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Resource

# The pros and cons of common actin labeling tools for visualizing actin dynamics during *Drosophila* oogenesis

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ABSTRACT

Dynamic remodeling of the actin cytoskeleton is required for both development and tissue homeostasis. While fixed image analysis has provided significant insight into such events, a complete understanding of cytoskeletal dynamics requires live imaging. Numerous tools for the live imaging of actin have been generated by fusing the actin-binding domain from an actin-interacting protein to a fluorescent protein. Here we comparatively assess the utility of three such tools – Utrophin, Lifeact, and F-tractin – for characterizing the actin remodeling events occurring within the germline-derived nurse cells during *Drosophila* mid-oogenesis or follicle development. Specifically, we used the UAS/GAL4 system to express these tools at different levels and in different cells, and analyzed these tools for effects on fertility, alterations in the actin cytoskeleton, and ability to label filamentous actin (F-actin) structures by both fixed and live imaging. While both Utrophin and Lifeact robustly label F-actin structures within the *Drosophila* germline, when strongly expressed they cause sterility and severe actin defects including cortical actin breakdown resulting in multi-nucleate nurse cells, early F-actin filament and aggregate formation during stage 9 (S9), and disorganized parallel actin filament bundles during stage 10B (S10B). However, by using a weaker germline GAL4 driver in combination with a higher temperature, Utrophin can label F-actin with minimal defects. Additionally, strong Utrophin expression within the germline causes F-actin formation in the nurse cell nuclei and germinal vesicle during mid-oogenesis. Similarly, Lifeact expression results in nuclear F-actin only within the germinal vesicle. F-tractin expresses at a lower level than the other two labeling tools, but labels cytoplasmic F-actin structures well without causing sterility or striking actin defects. Together these studies reveal how critical it is to evaluate the utility of each actin labeling tool within the tissue and cell type of interest in order to identify the tool that represents the best compromise between acceptable labeling and minimal disruption of the phenomenon being observed. In this case, we find that F-tractin, and perhaps Utrophin, when Utrophin expression levels are optimized to label efficiently without causing actin defects, can be used to study F-actin dynamics within the *Drosophila* nurse cells.

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## Introduction

*Drosophila* oogenesis or follicle development consists of 14 morphologically defined stages (reviewed in (Spradling, 1993)). Each egg chamber or follicle is comprised of 16 interconnected germline cells – 15 nurse cells and one oocyte – and approximately 1000 somatic cells termed follicle cells. Production of a viable egg requires dynamic remodeling of the actin cytoskeleton in both the somatic and germline cells. Thus, *Drosophila* oogenesis is an ideal system for studying the actin cytoskeleton and has been widely used to identify and elucidate the functions of actin binding proteins and regulatory factors (reviewed in (Hudson and Cooley, 2002)). Here we focus on events occurring within the germline-derived nurse cells. The main purpose of the nurse cells is to supply organelles, mRNA, and proteins to the oocyte, thereby providing the oocyte with everything needed to complete embryogenesis.

Spatial and temporal regulation of actin remodeling is required for this multi-stage transport process that is essential for the production of a viable egg. Transport occurs through the ring canals, remnants of incomplete cytokinesis that connect the nurse cells to each other and the oocyte. In the earlier stages of follicle development (prior to stage 10B (S10B)) the transport of materials from the nurse cells into the oocyte is slow (reviewed in (Hudson and Cooley, 2002)). Little actin remodeling is seen within the nurse cells during these stages. Intriguingly, we have observed that some actin

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remodeling is occurring at the ring canals connected to the oocyte in the posterior nurse cells during stage 9 (S9) (Spracklen et al., 2014). This normally results in minimal actin filaments near the ring canals (see Fig. 1A and A′), but occasionally results in more extensive actin filament and/or aggregate formation (see Fig. 1B and B′). Such remodeling is regulated as we have found that genetic loss of the *Drosophila* COX-like enzyme, Pxt, results in a significant increase in the prevalence of these early actin structures (Spracklen et al., 2014).

During S10B, the nurse cells undergo dynamic actin remodeling to facilitate the second, rapid phase of transport that occurs during stage 11 (S11), termed nurse cell dumping. Nurse cell dumping requires two distinct actin remodeling events during S10B: 1) the strengthening of nurse cell cortical actin, which ultimately undergoes an actomyosin based contraction to squeeze the nurse cell cytoplasm into the oocyte (Wheatley et al., 1995) and 2) the generation of parallel actin filament bundles that traverse from the nurse cell membrane, inward towards the nucleus, to form a cage that prevents the nucleus from plugging the ring canals during contraction (Guild et al., 1997; Huelsmann et al., 2013). The system of nurse cell dumping has been widely used to both identify actin regulators and define their functions in cytoskeletal remodeling (Cooley et al., 1992; Gates et al., 2009; Mahajan-Miklos and Cooley, 1994a, b). We have shown that Pxt, and thus prostaglandins (PGs) are required to regulate these dynamic remodeling events (Groen et al., 2012; Tootle and Spradling, 2008). Such studies have relied on analyses of actin structures using fixed samples labeled with phalloidin to visualize filamentous actin (F-actin).

While fixed imaging has provided important insights into the actin cytoskeletal remodeling events occurring within the *Drosophila* germline, many questions remain regarding the spatial and temporal regulation of this remodeling. For example, it has previously been shown that the remodeling during S10B begins along the nurse cell oocyte boundary and then progresses anteriorly (see Fig. 1D–F) (Guild et al., 1997). However, this spatial and temporal progression is not widely appreciated and the mechanisms underlying this regulation remain poorly understood. Specifically, the roles of particular actin regulators in establishing spatial and temporal regulation over these cytoplasmic actin remodeling events remain unknown. Additionally, fixed analyses cannot be used to assess nurse cell actin filament bundle dynamics, or how pharmacologic or genetic perturbations alter those dynamics. One means of addressing these knowledge gaps is to utilize live imaging.

One actin cytoskeleton live imaging reagent routinely used in *Drosophila* is the GFP-tagged actin binding domain of Moesin, GFP-Moe (Edwards et al., 1997). GFP-Moe has successfully been used to examine actin dynamics in the *Drosophil*a embryo during dorsal closure (Peralta et al., 2007; Toyama et al., 2008), tracheal morphogenesis (Kato et al., 2004), and hemocyte migration (Zanet et al., 2009). It has also been used to label actin dynamics

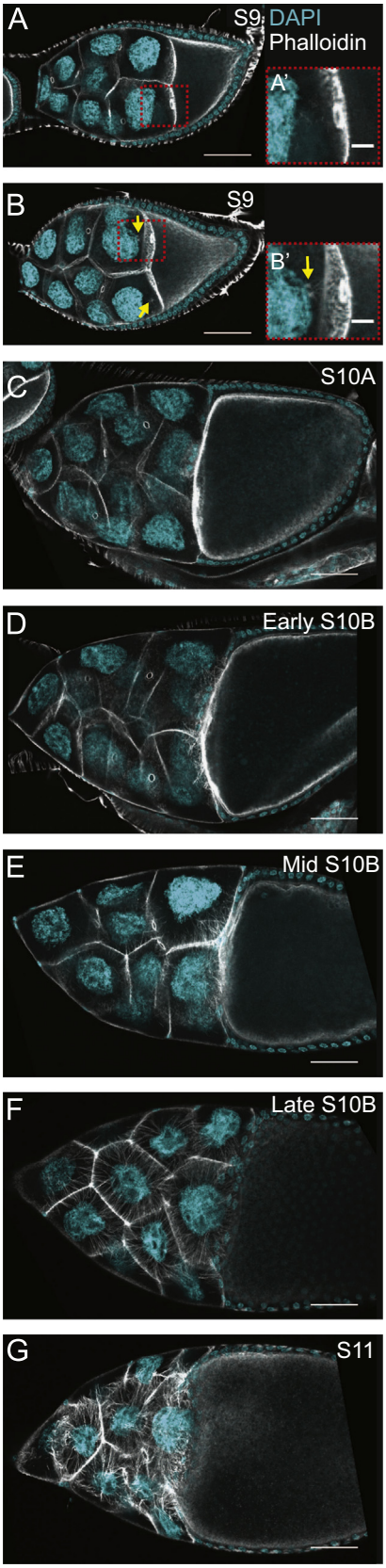


**Fig. 1.** Actin remodeling is temporally and spatially regulated during mid-oogenesis. (A–G) Maximum projections of 3–5 confocal slices of fixed and stained wild-type (*yw*) follicles, staged as indicated, taken at 20× magnification. Anterior is to the left. F-actin (phalloidin)=white, DNA (DAPI)=cyan. (A–B′) S9. (C) S10A. (D–F) S10B. (G) S11. During S9, the border cells and main-body follicle cells are undergoing migration and the nurse cell cytoplasm is largely devoid of actin filament structures (A–A′), but occasionally there are actin filament and aggregate structures emanating from the ring canals in the posterior nurse cells adjacent to the oocyte (B–B′). Both the border cell and main-body follicle cell migrations are completed by S10A and the nurse cells lack cytoplasmic F-actin structures (C). During S10B, dynamic actin remodeling is occurring within the nurse cells. Actin filament bundles first form in the posterior nurse cells, at the nurse cell-oocyte boundary (D). These bundles continue to elongate and bundle formation initiates on all of the nurse cell membranes that are directly attached to their neighboring nurse cell by a ring canal (E). At the completion of S10B, the bundles are uniformly distributed along the nurse cell membranes and extend all the way to the nucleus (F). During S11, the cortical actin contracts to squeeze the cytoplasmic contents of the nurse cells into the growing oocyte (G). Scale bars=50 μm, except in A′ and B′, where scale bars=10 μm.

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