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# Rapid progression through the cell cycle ensures efficient migration of primordial germ cells – The role of Hsp90

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ABSTRACT

Zebrafish primordial germ cells (PGCs) constitute a useful *in vivo* model to study cell migration and to elucidate the role of specific proteins in this process. Here we report on the role of the heat shock protein Hsp90aa1.2, a protein whose RNA level is elevated in the PGCs during their migration. Reducing Hsp90aa1.2 activity slows down the progression through the cell cycle and leads to defects in the control over the MTOC number in the migrating cells. These defects result in a slower migration rate and compromise the arrival of PGCs at their target, the region where the gonad develops. Our results emphasize the importance of ensuring rapid progression through the cell cycle during single-cell migration and highlight the role of heat shock proteins in the process.

## 1. Introduction

Cell migration plays key roles in embryonic development (reviewed in Scarpa and Mayor, 2016; Solnica-Krezel and Sepich, 2012), as well as during adult life in processes such as wound healing and immune response (reviewed in Shaw and Martin, 2016). When misregulated, cell migration is associated with pathological consequences, for example in the context of inflammatory disorders and cancer (reviewed in Friedl and Gilmour, 2009).

Cell motility depends on parameters such as adhesion, polarized actin polymerization and regulated myosin activity and can be directed by cues and physical conditions in the environment (reviewed in Collins and Nelson, 2015; Haeger et al., 2015; Mayor and Etienne-Manneville, 2016). Certain cells divide during their migration, for example collectively migrating cells during early gastrulation and wound healing (Matus et al., 2015; Solnica-Krezel and Sepich, 2012; Martin and Parkhurst, 2004). In such cases, despite the fact that during cell division the cell cannot actively migrate, cell division is not expected to strongly interfere with the speed of migration, as the dividing cells can be carried along with the non-dividing cells. Indeed, the migration of the posterior lateral line primordium (pLLP) cell cluster is not significantly affected by modulating its proliferation rate (Valdivia et al., 2011). Nevertheless, even in such cases, an inverse correlation between migration speed and cell division rate was observed, for example in the process of wound healing (Park et al., 2017). In contrast, single-migrating cells that rely on forces generated within them often do not divide during the process of migration (e.g. mature blood cells and a number of invading cancer cells) (Kohrman and Matus, 2017; Matus et al., 2015; Metcalf, 1989), or have to ensure that cell division phases do not extend to a level that affects the ability of the cells to reach their target and perform their tasks.

An useful *in vivo* vertebrate model for studying the coordination of single cell migration with cell division is that of zebrafish primordial germ cells (PGCs), a model allowing high-resolution live imaging of cell movements (reviewed in Barton et al., 2016; Raz, 2003), while employing a range of genetic tools (Gagnon et al., 2014; Hwang et al., 2013). Relevant for this work, PGCs divide during the migration process (Weidinger et al., 1999). As mentioned above, cell division slows down the displacement of the cells, a point that constitutes a major challenge for the PGCs that have to follow a dynamically changing distribution of a guidance cue and to overcome concurrent tissue movements (Boldajipour et al., 2008; Doitsidou et al., 2002; Weidinger et al., 2002). While the molecular mechanisms controlling PGC polarity and guidance were extensively studied in past years (Blaser et al., 2006; Boldajipour et al., 2008; Doitsidou et al., 2002; Kardash et al., 2010; Meyen et al., 2015; Tarbashevich et al., 2015), the effect of cell cycle progression on primordial germ cell migration was not studied before.

In this work we focused on the functional characterization of a protein enriched in the PGCs during migration, the Heat shock protein 90, alpha, class A member 1, tandem duplicate 2 (Hsp90aa1.2), in scopes of cell division dynamics and migration. We find that

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Hsp90aa1.2 function ensures efficient progression through the cell cycle and proper cell polarization, such that PGCs in which Hsp90aa1.2 activity is reduced show a reduction in the efficiency of arrival at their target, the region where the gonad develops. These results demonstrate the importance of the regulation over the cell cycle in single-cell migration and the role of heat shock proteins in the process.

## 2. Materials and methods

### 2.1. Animals

Zebrafish (*Danio rerio*) were raised and maintained as previously described (Kimmel et al., 1995). Embryos of AB background and of fish carrying *kop.EGFP-F-nos3'UTR* (Zfin: er1Tg), *kop.LifeActEGFP-nos3'UTR* (Zfin: mu4Tg) and *βactin:H2AmCherry* (Zfin: e103Tg) were used as wild-type.

### 2.2. PGC sorting and RNA isolation and analysis

RNA was extracted from FACS-isolated PGCs using PicoPure RNA Extraction Kit (Arcturus). The RNA concentration and purity were measured using a Nanodrop spectrophotometer (Thermo Fisher Scientific) and quality was checked using an Agilent Bioanalyzer (software version B.02.07.SI532) on a RNA Nano Chip (Agilent Technologies). Affymetrix GeneChip Zebrafish Genome microarray analysis (Affymetrix) was performed using the manufacturers´ instructions. 100 ng of total RNA were used for sample preparation, following the Affymetrix 3´-IVT Express Kit instructions. The probes were hybridized overnight to Affymetrix GeneChip Zebrafish Genome arrays. Following hybridization, the arrays were washed and stained using the Affymetrix GeneChip Fluidics Station 450 and scanned using the Affymetrix GeneChip Scanner 3000 7G. Statistical analyses of microarray data were performed using the Partek Genomics Suite software (Partek). PGC sorting, high-throughput sequencing and analysis is described in Paksa et al. (2016), GEO accession number GSE77077.

### 2.3. Whole-mount in situ hybridization (WMISH)

RNA expression patterns were determined as previously described (Thisse and Thisse, 2008) using an antisense RNA concentration of 200 ng per reaction.

### 2.4. Microinjection into zebrafish embryos

A 2 nl solution including morpholino antisense oligonucleotides (MO) (Gene tools LLC) and *in vitro* transcribed mRNA (mMessage mMachine (Ambion)) was injected into the yolk of one-cell stage embryos.

MOs targeting the following sequences (5'–3' direction) were utilized at 0.1 mM:

Control: CCTCTTACCTCAGTTACAATTTATA;

Hsp90aa1.2: TTATTCGCTGTATTTCCTCTTCTCC.

### 2.5. Plasmids used for in vitro transcription of mRNA

| Plasmid name and internal identification number | Transcribed RNA utilized for | 2 nl RNA injected at concentration |
|---|---|---|
| EGFP-F nos1 3'UTR (493) | Labeling PGC membrane with EGFP. | 60 ng/μl |
| DsRed-ex1 3'UTRnos3 (666) | Control in rescue experiments at 24 hpf, expression of DsRed-ex in PGCs. | 50 ng/μl |
| GFP-clip170-H.nos3'UTR (779) | Labeling microtubules and MTOC in PGCs. | 75 ng/μl |
| ECFP-m-nos (886) | Labeling PGC membrane with ECFP. | 70 ng/μl |
| mCherry-F-nos (A906) | Labeling PGC membrane with mCherry-F. | 60 ng/μl |
| Abp140-17aaRuby-nos (B007) | Labeling actin in PGCs. | 70 ng/μl |
| mCherry H2B (B325) | Labeling all nuclei. | 30 ng/μl |
| PAGFP-nanos (B671) | Control in Fucci experiments. Expresses photo-activatable version of GFP. | 66 ng/μl |
| mCherry-HSP90aa1.2-nos3 3'UTR (C191) | Expression of Hsp90aa1.2 fusion in PGCs. Used in rescue experiments at 24 hpf. | 200 ng/μl |
| hsp90aa1.2-nos3 3'UTR (C653) | Expression of Hsp90aa1.2 in PGCs. Used in rescue experiments. | 200 ng/μl |
| mCherry-hsp90aa1.2-hsp90aa1.2 3'UTR (D024) | Expression of an Hsp90aa1.2 protein fused to mCherry with *hsp90aa1.2* 3'UTR for sub-cellular localization studies and rescue experiments. | 100 ng/μl |
| mCherry-zCdt1(1/190)-nos3 3'UTR (D339) | Used in Fucci experiments, labels G1 phase of the cell cycle. | 50 ng/μl |
| mVenus-zGeminin (1/100)-nos3 3'UTR (D340) | Used in Fucci experiments, labels S/G2/M phases of the cell cycle. | 50 ng/μl |
| BFP-nls-globin3'UTR (D846), GFP-nls-nanos3'UTR (344) | Used in translation control experiments. | 10 ng/μl each |
| mCherry-hsp90aa1.2-globin 3'UTR (D995) | Expression of Hsp90aa1.2, mRNA localization/stability experiments. | 200 ng/μl |

### 2.6. Generation of zebrafish mutants using the CRISPR/Cas9 system

The mutagenesis was performed according to Gagnon et al. (2014). Three mutant lines were generated as described in the results section.

### 2.7. Microscopy

Embryos after WMISH were embedded in glycerol and imaged in brightfield. For live imaging embryos older than 20 hpf were anaesthetized in 0.64 mM Tricaine prior to microscopy. Epifluorescence and brightfield microscopy was applied for determining the 20–24 hpf phenotypes, images captured at 5× magnification, determination of PGC number and number of ectopic PGCs was performed at 20× magnification. For determining the dynamic migration parameters and for following the cell cycle employing the Fucci Cell Cycle Sensor, epifluorescence time-lapse microscopy at 10× magnification, 2 min/frame or 3 min/frame in 6–9 hpf embryos was performed. For the dynamic migration parameters 70 min migration tracks were analyzed using the MetaMorph (Molecular Devices) and Imaris (Bitplane) software packages. The drift of the somatic environment was corrected using the Imaris software. For long-term imaging of the cell cycle,