The Role of SPATA5 and SPATA5L1 in replisome turnover

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Overview

SPATA5 and SPATA5L1 are poorly characterized proteins belonging to the AAA+ ATPase superfamily. Our lab first identified SPATA5/5L1 as part of a four membered complex with likely cofactors C1ORF109 and CINP, all of which are required for complex stability and cell survival. SPATA5/5L1 were shown to be crucial for genome stability, cell cycle progression, and cell viability. SPATA5/5L1 show high sequence similarities to p97, a well characterized AAA+ ATPase. Through unfoldase activity, p97 regulates protein dynamics and turnover at chromatin in DNA damage repair, DNA replication, and cell cycle progression[1,2,3,4,5]. Prior work from our lab showed that the SPATA5/5L1 complex localizes to chromatin and interacts with replisome components. SPATA5/5L1 also mediate proteolytic cleavage of replisome components PolD3 and RFC1. Given these observations, SPATA5/5L1's similarity to p97, and the importance of SPATA5/5L1 for cellular functioning, we wondered if the cleavage of PolD3 and RFC1 are processes through which SPATA5/5L1 regulate turnover of chromatin-associated replisome proteins. We ask the following questions: 1) What is the stability of full length PolD3/RFC1 versus PolD3/RFC1 cleavage products? 2) What happens to the PolD3/RFC1 cleavage products? 3) When does cleavage of PolD3/RFC1 happen? Using a Halo tag approach followed by pulse-chase experiments, we investigate SPATA5/5L1's potential role in protein turnover and its larger role in DNA replication and genome stability.

References

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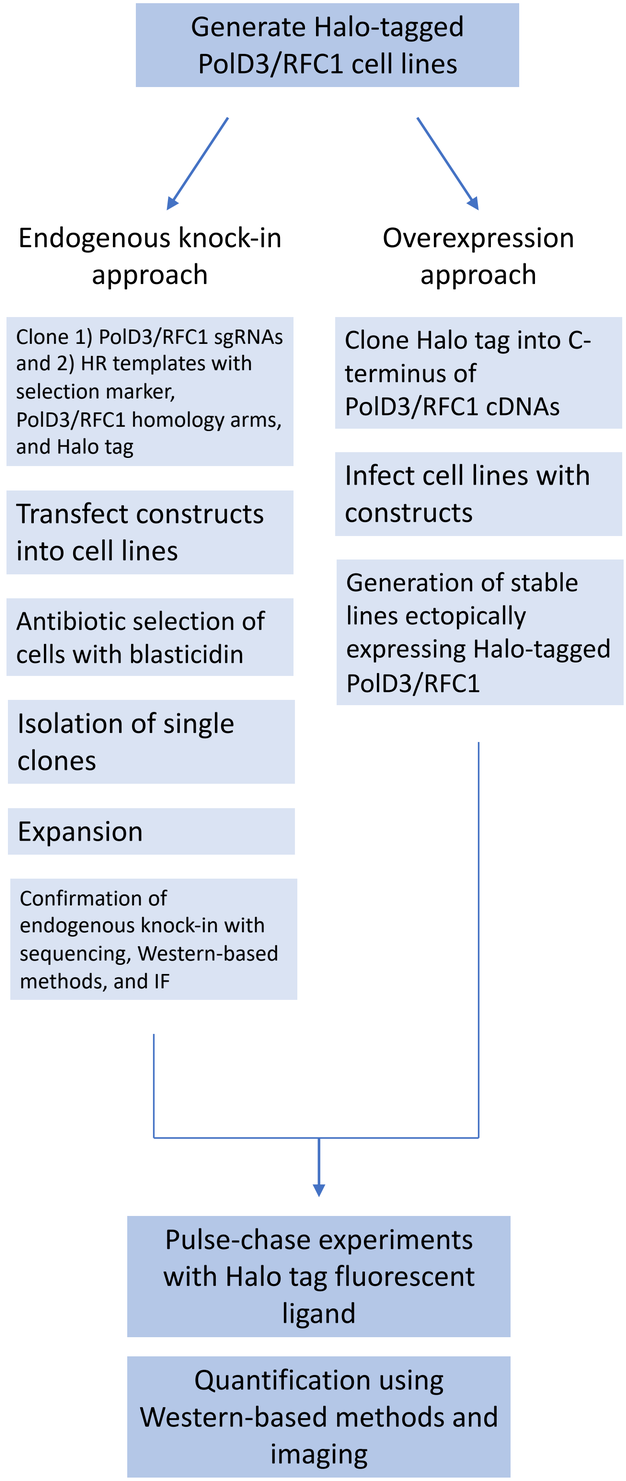
Methods



Results

Fig. 1 Diagram of the HR template used for the endogenous knock-in approach with PolD3 and RFC1 homology arms, the Halo tag sequence, and the blasticidin resistance marker. PolD3 and RFC1 sgRNAs will induce nicks in endogenous PolD3 and RFC1. Through homologous recombination, the HR templates will be used to endogenously tag PolD3 and RFC1 with Halo tag at the C-terminus.

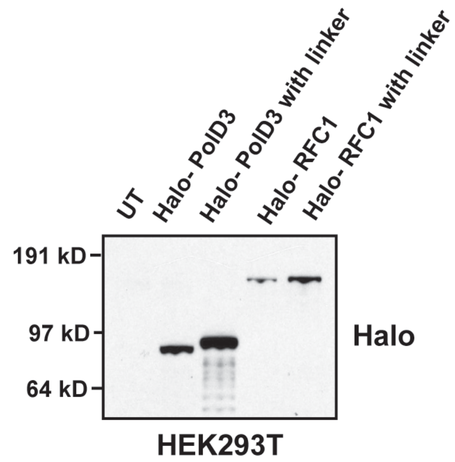


Fig. 2 Western blot showing expression of PolD3-Halo and RFC1-Halo overexpression constructs in HEK293T cell lines, probed with Halo primary antibody. While all constructs express well, constructs with linker sequences between the protein of interest and the Halo tag show higher expression.

Future Directions

- Pulse cell lines with Halo tag fluorescent ligand
- Couple pulse experiments with +/- calpeptin block, proteasome inhibitor bortezomib, and cell synchronization by double thymidine block.
- Use Western-based assays and fluorescent imaging to track quantity of fluorescently labeled proteins

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