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Review

Drosophila metalloproteases in development and differentiation: The role of ADAM proteins and their relatives

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ABSTRACT

ADAM metalloproteases are membrane bound glycoproteins that control many biological processes during development and differentiation, mainly by acting as ectodomain sheddases. The *Drosophila* genome contains five genes that code for classical ADAM proteins which are characterized by a highly conserved domain structure with the respective catalytic domains facing the extracellular space. More than 50 genes encode related proteins such as those that have lost their primary enzymatic activity while retaining, e.g., their adhesive properties. The physiological relevance of many *Drosophila* ADAMs and their relatives is still unknown, however for others, a striking role during organogenesis and tissue maintenance has been demonstrated during the last few years. We have carried out genetic screenings combined with candidate approaches, aiming to identify new components involved in cardiogenesis and muscle differentiation. Herein we summarize our results with a particular focus on metalloproteases with known or potential roles in tissue differentiation.

Introduction

The architectural simplicity of the insect heart, the availability of molecular genetic tools, and the possibility of visualizing cardiogenesis and cardiac function in living animals have proven that the *Drosophila* heart is a valuable model for understanding principle mechanisms of organogenesis. Many regulatory cascades and signaling pathways act similarly in fly and vertebrate cardiogenesis and thus the insect system has great impact on our current knowledge of human heart development and cardiac malfunctions (Ocorr et al., 2007; Taghli-Lamallem et al., 2008; Wessells and Bodmer, 2007; Zaffran and Frasch, 2002). Since our research on metalloproteases has aimed at identifying components that are critical for cardiogenesis, we briefly summarize the key events taking place during *Drosophila* heart formation. The heart differentiates during embryogenesis with pumping activity starting shortly before hatching of 1st instar larvae. At this stage, the *Drosophila* heart consists of at least three major cell types with distinct functions: cardiomyocytes, pericardial cells and the so-called alary muscles, which suspend the heart tube towards the body wall. Exactly 104 cardiomyocytes build the heart tube and provide contractility that causes hemolymph to stream into the body cavity of the animal. Hemolymph, the blood of insects, enters the heart lumen through specific openings formed by ostia cells – a subtype of cardiomyocytes – and leaves the heart anteriorly through an outflow tract (Molina and Cripps, 2001; Zmojdzian et al., 2008). The sarcomeres of the cardiomyocytes have a circular orientation, which allows diastolic and systolic contractions and thereby pumping activity. The larval heart is accompanied by pericardial cells that play an important role in detoxification and heart physiology (Das et al., 2007; Weavers et al., 2009). The progenitors of the wing hearts also originate from a distinct embryonic population of pericardial cells (Tögel et al., 2008). An additional major component of the fly circulatory system consists of seven pairs of alary muscles located symmetrically along the heart in a segmental arrangement. They attach dorsally to the heart tube and suspend the heart to the body wall (Bate and Martinez Arias, 1993; Curtis et al., 1999; LaBeau et al., 2009). The role of alary muscles for heart integrity and function is, however, not yet fully understood. In the adult fly, longitudinal muscles located on the ventral side of the heart tube may help to stabilize the architecture of the heart upon body movement (Shah et al., 2011). Fig. 1 illustrates the overall architecture of the *Drosophila* heart with its different components. Cross-sections through embryonic, larval, pupal and adult hearts visualize the increasing diameter of the heart lumen and the morphological changes that cardiomyocytes undergo in the course of differentiation.

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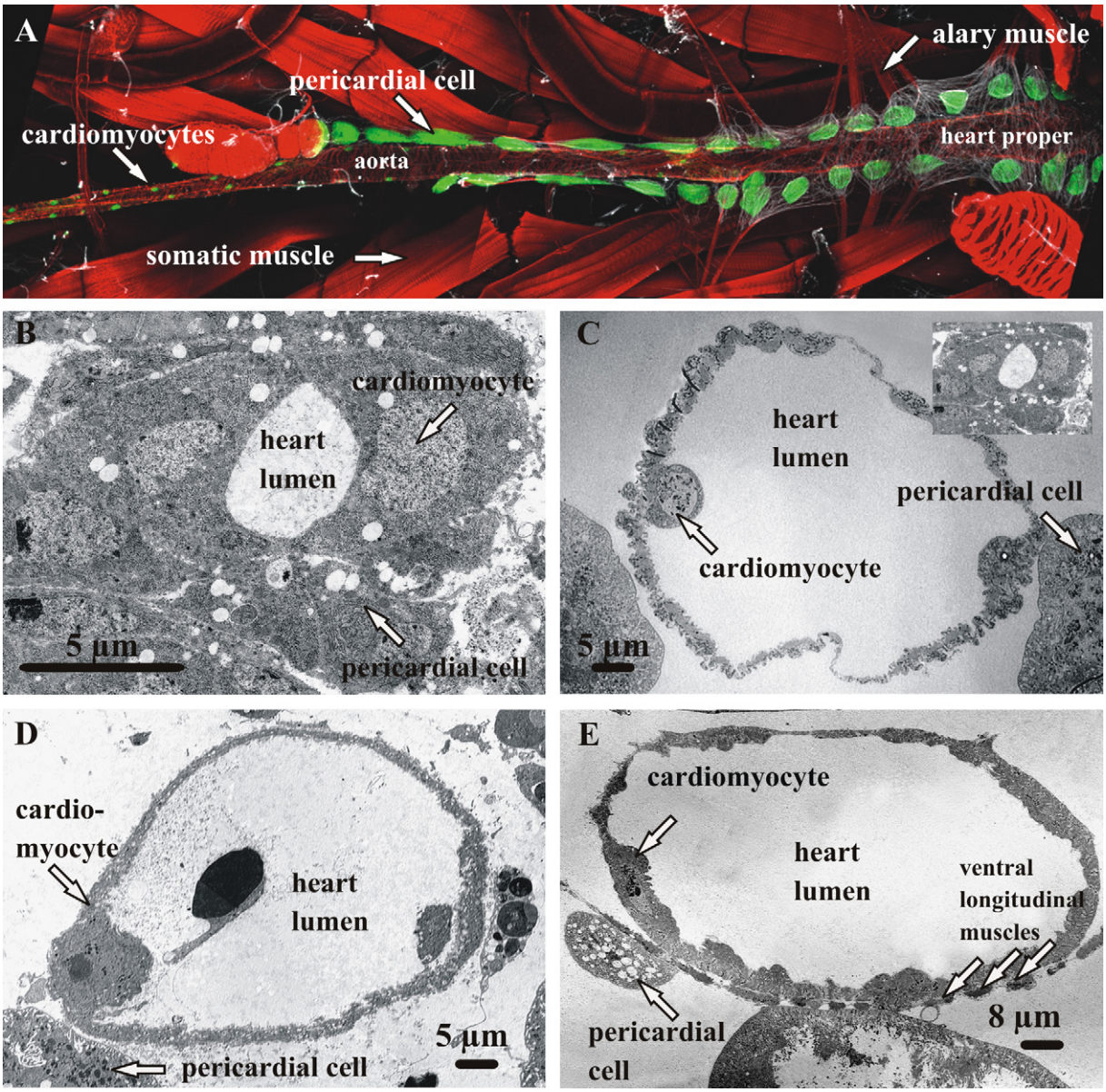

Fig. 1. (A) Triple staining for different components constituting the heart tube visualizes the overall architecture of the heart of a *Drosophila* 3rd instar larva. Actin filaments in somatic muscles, alary muscles and cardiomyocytes are stained by phalloidin (red). The nuclei of all cardiomyocytes and pericardial cells are visualized by *hand*C-GFP in green (Sellin et al., 2006). The distribution of the collagen Pericardin, detected by an anti-Pericardin antibody (EC11, DSHB, Iowa, USA) is shown in white. Transmission electron micrographs of cross-sections through the dorsal vessel of an embryo (B), a third larva (C), a pharate pupa (D) and an adult fly (E) illustrate the significant changes in cell shape and heart lumen diameter taking place after embryogenesis. A bundle of longitudinal muscles is located on the ventral side of the dorsal vessel. The inset in C is a copy of B at the same scale to illustrate the dimensions of the heart diameter. Specimens were prepared for TEM analysis as described (Lehmacher et al., 2009; Tepass and Hartenstein, 1994).

EMS screen for genes crucial for heart differentiation

Genetic screening approaches are one of the most powerful techniques available in the *Drosophila* system. As recently summarized, they have led to the identification of several proteins, e.g., transcription factors and signaling components, displaying essential functions in cardiogenesis (Reim and Frasch, 2010). In order to identify new genes involved in cardiogenesis and muscle differentiation, we previously have screened a collection of EMS-induced embryonic lethal mutants carrying mutations on the 2nd and 3rd chromosomes, respectively (Albrecht et al., 2006). While this collection was preselected for neuronal phenotypes (Hummel et al., 1999), several genes with crucial roles in processes other than neurogenesis have already been identified. For instance, screening this collection with markers visualizing the visceral mesoderm revealed an important role of Huckebein (Wolfstetter et al., 2009; Wolfstetter and Holz, 2011) and Jelly belly and Alk (Stute et al., 2004) for the differentiation of visceral muscles. Essential genes crucial for myoblast fusion have also been isolated from this collection, e.g., the gene *wasp* that encodes the Wiskott–Aldrich-syndrome protein (Schäfer et al., 2007) and the *kette* gene, which codes for an Actin-regulator (Schröter et al., 2004). Using an antibody against β3-Tubulin, which labels, amongst other cells, the four *tinman*-expressing cardiomyocytes of each abdominal hemisegment and second round screening with anti-Mef2, that stains all nuclei of heart tube forming cardiomyocytes, we identified mutants that display different types of heart malformations. Mapping several of these mutations revealed new alleles for genes involved in different cardiogenic pathways (Fig. 2). One category of heart mutants comprised animals being characterized by the failure to