Identification of Microprotein-Protein Interactions via APEX Tagging

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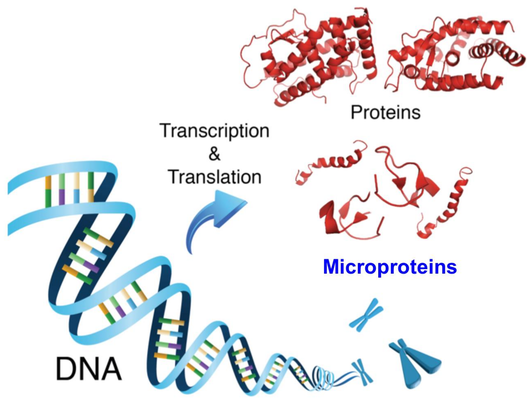
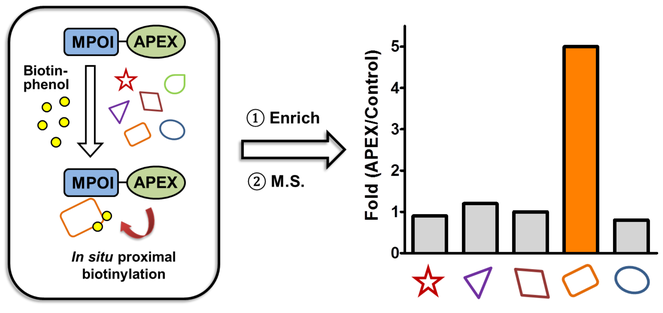
smORF/Microprotein



smORF	Length	Interacting protein	Biology
Bacteria			
SgrT	43 aa	Glucose transporter (PtsG)	Glucose metabolism
Flies			
Tal/Pri	11/32 aa	Ubr3	Development
Mice			
Mln	46 aa	Calcium transporter (SERCA1)	Muscle contraction and endurance
Human			
MRI/Cyren	69 aa	Ku70/Ku80	DNA repair
Nobody	68 aa	EDC4/Dcp1	mRNA decapping

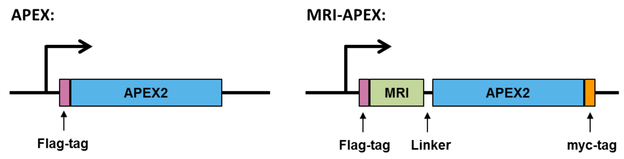
- Microproteins are a new class of molecules that are encoded directly from small open reading frames (smORFs)
- Microproteins have fundamental roles in biology, including metabolism, apoptosis, and development
- Most microprotein functions rely on microprotein-protein interactions

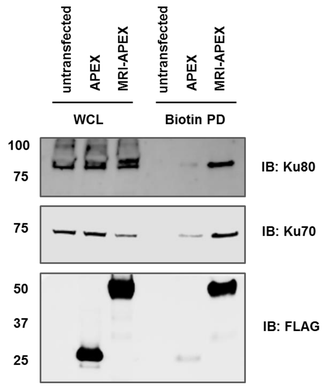
In situ APEX biotinylation

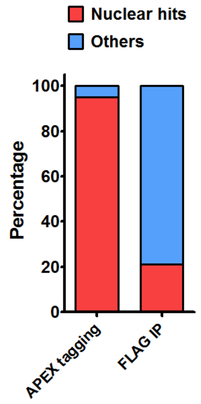


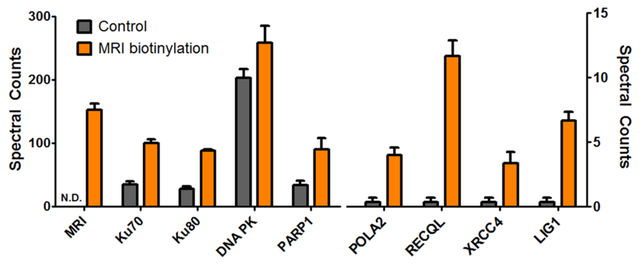
- An engineered ascorbate peroxidase (APEX) allows proximity biotin tagging in live cells
- APEX fusion to a microprotein of interest (MPOI) generates biotin phenoxyl radicals, and thus biotin labels surrounding proteins, which are likely microprotein interacting partners

Case study I: MRI-APEX



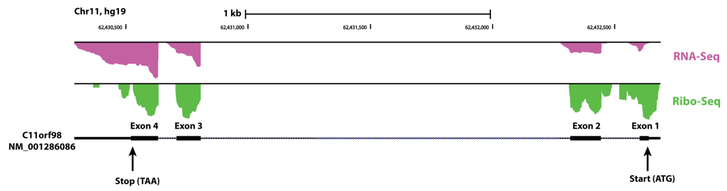




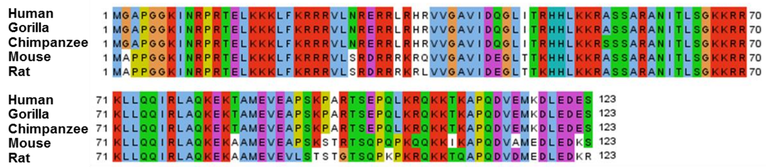


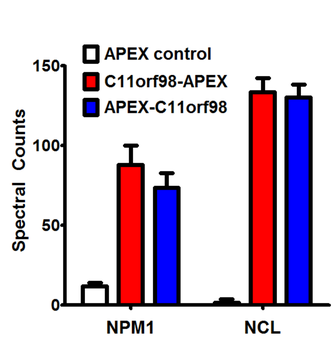
- APEX fusion to MRI microprotein successfully biotinylates and enriches Ku70 and Ku80
- Compared to FLAG IP, most MS hits in MRI-APEX tagging are nuclear proteins, which is consistent with its nuclear localization
- In addition to the Ku70/Ku80 heterodimer, MRI-APEX tagging also reveals many other proteins in the NHEJ DNA repair pathway

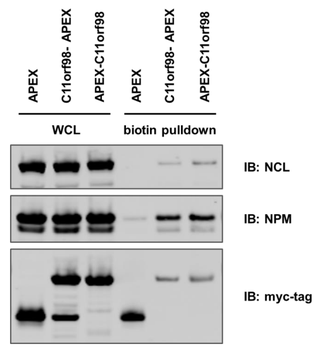
Case study II: C11orf98-APEX

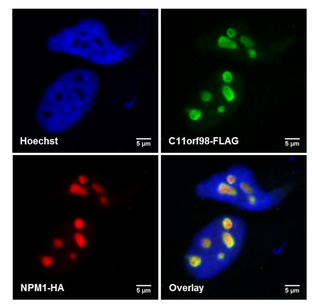


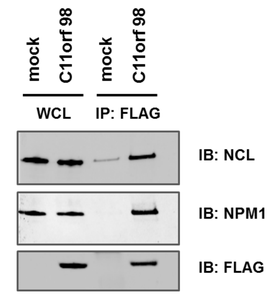
- C11orf98-MP is an uncharacterized 123 aa microprotein encoded by C11orf98 smORF
- It has robust RNA-Seq and Ribo-Seq coverage, and is conserved between human and mouse, suggesting a potential function











- APEX fusion to C11orf98 results in biotinylation and enrichment of many nuclear proteins
- Nucleolin (NCL) and nucleophosmin (NPM1) are the two most promising candidates as they have the highest fold enrichment and form a protein complex in the nucleus
- FLAG IP and immunofluorescence experiments validate the interaction between C11orf98-MP, NCL and NPM1

Conclusion

- APEX tagging is able to biotinylate and enrich microprotein interacting proteins in live cells
- Compared to FLAG immunoprecipitation, APEX tagging demonstrates greater fold increase of bona fide interacting proteins, while decreasing background non-specific interactions
- The apparent improvement in the APEX data supports the application of APEX to the remaining uncharacterized microproteins

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