In vivo cell biology using Gal4-mediated multicolor subcellular labeling in zebrafish

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The behavior of a cell is determined by the interplay of its subcellular components. Thus, being able to simultaneously visualize several organelles inside cells within the natural context of a living organism could greatly enhance our understanding of developmental processes. We have established a Gal4-based system for the simultaneous and cell type specific expression of multiple subcellular labels in transparent zebrafish embryos. This system offers the opportunity to follow intracellular developmental processes in a live vertebrate organism using confocal fluorescence time-lapse microscopy. Using this approach we recently showed that the centrosome neither persistently leads migration nor determines the site of axonogenesis in migrating neurons in the zebrafish cerebellum in vivo. Here we present additional in vivo findings about the centrosomal and microtubule dynamics of neuroepithelial cells during mitotic cleavages at early neural tube stages.

Key words: in vivo imaging, zebrafish, neuroepithelium, Gal4/UAS, subcellular labeling, mitosis

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In cells initiating mitosis, the microtubule cytoskeleton undergoes major remodeling and the centrosomes relocate within the cell to form the spindle poles of the microtubule-based spindle apparatus. Next, replicated chromosomes condense, the nuclear envelope breaks down and microtubules attach to kinetochores to pull sister chromatids apart. At the end of mitosis, cytokinesis separates the two daughter cells. Here, a contractile actomyosin ring drives constriction of the plasma membrane to form a narrow cytoplasmic bridge between the two reforming nuclei. Physical separation of the two daughter cells requires this bridge to be severed in a final step termed abscission. This cytoplasmic bridge consists of a thin tube of plasma membrane filled with two antiparallel bundles of microtubules with their plus-ends interdigitating at the midbody. Live imaging of cells in culture reveals that the bridge can persist for several hours before abscission occurs, through a mechanism that remains largely unknown.2 A current model suggests that the two centrioles separate and the mother centriole translocates to the intercellular bridge, bringing proteins located at the centrosome together with their interaction partners located at the midbody, hereby initiating abscission.3-6

The ability to visualize organelles inside cells within a living organism could greatly enhance our understanding of developmental events such as mitosis. Towards this goal, we have established a system for the expression of multiple subcellular labels in specific cells of a living zebrafish
In vivo time-lapse recording the interplay of plasma membrane, nucleus, microtubules and centrosome in neuroepithelial cells, which divide at the midline in the zebrafish hindbrain and anterior spinal cord at early neural tube stages (Sup. Vid. 1).13-16 The two centrioles of the centrosome are located at the apical membrane during nucleokinesis. Further, we found that the centrosome does not by its proximity determine the site of axonogenesis in these neurons.12

Here we have applied our multi-organelle labeling approach by co-injecting a Gal4 expression vector (pCSKalTA4) together with Medusa M1, M2 or M3 constructs12 at the one cell embryo. Owing to their transparency during embryonic stages and their fast and external development, zebrafish are an ideal model organism for in vivo microscopy.7,8 Our labeling technique is based on the Gal4-UAS system, in which Gal4 is a yeast-derived transcriptional activator that induces gene expression by binding to DNA binding sites called upstream activating sequences (UAS).

We have established so-called Medusa vectors (Fig. 1A), which contain UAS sites bidirectionally flanked by basal promoters driving expression of subcellularly targeted fluorescent proteins with distinct spectral properties. Thus, multiple organelles can be simultaneously demarcated for confocal fluorescence microscopy. Moreover, by placing Gal4 expression under tissue specific control, as already achieved in many stable transgenic Gal4-driver lines, the expression of organelle labels can be restricted to cell types of interest.9-11

We recently performed Medusa vector labeling of the centrosome, nucleus and membrane in migrating tegmental hindbrain nuclei neurons in the live zebrafish cerebellum, and demonstrated that the centrosome and nucleus exhibit an unexpected sort of leapfrogging behavior during nucleokinesis. Further, we found that the centrosome does not by its proximity determine the site of axonogenesis in these neurons.12

Here we have applied our multi-organelle labeling approach by co-injecting a Gal4 expression vector (pCSKalTA4) together with Medusa M1, M2 or M3 constructs12 at the one cell stage and following by in vivo time-lapse recording the interplay of plasma membrane, nucleus, microtubules and centrosome in neuroepithelial cells, which divide at the midline in the zebrafish hindbrain and anterior spinal cord at early neural tube stages (Sup. Vid. 1).13-16 The two centrioles of the centrosome are located at the apical membrane of
Figure 3. Centrosomal movements during cytokinesis. Dorsal views of an early neural tube stage zebrafish embryo expressing Medusa vector M1, which contains Centrin2-YFP (yellow centrosome), memmRFP (red membrane) and H2B-CFP (blue nucleus). After separation of the sister chromatids, the centrosome relocates from the spindle pole to the apical membrane. The centrioles of the centrosome (white arrow) remain together as they move towards the midbody of neuroepithelial cells.

Our system has proven to be a powerful tool for the study of developmental processes on a subcellular level in a living organism. Our initial applications of these in vivo cell biology tools have already revealed significant differences from current models, promising that there is much new and exciting to discover deep within a zebrafish.

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Note
Supplemental materials can be found at: www.landesbioscience.com/journals/cib/article/15037

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