Investigating the physiological roles of **ANGELs**

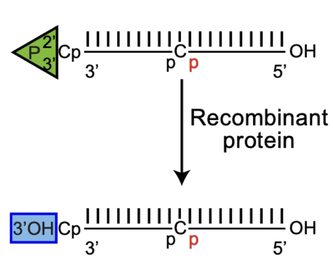
Ameya Khandekar, Paola Pinto, Stefan Weitzer and Javier Martinez
Max perutz Labs, Vienna BioCenter, and Center for Medical Biochemistry, Medical University of Vienna

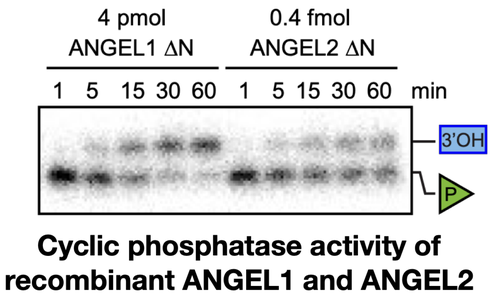
The ANGEL family: cyclic phosphatases, not deadenylases

1. ANGEL proteins possess a cyclic phosphatase activity

ANGEL proteins, restricted to metazoans and fungi, were classified as a yCCR4- related family of deadenylases (Dupressoir et al., 2001) based on homolgy. No enzymatic activity or role in mRNA degradation in vivo or in vitro was detected for any of the ANGEL proteins. Interestingly, recent work from the Martinez lab identified and re-defined the ANGEL family of proteins to be 2',3'-cyclic phosphatases capable of completely removing a 3' cyclic Phosphate at RNA ends (Pinto et al., 2020). A genome duplication early in vertebrate divergence produced two *ANGEL* paralogs- *ANGEL1* and *ANGEL2,* with ANGEL1 being a significantly weaker enzyme.

Mounting evidence in recent years suggests that cP-RNAs are involved in various biological processes and are physiologically relevant. However, general principles of how cP termini influence RNA biology are unknown. Therefore, studying enzymes that could influence these is crucial.





Cyclic phosphatase activity of recombinant ANGEL1 and ANGEL2

Having identified their biochemical activity in vitro, a major question that remains to be answer is:

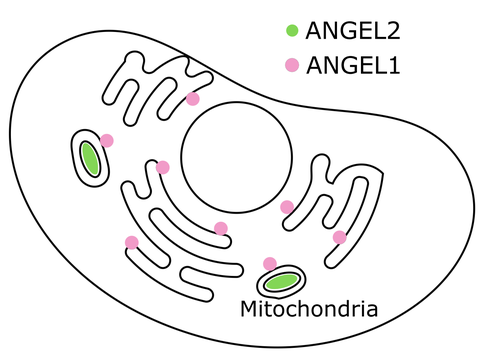
What is the physiological relevance of ANGEL proteins *in vivo*?

Hints so far

1. Sub-cellular Localisation

A previous report showed that ANGEL1 localised to the ER/ Golgi (Gosselin et al., 2013). Interestingly, a number of recent proximity labeling based studies suggest that it localises specifically to the cytosolic face of the ER and the OMM (Outer mitochondrial Menbrane). (Ting and Rhee labs)

The same proximity labeling studies reveal that ANGEL2 resides in the mitochondrial matrix. The confirmation of these data is currently underway.



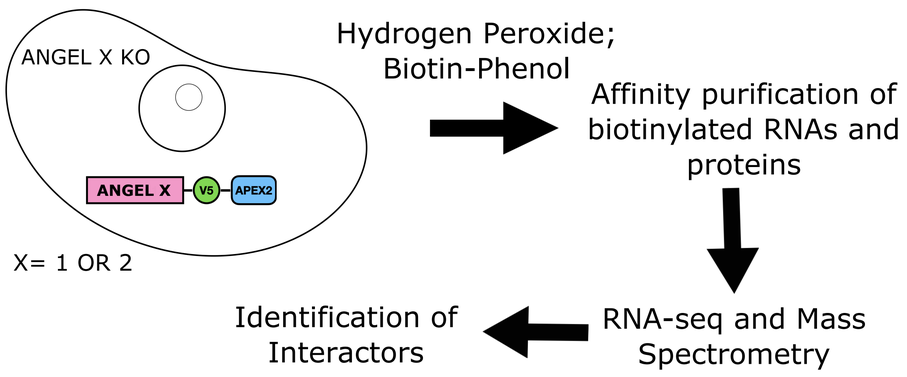
2.Known Interactions

The only confirmed protein that ANGEL1 interacts with is eIF4E through a conserved motif- YxxxxLΦ (where x is a variable amino acid and Φ is a hydrophobic residue) (Gosselin et al., 2013).

For ANGEL2, no such confirmed interaction has been found as yet.

Studying roles of ANGELs *in vivo* : current approaches

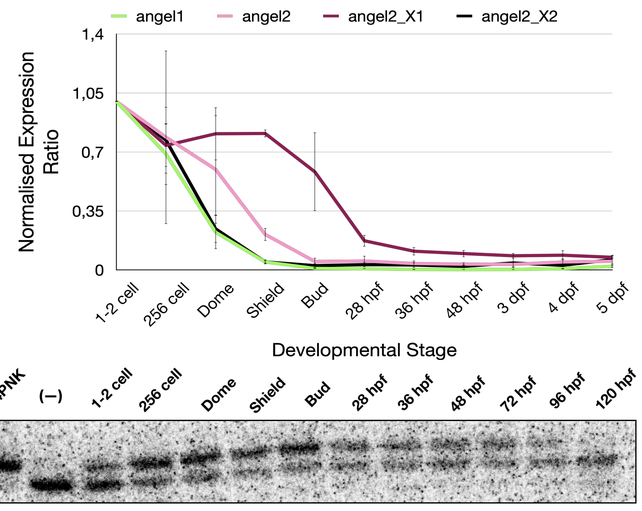
1. Proximity labeling as an aproach to identify RNA and protein interactors



To identify RNAs and proteins that interact with both the ANGELs, APEX2 mediated proximity labeling (Ting lab) is being carried out through a controlled over expression of APEX2-ANGEL constructs in a Knock out/ endogenous ANGEL deficient background. Identifying interactors and substrates could indicate the cellular processes ANGEL1 and 2 are involved in.

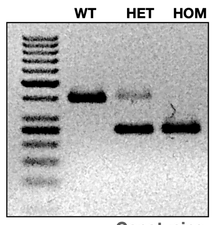
2. Setting up *Danio rerio* as a model system to study ANGELs

While the cell culture system offers numerous advantages, an organism allows us to ask more physiologically relevant questions, study different tissues and their interactions. Zebrafish have both paralogs- angel1 and angel2, which are expressed during early embryogenesis, their levels declining as development proceeds.

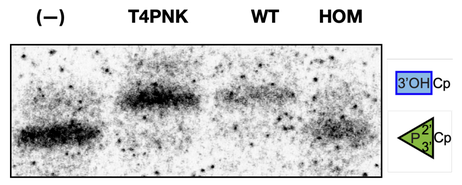


Embryonic extracts during early development also possess cyclic phosphatase activity which declines over time.

To assess the roles of angel1 and angel2, CRISPR Cas9 was used to create knock out (KO) fish lines for each of the genes. While the mutagenesis was successful, as shown below by the genotyping and activity assays for angel2 KO (HOM) fish, they do not exhibit any obvious phenotype. Analysis of transcriptional changes upon loss of angel2 is currently underway, along with the analysis of angel1 KOs and generation and analysis of double KOs.



Genotyping



cPase assay; 2 dpf,
50 embryos pooled; 1 mg/mL protein

Open questions

What is the effect of loss of angel1 in zebrafish? Is there a tissue specificity in the expression patterns of angel1 and angel2 in zebrafosh? Under which conditions does the role of angel2 become important?

What is the significance of ANGEL1's interaction with eIF4E? Could there be an impact on protein synthesis? Is its interaction with eIF4E uncoupled from ANGEL1's catalytic activity?

MEDIZINISCHE UNIVERSITÄT WIEN
MAX PERUTZ LABS VIENNA
ÖAW ÖSTERREICHISCHE AKADEMIE DER WISSENSCHAFTEN
FWF
Vienna BioCenter