SUMMARY

Removal of introns from pre-mRNA is an essential step of gene expression. The splicing reaction is catalyzed by a large RNA-protein complex termed the spliceosome. The splicing process is conserved from yeast to mammals. We chose the budding yeast, *Saccharomyces cerevisiae*, as a model system to study pre-mRNA splicing.

Introns are recognized during the early steps of spliceosome assembly with the formation of commitment complexes. This is mediated by the interaction of RNA and protein factors with conserved sequences in the pre-mRNA. The splicing factor BBP/SF1 participates in this recognition by interacting with the pre-mRNA branchpoint. This protein, which is essential in yeast, also interacts with the $U2AF^{65}$/Mud2p splicing factor.

We have analyzed the presence of BBP and Mud2p in yeast splicing complexes using gel supershift and co-precipitation assays. BBP and Mud2p are present in commitment complex 2 (CC2), but are neither detectable in commitment complex 1 (CC1) nor in pre-spliceosomes. These are the first commitment complex components that are shown to be released during or immediately after pre-spliceosome formation. Furthermore, genetic and biochemical depletion demonstrated that BBP/ScSF1 is required for CC2 formation. Interestingly, depletion of BBP or disruption of *MUD2* had no significant effect on (pre)-spliceosome formation and splicing *in vitro* but led to a transient accumulation of CC1. These observations support a model in which BBP/ScSF1 and Mud2p are recycled during transition from CC2 to pre-spliceosome.

To get further insight into the *in vivo* function of BBP/ScSF1, we generated 11 temperature-sensitive (ts) mutants and one cold-sensitive (cs) mutant in the *MSL5* gene, that codes for BBP/ScSF1, and analyzed their phenotypes. All mutants were blocked in the formation of commitment complex 2 (CC2) at non-permissive and permissive temperature. The ts mutants showed no defect in formation of mature splicesomes and splicing *in vitro*. The cs mutant was partially defective in (pre)-spliceosome formation, but *in vitro* splicing activity could be detected. *In vivo* splicing of reporters carrying introns weakened by mutations in the 5' splice site and/or in the branchpoint region was affected in all mutants. Interestingly, pre-mRNA leakage to the cytoplasm was strongly increased in the mutants (up to 40-fold compared to wild type). A combination of ms*l5* ts mutants with a disruption of *UPF1*, a gene involved in nonsense-mediated decay, resulted in a specific synthetic growth phenotype. This suggests that the essential function of SF1 could be related to the retention of pre-mRNA in the nucleus.