Using a microfluidic device to investigate the role of the furry (FRY) gene in Dictyostelium discoideum

HL Gray; Y Belotti; C Weijer

February 2014
Volume 5
Issue 1
Doctors Academy Publications

The Furry (FRY) gene is an evolutionary conserved gene that is present in yeast, Slime moulds, Drosophila, and humans. In Drosophila the FRY gene has shown to have a role in maintaining polarized cell extensions during the development and patterning of sensory neurons. The function of FRY in slime moulds which have no neurons and humans remains unknown. The aim of this study is the investigation of the role of FRY in Dictyostelium discoideum, where its location in the cortex might suggest a potential role in chemotactic cell migration. of the FRY gene in human cells.

DOCTORS
ACADEMY
Disseminating Medical Knowledge and Skills Globally

DOCTORS ACADEMY
Disseminating Medical Knowledge and Skills Globally
ISSN 2052-1715

WJMER

World Journal of Medical Education and Research

An Official Publication of the Education and Research Division of Doctors Academy

5th International Medical Summer School, It's fun to learn

A career in Military Medicine

An Introduction to Emergency Medicine

Anatomy of Trauma, Emergency Medicine and Operative Procedures

Introduction

The World Journal of Medical Education and Research (WJMER) (ISSN 2052-1715) is an online publication of the Doctors Academy Group of Educational Establishments. Published on a quarterly basis, the aim of the journal is to promote academia and research amongst members of the multi-disciplinary healthcare team including doctors, dentists, scientists, and students of these specialties from around the world. The principal objective of this journal is to encourage the aforementioned, from developing countries in particular, to publish their work. The journal intends to promote the healthy transfer of knowledge, opinions and expertise between those who have the benefit of cutting edge technology and those who need to innovate within their resource constraints. It is our hope that this will help to develop medical knowledge and to provide optimal clinical care in different settings. We envisage an incessant stream of information flowing along the channels that WJMER will create and that a surfeit of ideas will be gleaned from this process. We look forward to sharing these experiences with our readers in our editions. We are honoured to welcome you to WJMER.

Volume 5, Issue 1, 2014, World Journal of Medical Education and Research (WJMER). An Official Publication of the Education an d Research Division of Doctors Academy Group of Educational Establishments.

Electronic version published at	:	Doctors Academy, PO Box 4283, Cardiff, CF14 8GN, United Kingdom
Print version printed and published at	:	Abbey Bookbinding and Print Co., Unit 3, Gabalfa Workshops, Clos Menter, Cardiff CF14 3AY
ISBN	:	978-93-80573-33-5
Designing and Setting	:	Doctors Academy, DA House, Judges Paradise, Kaimanam, Trivandrum, 695018, Kerala, India
Cover page design and graphics	:	Sreekanth S.S
Type Setting	:	Lakshmi Sreekanth
Contact	:	wjmer@doctorsacademy.org.uk

Basic Science Research
DAUIN 20140033

World Journal of Medical Education and Research:
An Official Publication of the Education and Research Division of Doctors Academy

WJMER

Using a microfluidic device to investigate the role of the furry (FRY) gene in *Dictyostelium discoideum*

Ms Hannah-Leigh Gray
Medical Student
University of Dundee

Mr Yuri Belotti
PhD Research Student
University of Dundee

Professor Cornelius Weijer
Professor of Developmental Physiology and Head of Systems Biology
University of Dundee

Address for Correspondence:
Ms Hannah-Leigh Gray: h.y.gray@dundee.ac.uk

Keywords:

Furry gene, FRY gene, Dictyostelium discoideum, Microfluidic device, Cytoskeleton, Cell migration

Abstract

Background: *The Furry (FRY) gene is an evolutionary conserved gene that is present in yeast, Slime moulds, Drosophila, and humans. In Drosophila the FRY gene has shown to have a role in maintaining polarized cell extensions during the development and patterning of sensory neurons. The function of FRY in slime moulds which have no neurons and humans remains unknown. The aim of this study is the investigation of the role of FRY in Dictyostelium discoideum, where its location in the cortex might suggest a potential role in chemotactic cell migration. Due to the similarity of cell migration mechanisms between Dictyostelium and human neutrophils, this research could give insight into the role of the FRY gene in human cells.*

Experimental design: *The FRY gene was knocked out in wild type Dictyostelium cells and rescued by reintroducing the FRY gene into the knockout strain. These strains were examined and their movement behavior compared against wild type cells. A microfluidic device was used to provide a controlled environment for rapid single cell chemotactic movement analysis using a confocal microscope for observation. The images retrieved specified the phenotype of the cell and were used to calculate cell velocity.*

Results: *FRY was shown to have only a minor effect on the average cell velocity but a tail-like phenotype extending from the back of the cell was produced in the FRY knockout strain.*

Conclusion: *These findings could localize the FRY gene at the back of the cell and suggest that FRY is involved in regulating the integrity of the rear cytoskeleton. The cell migration mechanics of amoeba are similar to human neutrophils and further research could elicit the role of FRY in human cells.*

Introduction

The furry gene (FRY) is an evolutionary conserved gene present in humans, Drosophila and *Dictyostelium discoideum.* However its role in humans and *Dictyostelium* is largely unknown. Emoto et al (2004)[1] has shown that FRY is involved in dendritic branching of sensory neurons whilst Fang et al (2005)[2] theorize that FRY is involved in maintaining polarized cell extensions through acting on the cytoskeleton. Polarized cell extensions are an important part of cell migration of *Dictyostelium* and thus FRY could have a role in regulating cell extensions in these cells[3,4]. *Dictyostelium* is a social amoeba that lives as a single cell in soil. It has the ability to transform from a single cell to a multicellular organism though chemotaxis[5]. *Dictyostelium* cells are ideal candidates to investigate the role of genes, such as the FRY gene, and the study of cell migration. *Dictyostelium* has a haploid genome and show efficient homologous recombination, which makes genetic manipulation and analysis of phenotypes easier[6]. Furthermore they display a very similar mechanism of cell migration as human neutrophils. This is clinically relevant as the more we learn from Dictyostelium we can apply to

human neutrophils and hopefully therefore further our understanding of human biology. The aim of this experiment is to investigate the role of the FRY gene in the cell migration of *Dictyostelium*, and to investigate if the FRY gene had a role in the velocity of cell migration.

Experimental Procedure

Dictyostelium cells were harvested from an anexic cell culture. They were then starved for nutrients and placed in a controlled environment and exposed to periodic nanomolar pulses of the chemo-attractant cyclic adenosine monophosphate (cAMP). Three different clones of FRY knockouts (KO) were produced and their motility analysed separately. Clones rescued by reintroduction of the FRY gene were also analysed.

A polydimethysiloxane (PDMS) microfluidic chip sealed to a glass slide provides a controlled environment. The chip is composed of 2 main reservoirs on either side of the chip connected by narrow 2x5 µm channels 1.5mm in length. This allows *Dictyostelium* cells to be deposited on one side of the chip and cAMP on the other. This creates

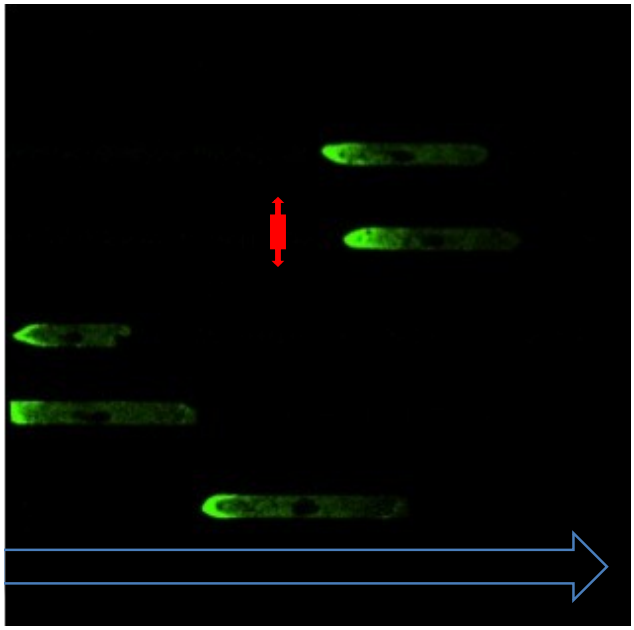

***Figure 1**: Myosin labelled GFP cells moving through the channels within the PDMS chip. The blue arrow indicates direction of movement. The red arrow indicates the width of the channel 5µm. The height of the channel was 2µm.*

a concentration gradient of cAMP which the cells can follow and chemotaxis through the connecting channels. This chip allows rapid single cell analysis of *Dictyostelium* cells moving through a confined space.

The cells were placed within the chip and after 60 minutes of exposure to cAMP the chip was imaged using a confocal microscope for 300 frames at 3-second time intervals. The images were analysed using Fiji software. The velocity of single cells was calculated using the time and distance points from the Fiji software software.

Statistical method

Cells were discounted from the statistical analysis if their velocity was not constant. A constant velocity was defined by using the coefficient of determination and a cut-off value of R^2 value of 0.95 or above was chosen. This was determined by creating graphs for each individual cell from their data points in time and distance in Excel 14.2.2. The velocity is extracted from the graph equation $y = mx + c$, where m is the velocity and c is the y intercept. The data was tested for normality by using a Shapiro-wilk test and Q-Q plots. This was done as the distribution of the data determines whether a parametric or non-parametric test should be used. Due to the distribution of the data a non-parametric one-way analyses of variance test called the Mann-Whitney-U Test was used to detect difference between the means of the data groups. This is the level of statistical difference between the data groups and was used to determine if there was a statistical significant difference between the cell groups velocities. If so this would suggest that the different in velocities was less likely to have occurred by chance. A p-value of less than 0.05 highlights a statistical significant difference. The data was statistically analyzed using SPSS statistics version 20.

Results

In total 168 cells were imaged and analysed and 17 discounted as the cell velocity was not constant.

Cell speed

The mean velocity for each cell group along with the 95% confidence interval (CI) and the range is shown in table 1. The control group velocity ranged from 5.34µm/min to 16.74µm/min with a mean velocity of 8.99µm/min. The FRY2B rescue group had the lowest average velocity at 3.37µm/min with the groups' velocities ranging from 1.49µm/min to 8.16µm/min. The FRY1B rescue group had the highest average velocity at 10.39µm/min with the data ranging from 3.84µm/min to 22.75µm/min.

Cell group Wild type	Number of cells analyzed 19	Number of cells dis-counted 1	Mean veloc-ity (µm/min) 8.99	95%CI 7.39-10.59	Standard deviation 3.32	Min-Max (µm/min) 5.34 16.74
FRY 1B KO	21	5	6.95	5.99-7.92	2.11	3.28-9.77
FRY 1B Rescue	27	0	10.39	8.66-12.13	4.38	3.84-22.75
FRY 2B KO	23	4	4.66	3.62-5.70	2.40	1.51-11.61
FRY 2B RESCUE	20	4	3.37	2.53-3.99	1.56	1.49-8.16
FRY5 KO	22	2	8.34	6.40-10.28	4.38	2.80-15.10
FRY5 RESCUE	19	1	6.98	5.35-8.60	3.37	1.49-8.16

The wild type group had a standard deviation of 3.32. Both FRY1B rescue and FRY5 knockout had the highest standard deviation of 4.38. The lowest standard deviation was for FRY2B rescue at 1.56.

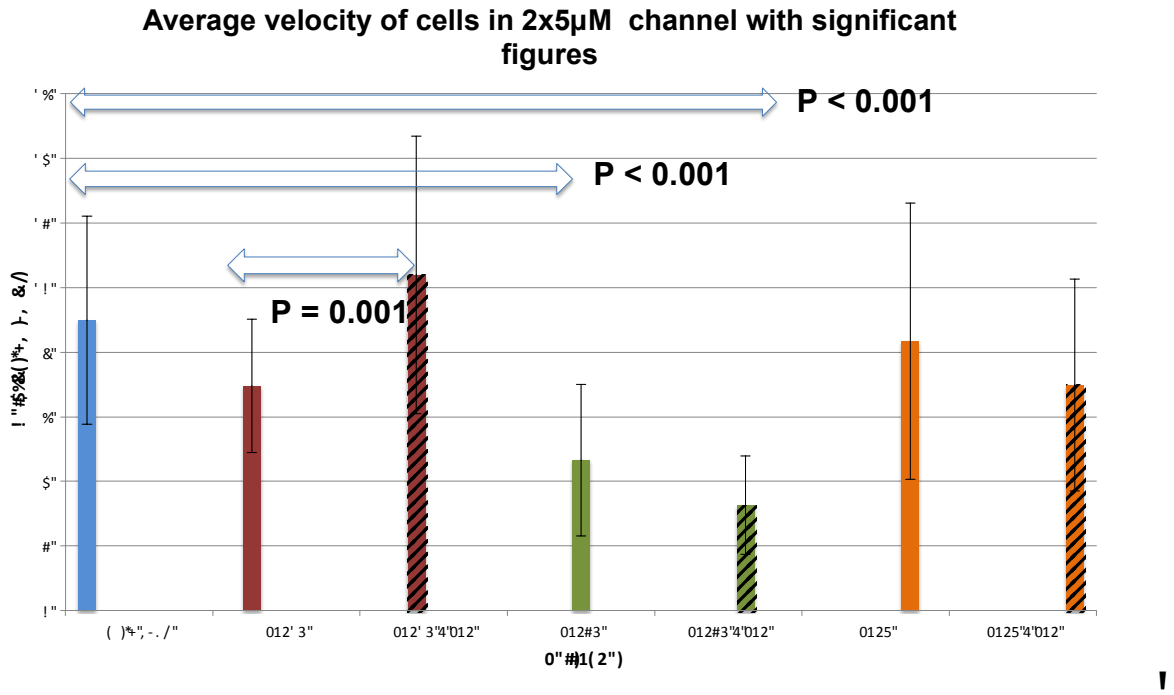

Figure 2: *Summary of cell velocity*

The most significant difference in cell speed was found between the wild type group and the FRY2B KO group, and between the wild type group and the FRY2B rescue group. A significant difference was found between the FRY1B KO group and the FRY1B rescue group.

Cell phenotype

A tail-like phenotype was observed at the back of cell in each gene knockout group and in a proportion of cell in the rescue cell groups. The wild type group had no cells displaying this phenotype. This phenotype is illustrated in Figure 3. The group with the highest percentage of cells was the FRY2B knockout cells with 86.96% of cells showing this phenotype. After reintroducing FRY into the FRY2B knockout cells the percentage of cells showing the phenotype was 40%. The FRY1B knockout and FRY5 knockout had a similar percentage of cells showing the phenotype at 76.19% in the FRY1B knockout group and 77.27% in the FRY5 knockout group.

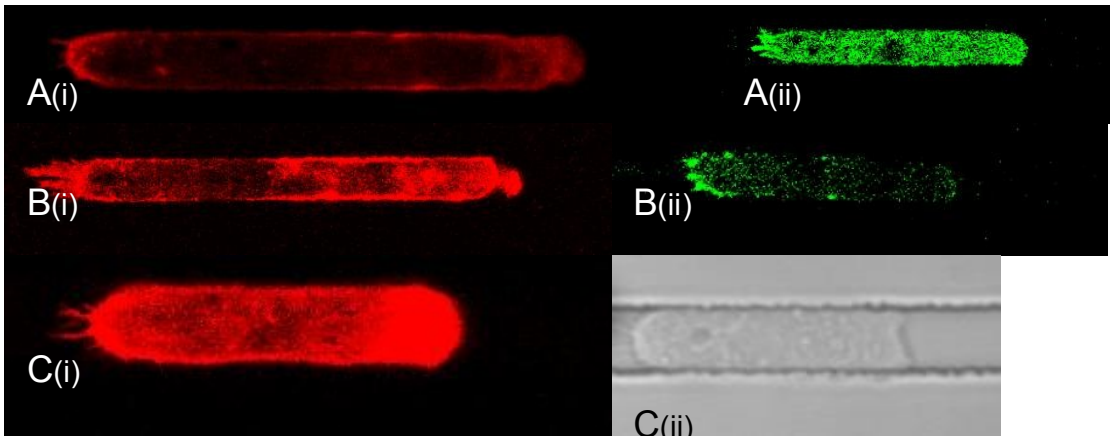

Figure 3: *Ai) FRY1B KO cell. Aii) FRY1B Rescue cell. Bi) FRY2B KO cell. Bii) FRY2B Rescue cell. Ci) FRY5 KO cell. Cii) FRY5 rescue cell.*

Discussion

Cell speed

FRY5 cells used in this study were believed to be true knockout cells and this knockout did not have a statically significant affect on the cell velocity. This could be due to the small sample size and therefore further experiments with an increased number of cells could enhance this finding. It has been suggested in previous research by Lammermann et al[7] that *Dictyostelium* are able to change their mechanics of cell migration between a 2 dimensional surface and a 3 dimensional surface. As FRY plays a role in maintaining polarized cell extensions in Drosophila it is possible that FRY could be involved in maintaining polarized cell extensions in *Dictyostelium*. These extensions may be adhesion dependent migration specific and the defect would not appear in the particular assay used in this experiment. Investigating *Dictyostelium* using different types of assays, for example a flat agar plate, may show the true role of the FRY gene.

Cell phenotype

In each knockout group a tail-like phenotype was observed extending from the back of the cell. This was not present in the wild type cells. This was also present in the rescued cells however this could be explained by the FRY gene being over transcribed when reintroduced into the cell, and therefore the tail phenotype remains present in a proportion of the cells within this group. Cell morphology defects have been found in previous studies of FRY knockouts in *Drosophila*. It is possible that FRY has a role in the morphology and the organization of the cytoskeleton in *Dictyostelium* as knocking the gene out causes changes in the structure of the cell. It is possible that FRY could localize at the back of the cell and maintain the structural integrity of the rear of the cell, hence the removal of FRY resulting in the tail-like phenotype.

Conclusion

Using a clear microfluidic chip we have been able to image and tract the movement of *Dictyostelium* cells in a confined space. It has been previously shown that the mechanisms of migration and the speed of the cell movement can change between a 2-dimensional and a 3 -dimensional environment. The advantage of using a microfluidic chip is that we can create different channel sizes to suit our requirements and thus control the amount of space the cell has to move in. This is relevant for many different types of cells for example tumour cells moving within the human body in metastasis or *Dictyostelium* moving in its natural environment in soil. FRY has not been show to alter the velocity of the cell once it has been knocked out but it has shown an interesting tail-like phenotype. FRY could be responsible for maintaining the integrity of the cytoskeleton at the rear of the cell and on removing the FRY gene this structural integrity at the back of the cell is lost. Removing the FRY gene has not directly affected the speed of cell migration in the 5x2 µm channels, but this could be due to the loss of FRY being compensated by other proteins involved in cell migration when the cell is migrating under these experimental conditions. Changing the assay used to analyse *Dictyostelium* migration, for instance by restricting the diameter of the channels further could give different results. Further research into this could discover the true role of the fry gene in *Dictyostelium*.

References:

1. Emoto K, He Y, Ye B, Grueber WB, Adler PN, Jan LY, et al. Control of dendritic branching and tiling by the Tricornered-kinase/Furry signaling pathway in Drosophila sensory neurons. Cell. 2004 Oct 15;119(2):245-56.
2. He Y, Fang X, Emoto K, Jan YN, Adler PN. The tricornered Ser/Thr protein kinase is regulated by phosphorylation and interacts with furry during Drosophila wing hair development. Mol Biol Cell. 2005 Feb;16(2):689-700.
3. Kortholt A, van Haastert PJ. Highlighting the role of Ras and Rap during Dictyostelium chemotaxis. Cell Signal. 2008 Aug;20 (8):1415-22.
4. Alberts J, Lewis, Raff, Roberts, Walter. Molecular biology of the cell. Fifth ed. New York: Garland Science; 2008.
5. Mir HA, Rajawat J, Pradhan S, Begum R. Signaling molecules involved in the transition of growth to development of Dictyostelium discoideum. Indian J Exp Biol. 2007 Mar;45(3):223-36.
6. Kelsey JS, Fastman NM, Blumberg DD. Evidence of an evolutionarily conserved LMBR1 domain-containing protein that associates with endocytic cups and plays a role in cell migration in dictyostelium discoideum. Eukaryot Cell. 2012 Apr;11 (4):401-16.
7. Lammermann T, Sixt M. Mechanical modes of 'amoeboid' cell migration. Curr Opin Cell Biol. 2009 Oct;21(5):636-44.

The World Journal of Medical Education & Research (WJMER) is the online publication of the Doctors Academy Group of Educational Establishments. It aims to promote academia and research amongst all members of the multi-disciplinary healthcare team including doctors, dentists, scientists, and students of these specialties from all parts of the world. The journal intends to encourage the healthy transfer of knowledge, opinions and expertise between those who have the benefit of cutting-edge technology and those who need to innovate within their resource constraints. It is our hope that this interaction will help develop medical knowledge & enhance the possibility of providing optimal clinical care in different settings all over the world.

DOCTORS ACADEMY
Disseminating Medical Knowledge and Skills Globally

ISSN 2052-1715

WJMER

World Journal of Medical Education and Research

An Official Publication of the Education and Research Division of Doctors Academy