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Invited review

Ca^{2+} signaling during vertebrate somitogenesis[1]

Sarah E WEBB, Andrew L MILLER[2]

Department of Biology, the Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong SAR, China

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[2] Correspondence to Dr Andrew L Miller.
Phn 852-2358-8631
Fax 852-2358-1559
E-mail almiller@ust.hk

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Abstract

A variety of Ca^{2+} signals, in the form of intercellular pulses and waves, have been reported to be associated with the various sequential stages of somitogenesis: from convergent extension and the formation of the paraxial mesoderm; during the patterning of the paraxial mesoderm to establish segmental units; throughout the formation of the morphological boundaries that delineate the segmental units, and finally from within the maturing somites as they undergo subsequent development and differentiation. Due to both the technical challenges presented in imaging intact, developing embryos, and the subtle nature of the Ca^{2+} transients generated, they have proved to be difficult to visualize. However, a combination of cultured cell preparations and improvements in explant and whole embryo imaging techniques has begun to reveal a new and exciting class of developmental Ca^{2+} signals. In this chapter, we review the small, but expanding, number of reports in the literature and attempt to identify common characteristics of the somitogenic Ca^{2+} transients, such as their mode of generation, as well as their spatial and temporal features. This may help to elucidate the significance and function of these intriguing Ca^{2+} transients and thus integrate them into the complex signaling networks that orchestrate early developmental events.

Embryonic Ca^{2+} signaling

It has been proposed that Ca^{2+} signaling, in the form of pulses, waves, and steady gradients, may play a crucial role in key pattern forming events during early development[1–3]. Webb and Miller[4] have suggested that in zebrafish, the complexity of embryonic Ca^{2+} signaling mirrors that of the developing embryo. Thus, early Zygotic and Cleavage Period Ca^{2+} signaling events take the form of relatively simple intracellular waves. Then during the Blastula to early Gastrula Periods, there is a transition from intracellular to more complex localized intercellular Ca^{2+} wave generation. This is followed, as gastrulation proceeds, by the generation of more extensive (including pan-embryonic) intercellular Ca^{2+} waves, which reflect the more wide-ranging morphological events occurring during this period, such as convergent extension and subsequent axis and germ layer formation. Evidence is beginning to accumulate to suggest that these Gastrula Period intercellular Ca^{2+} waves might help to regulate the coordinated movement of cells during gastrulation in both fish[5–7] and amphibian[8] embryos. Once the basic embryonic body plan has been established during gastrulation, it has been proposed that embryonic Ca^{2+} signaling then returns to more localized, intercellular events that are associated with the generation of specific structures such as the somites, tail, and various organ anlagen[4]. In this review, we consider the generation and possible function of the Ca^{2+} transients generated during somitogenesis, a fundamental and highly conserved mechanism used to establish basic embryonic pattern and form.

Somitogenesis

During vertebrate development, the somites are the first segmented structures to form as the paraxial mesoderm becomes divided into metameric subunits. The somites subsequently give rise to the dermis, the axial skeleton and the skeletal muscles. Individual pairs of somites, which are formed symmetrically on either side of the notochord, are generated in an anterior-to-posterior direction within the presomitic

mesoderm[9,10]. Figure 1 is a schematic illustration of a representative zebrafish embryo to show the anterior-posterior sequence of somite formation in the trunk. Brennan *et al*[11] have separated somitogenesis into four sequential developmental stages: (1)formation and specification of paraxial mesoderm; (2) patterning of the paraxial mesoderm to establish segmental units; (3) formation of morphological boundaries between segmental units; and (4) cellular differentiation within somites. These sequential stages will provide excellent reference points when describing and discussing a variety of Ca^{2+} signaling events that have been reported from different embryonic systems at different times throughout somitogenesis. From recent molecular and genetic studies, it is clear that many aspects of somitogenesis are conserved across animal species[12]. It is, however, also apparent that individual species possess unique features with respect to their somitogenic processes during this crucial developmental period. It is with this in mind, along with the small number of species so far examined, that we compare and contrast the various reports of Ca^{2+} signaling events associated with somitogenesis. When considering the number of reports relating to Ca^{2+} signaling during the four stages of somitogenesis outlined above, most relate to the last step in the process (ie, cellular differentiation within somites), then decline in numbers back towards the earliest of the somitogenic events. In spite of this, we will proceed by considering the signaling events in a chronological order with respect to somite development.

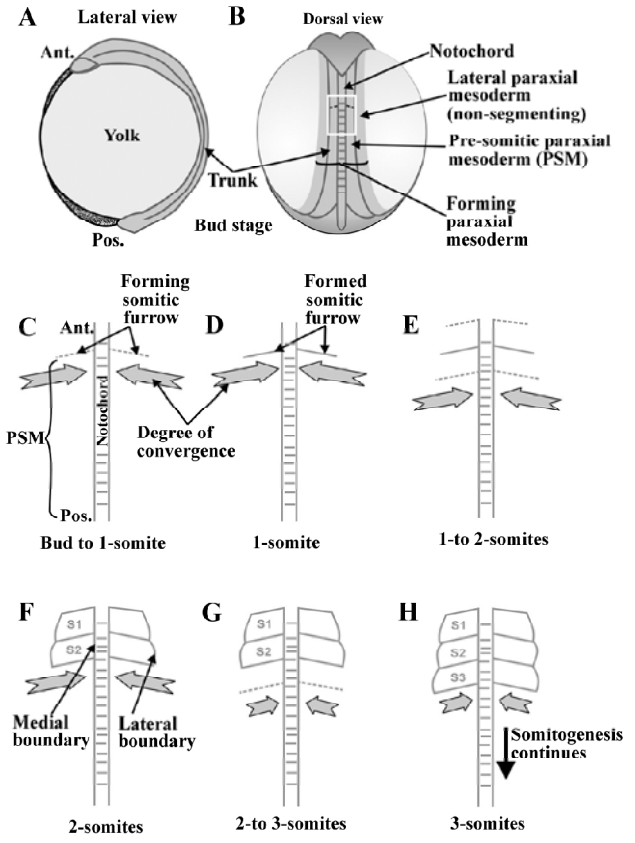

Figure 1. A schematic representation of a zebrafish embryo illustrating the formation of the first three somites. (A) Lateral and (B) dorsal views of an embryo at bud stage, just before the onset of somitogenesis. The formation of the first three somites, which occurs in the area of the trunk bounded by the white box in (B) are shown in more detail in panels (C to H). PSM is the presomitic paraxial mesoderm; S1 to S3 are somites 1 to 3; Ant. and Pos. are anterior and posterior, respectively.

Ca^{2+} signaling during the formation and specification of the paraxial mesoderm

The Ca^{2+} signaling events that occur during this period of development are the least well studied, and as a result, perhaps the least well understood. Where they have been visualized, however, they have been associated with morphogenic movements of the gastrulating embryo that are intimately linked to the establishment of the paraxial mesoderm. Furthermore, the fact that some of these Ca^{2+} signals exhibit characteristics similar to those that occur later, and are coincident with specific somitogenic events, suggests that Ca^{2+} transients generated during this early presegmentation period may indeed play a significant role in subsequent segmentation processes.

The most interesting of these Ca^{2+} transients are those reported in explants of gastrulating *Xenopus* embryos[8], where intercellular Ca^{2+} waves were visualized propagating through groups of cells undergoing convergent extension. Waves were only seen in explants that included the dorsal marginal zone, and were never observed from those that encompassed either the ventral marginal zone or prospective epidermis. The Ca^{2+} waves were reported to arise stochastically with respect to timing and position, and were often accompanied by a wave of contraction within the tissue. Most of the reported waves arose in the mesoderm, near the dorsal lip of the blastopore. Although convergent extension is more pronounced in the notochord, it also occurs in the ventrolateral mesoderm that forms the somites[13]. Using *in situ* hybridization to the muscle-specific marker *MyoD*, Wallingford *et al*[8] also showed that at the late gastrula stages, when the stochastic Ca^{2+} waves were detected, elongated arrays of *MyoD* (ie, prospective somites) flanked the notochord. In thapsigargin-treated embryos, however, the stochastic Ca^{2+} waves were suppressed and the lateral ex-

tension of *MyoD* on either side of the notochord was significantly restricted. It was proposed that this was due to a failure of convergent extension. In summary, the data presented by Wallingford *et al*[8] suggest that the Ca^{2+} transients visualized in *Xenopus* explants are not involved in determining cell fate, but that they do play an important role in controlling convergent extension. Thus, via this latter mechanism, they may play a role in defining the extent of the forming paraxial mesoderm.

Such propagating Ca^{2+} waves might prove to be a common feature of vertebrate convergent extension, and thus the formation of the paraxial mesoderm, as they have also been reported from the marginal, converging zone of the epibolizing blastoderm in developing zebrafish embryos[6,7].

Although evidence is accumulating to support a role for Ca^{2+} signaling in the specification of ventral cell fates prior to gastrulation[14,15], to date there is no clear evidence to directly link Ca^{2+} signaling to the specification of cell fates in the paraxial mesoderm.

Ca^{2+} signaling during patterning of the paraxial mesoderm to establish segmental units

Brennan *et al*[16] reported that highly localized waves of elevated intracellular Ca^{2+} propagated through blocks of anterior presomitic mesoderm (PSM) cells of approximately one somite's width just prior to somite formation. The precise role of this Ca^{2+} increase was not determined, but they proposed that Ca^{2+}-mediated cell communication via gap junctions might play a role in determining cellular boundaries during somitogenesis. Thus, unlike the wider-ranging convergent extension-related intercellular Ca^{2+} waves described in the previous section, these more localized waves, which occur just before the somitic boundaries are established, may help to pre-pattern the paraxial mesoderm in order to establish the subsequent segmental units.

Using the bioluminescent reporter aequorin, Créton *et al*[5] described a different type of Ca^{2+} wave associated with pre-patterning the paraxial mesoderm. They reported that an "ultraslow" Ca^{2+} wave, moving at ~ 0.07 μm/s, propagated along the trunk in an anterior to posterior direction and they suggested that it might be correlated in some way with the formation of the somites and neural keel.

It is interesting to note that there are no reports describing any ordered or regular series of Ca^{2+} signals generated in the PSM that correlate with any of the well reported patterns of gene expression[17]. This suggests that the pre-patterning of the paraxial mesoderm with respect to gene expression, results mainly from Ca^{2+} independent mechanisms.

Ca^{2+} signaling during the formation of morph ological boundaries between segmental units

Over 20 years ago, Chernoff and Hilfer[18] presented some of the first evidence to suggest that Ca^{2+} signaling might play some role in chick somitogenesis by culturing embryonic trunk explants on vitelline membranes in media that was either Ca^{2+}-free or which contained various Ca^{2+} agonists and antagonists. Whereas omitting Ca^{2+} in the culture medium resulted in an inhibition of somite formation and led to tissue dissociation, the application of the Ca^{2+} ionophore A23187, promoted the rapid, precocious completion of a new somite pair. Furthermore, treatment with the Ca^{2+} antagonists, verapamil and papaverine, gave similar results as the Ca^{2+}-free experiments and reversibly inhibited somitogenesis. These results suggested that in the chick, somite formation was Ca^{2+}-dependent, and the source of Ca^{2+} was extracellular. Somitogenesis was also inhibited in embryos treated with the calmodulin-binding antagonists, chlorpromazine and trifluoperazine, and by treatment with the microfilament and microtubule poisons, cytochalasin D and nocodazole, respectively. Taken together these results suggested that in the chick, Ca^{2+}-dependent somitogenesis was mediated by calmodulin and was dependent in some manner on a contraction event involving microfilaments that helped to define the anterior/posterior morphological boundaries between segmental units[18]. To date, unfortunately, there has been no attempt to directly visualize Ca^{2+} signaling during chick somitogenesis, and thus follow-up these interesting early experiments.

A series of Ca^{2+} transients have, however, been visualized in the developing myotome of *Xenopus* explants[19]. This represents the most detailed report to date with regards to Ca^{2+} signaling during the formation of somitic furrows. Using the fluorescent reporter fluo-3, Ferrari and Spitzer[19] examined Ca^{2+} transients in the intact myotome in stage 23/24 embryos, when 12 to 15 somites have already formed along the anterior-posterior (A-P) axis. For their analysis protocol, they distinguished four regions along the A-P axis, starting with differentiated anterior somites (AS), followed by maturing somites (MS), extending to segmenting somites (SS), and culminating in unsegmented paraxial mesoderm (UPM). This pattern of somite maturation was reflected in the expression of sarcomeric myosin, which was high in the AS and fell to undetectable levels in the most posterior region of the UPM. The pattern of Ca^{2+} transients detected, however, was seen to be the inverse of this myosin pattern, as myocytes in the AS produced no Ca^{2+} transients, while those in the UPM produced them with the highest frequency.

Furthermore, within SS, Ca^{2+} signaling activity was seen to be concentrated at the forming somitic furrows, where cells that were in the closest proximity to the forming somitic furrow were the most active and signaling coactivity was seen to be transmitted across the nascent somitic furrow. The Ca^{2+} transients did not, however, appear to be associated or produced by cell movements, as transient production was reported to be normal in the presence of the microfilament disrupting agent cytochalasin D. It was also demonstrated that the transients were generated by Ca^{2+} release from intracellular stores via ryanodine receptors (RyR). Blocking this release with ryanodine disrupted the formation of somitic boundaries. Ferrari and Spitzer[19] thus concluded that the spatiotemporal pattern of Ca^{2+} transients in the myotome indicated a role in the establishment or maturation of the somitic furrow. In addition, the *in vivo* Ca^{2+} transients from *Xenopus* myotome explants closely match transients recorded from cultured *Xenopus* myocytes with respect to incidence, duration and frequency. The latter have been shown to be necessary for myocyte differentiation in dissociated cell culture[20,21].

A variety of Ca^{2+} transients have also been reported in the trunk of intact zebrafish embryos during the Segmentation Period. While the study of trunk-generated Ca^{2+} signals in zebrafish is still in its infancy, these reports, as well as our own unpublished results (referred to subsequently as 'Leung *et al*, unpublished results') provide hints that Ca^{2+} may play a role in some, as yet unconfirmed, aspect of somitogenesis. Using the bioluminescent reporter aequorin, for example, Créton *et al*[5] reported localized intercellular Ca^{2+} transients along the trunk of segmenting zebrafish embryos and suggested that they might play a role in mediating the contraction events that result in the formation of somitic furrows. In addition, and also using aequorin imaging, Webb and Miller[4] reported that a number of transient localized Ca^{2+} signals appeared in the trunk during the segmentation period in zebrafish and they also suggested that the temporal and spatial characteristics of these signals might perhaps correlate with some aspects of somite formation. In both the above reports,[4,5] embryos were imaged from a lateral view using aequorin-based imaging (that has poor resolution in the z-axis[22]), thus neither group was able to conclude that Ca^{2+} transients were generated coincidently with the cutting off of each somite pair. More recently, we have used aequorin-based imaging to re-examine Ca^{2+} signaling during the segmentation period in zebrafish from a dorsal view that allowed us to visualize both sides of the embryonic mid-line (Figure 2, Leung *et al*, unpublished results). The most striking aspect of our new data regarding the Ca^{2+} transients generated during the formation of the first eight somite pairs, was the fact that unlike the physical process of cutting off somite pairs that occurs in a regular, predictable, and reproducible sequence[16,17], there was clearly no regular, reproducible pattern to the Ca^{2+} transients, both within individual embryos and when comparing one embryo with another. These Ca^{2+} transients were thus stochastic in nature and therefore resemble, in this respect, those reported during convergent extension (ie, during formation of the paraxial mesoderm) in explants from the dorsal marginal zone of *Xenopus* embryos[8]. We assigned these stochastic Ca^{2+} transients the general term of "forming somite signals" (FSS) as they were generated within the segmented paraxial mesoderm once the anterior/posterior somitic furrow boundaries had formed. Although stochastic in nature, there was a significant tendency for the FSS to occur within specific regions of the maturing somites, ie, ~75% of FSS were generated at or where the medial or lateral somitic boundaries were forming. Figure 2 shows two representative examples of embryos (from both dorsal and lateral views) displaying a number of FSS. We thus suggested that the medial and lateral FSS might provide an additional level of regulation that helps to define and maintain the medial and lateral somitic boundaries. The incubation of embryos from the 2-somite to the 6-somite stage with a variety of Ca^{2+} channel antagonists indicated that these stochastic Ca^{2+} transients were generated by Ca^{2+} release from intracellular stores via inositol trisphosphate receptors (IP_3R) and not by release via RyR or from extracellular sources, via Ca^{2+} channels in the plasma membrane (Leung *et al*, unpublished results). The application of the IP_3R antagonist, 2-APB, for example resulted in somites that were lengthened in the medio-lateral dimension. This therefore is unlike the situation reported for *Xenopus*[19] where Ca^{2+} release via RyR were responsible for generating the somitic Ca^{2+} transients.

As mentioned above, we suggest that there are several significant similarities between the characteristics (and thus possible developmental function) of the FSS that we described in early zebrafish somitogenesis (Figure 2) and the Ca^{2+} signaling reported during convergent extension in *Xenopus* embryos[8]. One of these is the common stochastic nature of the signals reported with respect to morphological events. This might in part be explained by a characteristic that is unique to the formation of early somites compared to the later ones. Rostral-caudal differences in somitogenesis have been described in teleosts such as the zebrafish[23] and the rosy barb, *Barbus chonchonius*[24], as well as in chick[25] and *Xenopus*[19]. These differences relate to the degree of post-gastrulation convergence that continues during the for-

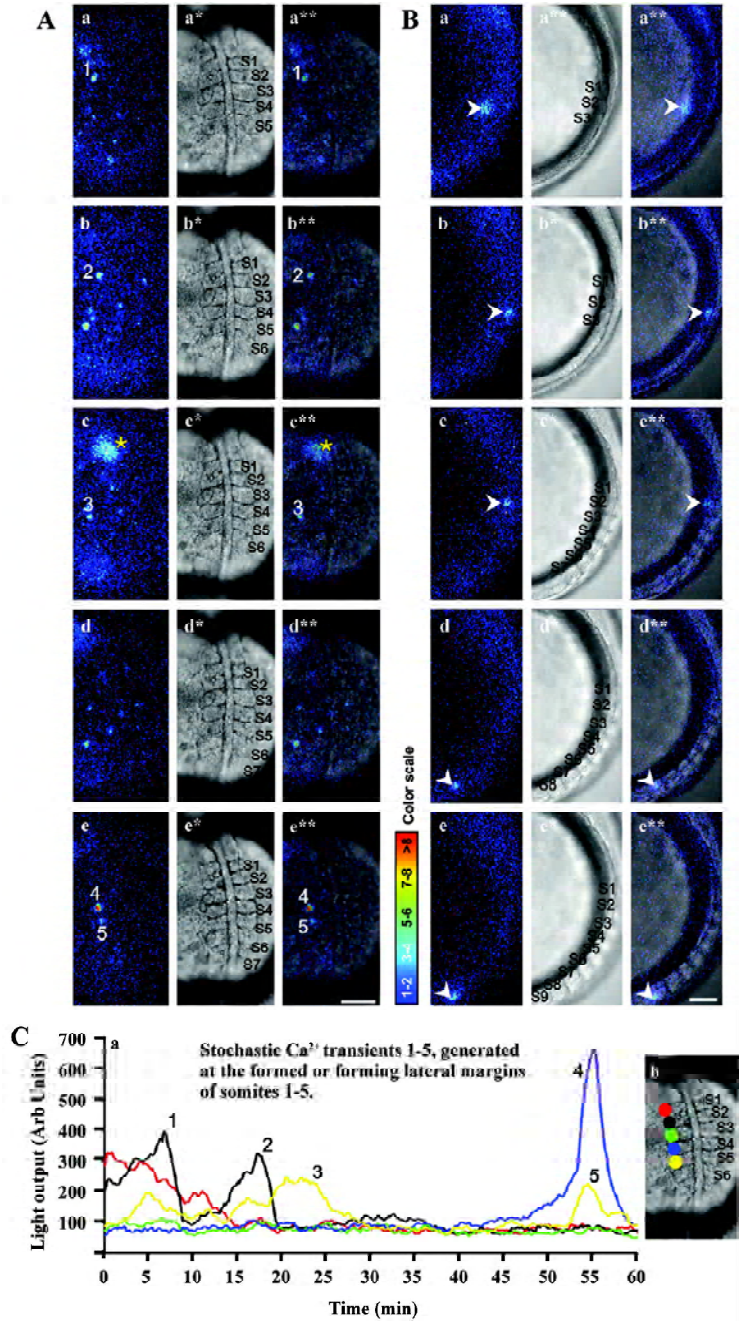

Figure 2. A stochastic pattern of Ca^{2+} transients is generated in the maturing somites during early somitogenesis in zebrafish. These are two representative examples of the Ca^{2+} transients observed in the trunk of zebrafish embryos from (A) a dorsal and (B) a lateral view (n=14, for each) during the formation of somites 1 to 8. In both (A) and (B), panels a to e represent aequorin-generated pseudocolor images where each image represents 120 s of accumulated aequorin-generated light, and panels a* to e* represent bright-field images that were acquired just prior to the respective aequorin-generated image. Panels a** to e** represent the aequorin images superimposed on the bright-field images to show the location of the Ca^{2+} signals more clearly. (A) and (B) clearly show the stochastic nature of the Ca^{2+} transients and the fact that they have a tendency to occur at the forming or formed lateral margins of somites. The color scale represents luminescent flux in photons/pixel. Scale bar is 100 μm. (C-a) A plot of the light output (arbitrary units) against time (mins) from the imaging sequence shown in (A). Traces were generated from data points representing 120 s of luminescence that were acquired every 10 s for five imaging fields, (shown in panel C-b; where the color of each trace matches that of the region of interest), each covering approximately 314 pixels at the lateral margin of somites 1 to 5. The numbers 1 to 5 on the graph represent the signals shown in panel A. The yellow asterisk in panel A-c and panel A-c** indicates an example of a Ca^{2+} transient generated in the hindbrain.

mation of the early somite pairs (Figure 1). This is not an issue with regards to later somite formation, as convergence has by then ceased. However, in early somite formation there is a continued movement of cells towards the embryonic midline that may, via cell-to-cell interaction, challenge the integrity of the medial boundary between the maturing somite and the notochord in addition to complicating the process of defining the lateral boundary once the required number of cells has converged into the forming somite. We suggest that the degree of these convergence-related challenges may vary between individual somites on either side of the embryonic midline within a single embryo and when each set of somite pair is cut off, with respect to the degree of convergence still taking place. Thus, if the FSS play some role in establishing and maintaining these medial and lateral somitic boundaries, their occurrence (and thus visualization) might well reflect this natural variability within individual embryos (as well as between embryos) and thus explain their

stochastic nature.

We also suggest that the cell movements that occur during convergence might also explain why the FSS are not seen until after the anterior/posterior somitic furrows are established as these represent restrictive boundaries that prevent the converging cells from spreading out of the forming somite in either an anterior or posterior direction. The onus, therefore, is then on maintaining the medial boundary followed, at the right time, by establishing and maintaining the lateral boundary. The fact that the medial and lateral FSS are not seen on a regular basis indicates that they are not the primary signal responsible for establishing or maintaining these essential boundaries. It does, however, suggest that they may represent a form of "policing" signal that is generated in response to occasions when the boundaries are challenged; for example when a cell or group of cells attempts to cross one of the boundaries or tries to leave the forming somite. The latter might explain the signals visualized from the middle of somites, or from already formed anterior or posterior boundaries. We did not observe any examples where a FSS was directly associated with the establishment of the anterior or posterior boundary of a somite, ie, a signal co-incident with a forming somitic furrow. This suggests that in zebrafish, unlike chick or *Xenopus*, this key event during somitogenesis appears to proceed largely via Ca^{2+}-independent processes.

To test our suggestion that the function of the FSS might be to help establish and maintain the medial and lateral boundaries of somites, we undertook experiments to modulate these signals via either the photo-release of Ca^{2+} (via uncaging NP-EGTA[26]) or photo-activation of a Ca^{2+} buffer (via uncaging of diazo-2[27]). Uncaging Ca^{2+} in the PSM of presumptive somites 3, 4 and 5 resulted in somites shortened along their medio-lateral dimension, whereas, photo-activating a Ca^{2+} buffer in a similar region, resulted in the elongation of somites along their medio-lateral dimension (Figure 3; Leung *et al*, unpublished results). This precocious and ectopic shortening of the somites by uncaging NP-EGTA to release Ca^{2+} supports our suggestion that an elevated domain of intracellular Ca^{2+} may indeed act as a "policing signal" that helps to establish the lateral boundary of the somite when other primary signals fail to invoke a response. We suggest that this is achieved via Ca^{2+}-sensitive elements that effect cell-to-cell contact and thus restrict cell movement. This, therefore, determines the lateral border and establishes the final somite dimension. The reverse is the case when it comes to activating the Ca^{2+} buffer whereby the imposed inability to generate the proposed policing "stop" signal results in somites that extend beyond their normal medio-lateral boundaries. Once again, we suggest that this could be mediated via Ca^{2+}-sensitive elements that regulate the contact characteristics between cells. When cells are free to move, they continue to converge into the forming somites and thus result in a lateral extension. These preliminary experiments therefore suggest a possible developmental function for the FSS, which is compatible with their stochastic nature. The fact that uncaging either Ca^{2+} or Ca^{2+} buffer had no effect on the establishment of the anterior/posterior somite boundaries supports our previous suggestion that in zebrafish these might form via Ca^{2+}-independent mechanisms.

In summary, the evidence to date clearly supports the proposition that Ca^{2+} signals may play a role during the formation of morphological boundaries between segmental units. What their precise function is, however, may vary both between species as well as the particular somitic boundary in question, ie, the anterior-posterior somitic furrow, or the medio-lateral somitic boundaries. The different signals that have been described in the paper are summarized in schematic form in Figure 4.

Ca^{2+} signaling during cellular differentiation within somites

From both *in vitro* and *in vivo* studies, evidence is accumulating from *Xenopus*[19–21], mouse[28–30], chick[31] and zebrafish[16] to indicate that Ca^{2+} signaling plays a role in myofibrillogenesis and the development and differentiation of myotubes (Figure 5). For example, Ferrari *et al*[20] reported that differentiating embryonic *Xenopus* trunk myocytes produced spontaneous Ca^{2+} transients during a restricted developmental window in primary culture. In addition, they showed that suppressing these Ca^{2+} transients disrupted myofibrillogenesis and the formation of sarcomeres. Subsequently, it was reported from cultured *Xenopus* embryonic myocytes, that these Ca^{2+} transients activated myosin light chain kinase (MLCK) in order to promote myosin thick filament incorporation into developing sarcomeres[21]. The involvement of MLCK in embryonic sarcomere development was supported by *in vitro* studies on sarcomere organization during cardiac hypertrophy in neonatal rats[32]. Furthermore, it was recently reported that spontaneous Ca^{2+} transients also appear to regulate patterned actin assemble during myofibrillogenesis in cultured *Xenopus* myocytes[33], where blocking the transients disrupted the assembly of actin thin filaments along with the associated z-disc affiliated proteins titin and capZ. Together, these data suggest that spontaneous Ca^{2+} transients may regulate one or more of the earliest steps in sarcomere differentiation.

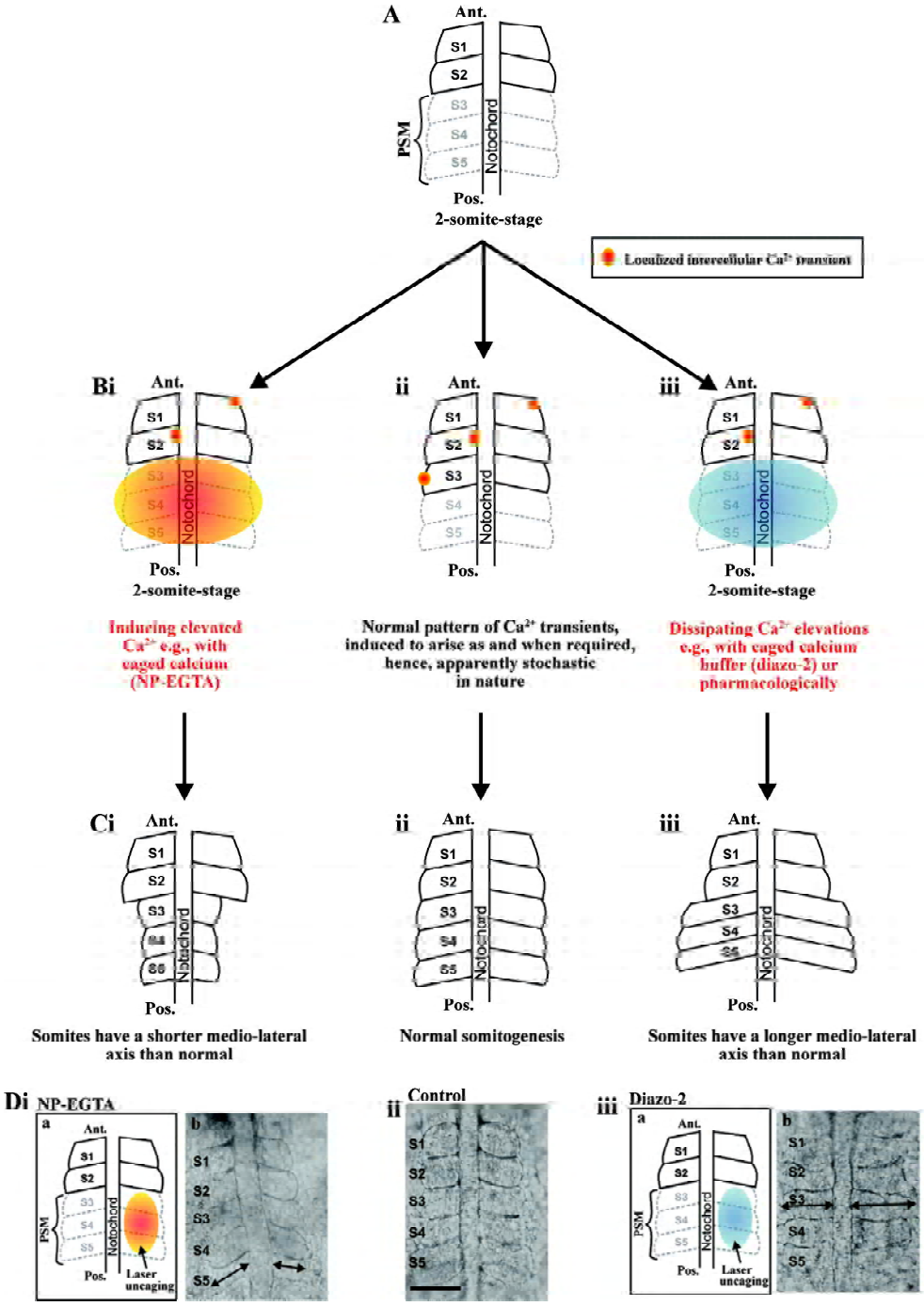

Figure 3. A schematic representation of the proposed role of the Ca^{2+} transients during early somitogenesis in zebrafish. We suggest that the normal stochastic pattern of Ca^{2+} transients might help to define and maintain the medial and lateral somitic boundaries during normal somite formation (see panels Bii and Cii). Indeed, evidence resulting from the modulation of somitic $[Ca^{2+}]_i$, within the paraxial mesoderm supports this suggestion. For example, the photo-release of caged Ca^{2+} (such as NP-EGTA; n=15) results in somites that are shorter in the medio-lateral dimension (see panels Bi and Ci), whereas the photo-activation of a caged Ca^{2+} chelator (diazo-2; n=15) results in somites that are longer in the medio-lateral dimension (see panels Biii and Ciii), respectively. Panels Di-Diii show representative examples of embryos that were loaded with NP-EGTA (panel Di) or diazo-2 (panel Diii) at the single-cell stage. When these photolabile compounds were uncaged in the presomatic mesoderm (PSM) along the right side of the notochord, the somites that subsequently developed were either shorter or longer than the intrinsic controls on the left side of the notochord, respectively. Panel Dii shows an untreated control embryo with normal somites. Bar is 100 μm.

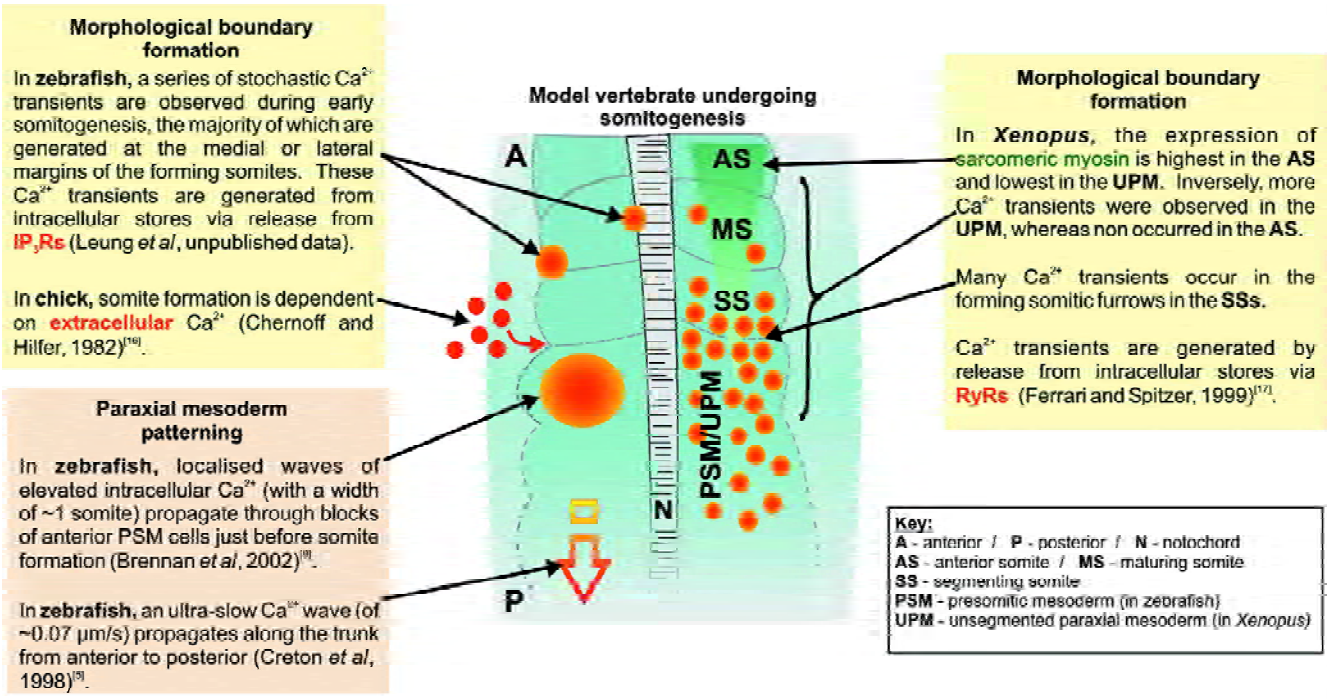

Figure 4. Schematic representation of the Ca^{2+} transients reported to occur during the patterning of the paraxial mesoderm and the formation of the morphological boundaries of the somites.

Following these cultured myocyte experiments, Ferrari and Spitzer[19] also examined the Ca^{2+} dynamics in maturing somites of *Xenopus* explants. They showed that the characteristics of the Ca^{2+} transients found in the cells of the maturing somites were remarkably similar to those found in cultured myocytes and were correlated with myocyte maturation. They also demonstrated that in *Xenopus* (unlike the chick), these Ca^{2+} transients were generated by Ca^{2+} release from intracellular stores. Furthermore, they identified the Ca^{2+}-release channels involved by showing the functional distribution of both IP_3R-activated stores and RyR-activated stores in intact myotome by eliciting Ca^{2+} elevations in response to photo-release of caged IP_3 and superfusion of caffeine. These experiments indicated that as in myocyte culture, Ca^{2+} transients *in vivo* depend on Ca^{2+} release mainly from RyR stores, and blocking release from these stores interferes with somite maturation[19].

Brennan *et al*[16] recently reported from intact zebrafish embryos, that Ca^{2+} signaling may also be required for the later development of slow muscle fiber formation between stages 16 to 22 h post fertilization (ie, at stages where functional neuromuscular contacts are first established). They also showed that acetylcholine drives initial muscle contraction and embryonic movement via the release of intracellular Ca^{2+} from RyR. When this activity-dependent pathway was inhibited, either at the level of the acetylcholine receptor or RyR, this did not disrupt slow fiber number, elongation or migration, but did affect myofibril organization. They proposed, therefore, that Ca^{2+} may be acting via the cytoskeleton to regulate myofibril organization and thus suggested a critical role for nerve-mediated Ca^{2+} signals in the formation of physiologically functional muscle units during development.

Conclusions

A number of different reports demonstrate that starting at the convergent extension events that precede the segmentation of the paraxial mesoderm, then during the segmentation process itself, and on through to the differentiation of the somites to form functional skeletal muscle, a range of different Ca^{2+} signals are generated from a variety of Ca^{2+} stores by a number of different Ca^{2+} release mechanisms. Although much has to be done to fully understand the developmental significance and function of these Ca^{2+} signals, it is already clear that they play a required role in controlling the generation of somites and their ultimate developmental fates. In several of the reports to date, the Ca^{2+} signals recorded have been to some degree stochastic. We thus suggest that such signals perhaps represent a new class of de-

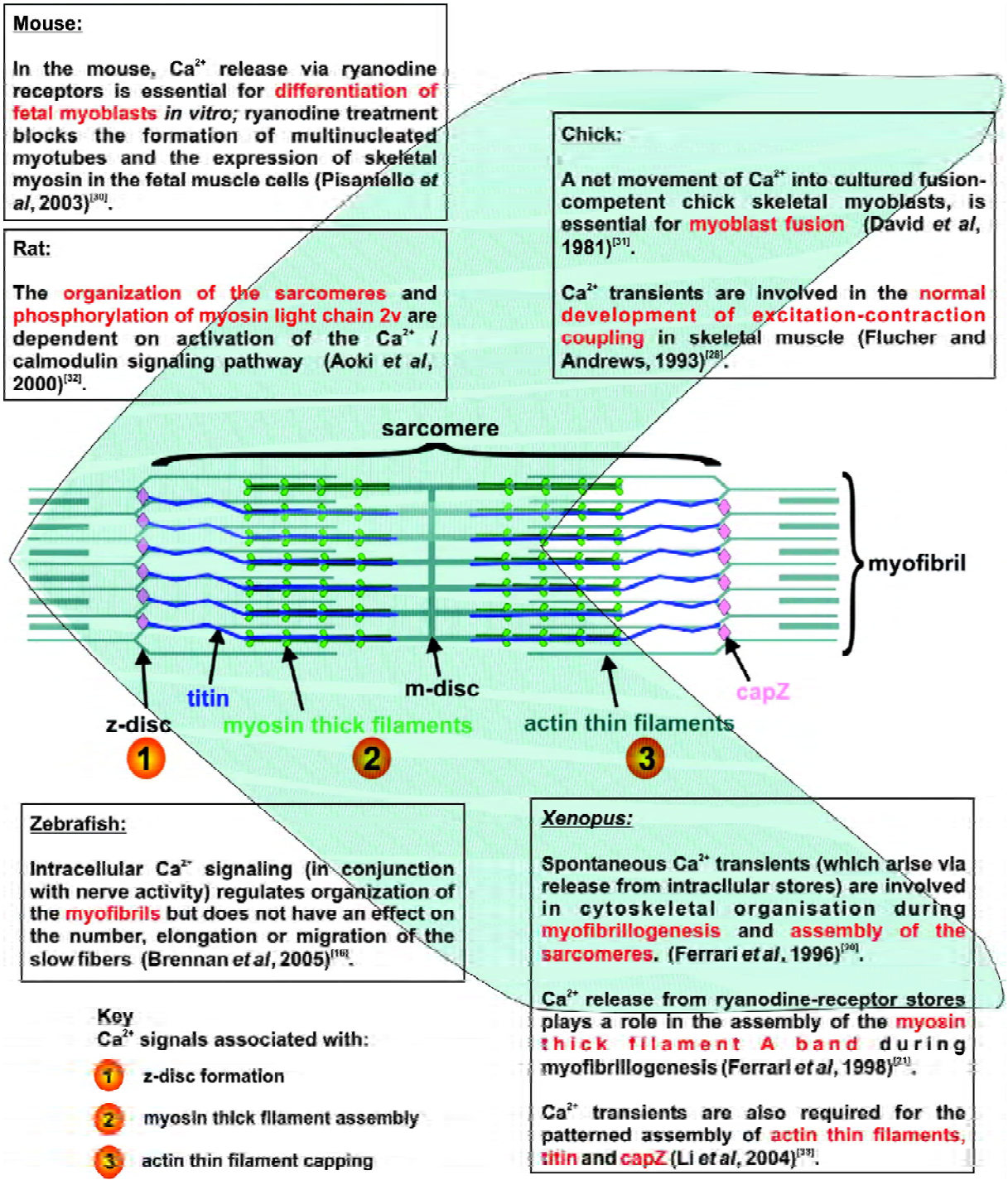

Figure 5. Summary of the possible downstream morphogenic targets of the Ca^{2+} transients reported to occur during cellular differentiation within the somites. Schematic of sarcomere modified from Li *et al*[31].

velopmental Ca^{2+} signaling, where Ca^{2+} is but one of several developmental regulators, which act in concert with other somitogenesis signaling pathways, such as the PDGF/PI3K and Wnt/Ca^{2+} pathways (reviewed by Ulrich and Heisenberg[34]). Both of these pathways have been clearly demonstrated to be connected with Ca^{2+} signaling. Moreover, genes in both pathways (for example the *wnt5* gene in the Wnt/Ca^{2+} pathway and the *PDGFR-α* gene in the PDGF/PI3K

pathway) are expressed in somites from the earliest stages of segmentation[35,36]. Thus, the physiological Ca^{2+} imaging data reviewed here, support the current genetic and molecular data, and thus provide additional evidence to suggest that Ca^{2+} signaling plays a significant role in somitogenesis.

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