© 2023. Published by The Company of Biologists Ltd | Development (2023) 150, dev201540. doi:10.1242/dev.201540

INTERVIEW

The people behind the papers – Lukasz Truszkowski and Erez Raz

Amoeboid cells can alter their migration mode depending on the substrate they encounter *in vitro*, but how this translates in an *in vivo* setting was unknown. Now, a paper published in Development describes changes in the migration mode of primordial germ cells when moving through different germ layers. We caught up with first author Lukasz Truszkowski and corresponding author Erez Raz, Professor at the University of Münster in Germany, to find out more about their research.

Lukasz Truszkowski (L) and Erez Raz (R)

Erez, can you give us your scientific biography and the questions your lab is trying to answer?

ER: I completed my PhD at the Weizmann Institute of Science in Israel in the lab of Benny Shilo, where we used *Drosophila* as a model for studying the role of the EGF receptor in embryonic development. At that time (the mid-1990s), genetic screens for embryonic phenotypes in zebrafish were being conducted in the USA (Boston, headed by Wolfgang Driever) and in Germany [Tübingen, headed by Janni (Christiane) Nüsslein-Volhard]. Zebrafish embryos offered many advantages, in particular the ability to follow processes in externally developing live embryos that are highly optically transparent. I therefore found the option of joining this new field of exploring early vertebrate development in this 'fresh' and evolving model very attractive, so moved to Boston for my postdoctoral training.

While many mutations affecting early zebrafish embryonic development were isolated in those screens, at the time it was very difficult to clone the genes responsible for the phenotypes. My project in Boston and later in my own lab at Freiburg University in Germany was to try and establish transposon-based transgenesis. The aim of developing the transposon tool was to employ it later for efficient generation of transgenic fish and for positional cloning of genes.

In an effort to improve the transgenesis efficiency, we then started studying primordial germ cells (PGCs), with the idea that learning more about the biology of this cell lineage would help this effort. With time, we became interested in the development of PGCs, irrespective of their potential benefit at the technology level. Here, we were helped by two molecular screens that were conducted in Europe at the time. One was a screen for gene expression patterns performed by Christine Thisse and Bernard Thisse, which from our perspective identified RNAs expressed in the germ cells that we could show play critical roles in controlling germ cell fate. Around the same time, we participated in a morpholino-based screen headed by Camila Esguerra, in which the molecule guiding the migrating germ cells towards the developing gonad was identified. Importantly, several of the proteins isolated in those screens turned out to be critical for the development of germ cells in all vertebrates that were checked, but have not been found in invertebrates. The results of these screens provided the lab with the questions that we have been following over the last 20 years, in Freiburg, at the Max-Planck Institute in Göttingen and then at the University of Münster, where the lab is currently located.

Lukasz, how did you come to work in Erez's lab and what drives your research today?

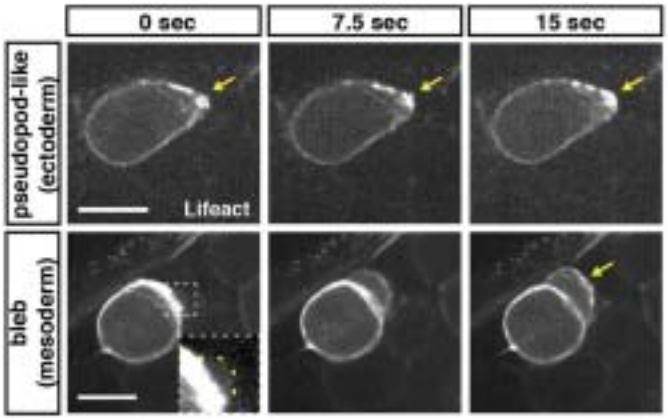
LT: When applying to graduate school in Münster, I chose to visit Erez's lab and talk to people in the group. I became enthusiastic about the experimental system, which offered the option of imaging migrating cells in a live organism. From my experiences during my master thesis, I knew that I enjoy imaging, so I decided to join the lab and extend my imaging expertise. Exploring the model of primordial germ cells, I became interested in the influence of environment on the function of cells. Here, I chose to investigate changes in migratory behaviour in response to changes in the environment.

What was known about how amoeboid cells respond to changes in substrates prior to your work?

LT & ER: Previous findings indicated that migrating amoeboid cells are very robust, so they can alter their migration mode in response to features in the environment and maintain their migration speed (e.g. Renkawitz et al., 2009). However, very few experiments exploring this topic were performed in an *in vivo* setting. Thus, we consider PGCs to be a potentially good new model for studying migration in different contexts in live tissues. In the course of the work, we made use of tools we have developed for manipulating PGCs and their environment, as well as tools and microscopy

L.T. & E.R.: Institute of Cell Biology, ZMBE, University of Münster, D-48149 Münster, Germany.
E.R.: Max Planck Institute for Molecular Biomedicine, D-48149 Münster, Germany.
E-mail: erez.raz@uni-muenster.de

Development (2023) 150, dev201540. doi:10.1242/dev.201540



Snapshots of migrating PGCs. Dashed square outlines the area shown in the inset (a zoomed-in, contrast-adjusted view of the early-stage bleb, with the yellow dashed line outlining the bleb contour). Yellow arrows indicate actin enrichment at the edge of protrusion. Scale bars: 15 µm.

techniques for evaluating the behaviour of the germ cells under different conditions.

Can you give us the key results of the paper in a paragraph?

LT & ER: To study PGC behaviour in response to different conditions in their environment, we converted all cells of zebrafish embryos into cells belonging to a single-germ layer, thereby generating different settings for migration. We found that PGCs were able to migrate in both environments at a similar speed. However, within the ectodermal environment they form primarily actin polymerization-driven protrusions, rather than hydrostatic-pressure powered blebs, which they generate more readily within the mesoderm. This shift is essential for maintaining the migration speed in the ectodermal environment. Relevant for this finding, we could show that cortical tension in surrounding cells is a key factor influencing bleb formation frequency and demonstrated that PGCs can sense and respond to purely physical stimuli.

Do you think that durotaxis could play a role in directed migration of PGCs?

LT & ER: In our previous publication (which provided the basis for this paper) we investigated the distribution of PGCs when they are not guided by the Cxcl12a chemokine (Gross-Thebing et al., 2020). In such a scenario, we did not observe enrichment of cells in specific domains. However, we did observe that PGCs did not enter notochord tissue, which is surrounded by ECM. Therefore, while we do not have evidence that durotaxis plays a major role in guiding the PGCs, it could, in principle, influence the positioning of the cells.

Time until coffee. Away from the lab, Erez's experiments are focussed on coffee!

When doing the research, did you have any particular result or eureka moment that has stuck with you?

LT: We were investigating which properties of the environment result in the observed change of blebbing frequency. Based on the literature, we hypothesized that cortical tension of the cells, which is different between the two environments, could be a factor that affects the protrusion type. Performing this experiment, I was really excited when I saw that manipulation of the contractility of cells in the environment does influence the frequency of bleb formation by the PGCs. As it was a challenging experiment, I was happy to see that the literature search and hard experimental work paid off.

I was really excited when I saw that manipulation of the contractility of cells in the environment does influence the frequency of bleb formation by the PGCs

And what about the flipside: any moments of frustration or despair?

LT: The experiments involving the introduction of dextran-based hydrogels into embryos yielded very exciting results – the PGCs were able to recognize the differences in stiffness in an adhesion-independent manner! However, as the cells were encountering the gel purely by chance, it took us over 9 months to generate enough data supporting this single statement. We could not find any way of increasing the efficiency of the experiment, so were forced to give up on continuing this line of experiments. Therefore, we could not provide more-detailed analysis of the response of the cells to the physical barrier. Nevertheless, we managed to share this result with the community in this publication, so there is some silver lining to the experience.

Lukasz, what is next for you after this paper?

LT: I have recently graduated and started applying for postdoctoral positions. Since I really enjoy studying the influence of environment (cellular or extracellular) on the behaviour and function of cells, I will explore this research theme further in the following steps of my career.

Erez, where will this story take your lab next?

ER: We will follow the story by conducting more thorough analysis of the behaviour of the cells under the same settings. In these future studies we will label many subcellular structures and organelles in the cells to determine what causes the cells to alter the proportion of different protrusion types in the different environments. We hope that those findings will provide us with knowledge and tools for analysing other cell types that migrate as single cells or in groups, and determine their flexibility in adapting to different conditions in the organism.

Finally, let's move outside the lab – what do you like to do in your spare time?

ER: Among other things, I buy green coffee beans from small farms in different parts of the world and roast them with a home roaster. This is done despite the complaints of other family members who do not like the smoke, but avidly drink the freshly brewed coffee.

Development (2023) 150, dev201540. doi:10.1242/dev.201540

LT: Just like the germ cells, I wander around in different environments – I enjoy traveling and hiking. Also, while being in the Raz lab, I caught the bug for playing board games and it will definitely remain an interest of mine in the future.

References

Gross-Thebing, S., Truszkowski, L., Tenbrinck, D., Sánchez-Iranzo, H., Camelo, C., Westerich, K. J., Singh, A., Maier, P., Prengel, J., Lange, P. et al. (2020). Using migrating cells as probes to illuminate features in live embryonic tissues. *Sci. Adv.* **6**, eabc5546. doi:10.1126/sciadv.abc5546

Renkawitz, J., Schumann, K., Weber, M., Lämmermann, T., Pflicke, H., Piel, M., Polleux, J., Spatz, J. P. and Sixt, M. (2009). Adaptive force transmission in amoeboid cell migration. *Nat. Cell Biol.* **11**, 1438-1443. doi:10.1038/ncb1992

Truszkowski, L., Batur, D., Long, H., Tarbashevich, K., Vos, B. E., Trappmann, B. and Raz, E. (2023). Primordial germ cells adjust their protrusion type while migrating in different tissue contexts in vivo. *Development* **150**, dev200603. doi:10.1242/dev.200603