The application of complementary luminescent and fluorescent imaging techniques to visualize nuclear and cytoplasmic Ca^{2+} signaling during *in vivo* differentiation of slow muscle cells in zebrafish embryos

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Intact zebrafish embryos were used as an *in vivo* animal model to investigate the role of Ca^{2+} signaling during the differentiation of slow muscle cells (SMCs) within forming skeletal muscle. Transgenic zebrafish were generated using an α-actin promoter that targeted apoaequorin expression specifically to muscle cells. Two distinct Ca^{2+} signaling periods (CSPs) were visualized in the developing SMCs: between ~17.5-19.5 hours post-fertilization (hpf) and after ~23 hpf, separated by a ~3.5 hour Ca^{2+} signaling quiet period. Further spatial characterization of these Ca^{2+} signals using confocal fluorescent microscopy and calcium green-1 dextran as a reporter, indicated that the earlier CSP displayed distinct nuclear and cytoplasmic components, whereas the later CSP was predominantly cytoplasmic. Both CSPs consisted of a series of oscillating Ca^{2+} waves generated at distinct frequencies, while the earlier CSP also displayed a slow rise then fall in the Ca^{2+} baseline-level. Imaging of cyclopamine- and forskolin-treated wild-type, or *smo*$^{-/-}$ mutant embryos, where SMCs do not form, confirmed the specific cell population generating the signals. Treating embryos with antagonists indicated that both IP_3Rs and RyRs are responsible for generating the temporal characteristics of the Ca^{2+} signaling signature, and that the latter plays a necessary role in SMC differentiation and subsequent myotome patterning (Cheung, *et al.*, 2010). Together, these data support and extend the proposition that specific spatiotemporal patterns of spontaneous Ca^{2+} signals might be used for different as well as combinatorial regulation of both nuclear and cytosolic signal transduction cascades, resulting in myofibrillogenesis in SMCs as well as myotome patterning (Webb & Miller, 2010).

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