Chapter 13. Labile filopodial extensions: signal-transmission, stem-cell exit and oncogenesis.

Many cells extend labile filopodia, including haemocytes, pathfinding neurones, stem-cells and carcinomas; with essential functions during wound healing and dorsal closure, in *Drosophila* [1 2 3 4]. Filopodial outgrowths consist of an actin microfilament core surrounded by a membrane sheath. Specialised "cytonemal" filopodia transport morphogen-associated exosomes during imaginal disc growth and in ovarian stem cells [5 6 7 8 9]. In the wing disc, apical cytonemes extend between the Wg-expressing cells around the D/V margin and the A/P compartment boundary [10]. Meanwhile, basal cytonemes transport Hh cross the A/P boundary and Dpp is trafficked across the disc lumen from peripodial cytonemes [11 12]. Thus, cytonemes may traffic morphogen cargos across compartmental boundaries, or between opposed epithelial surfaces [13].

In the wing disc, cytonemes from either side of the A/P boundary and are interconnected by a series of terminal "kissing complexes" [14]. Hh is transported from P > A, with a gradient of attenuated transmission associated with the stochastic collapse of extending cytonemes. The P > A movement must be driven by plus-end directed motor activity along P cytonemes and continued by minus-end motor activity after cargo-transfer to A cytonemes. It is unclear how this switch in motor activity is regulated, but kinase activation of the MyoVI (Jaguar, Jag) may be involved. Jag has high binding affinity for Hh, and is the only minus-end directed microfilament motor in *Drosophila* [15], see below Chapter **30**. In general, transport mechanisms may differ between different classes of filopodia and are not fully understood. Human adenocarcinomal filopodia can capture and transport quantum dots attached to the EGF receptor (EGFr-QDs). In this case, depolymerisation of the actin microfilament shaft transports EGFr-QDs towards the cell body [5]. Thus, exocytotic cargo vesicles may be captured on EGF receptors and carried by collapsing filopodia before endocytosis at the cell surface. Such a mechanism would enhance EGF uptake (and carcinomal growth) without requiring direct engagement with microfilament motor complexes. In contrast, fluorescently tagged MyosinX (MyoX-GFP) punctae can travel in either direction along the filopodia of HEK cells [8]; although it is unclear whether the fluorescence is tracking actively engaged motor assemblies, or the MyoX-GFP is transported as a vesicle cargo. Similarly it is uncertain, whether Hh and iHog-YFP MVBs are transported across the A/P boundary within cytoneme shafts, or as vesicles captured on their surface membrane, see [16 17]. A simple membrane-fusion mechanism appears to be ruled out, as fluorescent tags on exocytotic vesicles are not transferred to the filopodial shaft. In particular, red fluorescent vesicles (*mCd8-RFP*) remain discrete while transported along green (*mCd8-GFP*) filopodia in mixed (red/green) glioblastomal cell cultures, see [18]. In general, internal cargos may be attached to unconventional myosin motors, in combination with MyoII-driven cytoplasmic flux. Both cargo transport and microfibril remodelling require that some cortical microfibrils be anchored to the extracellular matrix. Meanwhile, exosomes bound to the EGF receptor might be coupled to an internal myosin motor assembly via a transmembrane linkage, such as Dystroglycan/Dystrophin. Such coupling would allow membrane flux around the membrane-spanning linker, with direct engagement of the motor protein heads on the internal microfilament core. In this context, Sisyphus (Sis, MyoXV) transports sensory and adhesion cargos along filopodial extensions, which remain straight despite apparent discontinuities in their F-actin core, see Fig. 3 of [18]. By implication, the rigidity of extending filopodial shafts may be dependent on an extracellular matrix sheath. Notably, exosomes from human fibroblast cultures stimulate protrusive filopodial growth from breast cancer cells; with Wnt signalling activated through Fz/Dsh and Pk/Vangl [19].

Summary:

Filopodial cytonemes may extend across twin-field boundaries, parasegmental AMSs, or between opposed epithelial surfaces. Morphogen-associated vesicles may be transported and released within the imaginal disc lumen or delivered directly to target cells. Filopodial extensions may deliver morphogens (and other vesicle cargos) during normal growth or capture EGF bound cargos during metastatic cancer proliferation.

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