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Review

Myoblast fusion: Experimental systems and cellular mechanisms

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ABSTRACT

Fusion of myoblasts gives rise to the large, multi-nucleated muscle fibers that power and support organism motion and form. The mechanisms underlying this prominent form of cell-cell fusion have been investigated by a variety of experimental approaches, in several model systems. The purpose of this review is to describe and discuss recent progress in the field, as well as point out issues currently unresolved and worthy of further investigation. Following a description of several new experimental settings employed in the study of myoblast fusion, a series of topics relevant to the current understanding of the process are presented. These pertain to elements of three major cellular machineries- cell-adhesion, the actin-based cytoskeleton and membrane-associated elements- all of which play key roles in mediating myoblast fusion. Among the issues raised are the diversity of functions ascribed to different adhesion proteins (*e.g.* external cell apposition and internal recruitment of cytoskeleton regulators); functional significance of fusion-associated actin structures; and discussion of alternative mechanisms employing single or multiple fusion pore formation as the basis for muscle cell fusion.

Contents

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1. Introduction

Myoblast fusion constitutes a particularly prominent form of cell-cell fusion, being a conserved mechanism for development and growth of skeletal/body-wall and limb muscles, major tissue systems whose proper function is a fundamental aspect of normal physiology and health of living organisms [1]. Elucidation of the mechanisms by which fusion of muscle cells is executed has been an active domain of investigation ever since the original demonstrations that the multi-nucleate nature of muscle fibers results from fusion rather than failure in cytokinesis (*e.g.* [2,3]). Many molecular players contributing to this process have been identified and characterized, and various models attempting to explain its cell-biological basis have been proposed (see [4–7] for recent comprehensive and insightful reviews). The purpose of the current review is to summarize and discuss a number of key recent advances in the field, which, while building upon prior work, expand the breadth of experimental systems in which the topic is investigated and add sophistication and depth to the description and understanding of the underlying mechanisms. Unresolved issues as well as some neglected areas will also be brought up, with the hope of stimulating discussion and further investigation.

The review is divided into two major sections. The first part will describe new and upcoming experimental settings for myoblast fusion research, including both variations within established systems (*e.g.*- study of *Drosophila* muscle groups distinct from the "classic" embryonic body-wall muscles, which have long served as the mainstay of myoblast fusion research in this organism), as well as novel settings such as the chick embryo, and will point out the advantages (and potential shortcomings) in pursuing these alternative approaches. The second, main part of the review is devoted to discussion of various recent advances made towards elucidation of the cell biological events and principles underlying the myoblast fusion process. More specifically, roles played by the cell-adhesion, actin polymerization and plasma membrane machineries in providing the context and executing fusion will be presented and critically assessed.

2. Expanding the spectrum of experimental systems used to study myoblast fusion

Study of myoblast fusion[1] has relied to a large extent on a small number of experimental frameworks. Two systems are particularly notable in this context:

- The *Drosophila* embryo, where screens and other forms of genetic investigation have identified most of the key molecules currently viewed as central to the process [4,8], and have established central paradigms, such as the concept of asymmetric fusion between dominant founder cells (FCs)/myotubes and "naive" fusion-competent myoblasts (FCMs) [9,10].
- Murine cell-culture [11], which provides both a mammalian cell setting and relative ease of manipulation and visualization.

Broadening the repertoire of relevant experimental systems is clearly an important goal, as it is likely to enable new investigative approaches, as well as a basis for revealing comparative analysis. Recent progress along these lines includes both expanded use of the existing systems and pioneering studies in potentially useful systems that have been neglected to this point.

[1] For simplicity, the term "myoblast" is used as a generic form describing individual myogenic cells, regardless of their state of differentiation.

2.1. *Drosophila*- new additions to the playbook

2.1.1. *Embryonic pharyngeal muscles*

Studies of myoblast fusion during *Drosophila* embryogenesis, the major *in vivo* model system in the field, have focused on the embryonic body-wall muscles, which develop in a segmentally-repeating pattern, and are eventually used for locomotion by the fly larva (see [12,13] for recent reviews). A technical challenge of this system is the difficulty of conducting live imaging in a tissue that undergoes considerable morphogenetic movement. The dorsal pharyngeal musculature (DPM), which mediates larval feeding, has now been shown to be a useful, complementary alternative in this regard. The DPM is considerably less motile than body wall muscles during development, thereby allowing for longer and more detailed imaging in living embryos. Importantly, the cellular principles and molecular elements involved in DPM myoblast fusion are highly similar to those mediating body-wall myogenesis [14,15], allowing for generalization of observations made in this tissue. Live imaging of the DPM has proved particularly useful for analyzing the dynamic events associated with formation of an invasive myoblast structure, composed of a large actin-rich base and emanating filopodia, at the interphase of fusing muscle cells ([15]; see below for further discussion of this key fusion-related structure). Furthermore, the amenability of the DPM to different transmission electron microscopy (TEM) methods [15] enables informative analysis of the structures recognized and documented using light microscopy, at ultrastructural resolution.

2.1.2. *Adult* Drosophila *flight muscles*

Being holometabolous insects, *Drosophila* flies undergo two separate periods of embryonic and pupal development, which give rise, respectively, to distinct larval and adult organs and tissues. Due to a variety of technical obstacles, study of pupal/adult myogenesis has lagged behind the embryonic system, but recent progress, primarily in the ability to visualize advanced developmental stages and apply genetic methods to their study [16–18], now makes adult fly myogenesis far more amenable to investigation. The appeal of this system is exemplified by the indirect flight muscles (IFMs), a set of particularly large muscle fibers that fill the adult thorax and power flight [19,20]. Both the developmental program and the mature, myofibrillar structure of the IFMs bear considerable resemblance to those of vertebrate skeletal muscles [21,22], so that study of this musculature is expected to yield observations and insight of general significance. Indeed, the topic of myoblast fusion has benefitted in recent years from the heightened interest in adult *Drosophila* myogenesis. A variety of studies, discussed in part below, have demonstrated the conservation of key aspects and principles of the embryonic fusion program, and have shed light on the regulation and execution of myoblast fusion during formation of the IFMs and additional adult muscles [23–28].

2.1.3. *Smooth testes muscles*

Recent studies have identified the muscles that surround the *Drosophila* testes [29] as an additional adult musculature that can provide an informative system for investigation of myoblast fusion. Although these are smooth, non-striated muscles, and therefore not an ideal model for skeletal myogenesis, they are composed of multinucleated myotubes generated through fusion. Importantly, initial observations demonstrate functional conservation of key molecular elements that participate in the embryonic body wall and adult flight muscle myoblast fusion programs [30]. The testes muscles are part of a well-studied, non-essential organ system, highly amenable to investigation by genetic and cell-biological approaches [31], and therefore hold the promise of serving as an intriguing, complementary model system for the study of myoblast fusion.