Developing plants as an expression system for mammalian ion channels

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Project description:

Membrane proteins are the target for approximately two thirds of currently marketed pharmaceuticals, and they play critical roles in infection and immunity in both animals and plants. Progress in drug development would be substantially accelerated if more human membrane protein structures could be determined, but this is a significant challenge; one of the many problems encountered when working with eukaryotic membrane proteins is low expression yield, in particular a low yield of correctly folded and stable protein. The best currently available expression systems for eukaryotic membrane proteins (mammalian and insect cell culture) are expensive to use and prone to infection, and so the development of a widely available and cheap system would be of significant benefit. Plants represent a potential solution to this challenge; they are cheap to grow, they can produce massive yields, and, by using a model experimental system such as Arabidopsis thaliana or Nicotiana benthamiana, there are a vast array of molecular tools already in place to simplify the cloning and expression of proteins.

The main aim of this project is to develop plant expression systems for eukaryotic membrane proteins, focusing on the P2X receptor family of ATP-gated ion channels. P2X receptors are found in vertebrates, where they sense extracellular ATP and play a wide variety of physiological roles including pain and taste sensation, control of blood vessel tone and innate immunity. There are seven P2X receptor subtypes in humans, and they are important targets for potential analgesic and anti-inflammatory drugs, but to date, only one human P2X receptor crystal structure has been determined (P2X3). Understanding P2X receptor 3D-structures would enable us to design subtype-selective small-molecule modulators, which may have significant therapeutic benefit, particularly in age-related inflammatory diseases such as arthritis, atherosclerosis and macular degeneration.

This project is an exciting opportunity to train in a new collaboration between experts in membrane protein expression and plant genetics, learning and applying a variety of techniques including molecular cloning, transgenic plant generation, plant culture, cell culture, protein expression, protein purification and functional assays using fluorescence imaging. Success would lead to the development of new tools to enable the production of large quantities of pure, folded membrane proteins for direct structural and functional studies, and be of significant benefit to the field of membrane protein structural biology.

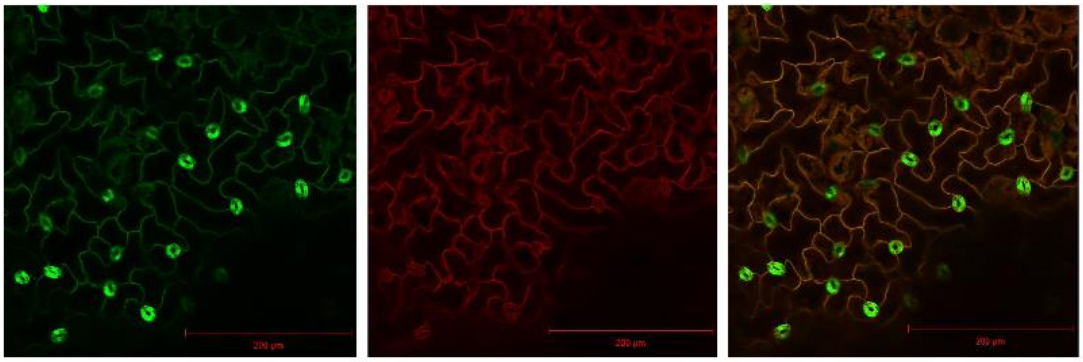

Fig 1. Expression of full-length P2X7-HIS-GFP in transgenic Arabidopsis leaf tissue counterstained with FM4-64. (A) GFP channel showing signal in epidermal pavement cells membranes and in stomata. (B) FM 4-64 channel showing plasma membranes. (C) Composite image of GFP and FM 4-64 channels.