Measuring the amount of the channel protein pannexin 1 across neuronal development

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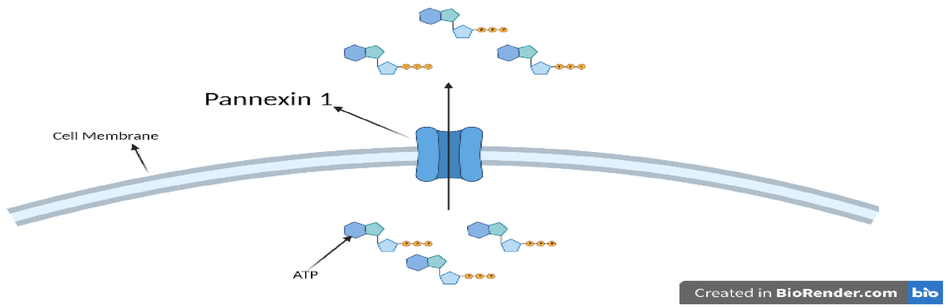
Pannexin 1 (PANX1) is a channel protein that regulates neuronal development

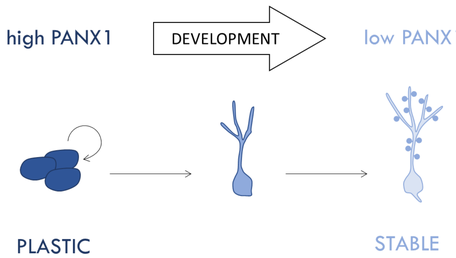
PANX1 forms regulated doorways, called 'channels', within cell membranes

- Allows anions, such as chloride and ATP, to pass through [1]
- PANX1 also interacts with the cell cytoskeleton [2]

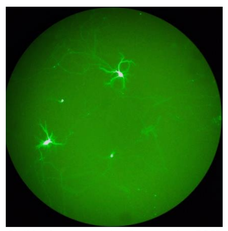


PANX1 regulates neuronal development

- PANX1 is highly expressed in neural precursor cells, and its expression decreases as these cells specialize [3,4]
- PANX1 expression decreases dramatically in the mouse cerebral cortex across the first four post-natal weeks [5,6], and is largely restricted to neurons [unpublished data]
- PANX1 plays a key role in the stabilization of nerve cell connections (synapses) [5,6]



How does PANX1 expression change across neuronal development?



DIV11 primary neural cells expressing EGFP. Image courtesy of Rachel Li, Swayne Lab

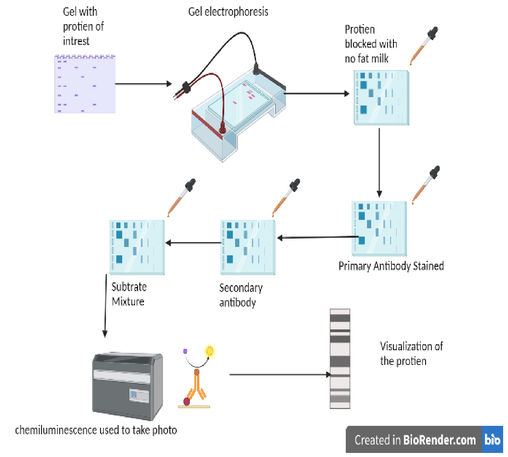
- Primary cortical neuron-astrocyte cultures are an easily-manipulated model to study the cellular and molecular mechanisms underlying neuronal development
- We therefore propose to use primary cortical neuron-astrocyte cultures to study the expression of PANX1 across neuronal development

References

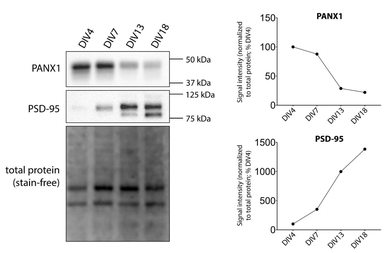
[1] Sanchez-Arias et al., Pharmacol Ther 2021; [2] Wicki-Stordeur and Swayne, Cell Commun Signal 2013; [3] Wicki-Stordeur et al., Neural Dev 2012; [4] Wicki-Stordeur and Swayne Cell Commun Signal 2013; [5] Sanchez-Arias et al., eNeuro 2019; [6] Sanchez-Arias et al., eNeuro 2020; [7] Hnasko, T.S., Hnasko, R.M. (2015). The Western Blot. In: Hnasko, R. (eds) ELISA. Methods in Molecular Biology, vol 1318. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-2742-5_9; [8] Bronstein I, Voyta JC, Murphy OJ, Bresnick L, Kricka LJ. Improved chemiluminescent western blotting procedure. Biotechniques. 1992 May;12(5):748-753. PMID: 1515143.

Quantifying PANX1 levels with SDS-PAGE and Western blot

- **SDS-PAGE** separates complex mixtures of proteins based on molecular weight [7]
- **Western blotting** uses antibodies to identify specific proteins, and provides a readout of protein 'intensity' from a set of samples [7,8]
- Measuring the resulting signal intensities gives a **semi-quantitative** (relative) readout of protein levels between samples when compared to a total protein stain, or loading control signal (stably-expressed protein)



Preliminary: PANX1 decreases across neuronal development



Example Western blot image from primary neural cultures at day *in vitro* (DIV)4 - 18, probed for PANX1 and PSD-95 (synaptic marker). Total protein signal (stain-free) is shown below. Densitometry analysis indicates a relative decrease in PANX1 levels across DIVs. Images courtesy of Becca Candlish and Leigh Wicki-Stordeur, Swayne Lab.

- Preliminary data indicate **PANX1 expression decreases** as primary neural cultures and synapses (PSD-95) mature
- However, further replicates are needed to confirm these findings

Learning Outcomes

- Gained knowledge of primary neural cultures, neuronal development, and SDS-PAGE/Western blot techniques
- Experienced how a scientific research lab operates
- Began developing skills in reading scientific literature
- Increased competency in important transferrable skills including workplace etiquette, effective communication techniques, and reading comprehension

Acknowledgements

This research was supported by the Valerie Kuehne Undergraduate Research Awards and the University of Victoria, and was funded by a Canadian Institutes of Health Research (CIHR) grant awarded to Dr. Leigh Anne Swayne. I am grateful for the supervision from Drs. Leigh Anne Swayne and Leigh Wicki-Stordeur in the Division of Medical Sciences, as well as to all other members of the Swayne Lab for their help and guidance.

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CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada