Supporting Information

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FLAG-Dap2	MDY**K**DDDD**K**EGGEEEVERIPDELFDT**KKK**HLLD**K**LIRVGIILVLLIWGTVLLL**K**SIPHHSNTPDYQEP
FLAG-Dap2TM	MDY**K**DDDD**K**EGGEEEVERIPDELFDT**KKK**HLLD**K**LIRVGIILEHP**K**SGTDSSL**K**SIPHHSNTPDYQEP
	*
FLAG-Dap2α	MDY**K**DDDD**K**EGGEEEVERIPDELFDT**KKK**HLLD**K**LIRFLLE**K**EL**K**YFG**K**ALEN**K**SIPHHSNTPDYQEP

Fig. S1. N-terminal 60 aa of Dap2, Dap2TM, and Dap2α. The FLAG tag (blue) that was inserted between the Met (1) and Glu (2) of Dap2, Dap2TM, and Dap2α. The transmembrane signal anchor sequence localized between amino acids 30 and 45 (1) is shown in red. Lysines which are amenable for chemical crosslinking are bold, and the positions at that the amber codon was introduced for photocross-linking are marked by an asterisk.

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Fig. S2. Experimental design to quantify the occupation of RNCs carrying specific nascent chains with SRP and other RPBs. (*A*) We have used a homologous yeast translation system to generate RNCs carrying nascent chains of a specific length by using truncated mRNAs lacking a stop codon in the translation reactions (2). The ribosome halts at the end of the truncated mRNA, and the nascent chain remains bound to the ribosomes as a peptidyl-tRNA. The only source of ribosomes, SRP, and other RPBs in the reactions was yeast translation extract, which contains a crude mixture of all soluble yeast proteins. Addition of purified SRP or other RPBs was omitted to maintain the relative stoichiometry of RPBs and ribosomes during translation and analyze samples that closely approximate the in vivo situation. In the course of the study nascent N-terminally FLAG-tagged versions of Dap2 or Pgk1 were used to pull down RNCs and associated factors. This method isolates those RNCs that carry a FLAG-tagged nascent chain. Empty ribosomes or RNCs involved in the translation of residual internal mRNAs remain in the supernatant. This approach allowed us to quantify the binding of SRP and other RPBs to RNCs with a single type of nascent chain by immunoblotting of the material isolated via the affinity matrix. (*B*) Experimental setup for a typical quantification reaction. In the experiment shown, RNCs carrying nascent FLAG-tagged (+FLAG) or non-tagged (−FLAG) Dap2, Dap2TM, or Dap2α of 54 aa were generated and analyzed for the binding of SRP. To that end, translation reactions (each 75 μL) were performed at 20 °C for 80 min and were terminated by the addition of cycloheximide to a final concentration of 200 μg/mL. Translation reactions were then added to 40 μL of anti-FLAG M2 affinity gel (αFLAG-beads, Sigma) resuspended in 500 μL of IP-buffer (20 mM Hepes-KOH, pH 7.4, 150 mM potassium acetate, 2 mM magnesium acetate, 50 μg/mL trypsin inhibitor, 1 mM PMSF, protease inhibitor mix: 1.25 μg/mL leupeptin, 0.75 μg/mL antipain, 0.25 μg/mL chymostatin, 0.25 μg/mL elastinal, 5 μg/mL pepstatin A). Reactions were incubated for 4 h at 4 °C on a shaker. Beads were separated from the supernatant and were twice washed with 500 μL of ice-cold IP-buffer. Washed αFLAG beads were incubated in SDS/PAGE sample buffer for 10 min at 95 °C and aliquots of the samples and of Rps9a and Srp54 were run on 10% Tris-Tricine gels, transferred to PVDF membrane and analyzed by immunoblotting using αRps9a and αSrp54 (2, 3). Signals obtained from the same exposure of a single gel are boxed. In case of the standard, numbers above the lanes give the amount of the respective purified protein loaded to the gel in picomoles. To determine the amount of RNCs and SRP isolated, the background signals (−FLAG) were subtracted from the signals obtained for the respective FLAG-tagged RNCs. Signals of the standard proteins were used to generate the standard curve shown on the right. Resulting values for Rps9a/b were divided by the factor 0.7 corresponding to the previously determined Rps9a/b deviation form the mean value of the 4 ribosomal standard proteins, Rps9a, Asc1, Rpl39, and Rpl17a (3). Purified His_6-Rps9a was supplemented with total yeast lysate as a carrier to avoid protein loss. Tagged and nontagged versions of Rps9a were clearly separated on SDS/PAGE. (*C*) Standard curves for the quantification of Srp54, αNAC, Ssb1, and Nat1 on RNCs carrying nascent Dap2-120. The experiments were performed as described in *B*. The immunoblots used to generate the standard curves are shown in Fig. 1*D*.

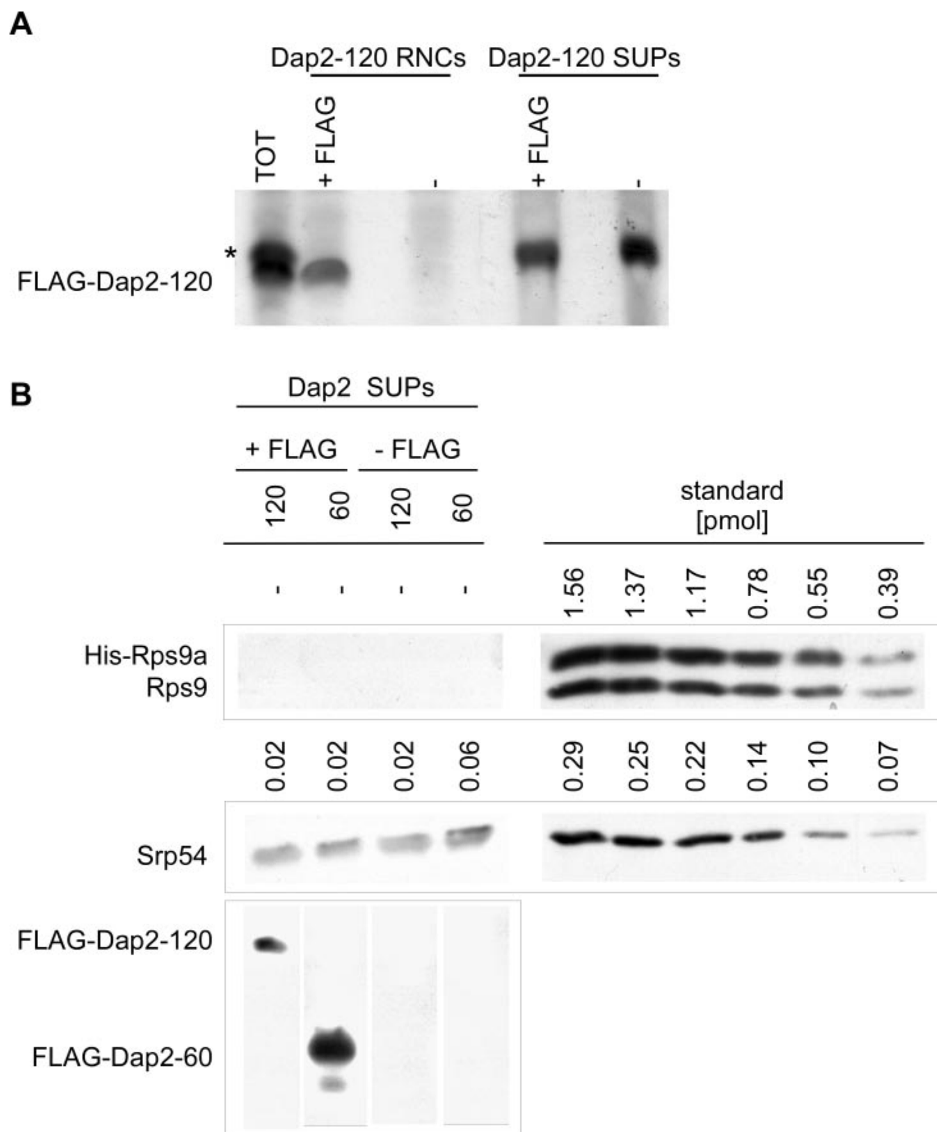

Fig. S3. SRP binds to FLAG-tagged chains only when they are attached to ribosomes as nascent chains. To test whether FLAG-tagged chains containing a SA were able by themselves to interact with SRP in our experimental set up we examined nascent chains after their release from ribosomes. SRP was coisolated together with neither FLAG-Dap2-120 nor FLAG-Dap2-60. This observation is in agreement with earlier data indicating that an SRP interacts with a signal sequence only in the context of the ribosome (4). (*A*) FLAG-tagged nascent polypeptides are quantitatively bound to ribosomes. After a translation reaction primed with FLAG-tagged Dap2-120 (+FLAG) or without the addition of mRNA (−) ribosomes and RNCs were isolated via a low-salt sucrose cushion as described (3). Half of the total reaction (TOT), the supernatant after centrifugation (SUPs), and the ribosomal pellet (RNCs) was analyzed by immunostaining with αFLAG antibody. The asterisk marks an αFLAG cross-reactive yeast protein that runs just above FLAG-Dap2-120 and remains in the supernatant after affinity purification on αFLAG-beads. (*B*) SRP does not bind to FLAG-tagged Dap2 released from ribosomes. FLAG-Dap2–120 or FLAG-Dap2-60 (+FLAG) released from ribosomes after in vitro translation were separated from RNCs and ribosomes by centrifugation through a low-salt sucrose cushion. Supernatants (Dap2 SUPs) were applied to native immunoprecipitation using αFLAG beads. The material recovered on αFLAG beads and purified standard proteins were analyzed by immunoblotting using αRps9, αSrp54, and αFLAG antibodies. Signals obtained from the same exposure of a single gel are boxed. In case of the standard, numbers above the lanes give the amount of the respective purified protein loaded to the gel in pmol. The background derived from an identically treated sample containing untagged Dap2–120 and Dap2–60 (- FLAG) was subtracted. FLAG-Dap2-120 and FLAG-Dap2-60 (+FLAG) isolated under these conditions were free of ribosomes or SRP.

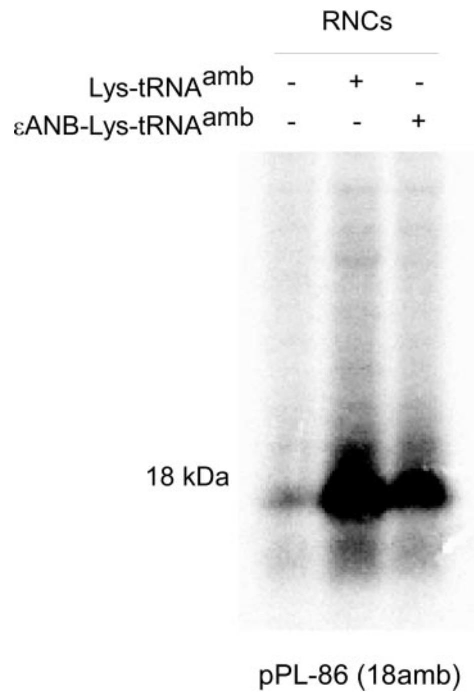

Fig. S4. Site-specific cross-linking in a homologous yeast translation system. Previous studies employing eukaryotic RNCs for site-specific cross-linking were based on either wheat germ translation (e.g., refs. 5 and 6) or reticulocyte lysate translation (e.g., ref. 7). Here, we have used a yeast translation system for the incorporation of a photo probe into a nascent chain. εANB-Lys-tRNA^{amb} was prepared as described (5, 6, 8, 9). We initially tested the incorporation of Lys-tRNA^{amb} and the εANB-Lys-tRNA^{amb} in pPL-86 (18 amb) lacking a stop codon (9). Translation was performed as described in *Materials and Methods* without addition of a suppressor tRNA, in presence of Lys-tRNA^{amb}, or in the presence of εANB-Lys-tRNA^{amb}. Lysine and εANB-lysine were effectively incorporated at the position of the amber codon and recovered in the ribosomal pellet after centrifugation through a sucrose cushion.

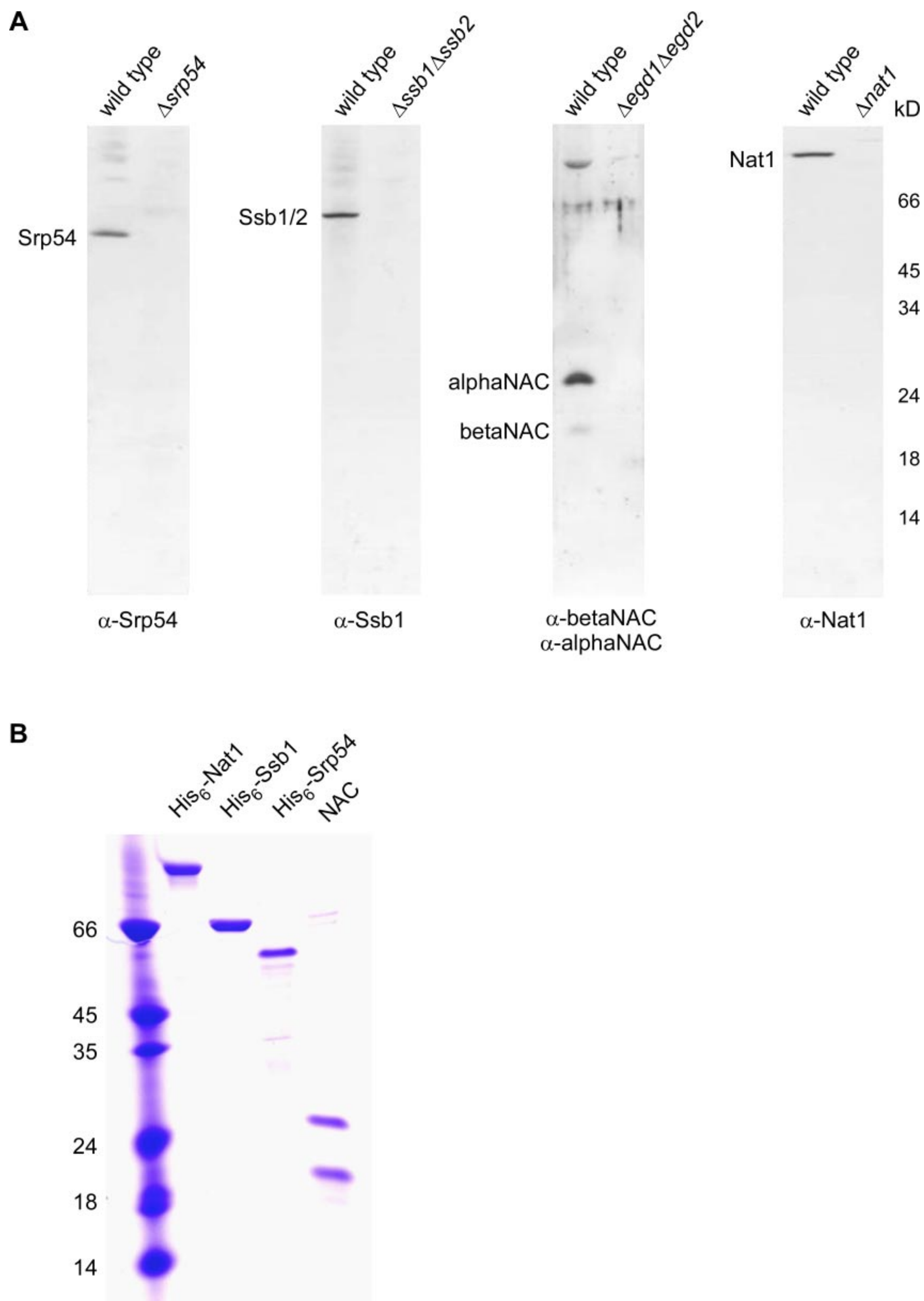

Fig. S5. Proteins and antibodies used for quantitative immunoblotting. (*A*) Polyclonal rabbit antibodies used in the pull-down experiments were tested for their specificity on total yeast extract derived either from wild-type or the respective deletion strains. Immunoblots were developed with ECL as described in *Materials and Methods*. (*B*) Purified proteins used for quantification of RPBs in the immunoblots. Shown is a Coomassie-stained gel with 2 μg of each of the standard protein. Nat1, Ssb1, and Srp54 were expressed as N-terminally His-tagged versions in *Escherichia coli* (3). NAC was purified from yeast (2).