744–746 (1992).
- Hann, B. C. & Walter, P. *Cell* **67**, 131–144 (1991).
- Ogg, S. C., Poritz, M. A. & Walter, P. *Molec. Biol. Cell* **3**, 895–911 (1992).
- Joly, J. C. & Wickner, W. *EMBO J.* **12**, 255–263 (1993).
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. *J. molec. Biol.* **215**, 403–410 (1990).
- Pearson, W. R. *Meth. Enzym.* **183**, 63–98 (1990).
- Higgins, D. G. & Sharp, P. M. *Comput. Appl. Biosci.* **5**, 151–153 (1989).
- Schuler, G. D., Altschul, S. F. & Lipman, D. J. *Proteins Struct. Funct. Genet.* **9**, 180–190 (1991).
- Christiansen, T. W., Sikorski, R. S., Dante, H., Shero, J. H. & Hieter, P. *Gene* **110**, 119–122 (1992).
- Guthrie, C. & Fink, G. R. (eds) *Guide to Yeast Genetics and Molecular Biology* (Academic, San Diego, 1991).

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## Interaction of *E. coli* Ffh/4.5S ribonucleoprotein and FtsY mimics that of mammalian signal recognition particle and its receptor

Joshua D. Miller, Harris D. Bernstein\* & Peter Walter†

Department of Biochemistry and Biophysics, University of California Medical School, San Francisco, California 94143-0448, USA

THE mechanism of protein translocation across the endoplasmic reticulum membrane of eukaryotic cells and the plasma membrane of prokaryotic cells are thought to be evolutionarily related<sup>1–7</sup>. Protein targeting to the eukaryotic translocation apparatus is mediated by the signal recognition particle (SRP), a cytosolic ribonucleoprotein, and the SRP receptor, an endoplasmic reticulum membrane protein<sup>8,9</sup>. During targeting, the 54K SRP subunit (*M*<sub>r</sub> 54,000; SRP54), a GTP-binding protein<sup>10–12</sup>, binds to signal sequences<sup>13,14</sup> and then interacts with the  $\alpha$ -subunit of the SRP receptor (SR $\alpha$ ), another GTP-binding protein<sup>12,15</sup>. Two proteins from *Escherichia coli*, Ffh and FtsY, structurally resemble SRP54 and SR $\alpha$ <sup>10,11,16</sup>. Like SRP54, Ffh is a subunit of a cytosolic ribonucleoprotein that also contains the *E. coli* 4.5S RNA<sup>17,18</sup>. Although there is genetic and biochemical evidence that the *E. coli* Ffh/4.5S ribonucleoprotein has an SRP-like function<sup>19–21</sup>, there is no evidence for an SR $\alpha$ -like role for FtsY. Here we show that the Ffh/4.5S ribonucleoprotein binds tightly to FtsY in a GTP-dependent manner. This interaction results in the stimulation of GTP hydrolysis which can be inhibited by synthetic signal peptides. These properties mimic those of mammalian SRP and its receptor, suggesting that the *E. coli* Ffh/4.5S ribonucleoprotein and FtsY have functions in protein targeting that are similar to those of their mammalian counterparts.

To test for an interaction between the Ffh/4.5S ribonucleoprotein (RNP) and FtsY, Ffh and 4.5S RNA were purified from overproducing strains and reconstituted into an RNP. FtsY was purified as a fusion protein with glutathione *S*-transferase (FtsY-GST) and immobilized on a glutathione-affinity resin, which was then incubated with Ffh/4.5S RNP in the presence of either GDP or the non-hydrolysable GTP analogue GMP-PNP. Nucleotide was included in these reactions because both Ffh and FtsY contain GTP-binding domains which might regulate their interaction. More than 90% of the Ffh co-fractionated with the FtsY-GST resin in the presence of GMP-PNP; most of the Ffh was recovered in the supernatant fraction in the presence of GDP (Fig. 1). Similarly, most of the Ffh was recovered in the supernatant fraction in the presence of the non-hydrolysable ATP analogue AMP-PNP (not shown), indicating that the interaction requires guanosine triphosphate. Free Ffh protein failed to bind to FtsY-GST resin even in the presence of GMP-PNP (Fig. 1), indicating that FtsY binding requires both Ffh and 4.5S RNA. Neither Ffh/4.5S RNP nor free Ffh bound to a control resin containing immobilized GST, indicating that binding is specific for FtsY (Fig. 1).

Interestingly, no binding was observed when the reaction shown in Fig. 1 was performed with GTP instead of GMP-PNP (not shown), indicating that GTP might be hydrolysed during the reaction. We therefore analysed the ability of these components to hydrolyse GTP. As shown in Fig. 2, purified Ffh hydro-

\* Present address: Genetics and Biochemistry Branch/NIDDK, National Institutes of Health, Building 10, Room 9D-15, Bethesda, Maryland 20892, USA.

† To whom correspondence should be addressed.