A Novel List of Proteins interact with Cld8 tails

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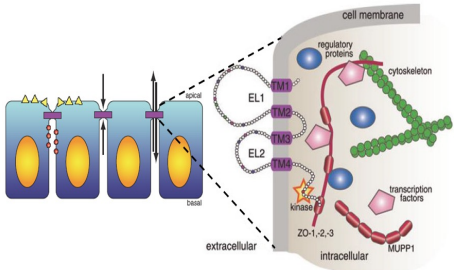
Introduction

Claudins are one of the main components of tight junction, which plays an important role in cells communication and movements during development. In earlier experiments, we found that removal of Claudins 3, -4, and -8 can cause defects in neural tube closure.

Claudin basic components:

- Two extracellular loops
- Four transmembrane domains
- A intracellular tail



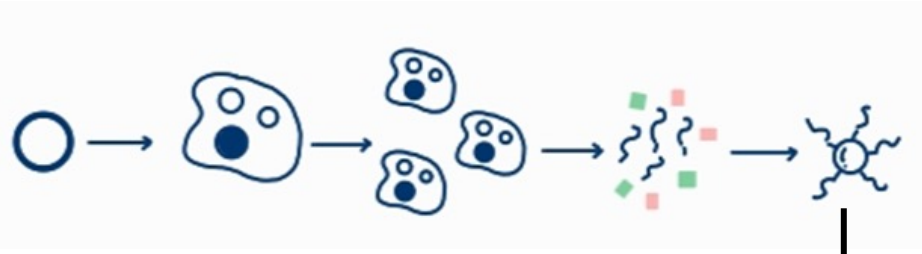
Problem: The role of intracellular tails of Cld8 is unknown

Goal: To discover the list of proteins interacting with CLd8 intracellular tails

Methods

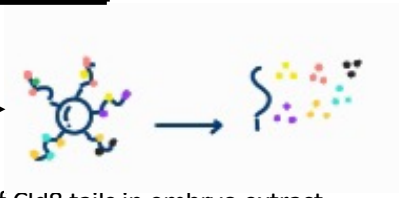
1. Preparation of Cld8 tails

- Plasmid carrying Cld8 tails gene and GST tag are incorporated in E.coli
- Grow the E.coli population and induce production of Cld8 tails
- Rupture the bacterial cells to collec proteins
- Purify Cld8 tails by pull-down specifically with GST beads



2. Preparation of chick embryo extract

- Incubate chicken eggs
- Dissect the eggs to harvest embryo
- Lysis embryo to collect embryo extracts



3. Incubation of Cld8 tails in embryo extract

- Mixing the Cld8 bound beads in the embryo extract
- Allow Cld8 to interact with the protein of interest
- Release the Cld8 and the bounded proteins from the beads
- Using Mass Spectrometry to identify the proteins

Expected Result

Summary of interactors: top-10 hits for each claudin (Total Spectrum Counts)

	Protein	Control	Cldn1	Cldn3	Cldn4	Cldn8	Cldn14	Function
Peptides for pull-down	Claudin-1	0	101	10	0	8	7	Peptide used for pull-down
	Claudin-3	0	0	20	0	1	2	Peptide used for pull-down
	Claudin-8	9	14	1	16	205	30	Peptide used for pull-down
	Claudin-14	0	0	0	0	0	27	Peptide used for pull-down
Tight Junction/cell adhesion	ZO1 (TJP1)	46	39	29	22	358	67	Scaffolding protein, member of tight junction
	ZO2 (TJP2)	12	32	2	7	150	18	Scaffolding protein, member of tight, adherens and gap junctions
	MPDZ (MUPP-1)	0	8	0	0	25	0	Scaffolding protein, member of tight, adherens and gap junctions
	Cingulin	49	39	31	20	49	85	Scaffolding protein, member of tight junction
	Destrin	2	8	14	4	7	3	Actin-depolymerizing protein. Severs actin filaments (F-actin) and binds to actin monomers (G-actin)
	MLLT4 (affadin-6)	5	2	2	5	34	5	Ras-protein that regulates the interaction of adherens junction to cytoskeleton

Impact

- Reveal the list of proteins interacting with Cld8
- Provide a reference for further research study Cld8 function in:
  - Regulating embryology development
  - Mechanism leading to multiple diseases
  - New area for researching novel therapy treating the related diseases