A role for COP9 signalosome component CSN-5 in stabilizing stem cell regulators FBF-1 and FBF-2

Emily Osterli (emily.osterli@umontana.edu), Xiaobo Wang, Mary Ellenbecker, Baisen Zeng, and Ekaterina Voronina
Division of Biological Sciences, Center for Biomolecular Structure and Dynamics, University of Montana

Abstract

The highly conserved COP9 (constitutive photomorphogenesis 9) signalosome complex can affect protein stability through a range of mechanisms including deneddylation, deubiquitination, and phosphorylation (Wolf et al., 2003). The most extensively studied mechanism of COP9 is its deneddylating activity on the cullin family of ubiquitin-ligases. Furthermore, the catalytic subunit of COP9, CSN-5, can interact with other cellular proteins beyond COP9 components (Shackleford & Claret, 2010). Our lab identified an interaction between CSN-5 and the RNA-binding proteins FBF-1 and FBF-2, both of which are required for stem cell maintenance in *Caenorhabditis elegans*. We also discovered that CSN-5 promotes the accumulation of FBF-1 and FBF-2 proteins in *C. elegans* stem and progenitor cells, therefore contributing to stem cell maintenance in the organism. Additionally, genetic analysis suggests that the CSN-5/FBF interaction functions in the context of COP9 holoenzyme because FBF-1 protein levels are also decreased in *csn-2* and *csn-6* mutant worms. Both COP9 subunits CSN-5 and CSN-6 have an MPN metalloprotease domain (Mpr1/Pad1 N-terminal), which is essential for integration into the COP9 complex, but only CSN-5 is catalytically competent (Cope et al., 2002; Lingaraju et al., 2014; Birol et al., 2014). Our preliminary results support the hypothesis that the metalloprotease domain of CSN-5 binds to the conserved RNA-binding domains of FBF-1 and FBF-2, thus identifying a protein complex that is evolutionarily conserved. Interactions between CSN-5 and other cellular proteins outside of the COP9 signalosome complex have not been extensively studied, but since the FBF stabilization depends on multiple subunits of the COP9 complex we expect the FBF/CSN-5 interaction to also occur when CSN-5 is incorporated into the COP9 complex. Analysis of the interaction between CSN-5 and the FBF proteins will elucidate how assembly of this protein complex is mediated and provide tools to further test our hypothesis that FBF/CSN-5 interaction stabilizes FBFs in stem and progenitor cells.

Background

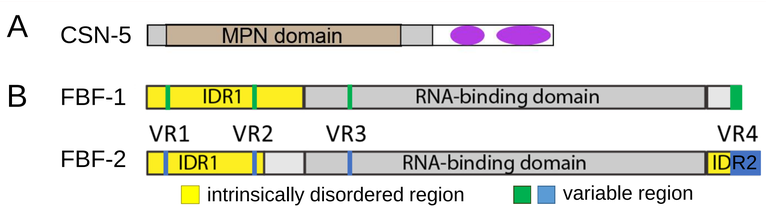


Figure 1. Schematics of CSN-5, FBF-1, and FBF-2. **A.** The N-terminal domain (grey) containing MPN catalytic core (brown) forms a stable heterodimer with the N-terminal domain of CSN-6 (Birol et al., 2014). C-terminal helices (purple ovals) are incorporated into the helical bundle stabilizing COP9 holoenzyme (Lingaraju et al., 2014). **B.** Schematics of RNA-binding proteins FBF-1 and FBF-2 with predicted intrinsically-disordered domains (IDRs), RNA-binding domains, and variable regions 1-4 (VR1-4).

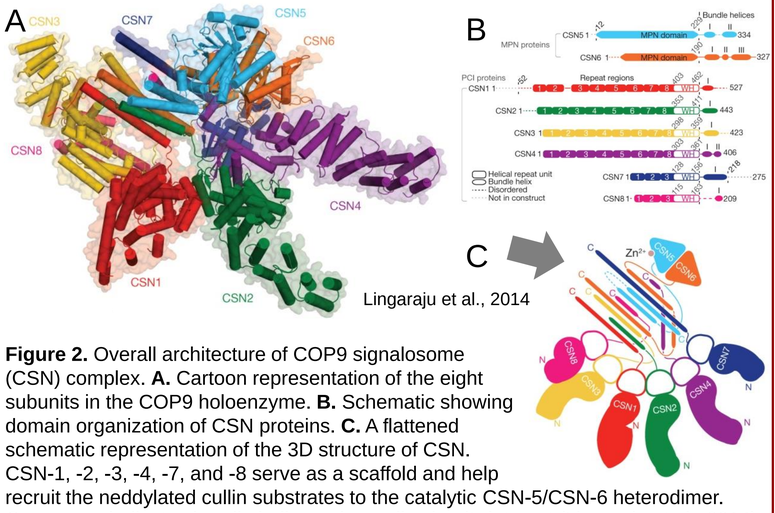


Figure 2. Overall architecture of COP9 signalosome (CSN) complex. **A.** Cartoon representation of the eight subunits in the COP9 holoenzyme. **B.** Schematic showing domain organization of CSN proteins. **C.** A flattened schematic representation of the 3D structure of CSN. CSN-1, -2, -3, -4, -7, and -8 serve as a scaffold and help recruit the neddylated cullin substrates to the catalytic CSN-5/CSN-6 heterodimer. Grey arrow indicates bundle helices where all subunits interact (Lingaraju et al., 2014).

Results

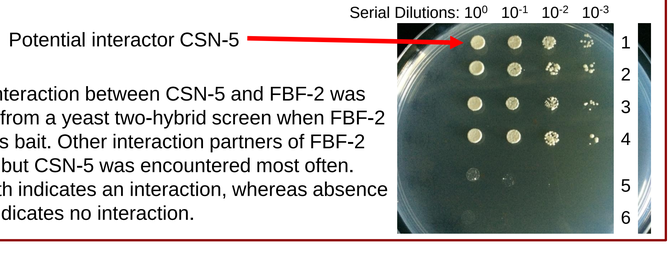


Figure 3. Interaction between CSN-5 and FBF-2 was discovered from a yeast two-hybrid screen when FBF-2 was used as bait. Other interaction partners of FBF-2 were seen, but CSN-5 was encountered most often. Yeast growth indicates an interaction, whereas absence of growth indicates no interaction.

Results

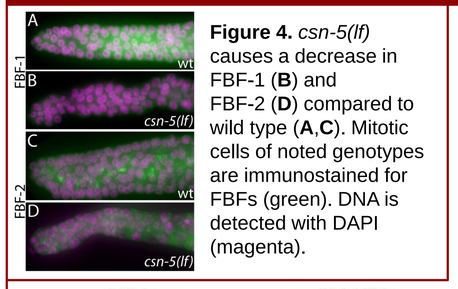


Figure 4. *csn-5(lf)* causes a decrease in FBF-1 (**B**) and FBF-2 (**D**) compared to wild type (**A**,**C**). Mitotic cells of noted genotypes are immunostained for FBFs (green). DNA is detected with DAPI (magenta).

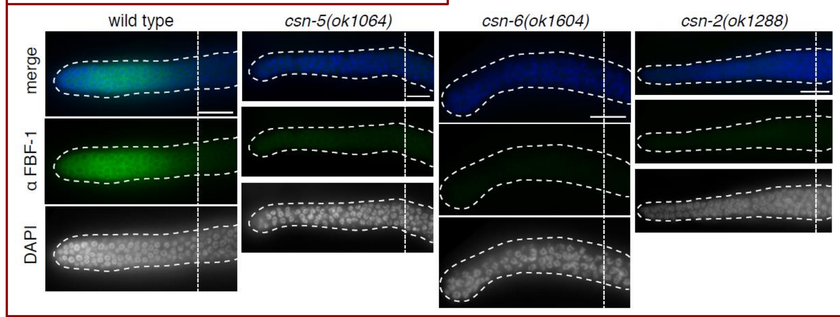


Figure 5. FBF-1 is degraded in several COP9 subunit mutants. Knocking down other subunits of the COP9 signalosome (*csn-6* and *csn-2*) yields the same phenotype seen in the *csn-5* mutant. Mitotic cells of noted genotypes are immunostained for FBF-1 (green) and DNA is detected with DAPI (white/blue in merge).

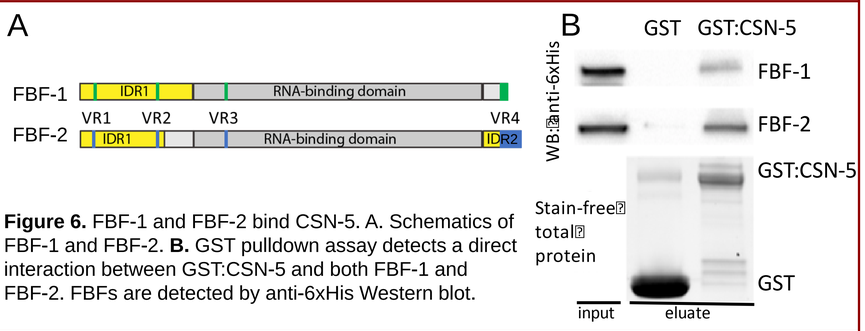


Figure 6. FBF-1 and FBF-2 bind CSN-5. A. Schematics of FBF-1 and FBF-2. **B.** GST pulldown assay detects a direct interaction between GST:CSN-5 and both FBF-1 and FBF-2. FBFs are detected by anti-6xHis Western blot.

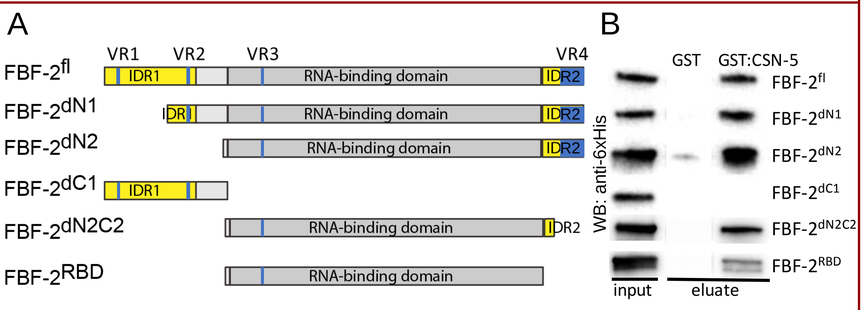


Figure 7. The interaction between CSN-5 and FBF-2 depends of the FBF-2 RNA-binding domain. **A.** Schematics of full length FBF-2 and FBF-2 truncation constructs with variable regions indicated. **B.** GST pulldown of FBF-2 constructs with GST:CSN-5. FBF constructs are detected by anti-6xHis.

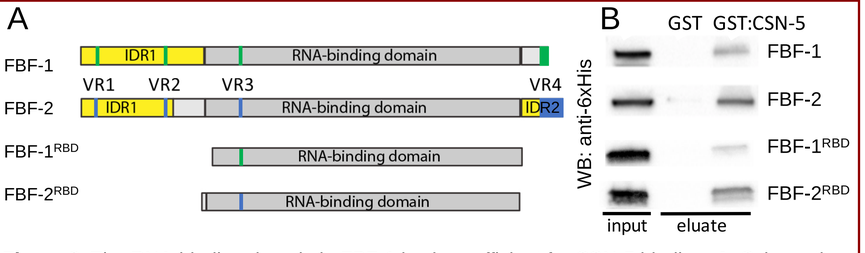


Figure 8. The RNA-binding domain in FBF-1 is also sufficient for CSN-5 binding. **A.** Schematics of full length FBF-1 and FBF-2 their respective RBD truncation constructs. **B.** GST pulldown of FBF constructs with GST:CSN-5. FBF constructs are detected by anti-6xHis.

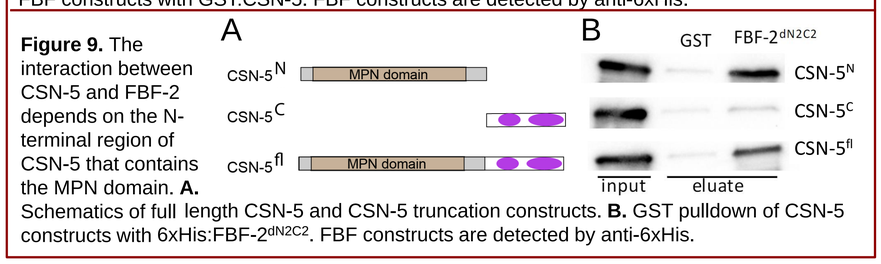


Figure 9. The interaction between CSN-5 and FBF-2 depends on the N-terminal region of CSN-5 that contains the MPN domain. **A.** Schematics of full length CSN-5 and CSN-5 truncation constructs. **B.** GST pulldown of CSN-5 constructs with 6xHis:FBF-2^{dN2C2}. FBF constructs are detected by anti-6xHis.

Results

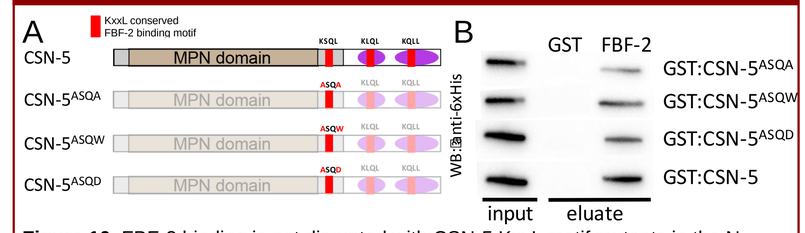
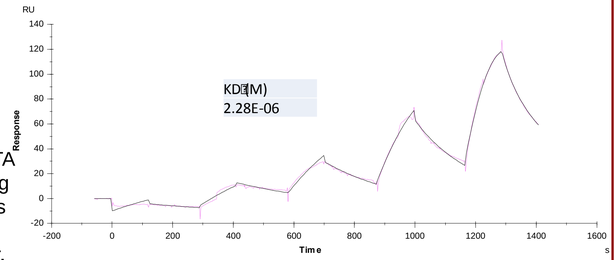


Figure 10. FBF-2 binding is not disrupted with CSN-5 KxxL motif mutants in the N-terminal domain. **A.** Schematic of full length CSN-5 with respective KxxL mutations. **B.** GST pulldown of FBF-2 with full length GST:CSN-5 and respective KxxL mutants. FBF-2 is detected by anti-6xHis. Three KxxL motifs are found in CSN-5 (red), mutation of the KxxL motif closest to the MPN domain does not disrupt FBF-2 binding.

Figure 11. FBF-2^{RBD} binds CSN-5 with micro-molar affinity. 6xHis:FBF-2^{RBD} (ligand/bait) is anchored on Ni-NTA chip with increasing flow concentrations of GST:CSN-5 (analyte/prey) over.



Discussion and Future Directions

Discussion:

1. Promotion of FBF accumulation in stem cells identifies a new role for COP9 signalosome.
2. Determining the FBF/CSN-5 interaction sites can help identify how CSN-5 is contributing to FBF activity and stability.
3. An interaction between the conserved domains of FBF and CSN-5 (RBD and MPN domain) would imply that this protein complex is evolutionarily conserved and the interaction may be relevant for homologous proteins in humans.
4. CSN-5^{MPN} has a sequence motif found in several FBF^{RBD} interacting proteins, KxxL (Qui et al., 2019 and references therein). Preliminary results suggest this KxxL motif doesn't dictate the interaction between FBF and CSN-5/6.
5. Affinity of CSN-5/FBF-2 interaction is similar to the previously published CSN-5 affinity with one of its other interaction partners, CSN-6. This suggests that the interaction is of a sufficient strength to be biologically relevant.

Future Directions:

- Generate GST:CSN-5^{N} KxxL mutants and determine if they can still bind FBF-2
- Quantify the amount of FBF protein/RNA in COP9 mutants
- Determine if the FBF/CSN interaction is conserved in human homologs
- Analyze CSN-5^{MPN}/FBF-2^{RBD} complex by MALS
  - In collaboration with University of Montana ISB core personnel

References

1. Wolf DA, Zhou C, Wee S. The COP9 signalosome: an assembly and maintenance platform for cullin ubiquitin ligases? Nat Cell Biol 2003;5(12):1029-33.
2. Shackleford TJ, Claret FX. JAB1/CSN5: a new player in cell cycle control and cancer. Cell Dev 2010;5:26.
3. Cope GA, Suh GS, Aravind L, Schwarz SE, Zipursky SL, Koonin EV, Deshaies RJ. Role of predicted metalloprotease motfi of Jab1/Csn5 in cleavage of Nedd8 from Cul1. Science 2002;298(5593):608-11.
4. Lingaraju GM, Bunker RD, Cavadini S, Hess D, Hassiepen U, Renatus M, Fischer ES, Thomä NH. Crystal structure of the human COP9 signalosome. Nature 2014;512(7513):161-5.
5. Birol M, Enchev RI, Padilla A, Stengel F, Aebersold R, Betzi S, Yang Y, Hoh F, Peter M, Dumas C, Echalier A. Structural and biochemical characterization of the Cop9 signalosome CSN5/CSN6 heterodimer. PLoS One 2014;9(8):e105688.
6. Qui C, Bhat VD, Rajeev S, Zhang C, Lasley AE, Wine RN, Campbell ZT, Hall TMT. A crystal structure of a collaborative RNA regulatory complex reveals mechanisms to refine target specificity. Elife 2019;8:e48968.

Acknowledgments:
We gratefully acknowledge the Ryckman lab for the use of Western gel documentation and UM's ISB core personnel for collaborating with us on this project. This work is funded from the NIH (R01GM109053) and the Pilot Grant from CBSD.