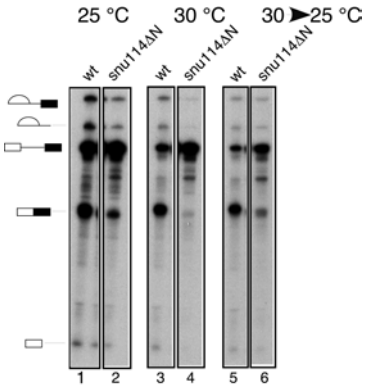
Supplementary data

The *in vitro* splicing defect observed at 30 °C (non-permissive temperature), is reversed by cooling to 25 °C

The temperature dependent *in vitro* pre-mRNA splicing defect was observed using two conditions. Both conditions could be used independently to obtain the results shown in Fig. 4A and 5. Protocol I; the inactivation in high K-PO_4 according to (Xie et al., 1998), leads to a specific, irreversible inactivation of snu114ΔN. The extracts were incubated in 120 mM K-PO_4 at 35 °C prior to the splicing reaction. The splicing reactions after thermal inactivation were performed for 25 minutes at 25 °C, under standard splicing conditions containing 60 mM K-PO_4. Protocol II; the inactivation according to (Kuhn et al., 1999), leads to a specific, reversible inactivation of snu114ΔN. In this case the splicing reactions were performed using standard splicing conditions but at higher temperature (30°C instead of 25°C).

Fig. S1 shows an example of splicing inactivation using protocol II. Splicing reactions were performed with wildtype and snu114ΔN extracts at 25 and at 30 °C (lanes 1-4). At 25 °C the splicing activity of the mutant extract is only slightly less than that of the wildtype (compare lane 2 and 1). However, while splicing at 30 °C is considerably decreased in the snu114ΔN extract, it is only barely reduced in the wildtype (compare lane 4 and 3). Nevertheless, this blockage of splicing in the mutant is reversed, although not completely, upon shifting the reaction from 30 back to 25 °C (lane 6), indicating that using these conditions the high temperature does not lead to a dead-end splicing reaction but rather to a reaction that is inhibited at 30 °C and can be reactivated by lowering the temperature.

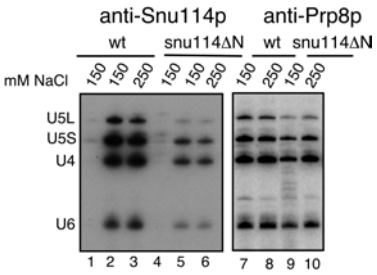


Supplementary Fig. 1. The *in vitro* splicing defect observed at 30 °C (non-permissive temperature), is reversed by cooling to 25 °C. Splicing reactions, including the pre-mRNA, were performed with wildtype and snu114ΔN extracts at 25 °C (lanes 1 and 2) and at 30 °C (lanes 3-6). After 25 minutes of incubation at 30 °C, aliquots of the reaction were shifted to 25 °C for 25 minutes (lanes 5 and 6). Indicated is the identity of the ^{32}P-labeled RNA-species (from top to bottom): intron-lariat-exon 2 intermediate, excised lariat-intron, pre-mRNA, mature mRNA and cleaved exon 1 intermediate.

snu114ΔN associates with tri-snRNP particles

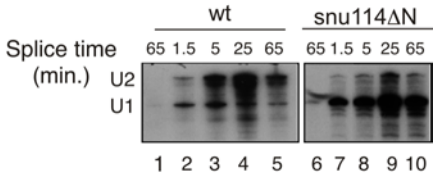
To understand whether the splicing defect observed with snu114ΔN extracts is due to loss of the deletion mutant protein from tri-snRNP particles and/or due to insufficient levels of properly formed tri-snRNP, we investigated wildtype and mutant tri-snRNPs by immunoprecipitation (Fig. S2). After immunoprecipitation, all of the reactions were washed with 150 or 250 mM NaCl. Subsequently, the precipitated RNAs were analysed by northern blotting using hybridisation with U6, U4 and U5 probes. Fig. S2 shows that snu114ΔN, although lacking ~130 amino acids, associates with the tri-snRNP particle, as determined by co-immunoprecipitation of all three RNAs by the anti-Snu114p antibodies (lanes 5 and 6). This immunoprecipitation is less efficient than with wildtype tri-snRNPs (lanes 2 and 3). This may be due to the limited recognition region that the anti-Snu114p antibody encounters in the deletion mutant snu114ΔN. The antibody we used in this experiment is directed against a mixture of three peptides, one of which encompasses 40 amino acids of the

N-terminus that are missing in snu114ΔN. Indeed, using an additional tri-snRNP antibody, anti-Prp8p, we show that the the tri-snRNP is immunoprecipitated to a good level from the deletion mutant extract (compare lanes 9 and 10 with lanes 7 and 8).



Supplementary Fig. 2. snu114ΔN associates with tri-snRNP particles.
Immunoprecipitation experiments were performed with anti-Snu114p (lanes 2-6) or anti-Prp8p antibodies (lanes 7-10), using either wildtype (lanes 2, 3 and 7, 8) or snu114ΔN extracts (lanes 5, 6 and 9, 10). In lanes 1 and 4 the non-immuneserum was used. After immunoprecipitation all of the reactions were washed with 150 or 250 mM NaCl. The RNA content of the precipitates was assayed by northern blot analysis using the U6, U4 and U5 probes indicated on the left.

Snu114p participates in spliceosome activation



Supplementary Fig. 3. Lighter exposure of the U1 and U2 RNAs shown in Fig. 4A. The level of the U1 RNA decreases between 25 and 65 minutes in the wild type (lane 4 and 5), while a time-dependent increase of the U1 RNA is seen in snu114ΔN extracts (lane 9 and 10).

Supplementary Methods

Antibody and immunoprecipitations

A rabbit was immunised with three peptides spanning positions 17-56, 507-546 and 969-1008 of Snu114p. The polyclonal antiserum obtained was specific for Snu114p, as tested by western blot analysis of purified yeast snRNPs and Snu114p. Protein-A-Sepharose antibody conjugates were prepared using 7 µl (anti-Snu114p or

NIS) or 2.5 µl (α-Prp8p) antibody per sample. Splicing extracts (400µg of protein), thermally inactivated or not, were incubated in a volume of 500 µl NET2-75 with 25 µl of antibody conjugates for 2 hours at 4 °C. The beads were washed with NET2-150 or 250. The co-precipitated RNAs were analysed by northern blot using radiolabelled probes specific for U4, U5 and U6 RNA.

Mutagenesis

To delete the N-terminus from Snu114p, a PCR fragment of 300 nucleotides was obtained from a plasmid containing the promoter and the ATG codon of *SNU114*. The PCR product was cloned into *pRS314/SNU114*. This construct gives a Snu114p protein lacking 128 amino acids at the N-terminus. Plasmid *pRS314/snu114ΔN* was sequenced and transformed into strain YPF8 having the wild-type Snu114 gene in plasmid *pRS316* (*URA3* marker) (Fabrizio et al., 1997). The plasmid-shuffling strategy was used. Selection was performed by several rounds of growth at 25 °C in a medium lacking tryptophan. Cells lacking the *URA3* plasmid were selected by growth on 5-FOA plates; cells that survived were grown up on rich medium and their growth phenotypes were analysed. A good temperature-sensitive strain was one which did not grow on –Ura plates at any temperature, and which grew in rich medium at 25 °C but not at 30 or 37 °C. Strain YPF36 [Matα, *trp1-Δ1; his3-Δ; ura3-52; lys2-801; ade2-101; snu114Δ::HIS, TRP1- pRS314/snu114ΔN*, Flu tag C-term, *ARS*, *CEN*], grew at 17, 25, 30 °C and died at 37 °C.

References

Fabrizio, P., Laggerbauer, B., Lauber, J., Lane, W.S. and Lührmann, R. (1997) An evolutionarily conserved U5 snRNP-specific protein is a GTP-binding factor closely related to the ribosomal translocase EF-2. *EMBO J.*, **16**, 4092-4106.

Kuhn, A.N., Li, Z. and Brow, D.A. (1999) Splicing factor Prp8 governs U4/U6 RNA unwinding during activation of the spliceosome. *Mol. Cell*, **3**, 65-75.

Xie, J., Beickman, K., Otte, E. and Rymond, B.C. (1998) Progression through the spliceosome cycle requires Prp38p function for U4/U6 snRNA dissociation. *EMBO J.*, **17**, 2938-2946.