Regulation of cellular postcodes by kinase enzymes

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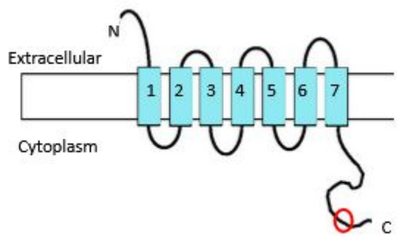
1. Introduction

Tmem184b

- **Tmem184b is a transmembrane protein** found in neurons and that was shown to have an influence on Alzheimer's disease but still very unknown.
- Present on the plasma membrane, later localizes to recycling endosomes and potentially lysosomes[2,8].



Its **C-terminal** includes a putative **dileucine motif** and **two nearby serine amino acids** that are phosphorylated (addition of a phosphoryl group) and could represent **signal peptides that regulates the trafficking** of the protein.

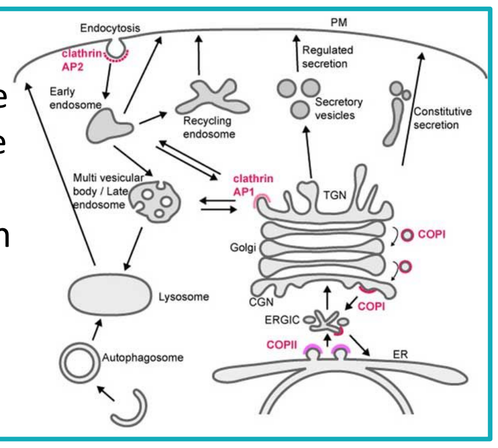
G-A-R-D-N- E-K-T-L-L-L -S-S -D-D-E-F

↓

[D/E]-X-X-X-L-[L/I]

What are signal peptides?

- **Short and specific sequence of amino acids** that ensure accurate trafficking and distribution of proteins into the right intracellular compartments.
- **Dileucine (LL)-based sequence motifs** are well-known signals that have **functions in the endocytosis and trafficking** of various membrane receptors and membrane proteins[1].



Aims and hypothesis

- Previous studies suggested that a **dileucine sequence AND phosphorylation are required for internalization** of the protein[9,10].
- Thus, I **hypothesis that Tmem184b's trafficking might be regulated by these two signals**.
- I am **looking at other proteins that have these similar features** to get a better idea of its function.

2. Analysis pipeline

- As the idea was to look at other proteins similar to Tmem184b to get a better idea of its function my main objective was to narrow down a list of proteins that had the same features as Tmem184b to compare, observe and identify potential pathways, behaviors and target kinases.
- The bioinformatic pipeline below was conducted in R[5], Python[6] and some online tools such as UniProt[12], ScanProsite[7], PhosphoSite[13] and NetPhos[14].

Secreted proteins from VerSeDa[3]
Number of proteins = 60,930

Secreted proteins from ProteinAtlas[4]
Number of proteins = 23,555

↓ Merge both lists
Number of proteins = 18,974

↓ Proteins with [D/E]-X-X-X-L-[L/I] motif
Number of proteins = 9,895

↓ Merge both lists
Number of proteins = 3,139

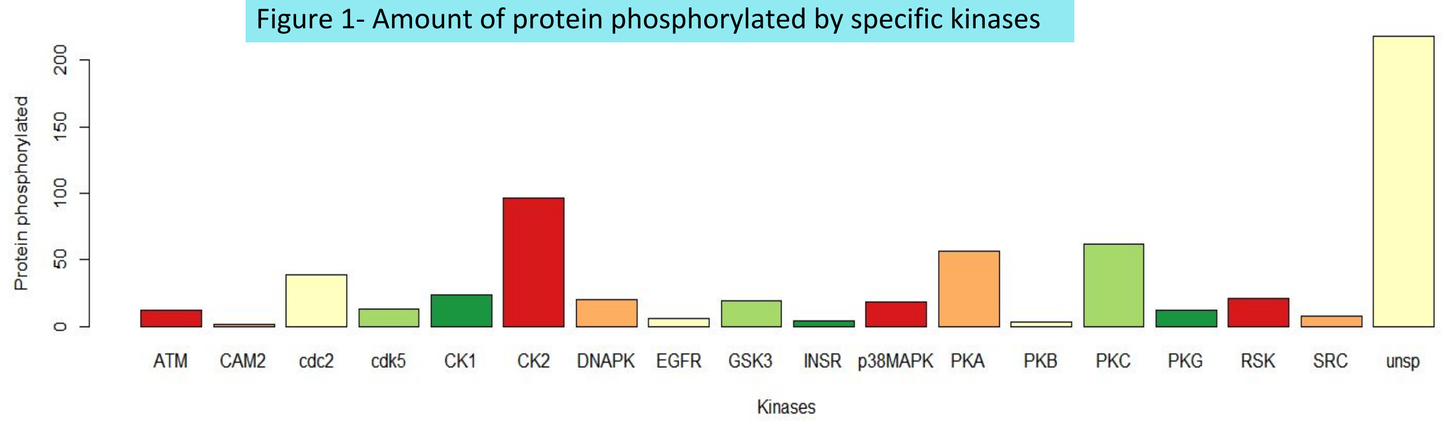
↓ Only taking proteins with di-leucine motif in the N/C-terminal
Number of proteins = 1,410

↓ Only taking proteins with phosphorylated residues 8 amino acids away from di-leucine motif
Number of proteins = 320

↓ Looking and analysing which kinase is phosphorylating the residues nearby the di-leucine motif for each protein (scores only >0.5)

3. Results

Figure 1- Amount of protein phosphorylated by specific kinases



- We can see that there is that there is a **tendency for residues nearby di-leucine motif in membrane proteins to be phosphorylated by CK2**.

CK2

- The serines of Tmem184b are phosphorylated by **casein kinase 2** (CK2)[7].
- Kinase = enzyme that catalyses the transfer of a phosphate group to a specific molecule.

What does this tell us?

- It gives us more information on a **potential form of regulation** by which CK2 activates di-leucine motif and affects trafficking.
- It suggests that it is a **conserved general mechanism**.
- It helps us support our hypothesis that the serines and the di-leucine motif of Tmem184b are linked and act together for protein trafficking.

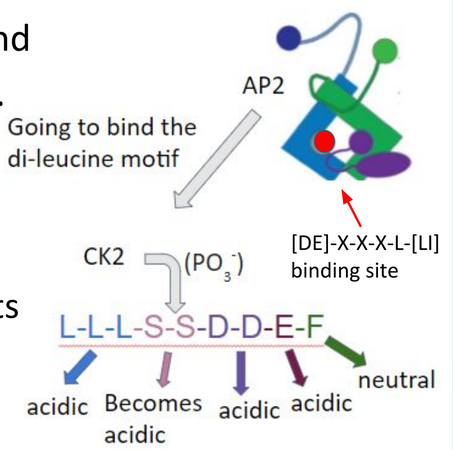
4. Summary and further experiment

Summary

- The C-termimus of Tmem184b includes a hypothesised di-leucine motif and phosphorylated serines that could represent signals that regulate the trafficking of the protein.
- Residues nearby di-leucine motif in membrane proteins tend to be phosphorylated by CK2 and show a **potential form of regulation** by which CK2 affects trafficking by activating the di-leucine motif.

Future work

- Di-leucine motifs can be activated by phosphorylation and increase ability to bind AP2 by creating an acidic patch[11].
- **CK2 phosphorylate the serines which could activate the di-leucine motif and reinforce AP2 binding to promote better internalization of the protein**.
- Further studies are needed through laboratory experiments (e.g. create mutants to see if the di-leucine signal depends on serine phosphorylation).



References
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