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Review

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Microcompartments in the *Drosophila* heart and the mammalian brain: general features and common principles

Abstract: Microcompartments are sub-organellar functional units and may have an important role in cellular physiology. They can act as highly dynamic or even transiently forming organizing compartments within cells. In this review, we would like to extend the concept of microcompartments as subcellular structures in individual cells in a way that it includes specializations that occur between different cells and between cells and components of the extracellular matrix. To develop the general features and properties of these structures, we will present two quite different examples – the development and maturation of the *Drosophila* heart and the dynamics of synaptic contacts in the mammalian brain. We argue that the molecular architecture, the function and the maintenance of these specializations follows common principles independent of the organ or the organism under investigation. They fulfill the criteria for being proper microcompartments, including their function as local units for the segregation of responses, their ability to serve as organizing platforms in a temporally and spatially highly restricted manner, and their regulation through instructions from neighboring cells or extracellular matrix components in a locally restricted and autonomous manner.

Keywords: *Drosophila*; extracellular matrix; heart; mammalian brain; spine; synapse.

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Introduction

Tissue development requires specialization, coordination and cooperation of and between assemblies of cells in a temporal and spatial manner in order to permit the emergence of novel organotypic features, such as the maintenance of blood flow by tubular structures or cognition in the brain. Tissue differentiation is often accompanied by characteristic morphological changes of the cells and by the formation of specializations within and between the cells. Such subcellular specializations can be viewed as microcompartments, whose formation and physiological function within or at the cells is instructed through interaction with other cells and components of the extracellular matrix (ECM). Thus, these microcompartments often form at or close to the plasma membrane and serve as an organizing platform for particular functionalities, such as adhesion, repulsion, local signaling or communication between and within cells in a tissue context. Dependent on the type of the tissue in which specialized cells fulfill distinct functions, microcompartments can be quite diverse, e.g., when migrating cells are compared with non-motile cells in an epithelium, or when mitotic cells are compared with postmitotic cells in the brain.

In this review, we will focus on microcompartments present in two distinct tissues: the *Drosophila* heart and the mammalian brain. We chose these examples to demonstrate that despite the diversity of the structure and function of microcompartments during tissue differentiation, they follow common principles with respect to their molecular architecture, physiological function and dynamics.

Cell contacts and microcompartments in the mammalian brain

Contacts between brain cells in the form of synapses constitute an essential feature for the proper function of the nervous system, which is designed for intense communication between cells. As a prerequisite for the development of synaptic contacts, neurons develop structural and

functional polarity into a signal-receiving (somatodendritic) and a signal-transducing (axonal) compartment. Development of cellular polarity is not a unique feature of neurons and occurs in a variety of different cell types including lumen-forming cells, such as during *Drosophila* heart development as described later in this review. The development of neuronal polarity has been described in numerous excellent reviews (e.g., Barnes et al., 2008; Barnes and Polleux, 2009) and will not be discussed here. Instead we intend to focus on chemical synapses, where communication occurs via release of neurotransmitters at the presynapse, which then bind to receptors on the postsynaptic site.

On average, a nerve cell receives input at about 1000 chemical synapses but it can be connected to as many as 150 000 presynapses in the most complex neurons, such as the Purkinje cells from the cerebellum. Learning and memory requires that individual synapses, rather than the entire cell, change both functionally and structurally, a feature that is called 'neuronal plasticity'. Thus, an important question that links cell biology to cognition is how individual cell contacts can be modified without affecting all other connections of the same cell.

Here, we argue that microcompartments, as common organizing platforms for particular functionalities between and within cells, underlie the formation and function of synaptic contacts in neurons. For this purpose, we would like to focus on the functionality of dendritic spines as postsynaptic specializations of dendrites, which share many common features with other types of microcompartments, such as microvilli or adhesive membrane zones. We will restrict our discussion to vertebrates and in particular to mice as the best studied model organism with respect to spine structure and functionality. It should, however, be noted that spines have been described also in invertebrates such as *Drosophila*, where they occur in a subset of processes along the dendrites of visual system interneurons (Leiss et al., 2009). *Drosophila* spines resemble the spines of vertebrates with respect to several criteria including size, density, enrichment for actin, excitatory synaptic input, and dynamicity. However they also show differences with respect to receptor composition (acetylcholine receptors instead of glutamate receptors) and their molecular composition is much less well understood. Spines are small protrusions from the dendritic membrane with a volume ranging from 0.01 to 0.8 μm^2 (Harris and Kater, 1994) and are the primary site of excitatory input in neurons. About 90% of the excitatory synapses in the mature brain end on spines. 'Spines' fulfill the criteria for being typical microcompartments in three important aspects that are also of importance in health and disease: (1) they function as a local microcompartment for the segregation of responses ('signal-segregating microcompartment'); (2) they serve as an organizing platform for molecular changes in a temporally and spatially highly restricted manner ('scaffolding microcompartment'); and (3) they are remarkably dynamic and their function is instructed through the interaction with other cells ('autonomously-responding microcompartment'). The aim of the following section is to develop this concept and to show how the three aspects relate to each other to mediate neuronal plasticity.

Spines as signal segregating microcompartments

Mature dendrites contain about 10 spines/µm of dendrites and a typical pyramidal cell of the CA1 region of the hippocampus, a region, which has been closely examined with respect to mechanisms of memory formation, harbors approximately 30 000 spines in total (Figure 1A). Spines come in a variety of different shapes and are usually categorized into three main types, mushroom, stubby and thin spines, dependent on the relative sizes of the head and neck of a species (Tackenberg et al., 2009) (Figure 1B). With respect to their functionality, much work has concentrated on the potential role of spines in locally regulating Ca^{2+} signaling as a function of synaptic activity and as a mediator of neuronal plasticity (Nimchinsky et al., 2002). Intracellular Ca^{2+} regulates diverse neuronal functions, including the modulation of ion channels, induction of neuronal plasticity and regulation of gene transcription. Increases in Ca^{2+} can come from three sources: glutamate receptors [mostly NMDA-type receptors (NMDARs)], voltage-sensitive Ca^{2+} channels, and release from internal stores (Figure 1C). The relative contribution made by each of the three is still a matter of debate and goes beyond the scope of this review article (for a detailed discussion, see for example Higley and Sabatini, 2008). In light of the diversity of the Ca^{2+} sources and responses, the obvious question arises as to how temporal and local specificity is achieved.

Spines may serve as important local microcompartments to segregate Ca^{2+}-responses in a nano- and microscale range but how does the segregation occur and which regulatory mechanisms operate? An important aspect appears to be the size and the geometry of the individual spine. Mushroom spines have a constricted, narrow neck compared to a large head, while stubby and thin spines have no obvious constriction between the head and attachment to the shaft (Figure 1D). The spine neck poses a barrier for diffusion and mediates the electrical

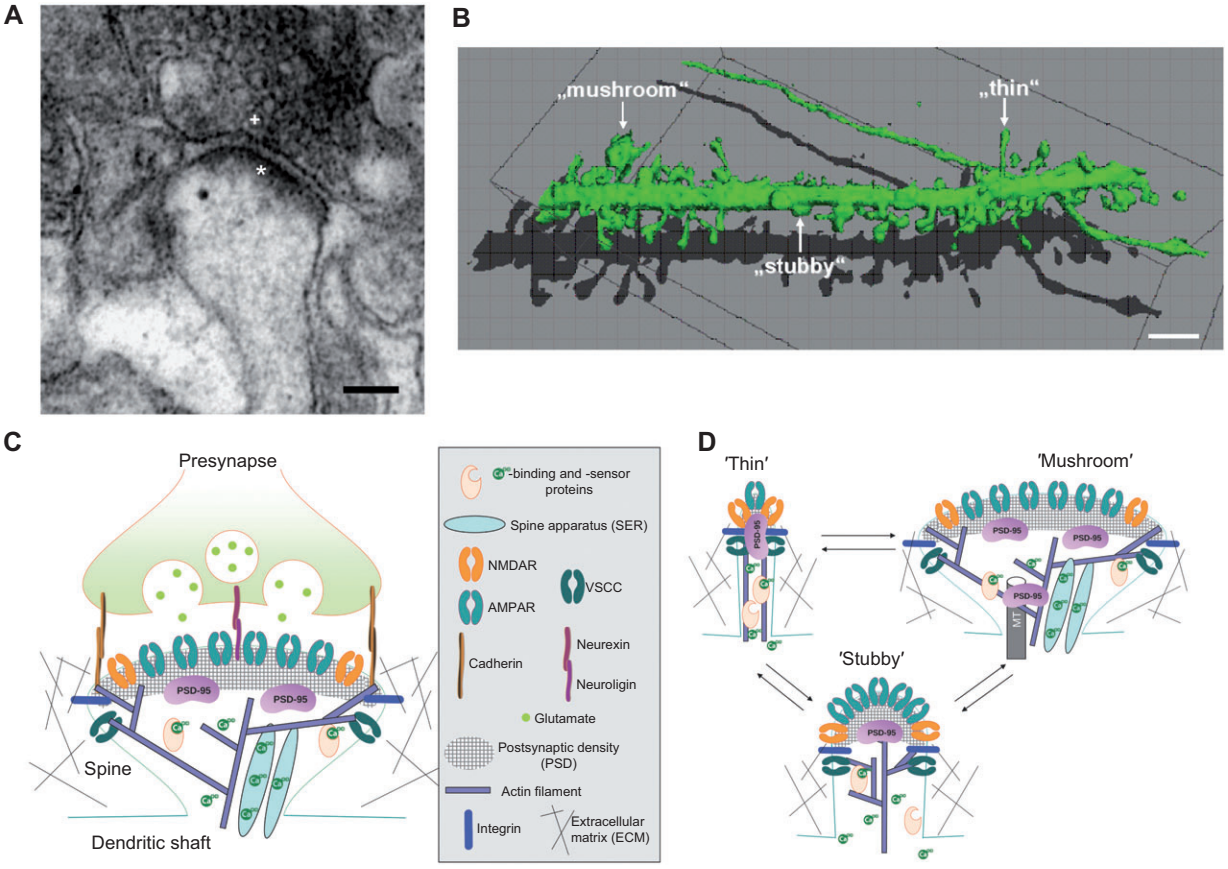

Figure 1 Dendritic spines serve as microcompartments in the brain.
(A) Electron micrograph showing a synaptic contact ending at a spine in the mouse brain. The postsynaptic density (*) and synaptic vesicles in the presynaptic bouton (+) are indicated. Scale bar, 100 nm. (B) Three-dimensional representation of a fluorescence micrograph showing a dendritic segment from a mouse brain slice with examples of different spine types. Scale bar, 5 µm. (C) Schematic representation showing the molecular components of a spine and proteins that mediate interactions of the synaptic junction. (D) Schematic representation showing changes in the distribution of molecular components in different spine types, which may affect their function as segregation and scaffolding compartments. In certain conditions, spines autonomously respond to extracellular signals by changing their morphology and composition.

and chemical isolation of each spine (Bloodgood and Sabatini, 2005). One of the obvious consequences of changes in spine morphology is that the diffusion of spine components into the dendritic shaft is altered. Long-term potentiation (LTP), which results in a strengthening of synaptic contacts, is thought to be associated with a shift from thin to mushroom spines, i.e., an increased electrical and chemical isolation, as discussed above. In turn, neurodegenerative changes are often associated with changes in spine morphology, in particular a shift from mushroom to stubby spines (Tackenberg and Brandt, 2009). Such a morphological change results in a reduced barrier for diffusion and thus could contribute to the malfunction of spines as signal segregation microcompartments in disease conditions. In addition to modulating the diffusion barrier, differentially sized spines may also have different ways of controlling Ca^{2+} homeostasis. Some spines contain a structure called a spine apparatus, which is formed by disks of smooth endoplasmic reticulum (SER). The SER represents a major internal store of Ca^{2+} and houses different Ca^{2+} pumps, thereby contributing to a tight regulation of the Ca^{2+} concentration (Nimchinsky et al., 2002). Large spines are more likely than smaller spines to contain SER, which may result in lower fluctuations and more stable Ca^{2+} concentrations in these spines. Further complexity arises through the presence of a variety of Ca^{2+}-binding and Ca^{2+}-sensor proteins with different concentrations and affinities for Ca^{2+}, which may create a highly competitive environment where Ca^{2+} sensors compete and affect signaling (Raghuram et al., 2012). It is evident that differences in spine geometry will affect the diffusion of different Ca^{2+} sensors in and out of spines, thus modulating the regulation of Ca^{2+} concentrations via Ca^{2+}-binding proteins.

Taken together, many lines of evidence indicate that spines act as local microcompartments for segregating Ca^{2+} responses via different mechanisms. An important aspect of this appears to be the size and shape of the spine, since as a chemical and electrical diffusion barrier it directly affects spine function and more indirectly influences the regulation of Ca^{2+} concentrations and fluctuations.

Spines as dynamic scaffolding microcompartments

Spines are not empty vessels containing certain Ca^{2+} concentrations but have a peculiar composition of protein complexes that serve as an organizing platform for molecular changes in a temporally and spatially highly restricted manner. Each functional spine contains a postsynaptic density (PSD), usually at the head where the synaptic junction is located (Figure 1A and C). Morphologically, the PSD is easily identified in electron microscopic images by its appearance as electron-dense thickening of the membrane. On a molecular level, the PSD consists of an intermingled network of molecules in which hundreds of components, including receptors, cytoskeletal components and diverse signaling molecules, interact.

The term 'scaffold proteins' was initially introduced in immune cells to refer to proteins that interact with multiple members of signaling cascades, thereby tethering them into complexes (Shaw and Filbert, 2009). Scaffold proteins may act by localizing signaling components to specific parts of the cell and insulating correct signaling proteins from competing proteins. In this respect, scaffold proteins could also have an important role in the PSD and in regulating local Ca^{2+} signaling. Scaffold proteins often contain multiple PDZ domains, such as the paradigmatic and well-characterized protein PSD-95 (Specht and Triller, 2008), a major component of the PSD. PDZ domains are known to mediate interactions between proteins and it has recently been suggested that tandem-arranged PDZ domains serve as supramodules that regulate the organization and dynamics of postsynaptic protein complexes (Feng and Zhang, 2009).

In order to couple postsynaptic function to changes in a temporally and spatially defined manner as required in mediating neuronal plasticity, the postsynaptic scaffolds have to be highly dynamic in spines. How can this be achieved in a morphologically relatively rigid structure, such as a PSD? One possibility is that the entire scaffold in the PSD provides a relative stable framework that ensures the stability of the postsynaptic protein complex, while individual scaffold or signaling molecules exchange at a fast rate thereby modulating the specific properties of the PSD. One way could be that certain proteins show a specific geometric distribution, which allows for a more dynamic exchange. This appears to be the case for the Ca^{2+}- and calmodulin-dependent kinase (CaMKII), which is associated with the cytoplasmic face of the PSD (Petersen et al., 2003), thus allowing for a fast translocation in response to synaptic activity. Such a mechanism could couple the function of spines as Ca^{2+}-segregating compartments to Ca^{2+}-dependent signaling, which is modulated by the PSD. PSD-95 contains three consecutive PDZ domains and may be associated with temporal regulation of the PSD, since it forms a dynamic equilibrium and its clustering correlates with the lifespan of excitatory synapses (Specht and Triller, 2008). Interestingly, the exchange rate suggests at least two separate pools of PSD-95, which may indicate the presence of posttranslational modifications, perhaps also Ca^{2+}-regulated phosphorylation and dephosphorylation cycles as regulators of exchange kinetics (Specht and Triller, 2008).

Scaffolding proteins may also have an important role in regulating neurotransmitter availability, thereby directly affecting synaptic strength. AMPA-type receptors (AMPARs) which largely mediate excitatory synaptic transmission in spiny synapses, exist in synaptic and extrasynaptic pools that may be in rapid exchange during synaptic plasticity (for a review, see Opazo et al., 2012). Extrasynaptic AMPARs are highly mobile and might serve as a readily-available pool for synaptic recruitment during LTP. Experimental evidence suggests that adjustable diffusional traps occur at the spine head that reduce the lateral diffusion of AMPARs and increase the availability of these receptors at the postsynapse. Recently, a complex between AMPARs and the transmembrane AMPA receptor regulator protein (TARP) stargazin has been reported. Stargazin contains a PDZ domain that can directly interact with PSD-95-like proteins (which contain multiple PDZ domains acting as protein-protein interaction mediating supramodules, as discussed before). Interestingly, the interaction between stargazin and PSD-95-like proteins can be modified by posttranslational modifications and is affected by phosphorylation through CaMKII (Opazo et al., 2010).

It is evident that with respect to the dynamics of the postsynaptic scaffold, the size and shape of the spine play an important role since they determine the geometry of the postsynaptic scaffold, influence the relative proportion of the cytoplasmic face of the PSD, and affect Ca^{2+} concentrations and fluctuations as discussed above.

Spines as autonomously responding microcompartments

The function of spines as independent microcompartments requires that they respond in a largely autonomous manner to locally-acting signals. This requires mechanisms by which extracellular signals are translated into intracellular changes to modify the shape and composition of individual spines. Newly-formed spines are usually thin and elongated with a small spine head. During development, the head grows and they develop towards a mushroom-like shape. As discussed before, spine size and shape is likely to play an important role in the function of spines as a signal segregating and dynamic scaffolding microcompartment. Spines have a largely actin-based cytoskeleton and local modulation of actin dynamics may play a central role in regulating spine shape. The Arp2/3 complex-induced nucleation of actin filaments and its regulation may have a central function in spine head growth and experimental evidence exists that links Arp2/3 complex activation to spine head growth during maturation (for a review, see Hotulainen and Hoogenraad, 2010). In addition motor protein, e.g., myosin II, activity and additional actin-binding proteins are likely to be involved in modifying the size and shape of spines (Figure 1D).

It has been demonstrated that transmembrane adhesion proteins such as neurexins, neuroligins, and cadherins are localized at specific positions of the synapse so that they affect the distribution and diffusion of other membrane proteins (Specht and Triller, 2008). Through such a mechanism, presynaptic cell adhesion molecules may signal to 'their' postsynapse and modulate features of the spine via homophilic or heterophilic interactions. Cadherins are known to interact with catenins, which serve as important regulators of the actin cytoskeleton.

Recently, the contributions of components of the ECM in regulating aspects of synaptic plasticity have been reported, which suggests that ECM molecules are also involved in the function of spines as autonomously responding microcompartments. ECM components are present in the extrasynaptic space surrounding spines and even extend into the synaptic cleft (for a review, see Dansie and Ethell, 2011) (Figure 1C). They include scaffolding proteins such as laminin, fibronectin and tenascin, as well as proteoglycans and matrix metalloproteinases, which may be involved in local remodeling of the ECM. The diversity of ECM molecules provides a set of molecular tools to mediate spatially and temporally restricted changes in the microenvironment of individual spines, which may regulate spine function. The most prominent accumulations of ECM molecules in the central nervous system are found in the so-called perineuronal nets, which may affect the lateral diffusion of membrane proteins thereby affecting synaptic plasticity through local restrictions in Ca^{2+} influx (for a review, see Wlodarczyk et al., 2011) and may also be involved in neurodegenerative processes (Morawski et al., 2012). In addition, ECM molecules are known to signal to cells through specific receptors. Currently, the best studied receptors in this context are the integrins, which possess a large combinatorial potential through combinations of different chains. Integrins are known to mediate the regulation of actin polymerization and stabilization, thereby linking the ECM to intracellular changes in cytoskeletal organization and dynamics. In addition, integrin-mediated changes in trafficking of synaptic AMPARs have been reported that directly affect the strength of individual synapses (Cingolani et al., 2008). A prototypic example of an ECM molecule that regulates NMDA receptors is reelin, which is also known to be involved in neuronal migration (for reviews, see Herz and Chen, 2006 and Dityatev et al., 2010). Reelin binds to the very-low-density lipoprotein receptor and apolipoprotein E receptor type 2, resulting in activation of a complex signaling pathway that is important for the induction of LTP and may also antagonize the suppressive effects of synaptic functions during neurodegenerative conditions (Förster et al., 2010).

Extracellular signals from cell adhesion molecules may translate into cytoskeletal changes, which then modulate spine shape. In support of this hypothesis, rapid actin-based changes in spine motility occur within seconds in cultured cells (Fischer et al., 1998) and within minutes in organotypic slice preparations (Dunaevsky et al., 1999). Activation of AMPARs blocked motility whereas NMDAR stimulation induced spine motility (Ackermann and Matus, 2003; Brünig et al., 2004), linking actin-based spine dynamics to glutamate receptor-dependent activation as it is occurring during neuronal plasticity. NMDARs are also involved in the induction of long-term depression, the weakening of a synapse, by activating the actin depolymerizing factor cofilin through the Ca^{2+}-regulated phosphatase calcineurin (Mulkey et al., 1994; Cummings et al., 1996; Nägerl et al., 2004). Recently it has been reported that microtubules may also be involved in conferring extracellular signals into intracellular morphological changes in a spine-autonomous manner. In a landmark publication, it has been shown that microtubules are present in at least some spines and that drug-induced changes in microtubule dynamics impair spine formation (Gu et al., 2008). In a live cell imaging study it has then be demonstrated that invasion of microtubules in spines occurs transiently and that neuronal activity enhances microtubule invasion both in number and duration (Hu et al., 2008), which provides

an additional and microtubule-dependent mechanism by which neuronal activity affects spine morphology. The important question that remains, however, is how extracellular signals may affect microtubule dynamics and whether microtubules in turn affect spine composition. Recent data have linked NMDAR activation as it occurs during LTP to the suppression of microtubule invasion (Kapitein et al., 2011). Interestingly, in the same study it has been shown that the effect on microtubules involves Ca^{2+} entry, which couples Ca^{2+}-signaling to the regulation of microtubule dynamics in spines. In a further study it has been demonstrated that an increase of PSD-95 in spines, as can be induced by treatment with the neurotrophic factor brain-derived neurotropic factor, requires microtubule invasions (Hu et al., 2011), which links the assembly, maintenance and dynamics of the PSD to microtubule dynamics.

Thus it is conceivable that extracellular signals such as cell adhesion molecules, neurotransmitters or neurotrophic factors locally modulate individual spines, which act as autonomous microcompartments. Via receptor-mediated mechanisms, signals are then translated into changes of actin filaments and microtubules, which in turn modulate spine shape and PSD composition. All of these mechanisms result in modulating the signalization properties of spines.

Microcompartments and cell contacts in the *Drosophila* heart

The *Drosophila* heart is the second example we have chosen to illustrate microcompartmentation and its role in organ functionality. The cells that constitute the heart harbor typical microcompartments that are found in many cells, as reviewed in the article by Holthuis and Ungermann (2013). *Drosophila* heart cells also display distinct microcompartments that are fundamental for the differentiation of the heart and its functionality as the main circulatory organ. Heart cells develop a unique type of cell polarity that results in compartmentation of the cell membrane with luminal and abluminal membrane domains and these membrane domains serve as organizing platforms for the assembly of an ECM with locally-distinct composition. We would like to argue that the ECM is not only influencing microcompartments, such as spines (as discussed above), but that the ECM of heart cells itself forms specializations that qualify as microcompartments with specific physical and functional properties due to their local composition.

Biological tubes in multicellular organisms are integral components of many organs and enable the transport and exchange of fluids and gases between tissues. Cells that contribute to tube formation display an obvious polarity in which the apical side of the cell faces the lumen and the basal side delineates the organ towards the body cavity. The apical-basal polarity in lumen-forming tissues is achieved by different mechanisms, for example by cavitation and hollowing in which cells establish the polarity *de novo*, or by wrapping and budding in which the pre-existing polarity of cells is maintained (reviewed in Baer et al., 2009). In all cases, the development of cell polarity and the formation of an internal luminal space is accompanied by the formation of microcompartments within the cells or at the surface, for example by the definition of zonula adherens junctions at the apical-lateral membrane domains required for tissue maintenance or the differentiation of microvilli at the apical side of the cell. The *Drosophila* heart is one of the organs in flies that form a biological tube by a mechanism that shares similarities to blood vessel tubulogenesis. In the following section we will summarize how the *Drosophila* heart lumen is established by a mechanism called 'cell assembly'. The key features of heart lumen formation are illustrated in Figure 2.

Heart lumen formation and establishment of microcompartments

The functionality of the *Drosophila* heart relies on the longevity of the cells constituting the heart due to the fact that there is no replacement of cells and no repair after mechanical damage. Cardioblasts originate from non-polarized cells of the early mesoderm under the instructive control of external signals, including bone morphogenetic proteins (BMP), fibroblast growth factor (FGF) and Wingless (Wg) (reviewed by Tao and Schulz, 2007; Bryantsev and Cripps, 2009), which finally lead to the expression of the transcription factor tinman that is thought to be a general key regulator of cardiomyocyte differentiation (Zaffran et al., 2006). Once cardioblasts are selected from the bilateral mesoderm, they start to migrate towards the dorsal midline of the embryo to form two rows of myoendothelial cells (Frémion et al., 1999). During migration, each cardioblast undergoes a mesenchymal-epithelial transition, giving rise to polarized cells that at the end of embryogenesis for the heart lumen, which is crucial for proper hemolymph transport in the animal. Essentially, the formation of the heart lumen depends on the dynamic control of cell shape changes, which forces the cells into a crescent-like shape. Two rows of opposing cardioblasts form the luminal space in between, a process that is driven by a series of events that starts with the recognition of two dorsal adhesive membrane

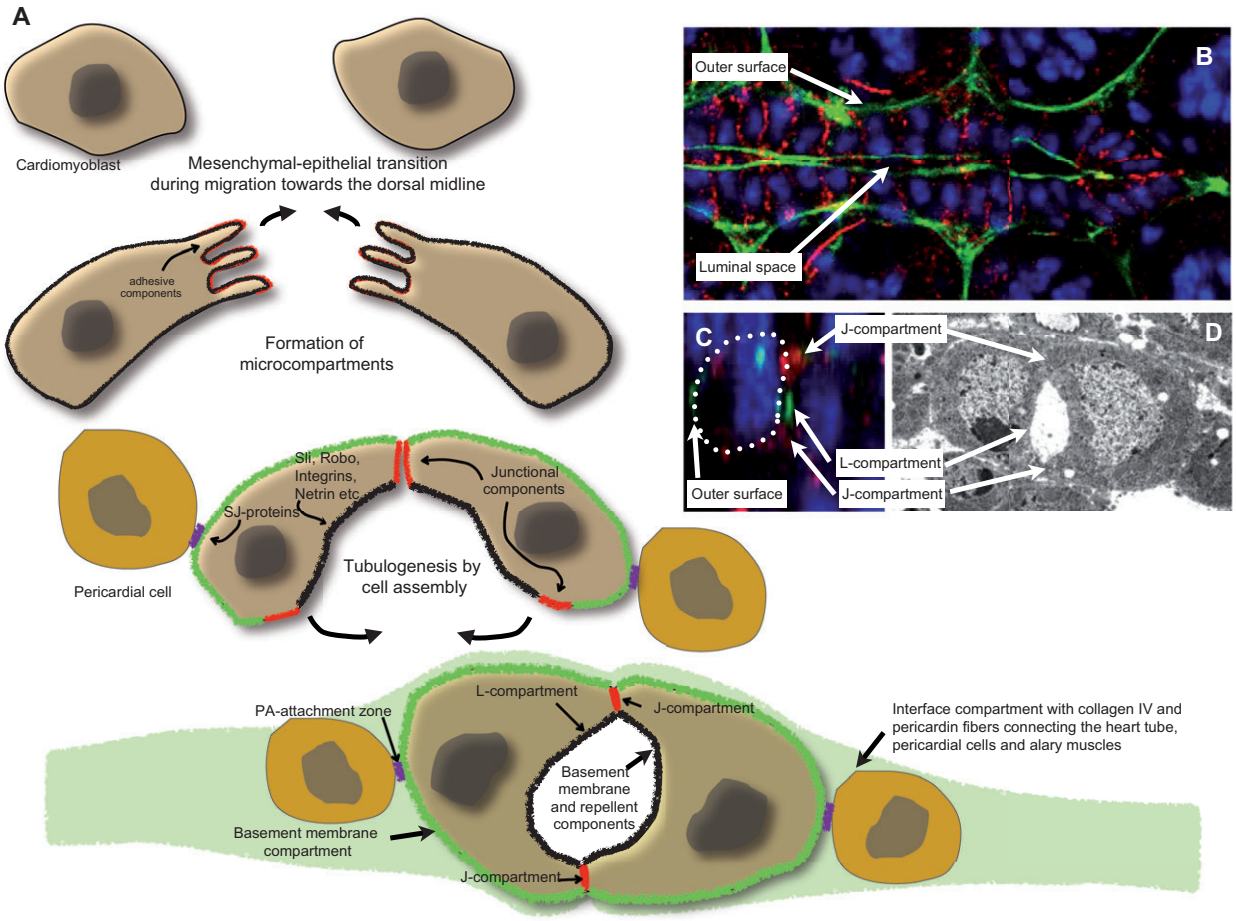

Figure 2 Heart tube differentiation in *Drosophila*.
(A) Schematic illustration of microcompartments in cardioblasts during heart lumen formation. At the end of embryogenesis, the heart tube is covered by a basement membrane (dark green) and a network of extracellular matrix fibers (light green) that connect the heart with associated tissues, such as pericardial cells and suspending muscles. (B) Fluorescence micrograph showing the heart of a stage-17 embryo stained for Draq5 (nuclei, blue), Armadillo/β-catenin (adherens junctions, red) and trol/perlecan (basal and luminal compartment, green). (C) Optical cross-section of the heart tube of the same embryo as in (B) illustrating the J- and L-compartment of cardioblasts. (D) Transmission electron micrograph of an ultra-thin sectioned heart at the end of embryogenesis.

compartments that confine the heart lumen at the dorsal side. At this time, cardioblasts adopt their crescent-like shape, which drives their bases to join ventrally, thereby forming an internal lumen (Medioni et al., 2008). Although cardioblasts undergo a clear transition from an unpolarized into a polarized state, they lack several attributes commonly associated with the definition of classical apical and basal membrane compartments (reviewed in Bryant and Mostov, 2008). The luminal membrane compartment, for example, which would be functionally considered as the apical side of the cardioblasts, harbors several components characteristic of basement membranes such as perlecan or dystroglycan that typically line the basal side of epithelial tissues (Qian et al., 2005; MacMullin and Jacobs, 2006; Santiago-Martinez et al., 2006; Medioni et al., 2008, 2009; Albrecht et al., 2011). At the same time, it lacks the expression of some well-known classical apical markers, including crumbs (Tepass et al., 1990) or β-heavy spectrin (Medioni et al., 2008). For instance, the lack of crumbs, which promotes the formation of apical membrane compartments in most epithelial tissues by defining the zonula adherens zone (Bulgakova and Knust, 2009), indicates that cardioblasts do not follow the classical apico-basal polarity concept of epithelial cells and are likely to use different molecular mechanisms to specify their polarity. Heart lumen formation by the assembly of mesenchymal cells in flies is reminiscent of the process that leads to formation of the luminal space in the mouse aorta, in which vasculogenesis is driven by cord hollowing. Here, the luminal membrane compartment of developing blood vessels displays characteristics of basement membranes as well (Iruela-Arispe and Davis, 2009; Strilic et al., 2009, 2010). In the mouse aorta, the luminal space

emerges by presenting de-adhesive surface-coating proteins (CD34-sialomucin) that promote the repulsion of the luminal membrane compartments. Similarly, the luminal membrane compartment of *Drosophila* cardioblasts displays repellent and adhesion molecules including netrins, uncoordinated5 (Unc5), slit (sli), roundabout (robo), all of which are crucial for lumen formation and maintenance (Qian et al., 2005; MacMullin and Jacobs, 2006; Medioni et al., 2008; Santiago-Martínez et al., 2008; Albrecht et al., 2011) that may activate pathways that lead to the presentation of as yet unknown luminal components crucial for the formation and stabilization of the luminal wall. Tenectin (Tnc), a recently discovered *Drosophila* mucin-like protein is secreted into the luminal space of the heart and other tubular organs and might serve as such a lumen stablizing component (Syed et al., 2012).

Recent studies have provided some insight into how the cardiac cells control the formation of polarity and the presence of adherent and repellent microcompartments at the luminal membrane. Syndecan, which belongs to the transmembrane heparan sulfate proteoglycans that interact with several extracellular ligands and cell surface receptors (Choi et al., 2011), turned out to be crucial for localization of the robo receptor and its ligand at the non-adherent luminal membrane compartment of heart cells (Knox et al., 2011). In *syndecan* mutant embryos, slit and robo become distributed over the entire cell surface and lumen formation is abolished. One current hypothesis is that the assembly of cell surface receptors and signals, including integrin and robo, at the luminal membrane compartment inhibit adhesion by excluding the accumulation of adhesion molecules such as cadherins within the non-adhesive domain (Medioni et al., 2008; Santiago-Martínez et al., 2008). How syndecan itself localizes to the luminal membrane compartment and by which mechanism it contributes to signaling pathways associated with the robo receptor remains an open question. We argue that polarization of the cells is accompanied by the formation of subcellular microcompartments, such as locally-restricted membrane domains displaying repellent and adhesive cues. Interestingly, the repellent and adhesion molecules slit, robo and others are present in *Drosophila* heart cells within the same cellular microcompartment (the luminal membrane domain), indicating an autocrine mode of action.

The ECM compartment is crucial for heart functionality

The data on the mechanisms of heart lumen formation indicate that the local deposition of ECM proteins and their membrane receptors may play a critical role in microcompartment function, e.g., in local signaling or in providing locally-restricted physical properties such as a non-adherent membrane domain. In support of such a hypothesis, the ECM of the heart is critical for heart morphogenesis and heart integrity. The cardiac lumen and the outer surface of the heart tube are covered by an ECM that provides specific physical properties, such as elasticity and protection, and builds a connecting interface between the heart tube and associated tissues. Similarly, the muscle cells constituting the vertebrate blood vessels produce a complex ECM, which defines the physical properties of the vascular system (reviewed in Wagenseil and Mecham, 2009). In flies, hemocytes and fat body cells secrete collagen IV, perlecan, and other structural proteins, which afterwards assemble at the surface of cardioblasts (and other tissues) and, mediated by integrins, link to the cytoskeleton (Bunt et al., 2010; Pastor-Pareja and Xu, 2011). Integrins are not only crucial for adhering structural ECM components but also for directing specific receptors and ligands (such as slit and robo) to the presumptive luminal membrane compartment of the heart (Vanderploeg et al., 2012). The ECM of heart cells is also compartmentalized. Whereas collagen IV and perlecan are present at the luminal domain and the outer surface, pericardin, a fibrillar collagen-like protein exhibiting similarities to type IV collagen but with an atypical repeat structure, is concentrated at the outer surface but is virtually absent at the luminal side. Animals that lack pericardin fail to undergo proper heart morphogenesis (Chartier et al., 2002) and the heart becomes nonfunctional upon aging due to the disintegration of the ECM structure at the outer surface of the heart tube (Drechsler et al., 2011). The collagen IV membrane receptor integrin is present at both sides of the heart cells, but at lower levels on the outer surface (Vanderploeg et al., 2012), so pericardin most likely requires other receptors to become specifically incorporated into the outer ECM of the cardioblasts. In a recent screen for ECM components affecting heart integrity, we identified an ADAMTS-like protein that is secreted by cardioblasts at the outer membrane compartment and serves as a pericardin-specific adapter (Drechsler et al., 2011). The formation of microcompartments within the ECM implies that the heart ECM structural composition becomes different at distinct local areas. This is the case as multiplexin, a collagen with thrombospondin and an endostatin domain, was recently discovered to be concentrated in the luminal ECM compartment of the heart (Volk and Woller, 2011) and is responsible for the differentiation of a defined heart luminal diameter. How multiplexin is directed to the luminal compartment, how it

Drosophila protein	Mammalian homologue	Localization in the late embryonic heart	Protein function or mutant phenotype in the heart	References (with a special focus on protein localization)
ECM constituents				
Viking (Vkg)	Collagen IV	Basal and L-compartment	Structural component of the basement membrane	(Urbano et al., 2009; Drechsler et al., 2011)
Cgc25c	Collagen IV	Basal and L-compartment	Structural component of the basement membrane	(Urbano et al., 2009; Drechsler et al., 2011)
Laminin A (LanA)	Laminin $\alpha3/\alpha5$	Basal and L-compartment	Multiadapter glycoprotein in basement membranes; mutants show PC detachment and disorganized CC	(Tepass and Hartenstein, 1994; Yarnitzky and Volk, 1995)
Nidogen (Ndg) (Entactin)	Nidogen	Basal and L-compartment	Multiadapter glycoprotein in basement membranes	(Drechsler et al., 2011)
Pericardin (Prc)	Similarities with collagens	Basal	Fibrillar component of some extracellular matrices; mutants show PC detachment and disorganized CC, DC defects	(Frémion et al., 1999; Chartier et al., 2002; Santiago-Martinez et al., 2006; Albrecht et al., 2011; Drechsler et al., 2011; Knox et al., 2011; Wang et al., 2012)
Terribly reduced optic lobes (Trol)	Perlecan	Basal and L-compartment	Basement membrane heparan sulfate proteoglycan core protein	(Medioni et al., 2008)
Wingblister (Wb)	Laminin $\alpha1/\alpha2$	n.d.	Multiadapter protein in extracellular matrices; mutants display PC detachment, heart disorganized	(Martin et al., 1999)
LamininB1 (LanB1)	Laminin $\beta1$	n.d.	Multiadapter protein in extracellular matrices; mutants show mis-localization of Prc, trol and collagen IV	(Urbano et al., 2011)
LamininB2 (LanB2)	Laminin γ	n.d.	Multiadapter protein in extracellular matrices; mutants show PC detachment, heart disorganized	(Wolfstetter and Holz, 2012)
Multiplexin (Mp)	Collagen XV/ XVIII	L-compartment	Collagen-like with thrombospondin and endostatin domains	(Volk and Woller, 2011)
Papilin	Papilin	Basal and L-compartment	Basement membrane proteoglycan-like sulfated glycoprotein	(Kramerova et al., 2003)
Membrane ECM receptors and signals				
Dystroglycan (Dg)	Dystroglycan	Basal and L-compartment	Links extracellular matrix components and the cytoskeleton; mutants affect lumen formation	(Qian et al., 2005; Santiago-Martinez et al., 2006; Medioni et al., 2008; Vanderploeg et al., 2012)
β PS1 integrin (Mys)	$\beta1$ Integrin	Basal (weak) and L-compartment (highly enriched)	Links extracellular matrix components and the cytoskeleton; mutants show a disorganized heart with gaps, no lumen formation	(Vanderploeg et al., 2012)
αPS3 Integrin (Scab)	$\alpha4/9$ Integrin	n.d.	Links extracellular matrix components and the cytoskeleton; mutants show a disorganized heart with gaps, no lumen formation	(Stark et al., 1997; Vanderploeg et al., 2012)
NetrinB (NetB)	Netrin B	Late: basal and L-compartment	Guidance function, binds e.g., slit; mutants display a disorganized heart lacking a proper lumen	(Albrecht et al., 2011)
Roundabout 3 (Robo3)	Robo2	Early: entire cell surface Late: L-compartment (highly enriched), lateral	Guidance function, binds e.g., slit; mutants display a disorganized heart lacking a proper lumen	(Qian et al., 2005; Santiago-Martinez et al., 2006, 2008; Albrecht et al., 2011; Knox et al., 2011; Vanderploeg et al., 2012)

(Table 1 continued)

Drosophila protein	Mammalian homologue	Localization in the late embryonic heart	Protein function or mutant phenotype in the heart	References (with a special focus on protein localization)
Syndecan (Sdc)	Syndecan	Lateral (weaker) and L-compartment (enriched)	Membrane-spanning heparan sulfate proteoglycan; mutants have defects in heart cell determination, no lumen formation	(Knox et al., 2011)
Toll (Tll)	Toll-like receptor 8	Early and late: lateral and L-compartment	Receptor of the interleukin superfamily; mutants show a disorganized heart with gaps	(Qian et al., 2005; Wang et al., 2005)
Uncoordinated 5 (Unc5)	Unc5	Early and late: basal and L-compartment	Guidance function; mutants show a disorganized heart lacking a proper lumen	(Albrecht et al., 2011)
Secreted signals				
Slit (Sli)	Slit	Early: entire cell surface Late: L-compartment (highly enriched), lateral	Ligand with guidance function, heart disorganized, no lumen formation	(MacMullin and Jacobs, 2006; Medioni et al., 2008; Santiago-Martínez et al., 2008; Albrecht et al., 2011; Knox et al., 2011; Vanderploeg et al., 2012)
Junctional proteins				
Armadillo	β-catenin	Early: baso-lateral, Late: lateral and J-compartment	Links cadherin to the cytoskeleton, cell junctions, adherens junction component	(Qian et al., 2005; Medioni et al., 2008; Albrecht et al., 2011)
Bazooka	Par-3	J-compartment	Zonula adherens proteins	(Santiago-Martínez et al., 2008)
Discs large (Dlg)	hDlg	Early: baso-lateral, Late: lateral and J-compartment	Membrane-associated guanylate kinase homolog, septate junction structure, cell polarity	(Qian et al., 2005; Albrecht et al., 2011; Knox et al., 2011)
Faint sausages (Fas)	Not clear	n.d.	Ig-like	(Haag et al., 1999)
Fasciclin III (FasIII)		Late: lateral and J-compartment	Integral membrane glycoprotein, cell adhesion, septate junctions	(Drechsler et al., 2011)
Lethal (2) giant larvae L(2)gl, Lgl	Lethal giant larvae homolog 2Llgl2 (Llgl2)	J-compartment		Schematically indicated in (Medioni et al., 2009)
Neurotactin (Nrt)	Neurotactin	J-compartment	Transmembrane glycoprotein acting as a chemokine	(Frémion et al., 1999)
Shotgun (Shg) (DE-cadherin)	Cadherin	Early: baso-lateral, Late: lateral and J-compartment	Transmembrane component of adherens junctions, cardioblasts fail to attach at the midline	(Haag et al., 1999; Santiago-Martínez et al., 2008)
Gγ1		n.d.	Heart integrity, CC–PC adhesion affected	(Yi et al., 2008)
G-oα47A		n.d.	Heart integrity, CC–PC adhesion affected	(Zaffran et al., 1995; Frémion et al., 1999; Yi et al., 2008)
Gβ13F		n.d.	Heart integrity, CC–PC adhesion affected	(Yi et al., 2008)
Neurexin IV (Nrx-IV), Sinuous (Sinu), Coracle (Cora), Nervana2 (Nrv2)	Neurexin, claudin, mammalian protein 4.1, β subunit of Na^+, K^+-ATPase	Basal and L-compartment, enriched at the PC–CC adhesive zone	Septate junction proteins, noncanonical Gγ1 pathway	(Yi et al., 2008)
Cytoskeletal proteins				
α-Spectrin		Early: baso-lateral, Late: lateral and J-compartment?	Constituent of the submembrane cytoskeleton of epithelial cells	(Frémion et al., 1999; Qian et al., 2005; Santiago-Martinez et al., 2006, 2008; Albrecht et al., 2011)

Table 1 Proteins that localize to microcompartments in *Drosophila* heart cells.
CC, cardioblasts; PC, pericardial cells; L-compartment, luminal membrane compartment; J-compartment, junctional membrane compartment; DC, dorsal closure; n.d., not determined.

is assembled into the ECM, and how it controls lumen formation remains to be elucidated. Table 1 summarizes information about proteins that localize to unique, several or all microcompartments in the *Drosophila* heart. It will be very useful to obtain a mechanistic understanding of how the different proteins are localized to their respective microcompartments and the functional implications associated with their distribution.

Conclusions

We have chosen two diverse examples for subcellular specializations in and between cells during tissue development. We argued that the molecular architecture, function and maintenance of these specializations follow common principles, which justify considering and studying them to be 'microcompartments'.

With respect to the dynamics of synaptic contacts, which underlie learning and memory in the brain, we showed that dendritic spines fulfill the criteria required to be proper microcompartments in three important aspects. They function as local microcompartments for the segregation of responses, they serve as organizing platforms for molecular changes in a temporally and spatially highly restricted manner, and they act dynamically and autonomously. With respect to the formation of microcompartments in the *Drosophila* heart, we showed that heart lumen formation is closely associated with transition from an unpolarized to a polarized state and also demonstrated the establishment of distinct membrane compartments that can be considered as microcompartments. In this system, the formation of microcompartments is different from the classical definition of apical and basal membrane compartments and appears to be associated with the formation of adherent and repellent specializations. Furthermore, local deposition of ECM components may be crucially involved in microcompartment function (as in the case of spines) or may qualify as a microcompartment (as in the case of the *Drosophila* heart), arguing that extracellular components also need to be considered.

We are convinced that a concept that takes into account microcompartments as dynamic and physiologically-relevant subcellular specializations between cells and components of the ECM could fruitfully guide experiments to unravel the role of these specializations as local functional units during tissue development, maintenance and aging, and disease.

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References

Ackermann, M. and Matus, A. (2003). Activity-induced targeting of profilin and stabilization of dendritic spine morphology. Nat. Neurosci. *6*, 1194–2000.

Albrecht, S., Altenhein, B., and Paululat, A. (2011). The transmembrane receptor Uncoordinated5 (Unc5) is essential for heart lumen formation in *Drosophila melanogaster*. Dev. Biol. *350*, 89–100.

Baer, M.M., Chanut-Delalande, H., and Affolter, M. (2009). Cellular and molecular mechanisms underlying the formation of biological tubes. Curr. Top. Dev. Biol. *89*, 137–162.

Barnes, A.P. and Polleux, F. (2009). Establishment of axon-dendrite polarity in developing neurons. Annu. Rev. Neurosci. *32*, 347–381.

Barnes, A.P., Solecki, D., and Polleux, F. (2008). New insights into the molecular mechanisms specifying neuronal polarity *in vivo*. Curr. Opin. Neurobiol. *18*, 44–52.

Bloodgood, B.L. and Sabatini, B.L. (2005). Neuronal activity regulates diffusion across the neck of dendritic spines. Science *310*, 866–869.

Brünig, I., Kaech, S., Brinkhaus, H., Oertner, T.G., and Matus, A. (2004). Influx of extracellular calcium regulates actin-dependent morphological plasticity in dendritic spines. Neuropharmacology *47*, 669–676.

Bryant, D.M. and Mostov, K.E. (2008). From cells to organs: building polarized tissue. Nat. Rev. Mol. Cell Biol. *9*, 887–901.

Bryantsev, A.L. and Cripps, R.M. (2009). Cardiac gene regulatory networks in Drosophila. Biochim. Biophys. Acta *1789*, 343–353.

Bulgakova, N.A. and Knust, E. (2009). The Crumbs complex: from epithelial-cell polarity to retinal degeneration. J. Cell Sci. *122*, 2587–2596.

Bunt, S., Hooley, C., Hu, N., Scahill, C., Weavers, H., and Skaer, H. (2010). Hemocyte-secreted type IV collagen enhances BMP signaling to guide renal tubule morphogenesis in *Drosophila*. Dev. Cell *19*, 296–306.

Chartier, A., Zaffran, S., Astier, M., Sémériva, M., and Gratecos, D. (2002). Pericardin, a *Drosophila* type IV collagen-like protein is involved in the morphogenesis and maintenance of the heart epithelium during dorsal ectoderm closure. Development *129*, 3241–3253.

Choi, Y., Chung, H., Jung, H., Couchman, J.R., and Oh, E.S. (2011). Syndecans as cell surface receptors: unique structure equates with functional diversity. Matrix Biol. *30*, 93–99.

Cingolani, L.A., Thalhammer, A., Yu, L.M., Catalano, M., Ramos, T., Colicos, M.A., and Goda, Y. (2008). Activity-dependent regulation of synaptic AMPA receptor composition and abundance by ß3 integrins. Neuron *58*, 749–762.

Cummings, J.A., Mulkey, R.M., Nicoll, R.A., and Malenka, R.C. (1996). Ca2+ signaling requirements for long-term depression in the hippocampus. Neuron *16*, 825–833.

Dansie, L.E. and Ethell, I.M. (2011). Casting a net on dendritic spines: the extracellular matrix and its receptors. Dev. Neurobiol. *71*, 956–981.

Dityatev, A., Schachner, M., and Sonderegger, P. (2010). The dual role of the extracellular matrix in synaptic plasticity and homeostasis. Nat. Rev. Neurosci. *11*, 735–746.

Drechsler, M., Schmidt, A., and Paululat, A. (2011). The ADAMTS-like protein Lonely heart is crucial for cardiac basement membrane formation and heart function. In: 22nd EDRC (Lisbon, Portugal: Instituto Gulbenkian de Ciência Oeiras), ORG24.

Dunaevsky, A., Tashiro, A., Majewska, A., Mason, C., and Yuste, R. (1999). Developmental regulation of spine motility in the mammalian central nervous system. Proc. Natl. Acad. Sci. USA *96*, 13438–13443.

Feng, W. and Zhang, M. (2009). Organization and dynamics of PDZ-domain-related supramodules in the postsynaptic density. Nat. Rev. Neurosci. *10*, 87–99.

Fischer, M., Kaech, S., Knutti, D., and Matus, A. (1998). Rapid actin-based plasticity in dendritic spines. Neuron *20*, 847–854.

Förster, E., Bock, H.H., Herz, J., Chai, X., Frotscher, M., and Zhao, S. (2010). Emerging topics in Reelin function. Eur. J. Neurosci. *31*, 1511–1518.

Frémion, F., Astier, M., Zaffran, S., Guillen, A., Homburger, V., and Sémériva, M. (1999). The heterotrimeric protein Go is required for the formation of heart epithelium in *Drosophila*. J. Cell Biol. *145*, 1063–1076.

Gu, J., Firestein, B.L., and Zheng, J.Q. (2008). Microtubules in dendritic spine development. J. Neurosci. *28*, 12120–12124.

Haag, T.A., Haag, N.P., Lekven, A.C., and Hartenstein, V. (1999). The role of cell adhesion molecules in *Drosophila* heart morphogenesis: faint sausage, shotgun/DE-cadherin, and *laminin A* are required for discrete stages in heart development. Dev. Biol. *208*, 56–69.

Harris, K.M. and Kater, S.B. (1994). Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. Annu. Rev. Neurosci. *17*, 341–371.

Herz, J. and Chen, Y. (2006). Reelin, lipoprotein receptors and synaptic plasticity. Nat. Rev. Neurosci. *7*, 850–859.

Higley, M.J. and Sabatini, B.L. (2008). Calcium signaling in dendrites and spines: practical and functional considerations. Neuron *59*, 902–913.

Holthuis, J.C.M. and Ungermann, C. (2013). Cellular microcompartments constitue general sub-organellar functional units in the cells. Biol. Chem. *394*, 151–161.

Hotulainen, P. and Hoogenraad, C.C. (2010). Actin in dendritic spines: connecting dynamics to function. J. Cell Biol. *189*, 619–629.

Hu, X., Viesselmann, C., Nam, S., Merriam, E., and Dent, E.W. (2008). Activity-dependent dynamic microtubule invasion of dendritic spines. J. Neurosci. *28*, 13094–13105.

Hu, X., Ballo, L., Pietila, L., Viesselmann, C., Ballweg, J., Lumbard, D., Stevenson, M., Merriam, E., and Dent, E.W. (2011). BDNF-induced increase of PSD-95 in dendritic spines requires dynamic microtubule invasions. J. Neurosci. *26*, 15597–15603.

Iruela-Arispe, M.L. and Davis, G.E. (2009). Cellular and molecular mechanisms of vascular lumen formation. Dev. Cell *16*, 222–231.

Kapitein, L.C., Yau, K.W., Gouveia, S.M., van der Zwan, W.A., Wulf, P.S., Keijzer, N., Demmers, J., Jaworski, J., Akhmanova, A., and Hoogenraad, C.C. (2011). NMDA receptor activation suppresses microtubule growth and spine entry. J. Neurosci. *31*, 8194–8209.

Knox, J., Moyer, K., Yacoub, N., Soldaat, C., Komosa, M., Vassilieva, K., Wilk, R., Hu, J., Vasquez Pas, L.D., Syed, Q., et al. (2011). Syndecan contributes to heart cell specification and lumen formation during *Drosophila* cardiogenesis. Dev. Biol. *356*, 279–290.

Kramerova, I.A., Kramerov, A.A., and Fessler, J.H. (2003). Alternative splicing of papilin and the diversity of *Drosophila* extracellular matrix during embryonic morphogenesis. Dev. Dyn. *226*, 634–642.

Leiss, F., Koper, E., Hein, I., Fouquet, W., Lindner, J., Sigrist, S., and Tavosanis, G. (2009). Characterization of dendritic spines in the *Drosophila* central nervous system. Dev. Neurobiol. *69*, 221–234.

MacMullin, A. and Jacobs, J.R. (2006). Slit coordinates cardiac morphogenesis in *Drosophila*. Dev. Biol. *293*, 154–164.

Martin, D., Zusman, S., Li, X., Williams, E.L., Khare, N., DaRocha, S., Chiquet-Ehrismann, R., and Baumgartner, S. (1999). Wing blister, a new *Drosophila* laminin alpha chain required for cell adhesion and migration during embryonic and imaginal development. J. Cell Biol. *145*, 191–201.

Medioni, C., Astier, M., Zmojdzian, M., Jagla, K., and Sémériva, M. (2008). Genetic control of cell morphogenesis during *Drosophila melanogaster* cardiac tube formation. J. Cell Biol. *182*, 249–261.

Medioni, C., Sénatore, S., Salmand, P.A., Lalevée, N., Perrin, L., and Sémériva, M. (2009). The fabulous destiny of the *Drosophila* heart. Curr. Opin. Genet. Dev. *19*, 518–525.

Morawski, M., Brückner, G., Jäger, C., Seeger, G., Matthews, R.T., and Arendt, T. (2012). Involvement of perineuronal and perisynaptic extracellular matrix in Alzheimer's disease neuropathology. Brain Pathol. *22*, 547–561.

Mulkey, R.M., Endo, S., Shenolikar, S., and Malenka, R.C. (1994). Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. Nature *369*, 486–488.

Nägerl, U.V., Eberhorn, N., Cambridge, S.B., and Bonhoeffer, T. (2004). Bidirectional activity-dependent morphological plasticity in hippocampal neurons. Neuron *44*, 759–767.

Nimchinsky, E.A., Sabatini, B.L., and Svoboda, K. (2002). Structure and function of dendritic spines. Annu. Rev. Physiol. *64*, 313–353.

Opazo, P., Labrecque, S., Tigaret, C.M., Frouin, A., Wiseman, P.W., De Koninck, P., and Choquet, D. (2010). CaMKII triggers the diffusional trapping of surface AMPARs through phosphorylation of stargazin. Neuron *67*, 239–252.

Opazo, P., Sainlos, M., and Choquet, D. (2012). Regulation of AMPA receptor surface diffusion by PSD-95 slots. Curr. Opin. Neurobiol. *22*, 453–460.

Pastor-Pareja, J.C. and Xu, T. (2011). Shaping cells and organs in *Drosophila* by opposing roles of fat body-secreted Collagen IV and Perlecan. Dev. Cell *21*, 245–256.

Petersen, J.D., Chen, X., Vinade, L., Dosemeci, A., Lisman, J.E., and Reese, T.S. (2003). Distribution of postsynaptic density

(PSD)-95 and Ca2+/calmodulin-dependent protein kinase II at the PSD. J. Neurosci. *23*, 11270–11278.

Qian, L., Liu, J., and Bodmer, R. (2005). Slit and Robo control cardiac cell polarity and morphogenesis. Curr. Biol. *15*, 2271–2278.

Raghuram, V., Sharma, Y., and Kreutz, M.R. (2012). Ca(2+) sensor proteins in dendritic spines: a race for Ca(2+). Front Mol. Neurosci. *5*, 1–12.

Santiago-Martinez, E., Soplop, N.H., and Kramer, S.G. (2006). Lateral positioning at the dorsal midline: Slit and Roundabout receptors guide *Drosophila* heart cell migration. Proc. Natl. Acad. Sci. USA *103*, 12441–12446.

Santiago-Martínez, E., Soplop, N.H., Patel, R., and Kramer, S.G. (2008). Repulsion by Slit and Roundabout prevents Shotgun/E-cadherin-mediated cell adhesion during Drosophila heart tube lumen formation. J. Cell Biol. *182*, 241–248.

Shaw, A.S. and Filbert, E.L. (2009). Scaffold proteins and immune-cell signalling. Nat. Rev. Immunol. *9*, 47–56.

Specht, C.G. and Triller, A. (2008). The dynamics of synaptic scaffolds. Bioessays *30*, 1062–1074.

Stark, K.A., Yee, G.H., Roote, C.E., Williams, E.L., Zusman, S., and Hynes, R.O. (1997). A novel alpha integrin subunit associates with betaPS and functions in tissue morphogenesis and movement during Drosophila development. Development *124*, 4583–4594.

Strilic, B., Kucera, T., Eglinger, J., Hughes, M.R., McNagny, K.M., Tsukita, S., Dejana, E., Ferrara, N., and Lammert, E. (2009). The molecular basis of vascular lumen formation in the developing mouse aorta. Dev. Cell *17*, 505–515.

Strilic, B., Kucera, T., and Lammert, E. (2010). Formation of cardio-vascular tubes in invertebrates and vertebrates. Cell Mol. Life Sci. *67*, 3209–3218.

Syed, Z.A., Bougé, A.-L., Byri, S., Chavoshi, T.M., Tång, E., Bouhin, H., van Dijk-Härd, I.F., and Uv, A. (2012). A luminal glycoprotein drives diose-dependent diameter expansion of the Drosophila melanogaster hindgut tube. PLoS Genet. *8*, e1002850.

Tackenberg, C. and Brandt, R. (2009). Divergent pathways mediate spine alterations and cell death induced by amyloid-beta, wild-type tau, and R406W tau. J. Neurosci. *29*, 14439–14450.

Tackenberg, C., Ghori, A., and Brandt, R. (2009). Thin, stubby or mushroom: spine pathology in Alzheimer´s disease. Curr. Alzheimer Res. *6*, 261–268.

Tao, Y. and Schulz, R.A. (2007). Heart development in Drosophila. Semin. Cell Dev. Biol. *18*, 3–15.

Tepass, U. and Hartenstein, V. (1994). The development of cellular junctions in the Drosophila embryo. Dev. Biol. *161*, 563–596.

Tepass, U., Theres, C., and Knust, E. (1990). Crumbs encodes an EGF-like protein expressed on apical membranes of *Drosophila* epithelial cells and required for organization of epithelia. Cell *61*, 787–799.

Urbano, J.M., Torgler, C.N., Molnar, C., Tepass, U., Lopez-Varea, A., Brown, N.H., de Celis, J.F., and Martin-Bermudo, M.D. (2009). Drosophila laminins act as key regulators of basement membrane assembly and morphogenesis. Development *136*, 4165–4176.

Urbano, J.M., Dominguez-Gimenez, P., Estrada, B., and Martin-Bermudo, M.D. (2011). PS integrins and laminins: key regulators of cell migration during Drosophila embryogenesis. PLoS ONE *6*, e23893.

Vanderploeg, J., Vazquez Paz, L.D., Macmullin, A., and Jacobs, J.R. (2012). Integrins are required for cardioblast polarisation in Drosophila. BMC Dev. Biol. *12*, 8.

Volk, T. and Woller, N. (2011). Multiplexin promotes the formation of the heart lumen by inducing enhanced membrane indentations and activation of the Robo/Slit pathway. In: 22nd EDRC (Lisbon, Portugal: Instituto Gulbenkian de Ciência Oeiras), ORG3.

Wagenseil, J.E. and Mecham, R.P. (2009). Vascular extracellular matrix and arterial mechanics. Physiol. Rev. *89*, 957–989.

Wang, J., Tao, Y., Reim, I., Gajewski, K., Frasch, M., and Schulz, R.A. (2005). Expression, regulation, and requirement of the Toll transmembrane protein during dorsal vessel formation in Drosophila melanogaster. Mol. Cell. Biol. *25*, 4200–4210.

Wang, S., Meyer, H., Ochoa-Espinosa, A., Buchwald, U., Onel, S., Altenhein, B., Heinisch, J.J., Affolter, M., and Paululat, A. (2012). GBF1 (Gartenzwerg)-dependent secretion is required for *Drosophila* tubulogenesis. J. Cell Sci. *125*, 461–472.

Wlodarczyk, J., Mukhina, I., Kaczmarek, L., and Dityatev, A. (2011). Extracellular matrix molecules, their receptors, and secreted proteases in synaptic plasticity. Dev. Neurobiol. *71*, 1040–1053.

Wolfstetter, G. and Holz, A. (2012). The role of LamininB2 (LanB2) during mesoderm differentiation in Drosophila. Cell Mol. Life Sci. *69*, 267–282.

Yarnitzky, T. and Volk, T. (1995). Laminin is required for heart, somatic muscles, and gut development in the Drosophila embryo. Dev. Biol. *169*, 609–618.

Yi, P., Johnson, A.N., Han, Z., Wu, J., and Olson, E.N. (2008). Hetero-trimeric G proteins regulate a noncanonical function of septate junction proteins to maintain cardiac integrity in Drosophila. Dev. Cell *15*, 704–713.

Zaffran, S., Astier, M., Gratecos, D., Guillen, A., and Sémériva, M. (1995). Cellular interactions during heart morphogenesis in the Drosophila embryo. Biol. Cell *84*, 13–24.

Zaffran, S., Reim, I., Qian, L., Lo, P.C., Bodmer, R., and Frasch, M. (2006). Cardioblast-intrinsic Tinman activity controls proper diversification and differentiation of myocardial cells in Drosophila. Development *133*, 4073–4083.

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