Royal Holloway
University of London

Development of a first-choice non-animal model for bipolar disorder research

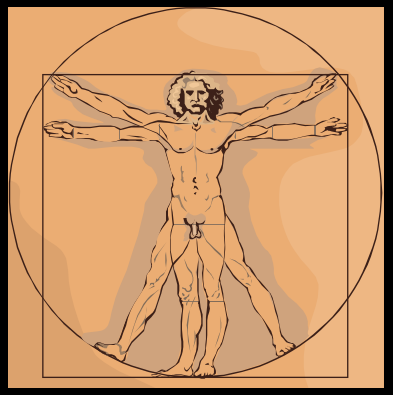
Prof Robin SB Williams
Centre for Biomedical Science
Royal Holloway University of London

Anna Frej
Dr Hadwen Trust for
Humane Research

Great Steps Forward in Medical Science

Discovery of DNA

Sequencing of the human genome



Molecular cell biology

...enabled us to use cells expressing a human/animal protein of interest for biomedical research without using animals!

... This project will develop this approach...

Bipolar disorder – an important and intractable condition

Background

- a devastating neurological condition that causes cyclic variation in mood
- reduced quality of life & increases suicide (15%)
- World wide occurrence up to 4%
- Cost estimates £4.6 billion annually in UK
- Current treatments:
  - Lithium
  - Valproic acid

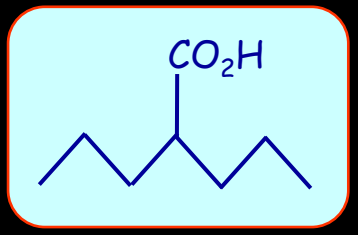
  } Limited efficacy + side effects
- Unknown mechanism of action...

Research

- Pharmacology

↓

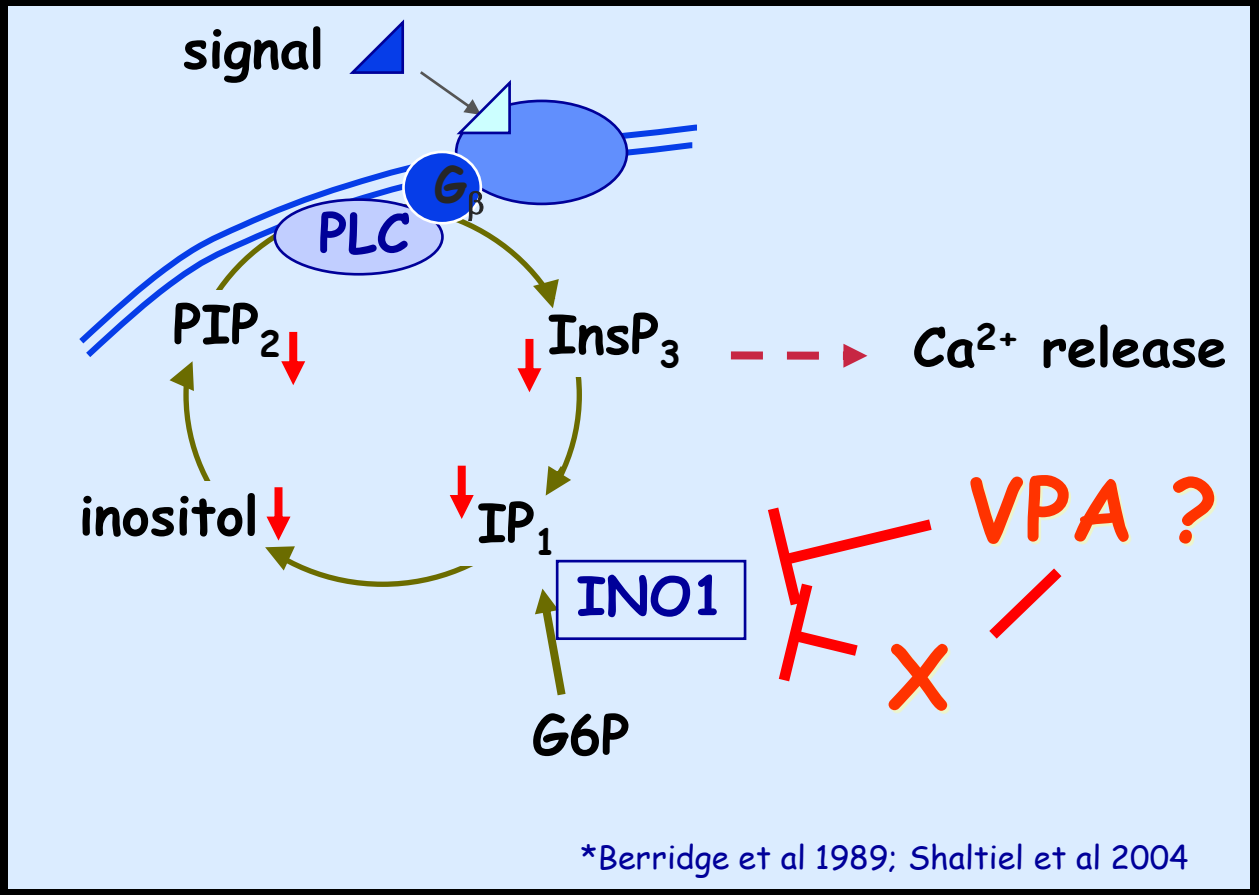
Valproic acid (VPA)



- Possibly targets inositol synthase (INO1) enzyme
- Reduces inositol in neurons

Ludtmann, Bockeler & Williams Seminars in Cell & Developmental Biology 2011, 22, 105–113
Williams et al, 2002, Nature 417, 292-5; Eickholt et al & Williams, 2005, Mol Pharm, 67, 1-8

Bipolar disorder – and the inositol depletion theory

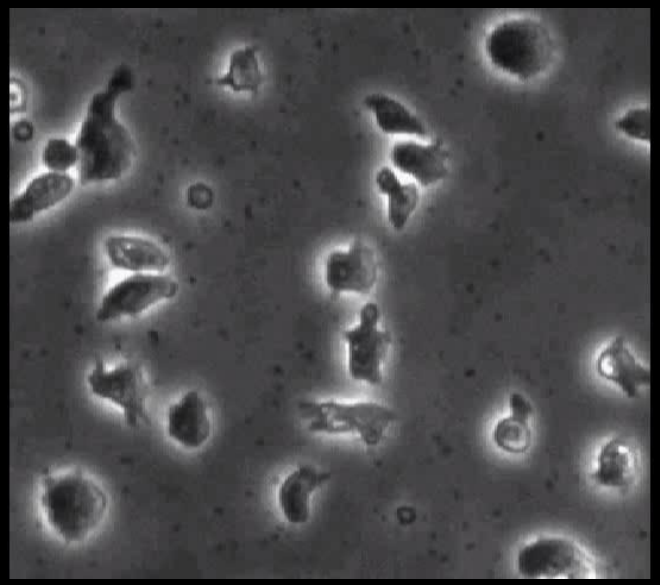


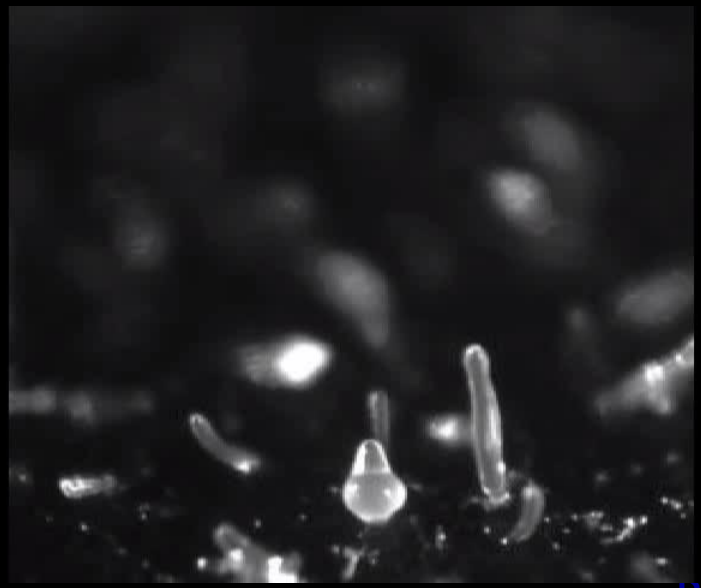
*Berridge et al 1989; Shaltiel et al 2004

Research in this area is predominantly based upon using primary rat neurons

How can we do this neuroscience and neuropharmacology research without using animals?

Dictyostelium: A eukaryotic model

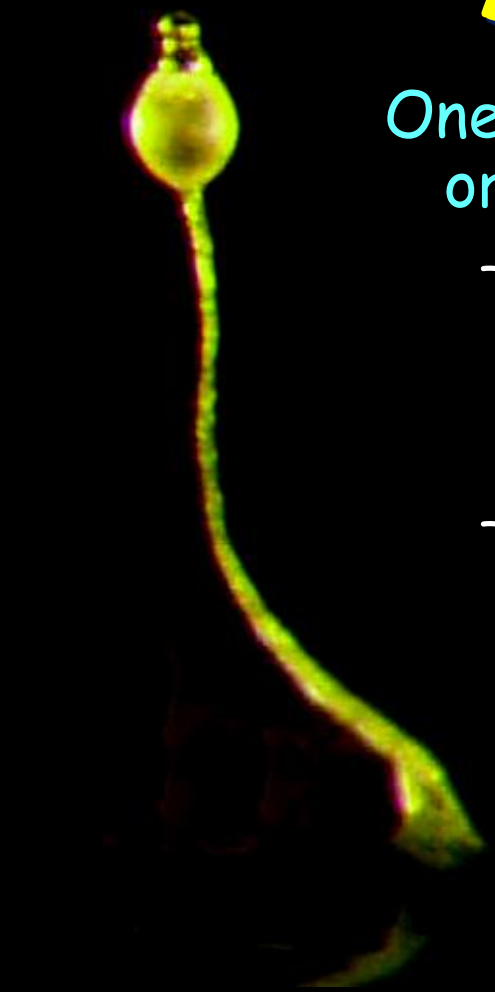




Rick Firtel / Rob Kay

- Social amoeba, *Dictyostelium discoideum*
- Contains only one copy of every gene (haploid) and contains 12500 genes
- Unicellular part of life cycle allows gene knockouts and isolation of isogenic lines for biochemical analysis
- Many common signalling pathways and protein binding partners to mammalian systems

Boeckeler and Williams 2007 Encyclopaedia Life Sciences



Dictyostelium …

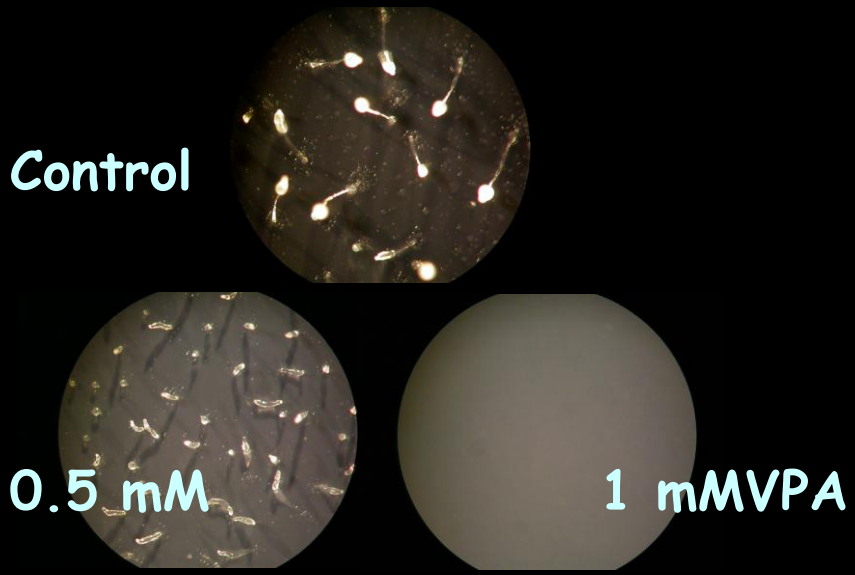
One of eight NIH-listed non-mammalian model organisms for biomedical research

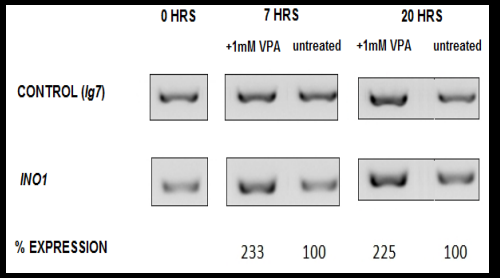
- More simple than other models such as *C. elegans* and *D. melanogaster*, *D. rerio* (zebrafish), *Xenopus*
  - » Use of isogenic clonal lines is advantageous for biochemistry and cell signalling analysis
- More complex than *S. cerevisiae, S. pombe* or *N. crassa* as has cellular movement, rudimentary development mechanisms and related signalling pathways

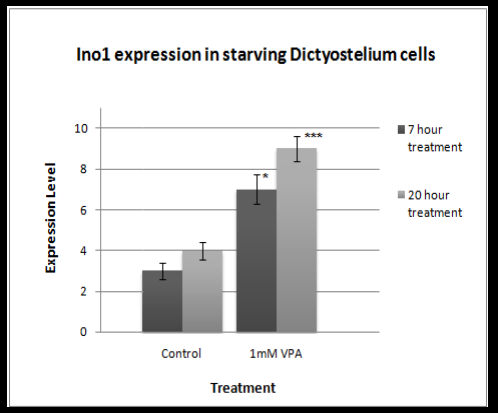
*Williams et al Trend Mol Med 2006

VPA and *Dictyostelium* development

- *Dictyostelium* development is sensitive to VPA
- VPA causes inositol depletion-like increase in ino1 expression







Suggests VPA works through inositol depletion in *Dictyostelium*

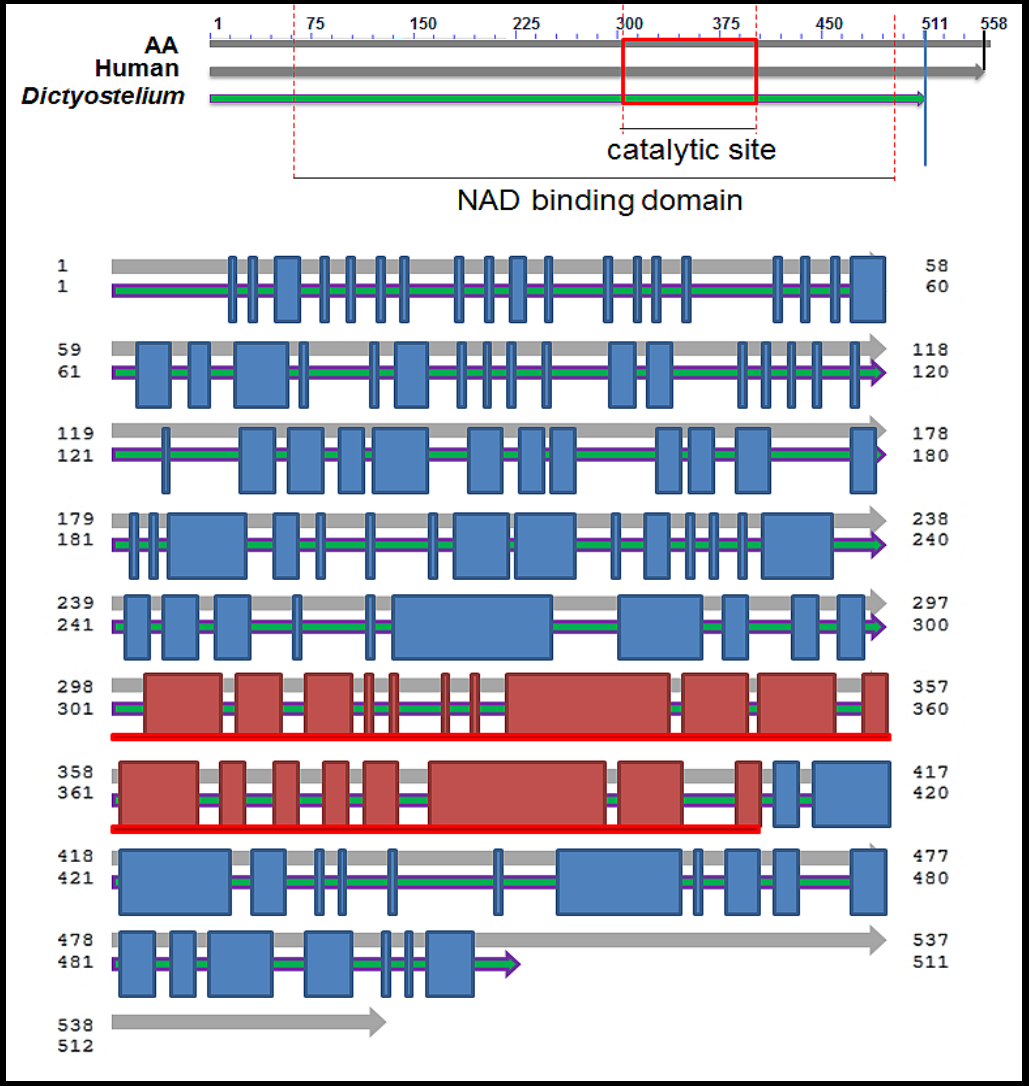
- VPA concentrations in patient blood are 0.4-0.7mM

The human and *Dictyostelium* INO1 proteins are highly similar

- Comparing Human and *Dictyostelium* Inositol synthase enzyme
  - Approx same size
  - High identity (58%)
  - High similarity (82%)

↓

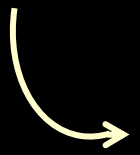
Suggests a conserved cellular role in humans and *Dictyostelium*



(ISYNA1)

We set out to ...

Develop *Dictyostelium* as a non-animal model for neuroscience and molecular pharmacology research

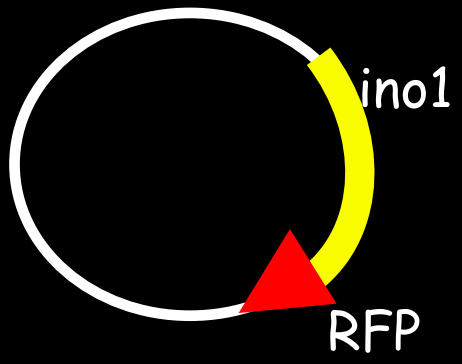


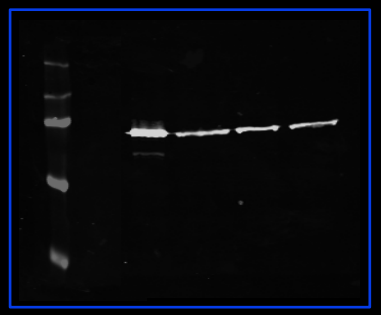
Investigate the role of Valproic acid in INO1 regulation and develop improved treatments

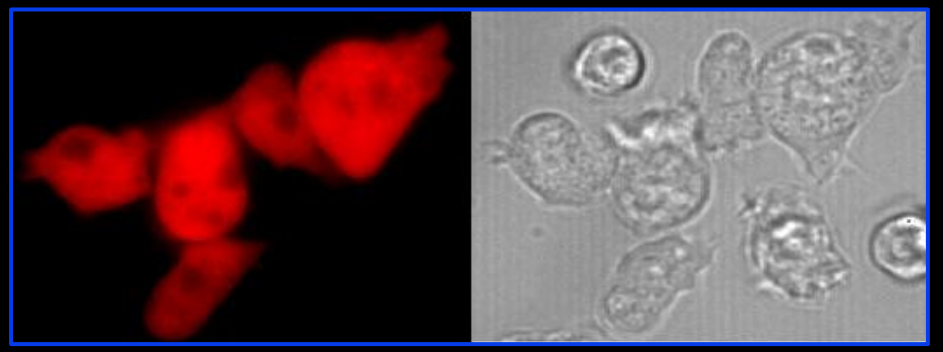
...using a non-animal model

Tools for analysis 1: Increasing protein activity (over-expression)

Dictyostelium ino1 expressing plasmid → INO1 stable in *Dictyostelium* cells → Visualisation of INO1 in *Dictyostelium* cells





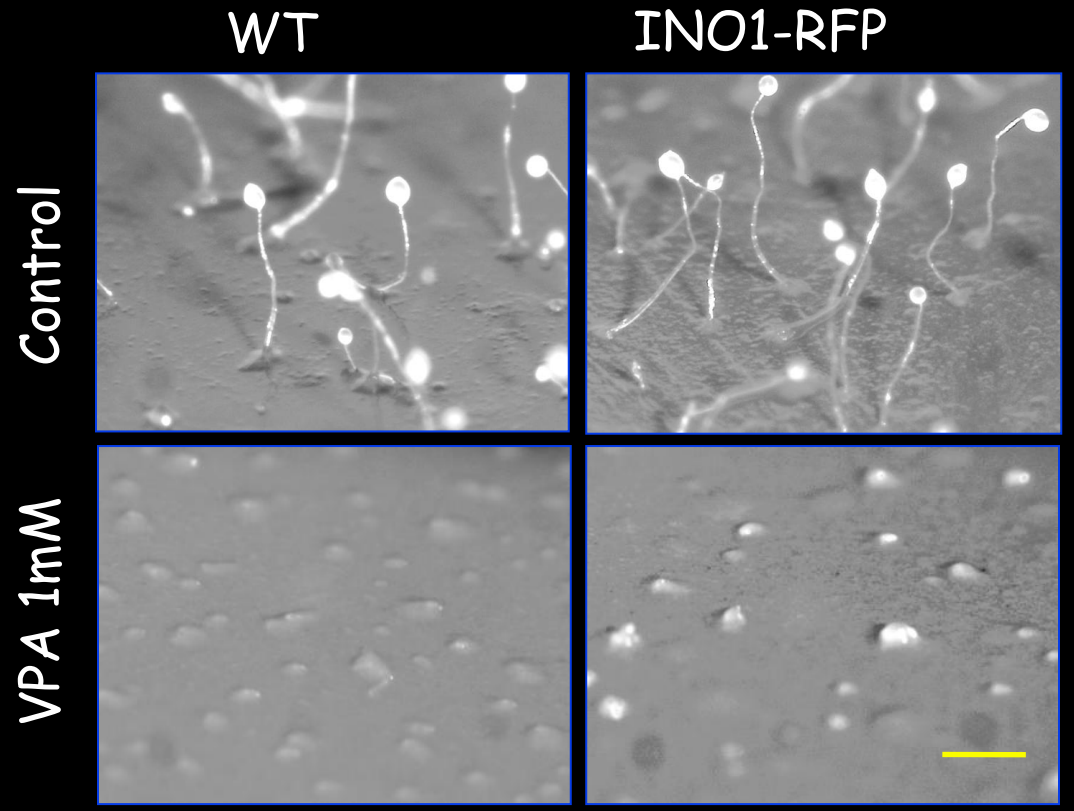


INO1-RFP

Multiple gram weights of homogeneous cell strain for developmental and biochemical analysis

Analysing effects of INO1 over-expression

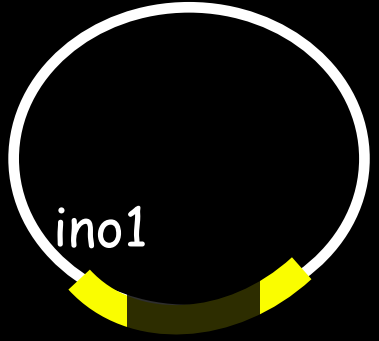
- If VPA functions by direct inhibition of INO1, more INO1 activity should give rise to resistance to VPA ...
  ... repeat development assays



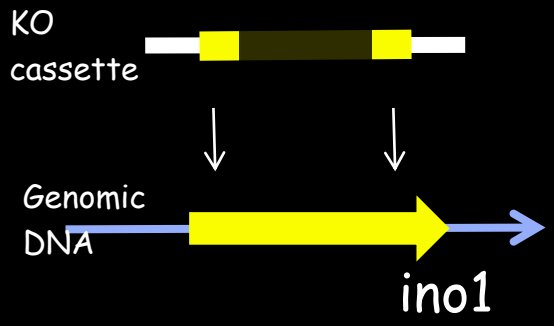
Elevated INO1 levels does not increase resistance to VPA during development

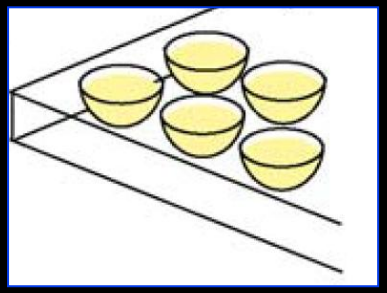
Tools for analysis 2: Removing protein activity (knock-out)

Dictyostelium ino1 Knock-out plasmid → KO gene in *Dictyostelium* cells → Isolate pure strain *Dictyostelium* KO cells

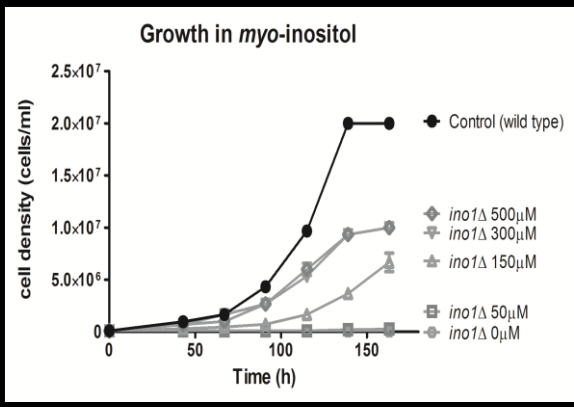


Deleted ino1
Central region



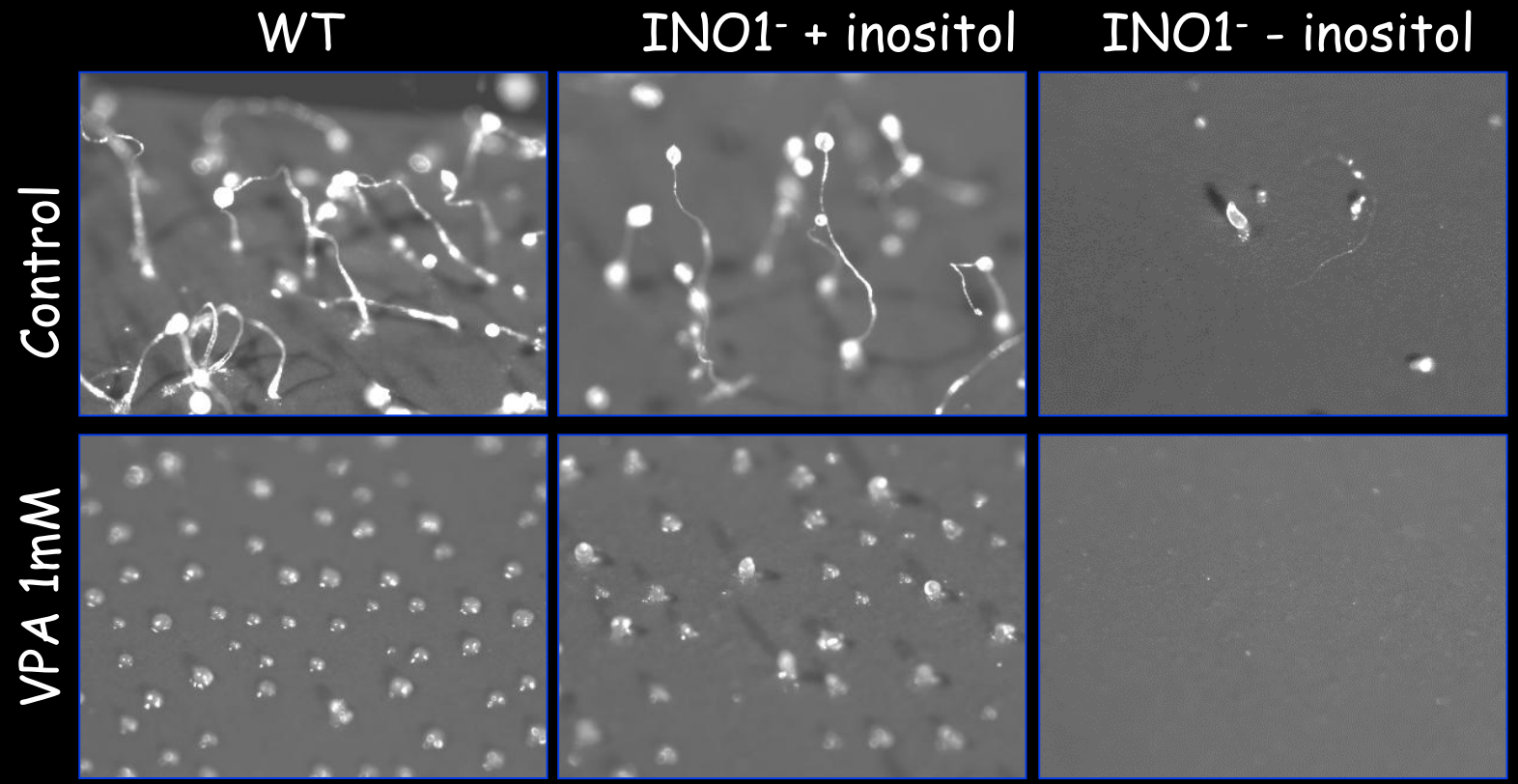


Multiple gram weights of homogeneous cell strain for developmental and biochemical analysis



Analysing effects of INO1 knock-out

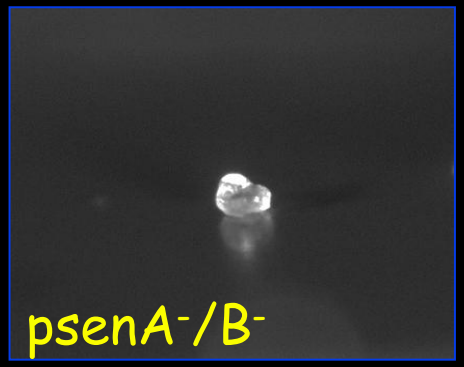
- If VPA functions by direct inhibition of INO1, removal of INO1 protein should negate the effect of VPA...
  ... repeat development assays

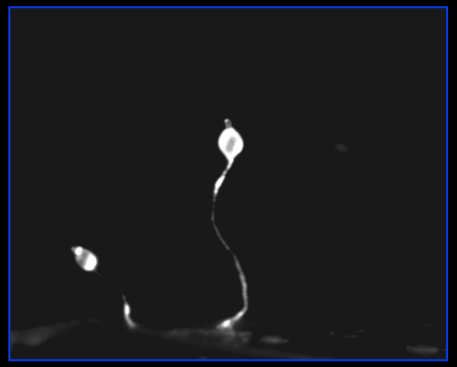


Eliminating INO1 does not give resistance to VPA during development

Work in progress: Humanising the research

- We have recently shown, in *Dictyostelium*, the human presenilin protein is fully functional (in development) - enabling biochemistry and pharmacology studies
- We are currently employing this approach – using the human ino1 gene – in this project...





Human presenilin 1

GFP

Human ino1

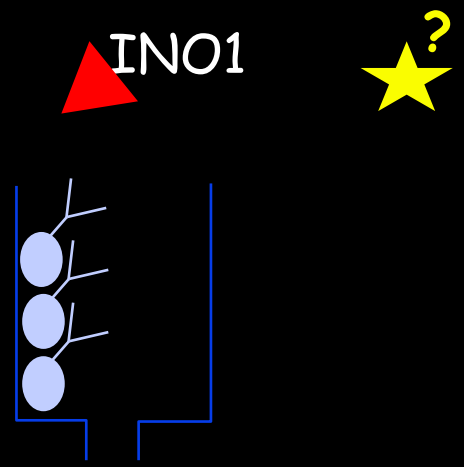
RFP

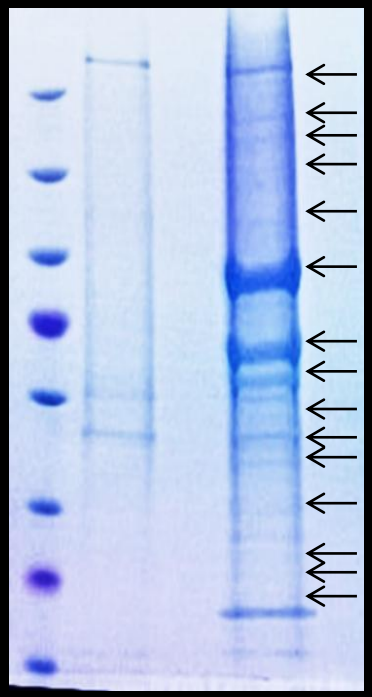
Into ino1⁻ cells on Monday!!!

Work in progress: Indentifying proteins that regulate INO1 activity

- If VPA functions by in-direct inhibition of INO1, proteins that bind to INO1 may be the target of VPA … but what are these proteins …

PULL DOWN APPROACH





PUTATIVE BINDING PARTNERS

- Inositol transcription regulating complex
- Glycolysis proteins known to be involved in inositol synthesis
- Cellular process (phagocytosis components)

Preliminary data

Work in progress: developing a high-throughput screen to identify new bipolar disorder treatments

- Bipolar disorder drugs (including VPA) work through inositol depletion
- How can we identify new compounds for bipolar disorder treatment?

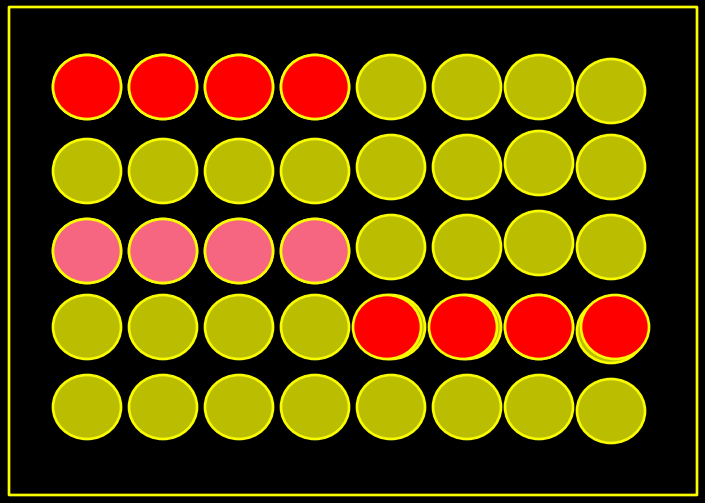
↓ Inositol depletion (via VPA)

Knock-in RFP into ino1 in cell line

→

VPA

?



?

Summary

- Understanding the molecular actions of pharmacologically relevant compounds is possible in non-animal models (with numerous advantages)
- We have shown VPA is unlikely to directly target (*Dictyostelium*) INO1 (human to follow...)
- We will:
  - understand how valproic acid functions
  - discover improved treatments using *Dictyostelium*
- Humanisation of non-animal models will allow a new era of biomedical research without animal experimentation

Funding Dr Hadwen Trust for Humane Research

The workers Anna Frej

Collaborators Robert Insall and Douwe Veltman (Beatson Institute)
Grant Churchill (Oxford)

The DHT is the UK's leading medical research charity that funds and promotes exclusively human-relevant research that encourages the progress of medicine and the replacement of animals in research.