Seminars in Cell & Developmental Biology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Auto-fusion and the shaping of neurons and tubes

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ARTICLE INFO

Article history:
Received 25 April 2016
Received in revised form 15 July 2016
Accepted 15 July 2016
Available online xxx

Keywords:
Auto-fusion
Fusogen
Dendrite morphogenesis
Neurons regeneration
Seamless tube

ABSTRACT

Cells adopt specific shapes that are necessary for specific functions. For example, some neurons extend elaborate arborized dendrites that can contact multiple targets. Epithelial and endothelial cells can form tiny seamless unicellular tubes with an intracellular lumen. Recent advances showed that cells can auto-fuse to acquire those specific shapes. During auto-fusion, a cell merges two parts of its own plasma membrane. In contrast to cell–cell fusion or macropinocytic fission, which result in the merging or formation of two separate membrane bound compartments, auto-fusion preserves one compartment, but changes its shape. The discovery of auto-fusion in *C. elegans* was enabled by identification of specific protein fusogens, EFF-1 and AFF-1, that mediate cell–cell fusion. Phenotypic characterization of *eff-1* and *aff-1* mutants revealed that fusogen-mediated fusion of two parts of the same cell can be used to sculpt dendritic arbors, reconnect two parts of an axon after injury, or form a hollow unicellular tube. Similar auto-fusion events recently were detected in vertebrate cells, suggesting that auto-fusion could be a widely used mechanism for shaping neurons and tubes.

Contents

1. Introduction—cell shaping by auto-fusion

Cells adopt specific shapes that are necessary for specific functions. For example, neurons extend elongated axons and arborized

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http://dx.doi.org/10.1016/j.semcdb.2016.07.018
1084-9521/

dendrites to contact their partners and transmit and receive electro-chemical signals [1]. Epithelial and endothelial cells can form hollow tubules to transport gases and fluids [2]. Pioneering studies in *C. elegans* showed that cell auto-fusion is an important mechanism that contributes to cell shaping [3–6]. Auto-fusion is the process whereby a cell merges two parts of its own plasma membrane using a mechanism similar to cell–cell fusion. In this review, we describe how cells can fuse their own membrane to acquire specific shapes. We compare auto-fusion to endocytic processes such as macropinocytosis ("cell gulping"), which also contribute to cell shaping.

2. Types of fission, fusion and fusogens

Membrane merging occurs during two different processes, fission and fusion [7] (Fig. 1A). Cell fission converts a single cytosolic compartment into two independent compartments surrounded by lipid bi-layers; examples include cytokinesis, vesicle endocytosis and budding of membrane-bound extracellular vesicles. Cell fusion is traditionally the merging of two compartments into one, as in vesicle fusion and exocytosis during secretion, virus-cell fusion during infection, gamete fusion during fertilization, or cell–cell fusion to form a syncytium. But fusion can also be used cell autonomously to form specific structures, as we will discuss.

Fission and fusion events can be further categorized as endoplasmic or exoplasmic, depending on whether initial membrane contacts occur between cytosol-proximal monolayers or external monolayers [7] (Fig. 1A). This distinction is important mechanistically, because each monolayer exposes different sets of lipids and proteins. Endocytosis, cell–cell fusion, and auto-fusion are all exoplasmic events.

2.1. Role of fusogens in membrane merging

A general pre-requisite for all types of membrane merging is that the two membranes involved are brought in very close proximity. In addition to cytoskeletal forces, this often involves specific transmembrane proteins that mediate membrane recognition to confer specificity, and help to overcome repulsive electrostatic and hydration forces to allow direct contact between the bilayers [8]. Upon sufficiently close contact, the exposed lipid leaflets can merge to form a hemi-fusion intermediate, which then is resolved to form a fusion pore that can expand to complete the fusion process [8].

Fusogens are membrane proteins that are both necessary and sufficient to promote membrane merging [8]. Some fusogens are exposed on only one of the fusing membranes and interact with other partners in the opposite membrane, making a heterotypic interaction. Other fusogens must be present on both membranes and require a homotypic interaction to mediate fusion.

Endoplasmic and exoplasmic merging events rely on different classes of fusogens with different orientations in the membrane. In the case of endoplasmic fusion, one major class of fusogens is the well-studied SNARE family; heterotypic interactions between V-SNARES and T-SNARES on different membranes promote fusion [9]. SNAREs have also been implicated in endoplasmic fission [10]. Other endoplasmic fusogens include atlastin, involved in Endoplasmic Reticulum fusion [11,12], and mitofusins 1 and 2 and OPA1, involved in mitochondria membrane fusion [13–15]. Many of the known exoplasmic fusogens are viral, and belong to three distinct structural classes [8]. Only a few exoplasmic fusogens have been identified in animals, including mammalian Syncytins and *C. elegans* Epithelial Fusion Failure-1 (EFF-1) and Anchor cell Fusion Failure-1 (AFF-1) [16–19], which are structurally related to viral class I and class II fusogens, respectively [20–23]. HAP2/GCS1 is a potential fusogen present in gametes of plants, protozoa, amoebae and some invertebrate animals [24–26].

2.2. Exoplasmic fission

Exoplasmic fission encompasses many mechanistically- and morphologically-distinct types of endocytosis. Clathrin-dependent endocytosis and related forms of micropinocytosis involve formation of small coated vesicles (~100 nm) that internalize materials from the cell surface [27]. In most cases, the small GTPase dynamin is required to pinch off the vesicle neck to separate it from the plasma membrane, and dynamin is sufficient for such scission in vitro [27]. Macropinocytosis and phagocytosis involve formation of larger, uncoated vesicles (>0.2–5 μm) for internalization of bulk membrane, external fluids and particles [27]. Scission of these larger vesicles can be dynamin- independent, but no membrane fusogens are known to be involved.

Macropinocytosis can influence cell shaping during development. For example, macropinocytosis internalizes plasma membrane during neuronal growth cone collapse and axon turning [28,29]. Macropinocytosis also is one of several proposed mechanisms involved in formation of small capillary tubules in the vertebrate vascular system [30–33] (see below).

2.3. Exoplasmic fusion and the FF family of fusogens

Exoplasmic cell–cell fusion can involve cells with distinct genetic material, as in the fusion of gametes during sexual reproduction or fusion of enveloped viruses to the host cell during infection, or it can involve cells with identical genetic material. Many examples of cell–cell fusion come from development, where it is used to form syncytia that contain multiple nuclei in a common cytoplasm. In mammals, cell–cell fusion occurs to form myoblasts, eye lens, osteoclasts, and syncytiotrophoblasts [34]. Trophoblast fusion requires Syncytins, which are encoded by elements originated from retroviruses, and are related to the class I viral fusogens [16,35,36]. With the exception of Syncytins, the mammalian cell–cell fusogens remain unidentified.

Cell-cell fusion is a central mechanism in the development of the nematode *C. elegans*. Indeed, the adult hermaphrodite harbors 44 syncytia containing 300 of the total 959 nuclei [37]. Many cell-fusions in *C. elegans* require two related fusogens, EFF-1 and AFF-1 [18,19], which define the FF fusogen family. Based on sequence analysis, the FF fusogens are only present in nematodes, some arthropods, a ctenophore and a protist [38]. However, FF fusogens are structurally related to class II viral fusogens [22,23], so it is conceivable that structural homologs exist in other species. During most characterized fusion events, identical FF fusogens are required on each fusing membrane, suggesting a homotypic mode of action [5,19,39]. Ectopic expression of EFF-1 or AFF-1 promotes ectopic cell fusion *in vivo* [19,40,41], and each can generate syncytia when expressed in cultured insect or vertebrate cells [19,38,39].

In vivo, FF fusogen expression and localization are regulated to ensure that cells fuse only at the right place and time. Various signaling pathways and transcription factors turn on FF fusogen expression in cells that are destined to fuse [5,19,42–46]. Other factors appear to influence FF protein localization or fusogenic activity [47–49]. In the hypodermis, RAB-5- and dynamin-dependent endocytosis keep EFF-1 localized predominantly to intracellular vesicles, so that EFF-1 is observed on the plasma membrane only rarely and transiently even in fusing cells [49]. AFF-1 localizes to both intracellular vesicles and the plasma membrane when overexpressed [19], but its endogenous localization has not yet been described.

The last type of exoplasmic fusion is auto-fusion. Phenotypic characterization of *C. elegans eff-1* and *aff-1* mutants revealed that FF-mediated fusion of two parts of the same cell can be used to

Please cite this article in press as: F. Soulavie, M.V. Sundaram, Auto-fusion and the shaping of neurons and tubes, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.07.018