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Functions of BCD1 in box C/D snoRNP biogenesis

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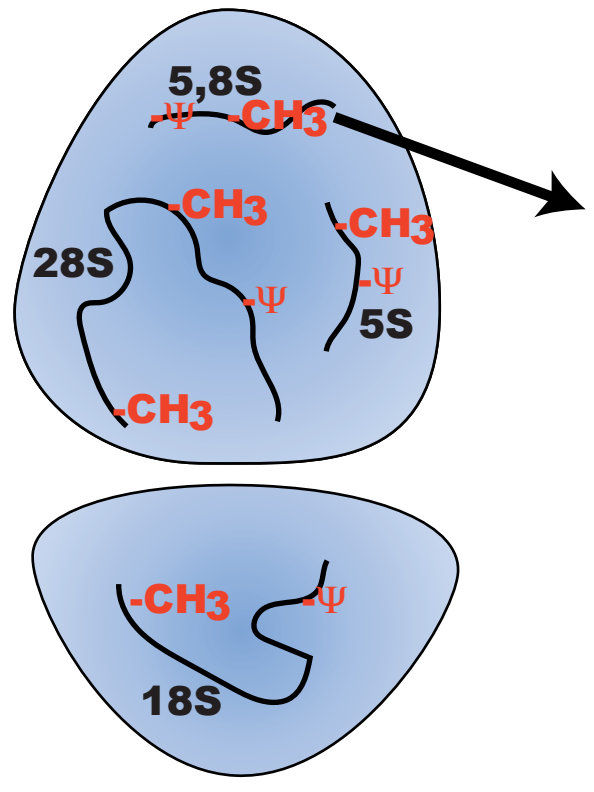
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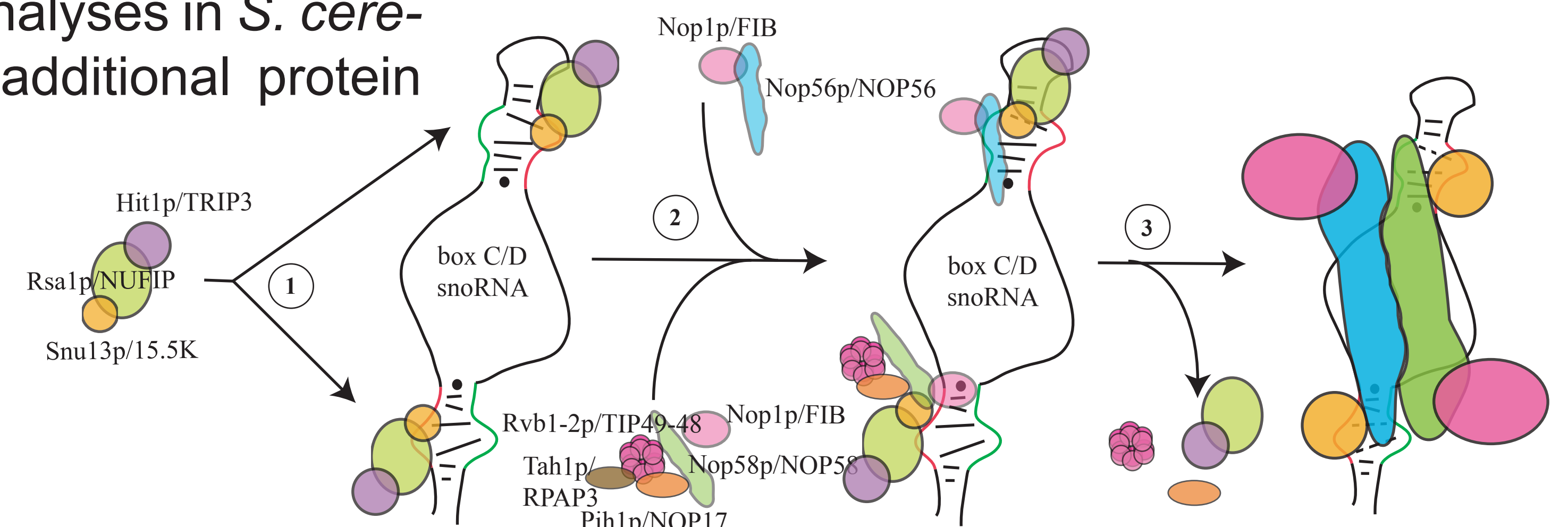
Box C/D snoRNP

2'-O-methylation

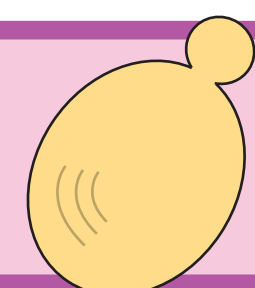


Ribosome biogenesis is dependent on the action of small RNA protein complexes called small nucleolar ribonucleoproteins (snoRNPs). These snoRNPs perform specific enzymatic processes that induce chemical modifications on target rRNAs. The box C/D snoRNPs are formed by association of a box C/D snoRNA with a set of four invariant proteins, and catalyze rRNA 2'-O-methylation. Biogenesis of these RNPs relies on the action of several protein complexes which constitute a specific assembly machinery. Rsa1p/NUFIP, Hit1p/TRIP3, and components of the R2TP complex are the best characterized protein actors of this machinery^{1,2,3,4}. Here, we present functional analyses in *S. cerevisiae* and in mammalian cell lines of an additional protein

factor which may participate in box C/D snoRNP biogenesis. Indeed, the Bcd1p/BCD1 protein (Box C/D snoRNA protein 1) is such a candidate as it is essential for the *in vivo* stability of box C/D snoRNAs in yeast⁵. Moreover, Bcd1p/BCD1 was shown to associate with snoRNP core proteins and assembly factors in yeast and human. Despite these observations, the precise role of Bcd1p/BCD1 in the biogenesis of box C/D snoRNP is still unknown.

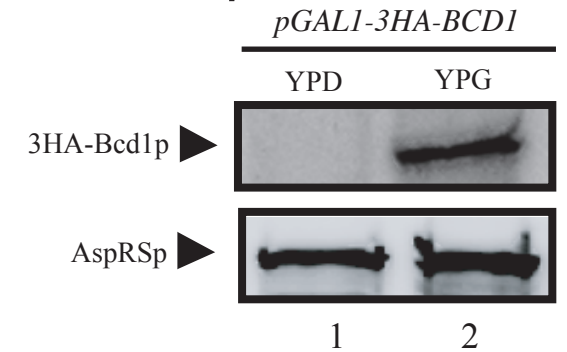


Experiments using yeast

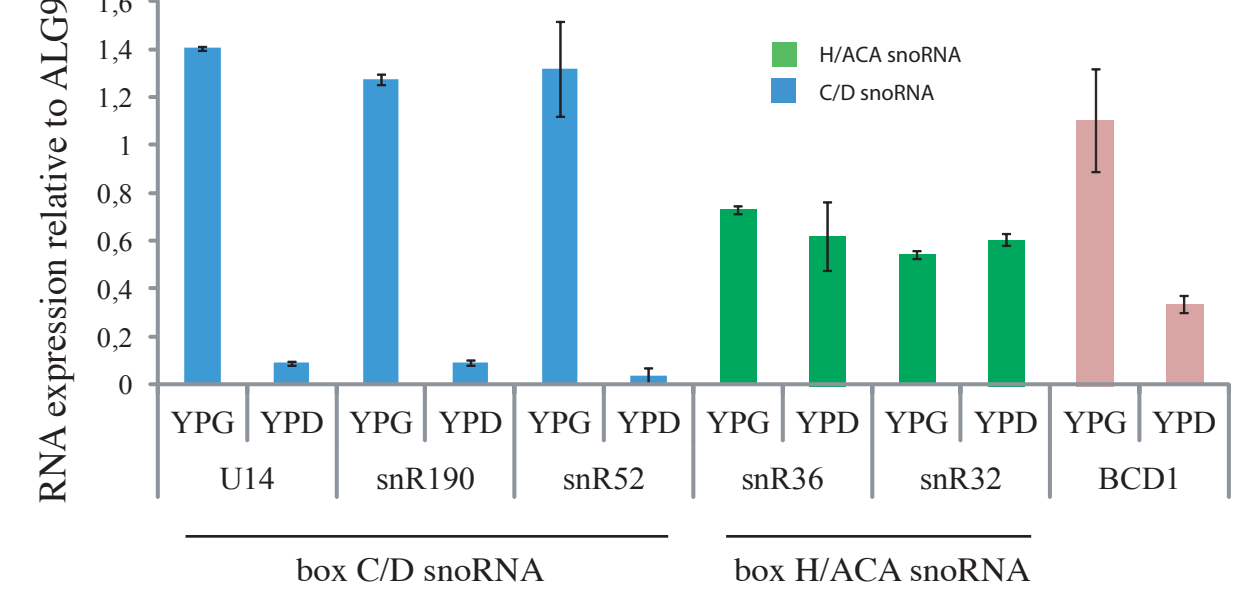


snoRNA steady state is affected by the absence of Bcd1p

Bcd1p is efficiently repressed by glucose in the *pGAL1-3HA-BCD1* strain



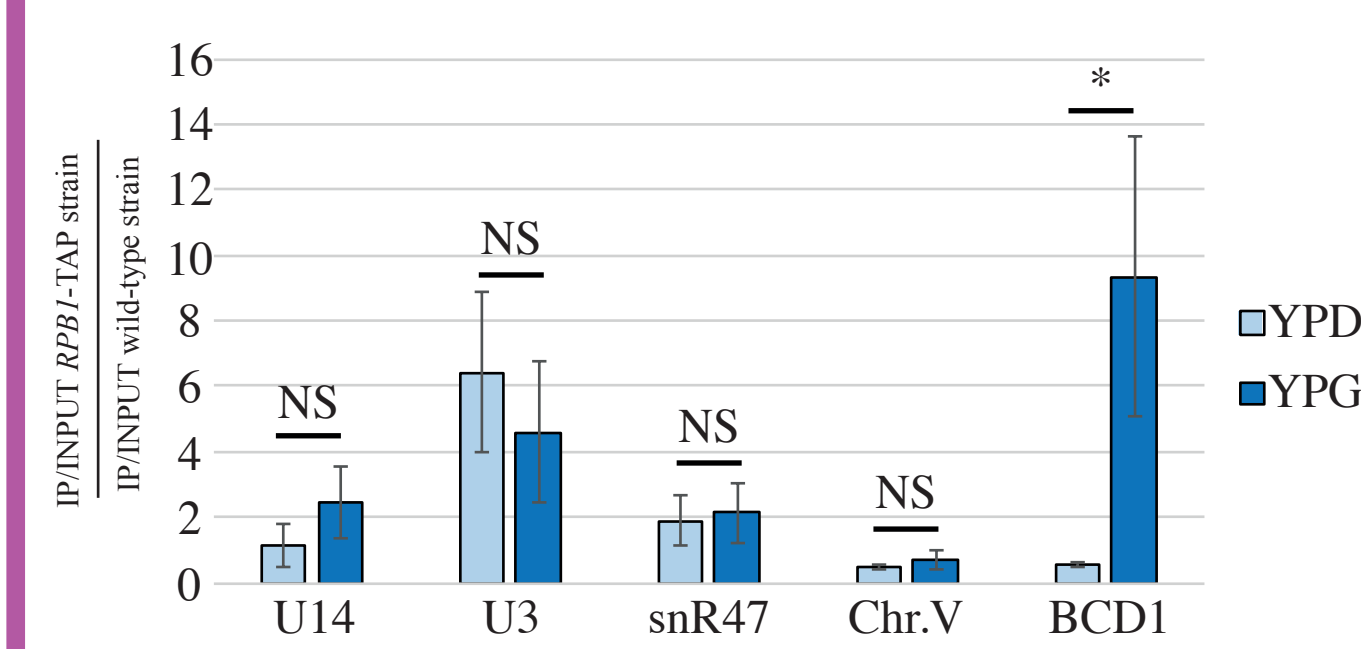
Bcd1p is required for box C/D snoRNA stability



(A) YPG medium containing Galactose allowed Bcd1p expression. YPD medium containing Glucose repressed Bcd1p expression. (B) snoRNA expression was measured by RT-qPCR in presence or in absence of Bcd1p (YPG or YPD) in *pGAL1-3HA-BCD1* strain in exponential growth phase.

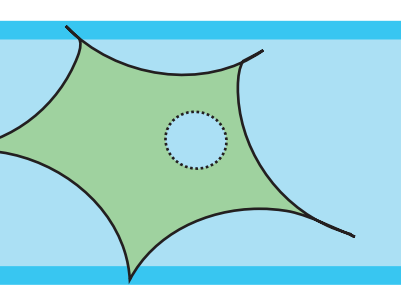
Bcd1p is not involved in box C/D snoRNA transcription

Rpb1p recruitment on box C/D snoRNA loci is not affected by Bcd1p depletion

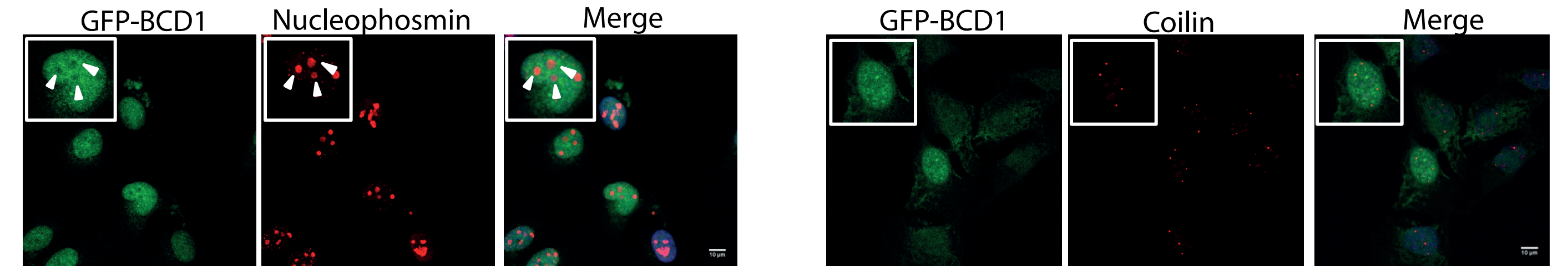


ChIP experiment on *TAP-RPB1;pGAL1-3HA-BCD1* strain in presence (YPG) or absence (YPD) of BCD1 gene expression for 2 hours (n=3). Rpb1p is the major subunit of RNA polymerase II in yeast.

Experiments using human cell lines

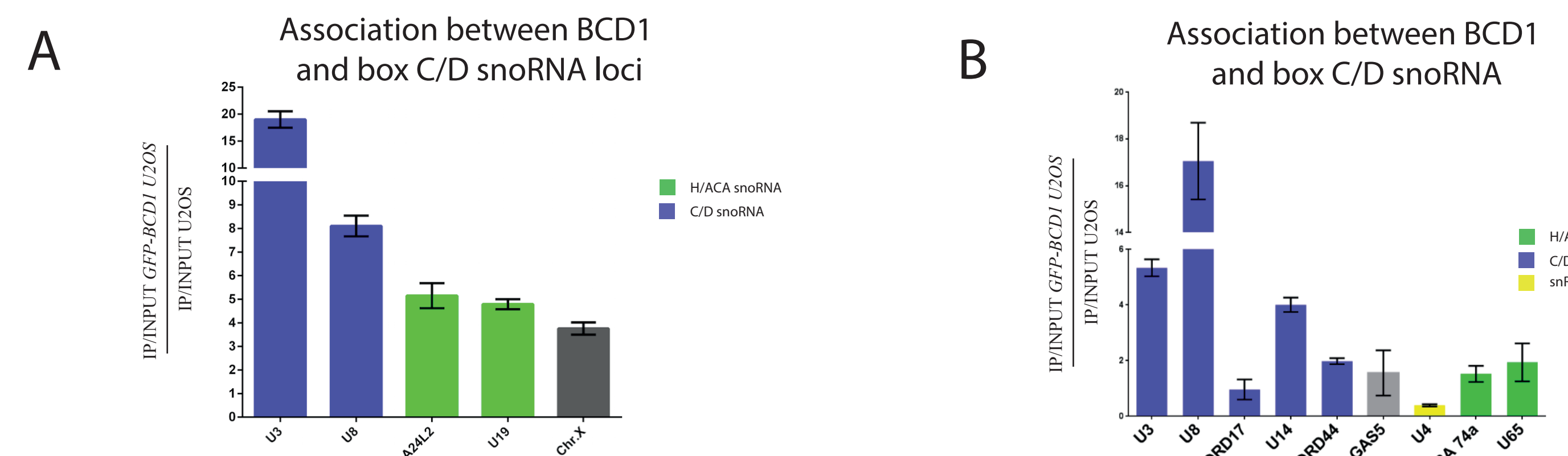


BCD1 is principally found in the nucleus, but is excluded from the nucleolus



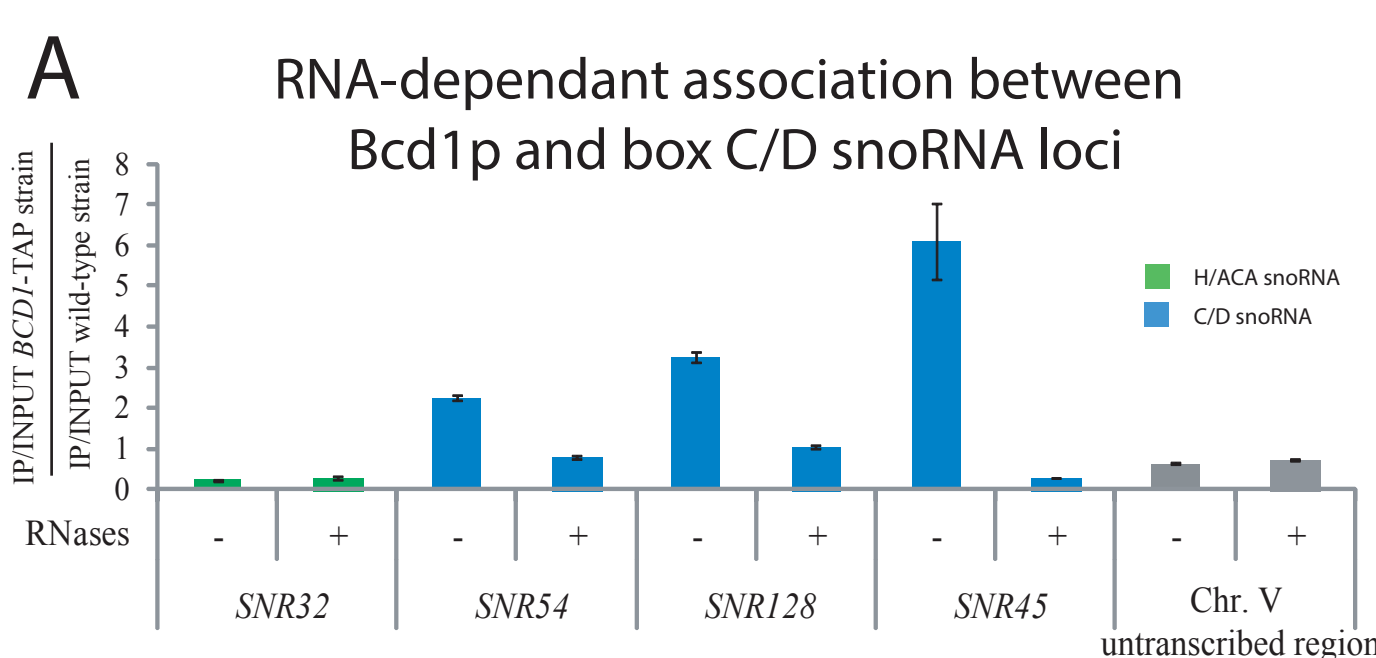
Immunofluorescence were performed on GFP-BCD1 U2OS cell line. GFP-BCD1 were co-localized with (A) Nucleophosmin which is present in the nucleolus, and (B) Coilin which is present in the Cajal bodies. The GFP-BCD1 U2OS cell line was generously providing by E. Bertrand lab².

BCD1 is associated with snoRNAs at their site of transcription

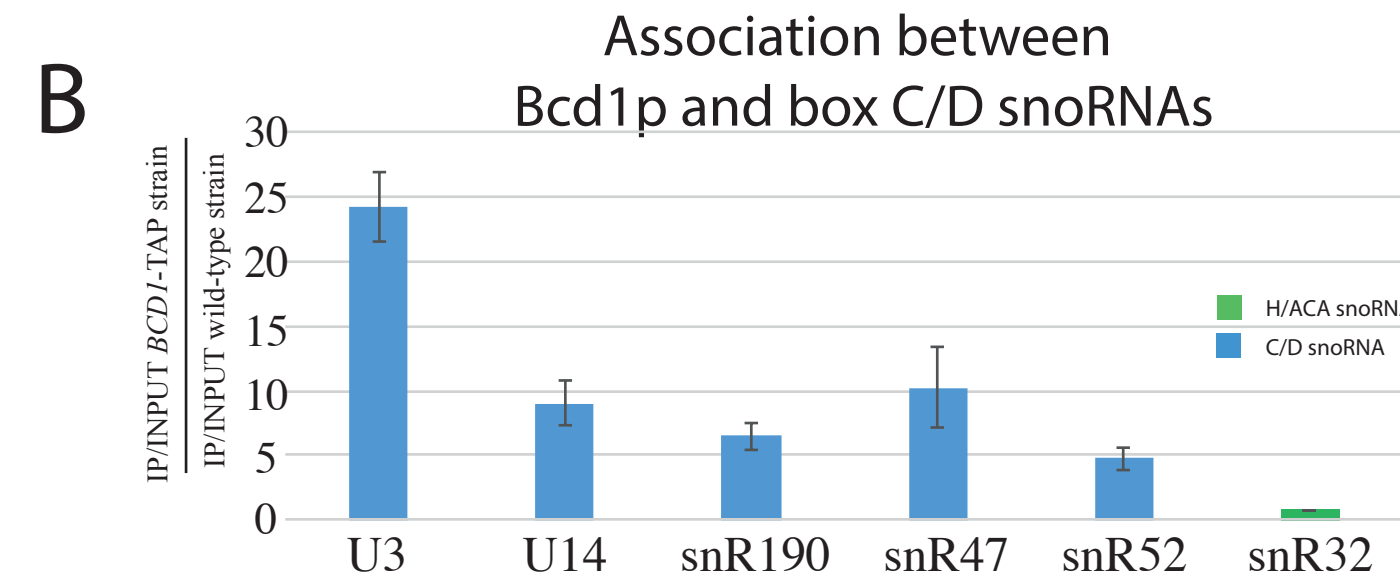


ChIP (A) and RIP (B) experiments on GFP-BCD1 U2OS cell line at 80% confluence. Representative experiments are shown. Chr. X is an untranscribed region of U2OS genome. GAS5 is the host gene of SNORD44.

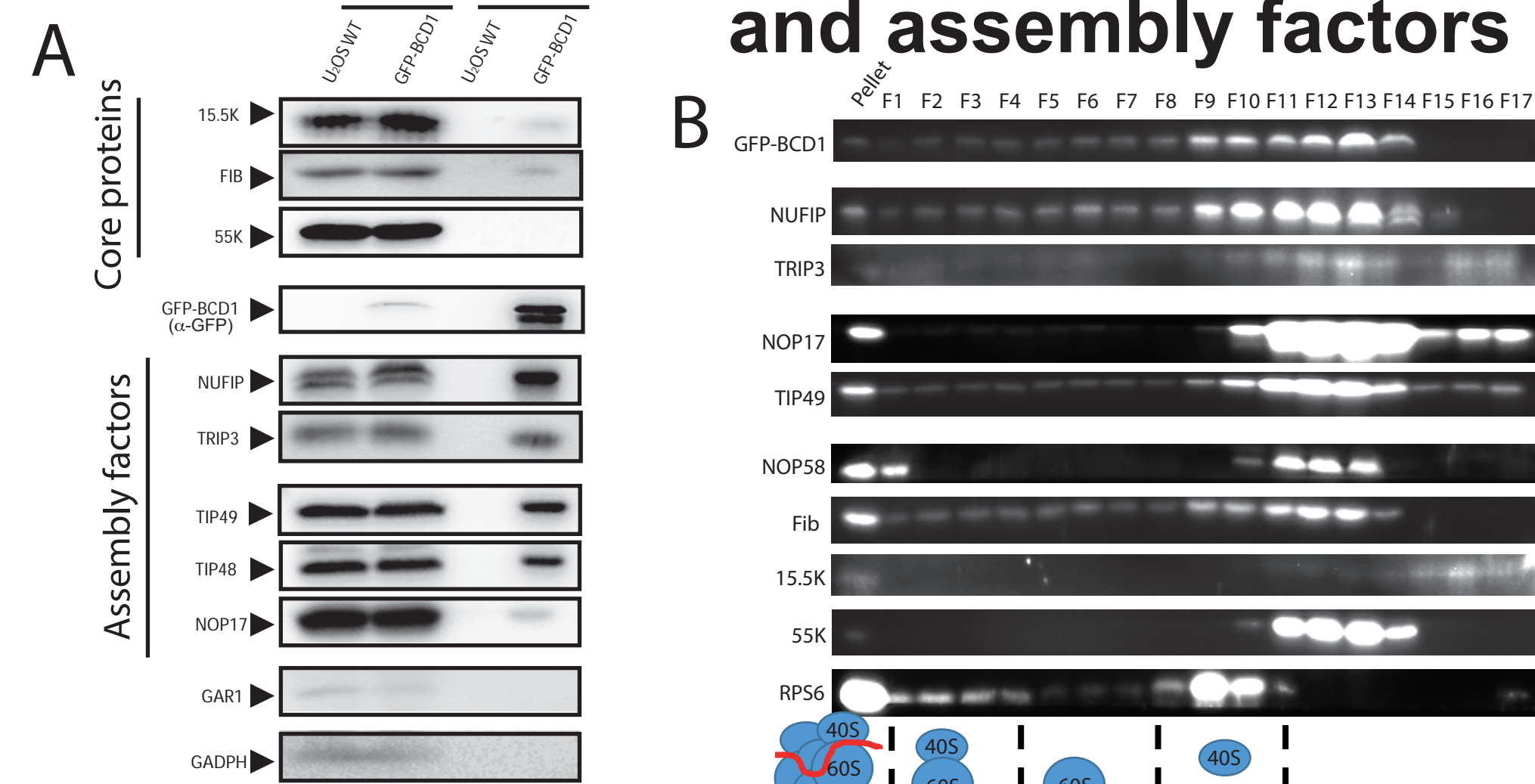
Bcd1p associates with box C/D snoRNAs at their site of transcription



ChIP (A) and RIP (B) experiments on TAP-BCD1 strain at exponential growth phase.



BCD1 is associated with box C/D snoRNP core proteins and assembly factors



(A) Co-immunoprecipitations were performed on cellular extracts from GFP-BCD1 U2OS cells at 80% confluence. Immunoprecipitation was done using α -GFP beads. (B) Sedimentation on 10-30% glycerol gradient of total cellular extracts from GFP-BCD1 U2OS cells.

Take Home Message :

The Bcd1p/BCD1 protein was proposed to be involved in box C/D snoRNP biogenesis. Our data suggests that Bcd1p/BCD1 is involved at early stages of box C/D snoRNP biogenesis, since it is recruited during snoRNA transcription.

Coming next :

-Using the yeast model, we will precise the effect of Bcd1p depletion on (i) the maturation of box C/D snoRNA and (ii) on the recruitment of the other assembly factors. Conversely, we will test the requirement of the latter for Bcd1p recruitment on box C/D snoRNAs. Data obtained by these experiments will be confirmed and precised using mutated proteins defective in specific protein-protein interactions.

- By using siRNA and inducible cell lines, we will use similar strategies to decipher BCD1 functions in human cell lines.

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