doi: 10.1387/ijdb.103160cc

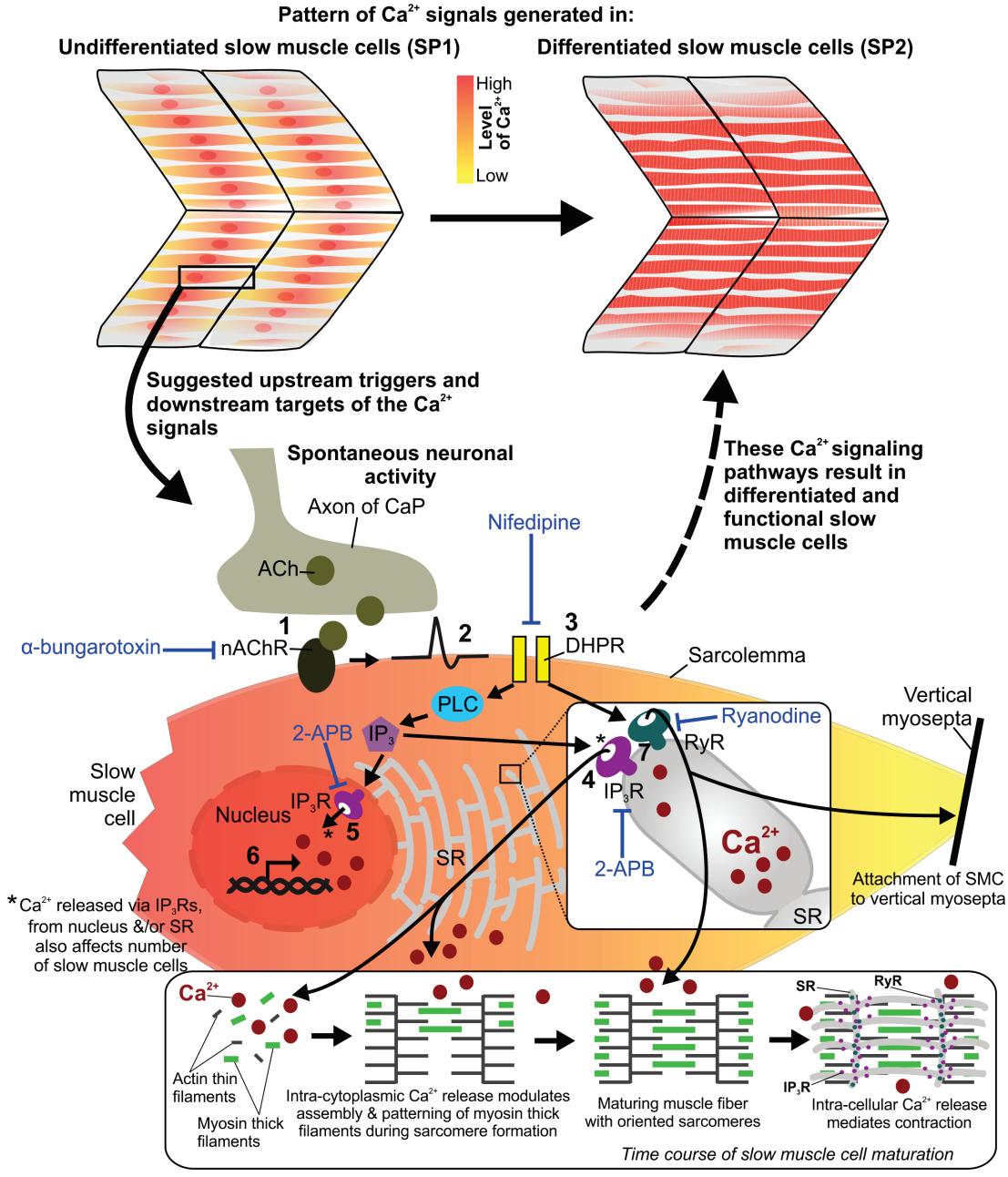
THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

SUPPLEMENTARY MATERIAL

corresponding to:

Visualization, characterization and modulation of calcium signaling during the development of slow muscle cells in intact zebrafish embryos

CHRIS Y. CHEUNG, SARAH E. WEBB, DONALD R. LOVE and ANDREW L. MILLER*



Supplementary Fig. S1. Schematic diagram of the proposed signaling cascade for the generation of the SP1 Ca^{2+} signals in SMCs during myofibrillogenesis. *At ~17.5 hpf, (i.e., when the SP1 Ca^{2+} transients begin), the myotomes are innervated by the caudal primary (CaP) axons (Eisen* et al., *1986; Myers* et al., *1986; Westerfield* et al., *1986). Acetycholine (ACh) released from the axon binds to nicotinic acetylcholine receptors (nAChRs) on the surface of the SMCs* **(1)**, *and thus induces a depolarization of the sarcolemma* **(2)**. *This depolarization activates dihydropyridine receptors (DHPRs* **(3)**, *that in turn stimulate the release of Ca^{2+} (see red circles) from intracellular stores, i.e., the sarcoplasmic reticulum (SR) and perinuclear cisternae. The SP1 Ca^{2+} signals are generated by Ca^{2+} release via both IP_3Rs and RyRs. It has been proposed that DHPRs can activate the production of IP_3 from phospholipase C (PLC) and then induce Ca^{2+} release via IP_3Rs from the SR* **(4)** *and the perinuclear cisternae* **(5)**; *(Jaimovich* et al., *2000; Powell* et al., *2001; Araya* et al., *2003). Moreover, it has been shown that elevations of nuclear Ca^{2+} can activate gene expression in skeletal muscle cells* **(6)** *(Carrasco* et al., *2003; Cárdenas* et al., *2005). We suggest that the Ca^{2+} released via IP_3Rs might play a role in regulating the expression of some important proteins that are involved in sarcomere assembly. In addition, it has been proposed that direct coupling of DHPRs with juxtaposed RyRs can trigger Ca^{2+} released through RyRs* **(7)**; *(Schneider and Chandler, 1973). We suggest that the Ca^{2+} released via RyRs maintains the integrity of the sarcomeres and regulates muscle contraction. Inhibiting the nAChRs, DHPRs, IP_3Rs and RyRs with α-bungarotoxin, nifedipine, 2-APB and ryanodine, respectively (indicated in blue) can block the SP1 signals generated in SMCs and thus disrupt different aspects of muscle development.*

Movie 1 *Representative example of the Ca^{2+} signals generated in some of the slow muscle cells in somite 8 (S8) of a wild-type embryo at ~17.5 hpf (i.e., the 17-somite stage), as visualized by confocal microscopy using calcium green-1 dextran. Anterior is to the left and dorsal is to the top.*