ABSTRACT

U5 small ribonucleoprotein particle (U5 snRNP) is a crucial component of the spliceosome, the complex responsible for pre-mRNA splicing. Despite the importance of U5 snRNP, not much is known about its biogenesis. When we depleted one of the core U5 components, protein PRPF8, the other U5-specific proteins do not associate with U5 snRNA and the incomplete U5 was accumulated in nuclear structures known as Cajal bodies. To further clarify the role of PRPF8 in U5 snRNP assembly, we studied PRPF8 mutations that cause an autosomal dominant retinal disorder, retinitis pigmentosa (RP). We prepared eight different PRPF8 variants carrying RP-associated mutations and expressed them stably in human cell culture. We showed that most mutations interfere with the assembly of snRNPs which consequently leads to reduced efficiency of splicing. The mutant PRPF8 together with EFTUD2 are stalled in the cytoplasm in a form of U5 snRNP assembly intermediate. Strikingly, we identified several chaperons including the HSP90/R2TP complex and ZNHIT2 as new PRPF8's interactors and potential U5 snRNP assembly factors. Our results further imply that these chaperons preferentially bind the unassembled U5 complexes and that HSP90 is required for stability of U5 proteins PRPF8 and SNRNP200. Finally, we provide evidence that the R2TP complex is important for proper maturation of U5 snRNPs and it is responsible for retention of mutated PRPF8 in the cytoplasm. We propose that the HSP90/R2TP chaperone system both promotes and controls the assembly U5 snRNP particles.