Mapping the Lateral Somitic Frontier in the Zebrafish Body Wall

by

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Acknowledgements

The words “Science will make you Stronger” are written on a blackboard in our lab. At first, I imagined them being written by a bitter graduate student in the wake of a failed experiment, as if to say “at least I’m building character.” There is no doubt that science will make you stronger, but as my time at Wesleyan has taught me, this strength is more than just the ability to withstand failure. The past three years have been an incredible period of growth for me, particularly in my fascination with the natural world and the pursuit of discovery. I would like to take this space to name a few of the people who have been so influential and motivational for me along the way.

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Introduction

1) The influence of development on the evolution of morphology

Natural selection is not the only force at work in shaping the diversity of life we see today. Natural selection acts on standing variation in a population – variation which is established through developmental processes. Development, as we understand conceptually, is not capable of producing all phenotypes equally. Constraints to what can develop play a large role in determining what can evolve. To understand exactly how large a role this is, we compare the development of different species to relate developmental events to morphological change.

First and foremost, developmental constraints stem from inherent physical properties of cells. There is increasing evidence that changes to the genome do not have a one-to-one correspondence to changes in morphology (Lewontin 1985, Alberch 1991, Pigliucci 2010). Rather, genetic interactions serve to bias an organism towards a certain developmental path (Oster and Alberch 1981). Environmental influences similarly affect the behavior of cells, and therefore the developmental outcome that is reached (West-Eberhard 2003).

Regardless of whether these developmental biases stem from genetic or environmental influences, natural selection acts on the phenotype that is produced. The evolutionary history of an organism is the result of natural selection preferencing certain developmental outcomes. The extent to which developmental constraints and
natural selection can explain the overall form of an organism, however, is still poorly understood (Wagner 2000).

Comparing the development of different species gives us clues to how developmental events changed as the species diverged. We are not determining the cause of these changes, but rather identifying what sorts of changes in tissue interactions tend to occur – what sorts of changes are relatively “easy” to evolve and vary considerably between species, and what sorts of changes are “difficult” to evolve and can be isolated to one or a few specific phylogenetic events.

One change that was predicted to be quite difficult to evolve is a change between taxa in the embryonic cell lineage that gives rise to a certain aspect of morphology (True and Haag 2001). Until recently, the morphological changes in homologous structures were assumed to correspond to changes in the degree or nature of each tissue’s contribution to a structure. Mounting evidence, however, indicates that changes in which embryonic tissue lineages contribute to a structure occur surprisingly frequently (True and Haag 2001).

As an example, a recent study found that a change in embryonic origin appears to have occurred in the *Xenopus* skull. The *Xenopus* skull has bones structurally homologous to those in salamanders, birds, and mammals, yet these bones are derived from different neural crest streams (Piekarski et al. 2014). Based on their morphology, the *Xenopus* bones were predicted to share a developmental system with other tetrapods, yet lineage-mapping experiments identified that the bones are from the hyoid and branchial neural crest streams in *Xenopus* and the mandibular
stream and non-neural crest populations in other tetrapods (Piekarski et al. 2014). The morphological divergence seen in the *Xenopus* skull is no more significant than divergence seen in any other tetrapod’s skull morphology, yet its developmental system in *Xenopus* is distinct.

This change in developmental system is only evident in the embryonic phenotype. It is hidden from natural selection, or ‘cryptic’, in the adult. As long as the change does not impair development, such a cryptic change might have little consequence for a species’ fitness. If however, this developmental change made possible future morphological innovation, then descending lineages may benefit from a more diverse spread of functional morphologies (Gerhart and Kirshner 2007). The question then becomes: how might a cryptic developmental change facilitate morphological change? Comparing the development of closely related but morphologically diverse species might reveal how cryptic changes in development correspond to functional changes in morphology. Vertebrates are one such group of related but diverse species.

Richard Owen’s vertebrate archetype is an early illustration of two opposing forces of conservation and elaboration at work in the vertebrate form (Owen 1849). A generic vertebrate is depicted as a series of vertebral-based units, where different structures branch off at different anterior-posterior regions. The differential modification of each of these units can theoretically recapitulate any vertebrate skeleton. This archetype demonstrates both the surprising conservation of anatomical modules and the remarkable degree to which these modules can be differentially
modified between species. This differential modification, or modularity, can be described at an evolutionary scale by identifying anatomical modules of the vertebrate form based on how they have changed between species (Schlosser and Wagner 2004).

Modularity also has an underlying developmental basis. The differential modification of developmental patterning in one region of the embryo versus another underlies modularity in adult anatomy. Modularity in patterning is commonly thought of as happening along the antero-posterior (A-P) axis, where cranial, cervical, thoracic, lumbar, sacral, and caudal levels each develop different structures that have specific functions to the animal. Morphological differences along the A-P axis correspond to changes in Hox gene expression, where each somite has a specific Hox code (Kessel and Gruss 1991, Burke et al. 1995).

Although less distinct than modularity along the A-P axis, there is also modularity between the dorsal and ventral compartments of the body. Vertebrates, aptly named for their vertebral column, have a relatively conserved body axis interfacing with a diverse array of locomotory structures.

We hypothesize that modularity between the dorso-medial and ventro-lateral patterning environments is essential for the integrated musculoskeletal innovations that are central to vertebrate diversity. The boundary between these two environments is the Lateral Somitic Frontier (LSF).

2) The Lateral Somitic Frontier in embryogenesis
Two populations of mesoderm give rise to the post-cranial musculoskeletal system: the somites and the lateral plate mesoderm (LPM). Somitic mesoderm is the source of all the skeletal muscles of the body. Somite transplant experiments have revealed that both somitic and LPM provide connective tissue lineages for developing somite-derived muscle (Chevallier et al. 1977, Ordahl and Le Douarine 1992, Christ and Ordahl 1995). These environments consist of three types of connective tissue (CT): specialized CT (bone and cartilage), dense regular CT (tendon), and dense irregular CT. Dense irregular connective tissue is made up of extracellular matrix-secreting fibroblasts found in high concentration surrounding muscles and at much lower concentration between muscle fibers (Nassari et al. 2017).

The discovery of a lateral plate-derived environment for somitic muscle suggests there is a boundary between somitic and lateral plate-derived connective tissue environments, yet the significance of such a boundary is just beginning to be appreciated. The Lateral Somitic Frontier (LSF) delineates the transition between the two connective tissue environments: primaxial (somitic) and abaxial (lateral plate) (Burke and Nowicki, 2003, and Figure 1).

Pectoral and pelvic paired appendages of both finned and limbed vertebrates originate from outgrowths of the LPM and its overlying ectoderm, collectively referred to as the somatopleure (Chevallier et al. 1977). The outgrowths of somatopleure are subsequently invaded by somite-derived migrating muscle precursors (MMPs) to contribute the appendicular muscles. As the connective tissue
environment for these appendicular muscles is lateral plate-derived, these structures are abaxial.

In the body wall, the primaxial-abaxial distinction is less clear-cut and does not align with the epaxial-hypaxial distinction (Figure 1C). Fate-mapping has revealed that the primaxial domain comprises the ribs, vertebrae, and epaxial vertebral muscles as well as the hypaxial intercostals. The abaxial domain in the body wall comprises the ventro-lateral region to differing extents depending on the lineage (Nowicki et al. 2003, Durland et al. 2008, Tulenko et al. 2013).

To map the LSF, we track the contribution of LPM during body wall formation. Body wall formation consists of a primary and secondary stage. In amniotes, primary body wall formation occurs when body folds lift the embryo off the yolk (Sadler and Feldkamp 2008). In anamniotes that engulf their yolk, an analogous but less-distinct process occurs as the lateral plate mesoderm expands ventrally to surround the yolk to form the primary body wall (Kaneko et al. 2014). In either case, this process establishes a population of LPM in the ventral body wall, well before the secondary body wall, the muscular body wall, has begun to develop.

As the muscular body wall begins to form, beginning dorsally and progressing ventrally, myogenic precursors either displace or infiltrate lateral plate mesenchyme. The cellular mechanisms of these two contrasting behaviors remain to be fully described, but an epithelial-to-mesenchymal transition (EMT) of myogenic precursors may be the essential step for their incorporation into lateral plate mesenchyme.
Dermomyotome extension is believed to be the primitive form of body wall muscle development. This is the mechanism used at all A-P levels in the body wall of the basal vertebrate, the lamprey (Kusakabe and Kuratani 2005, Tulenko et al. 2013). During lamprey body wall formation, the dermomyotome remains as an epithelium and forms an entirely primaxial body wall. All LPM is displaced to the ventral midline and the interior of the body wall muscle (Tulenko et al. 2013).

De-epithelialization of muscle precursors via EMT has most extensively been characterized in the limbs. In limb-level somites, de-epithelialization of the leading edge of the dermomyotome is induced by the binding of scatter factor/HGF, which is secreted by the lateral plate, to the receptor c-met, which is expressed by the dermomyotome (Dietrich et al. 1999). This prompts the de-epithelialization of migratory muscle precursors (MMPs) from the dermomyotome (Brand-Saberi et al. 1996, Dietrich et al. 1999). These MMPs express lbx1 and the chemokine receptor CXCR4 and migrate toward expression of the CXCR4 ligand SDF1 in regions of the lateral plate mesenchyme where they will differentiate into muscle (Vasyutina et al. 2005).

Whether a similar de-epithelialization occurs in abaxial muscles at inter-limb levels remains to be understood. In teleosts, some body wall muscle progenitors express the migratory muscle precursor (MMP) marker lbx1 and differentiate separate from the dermomyotome. This indicates that they are formed similarly to how appendicular muscles are formed, by somite cells that migrate a distance from the dermomyotome before differentiating (Windner et al. 2011). Because cells
differentiate close to the dermomyotome and appear to retain some organization as they migrate, there is debate as to whether or not they are formed from true MMPs (Haines et al. 2004). Further research is required to determine if other lineages express \(lbx1\) in body wall muscle and if this form of body wall formation is distinct from what occurs in the limbs.

There is increasing evidence that recruitment and differentiation of muscle precursors is coordinated by the surrounding connective tissue environment (Noden 1983, Kardon 1998, Marrel et al. 2015, reviewed in Nassari et al. 2017). The LSF delineates the boundary between two connective tissue environments of different embryonic origin: somitic and lateral plate. During gastrulation, somitic and lateral plate mesoderm are specified as separate mesoderm populations and acquire different gene expression profiles (Sasaki and Hogan 1993, Psychoyos and Stern 1996, Chapman et al. 1996, Yamaguchi et al. 1999, Mahlapuu et al. 2001). We propose that the primaxial and abaxial domains provide developing muscle with different cell signaling environments, which independently affect musculoskeletal patterning in the primaxial and abaxial domains.

Early somite transplant experiments in chick illustrate how distinct patterning environments differentially influence musculoskeletal development (Kieny et al. 1972, Nowicki and Burke 2000). Heterotopic transplants of segmental plate from one anterior-posterior (A-P) region to a different one (cervical to thoracic, thoracic to brachial, thoracic to cervical) found the transplanted tissue contributed to both primaxial and abaxial structures at the different A-P levels.
The primaxial structures that develop retain their A-P identity from their position in the donor. In a cervical to thoracic transplant, for example, primaxial ectopic neck muscles and cervical vertebrae develop from the graft. Additionally, transplanted somites retained their donor hox gene expression (Burke and Nowicki 2000). Importantly, transplanted somites gave rise to both muscle and connective tissue lineages in the primaxial domain, whereas in the abaxial domain, transplanted cells only contributed to muscle.

The results for the primaxial domain are in stark contrast to the graft cells’ behavior in the abaxial domain. Transplanted cells that cross the LSF contribute to the appropriate abaxial muscles at neck, limb, and thoracic levels, and there is no ectopic hox gene expression in the abaxial domain (Burke and Nowicki 2000). Importantly, the graft does not contribute to abaxial connective tissue, and one interpretation of these results is that muscle precursors are relatively naïve and rely on their surrounding connective tissue for A-P patterning information. Muscle precursors crossing the LSF entered the native connective tissue environment and differentiated appropriately whereas cells that remained in the primaxial domain differentiated into ectopic muscles in the host.

Many other studies benefit from using the LSF framework to explain the morphological variation they observe across different vertebrate lineages. Changes in the abaxial domain have been suggested in the formation of the mammalian diaphragm (Bucholtz et al. 2012), the modification of the avian sternum (Bickley and Logan 2014), human body wall development (Mekonen et al. 2015), the acquisition
of paired appendages (Johanson 2010, Tulenko et al. 2013), and the loss of appendages in many squamates (Head and Polly 2015), whereas changes in the primaxial domain have been implicated in the formation of the turtle shell (Burke 1989, Nagashima et al. 2007, Rice et al. 2015).

Importantly, each of these innovations occurred primarily on one side of the LSF. This supports our hypothesis that primaxial and abaxial domains are two independent patterning environments. If aberrant cellular behavior were to arise due to differential patterning in either the primaxial or abaxial domain, but not both, then a plastic developmental response in the other domain would be sufficient to accommodate the new morphology. Based on our hypothesis, we expect differential modification to the primaxial or abaxial domain to frequently mediate the evolution of new vertebrate body plans.

3) Present study

The LSF in the body wall of ray-finned fishes is virtually unstudied. Here, we characterize the LSF in the zebrafish body wall using transgenic methods to fate-map lateral plate-derived tissue. The LPM population in zebrafish is quite sparse, with very few known genetic markers, making visualization of LPM a challenge. We take advantage of one of the many transgenic lines of zebrafish to conduct a cre-recombinase based fate-mapping of lateral plate derived tissue.

The paired fins are the most well-studied lateral plate derived structures in zebrafish. Both pectoral and pelvic paired fins have been shown to be formed via
outcroppings of somatopleure, homolgous to limb formation in tetrapods (Ahn et al. 2002). The body wall, however, has not been extensively studied.

The zebrafish body wall is made of two muscle groups, the posterior hypaxial muscle (PHM, black arrows in Fig. 2A) and extensions from the myotome (red arrows in Fig. 2A) that form the superior and inferior obliques. The PHM attaches to the cleithrum anteriorly and becomes continuous with the trunk myotome posteriorly (Haines et al. 2004, Windner et al. 2011). Fate mapping experiments in zebrafish indicate that somites 1-4 do not contribute to the PHM (Nyet et al. 2000, Minchin et al. 2013). Based on expression of lbx1 and other genetic markers of migratory muscle precursors (MMPs) spreading anteriorly from somites 5 and 6, these somites are thought to be the source of MMPs that form the PHM (Haines et al. 2004, Windner et al. 2011).

The PHM forms relatively quickly in late embryonic/early larval zebrafish, as compared to the extending myotome, which does not reach the ventral body wall until late juvenile stages. In the adult, the trunk myotome extends over all but the anterior-most portion of the PHM, and the two muscles are relatively indistinguishable in whole mount (Fig. 2 B and C). The PHM gives rise to the anterior two segments of the obliquus inferioris muscle while the posterior obliquus inferor is and the entire obliquus superioris are formed through direct myotomal extensions (Windner et al. 2011).

In the ventral body wall, beginning around 7mm, some muscle fibers form disjoint from the main myotome (Fig. 2B, white arrows). These fibers are sometimes
termed the “abdominal rectus” and will go on to form the anterior infracarinalis. The anterior infracarinalis run along the ventral body wall on either side of the ventral midline and attach to the anterio-ventral pelvic girdle. The anterior infracarinalis becomes continuous with the trunk myotome anteriorly (Winterbottom, 1973).

In the following Methods section we describe our fate-map technique in detail. We then go on to describe the distribution of fate-mapped cells in the embryonic, larval, and juvenile zebrafish during primary and secondary body wall formation. Finally, we discuss the significance of our results in zebrafish in a comparative context, hypothesize as to underlying cellular mechanisms, and describe future experiments to build off of this work.
Materials and Methods

Raising fish

Two transgenic lines were used in this study: *draculin:creERT2* (*drl:creERT2*, described in Mosimann et al. 2015) and *ubiquitin:loxP-EGFP-STOP-loxP-mCherry* (*ubi:Switch*, described in Mosimann et al. 2011). Both lines were obtained from Christian Mosimann at Boston Children’s Hospital in Boston, MA and subsequently out-crossed to the Wesleyan University strain of wildtype fish. Presence of the *drl:creERT2* transgene was determined using fluorescent light as the *alpha-crystallin:Venus* reporter transgene has been stably incorporated in the *drl:creERT2* line. Presence of the *ubi:Switch* transgene was also determined using fluorescent light, as all cells in a *ubi:Switch* fish produce EGFP. Both the *drl:creERT2* and *ubi:Switch* transgenes exhibit simple Mendelian inheritance, and all transgenic individuals of the out-crossed generation appear to be heterozygous (based on the proportion of their offspring that retain the transgene). All experiments on wildtype fish were conducted with the Wesleyan university wildtype strain.

*draculin:creERT2 x ubiquitin:Switch hybrid system*

There are no known genetic markers constitutively expressed throughout the LPM. To address this, we take advantage of the cre recombinase-based fate mapping system. Using the *Tg(drl:cre)* and *Tg(ubi:loxP-EGFP-STOP-loxP-mCherry)* constructs, we genetically induce constitutive mCherry expression in cells expressing
*draculin* during early embryonic stages. Activation of cre recombinase is mediated by the timed addition of Tamoxifen to the embryo medium.

Hybrid offspring were generated by crossing two transgenic parental lines: *drl:creERT2* fish have a transgene encoding the enzyme cre-recombinase coupled to an estrogen receptor with a T2 mutation (CreERT2) downstream of a 6kbp *draculin* promoter sequence. *ubi:Switch* fish have a *loxP-EGFP-STOP-loxP-mCherry* construct downstream of a ubiquitously active promoter, making the construct constitutively transcribed in every cell. The presence of the STOP codon prevents the transcription of *mCherry* in single-transgenic *ubi:Switch* individuals. Thus, the F0 parental *ubi:Switch* fish are producing EGFP in every cell at all stages of development.

The enzyme cre-recombinase splices together two *loxP* sites, excising out the DNA sequence between them. In the presence of cre-recombinase, the *ubi:loxP-EGFP-STOP-loxP-mCherry* construct is converted to *ubi:mCherry*. Simply put, a *ubi:Switch* cell will “switch” from constitutively producing EGFP to constitutively producing mCherry in the presence of cre-recombinase. The CreERT2 construct, however, remains inactive in the cytoplasm until its ligand, a Tamoxifen metabolite, triggers its import into the nucleus. This allows for the timed activation of cre-recombinase activity. In this study, we use Tamoxifen (*trans-4-OHT*) as the CreERT2 ligand, but another Tamoxifen metabolite, Endoxifen, has been seen to function equally well, with the added benefit of being more stable in solution (Felker et al. 2016).
Cre-recombinase activity was induced in *drl:creERT2;ubi:Switch* hybrid embryos by adding Tamoxifen to their embryo medium at a concentration of 5µM at various embryonic stages: fertilization, high, shield, or bud stage (Kimmel et al. 1995). Activating cre-recombinase at different embryonic stages resulted in different proportions of lateral plate mesoderm expressing mCherry (see Results and figure 1). To control for this, the optimal embryonic stage for activating cre-recombinase was determined to be high stage, and all subsequently studied fish were dosed with Tamoxifen at high stage.

*Significance of mCherry expression in drl:creERT2;ubi:Switch hybrids*

The *draculin* (*drl*) gene encodes a 411 amino acid zinc-finger transcription factor, and is first expressed in zebrafish at late blastula stage around the blastoderm margin (Herbomel et al. 1999, Hsia and Zon 2005). During gastrulation, *drl* expression is seen in the ventral and ventro-lateral hypoblast and the presumptive prechordal plate (Herbomel et al. 1999). This expression is reduced to the lateral mesoderm by the end of gastrulation, and is further reduced to anterior and posterior subsets of the lateral mesoderm by the onset of somitogenesis (Herbomel et al. 1999). During somitogenesis, *drl* expression becomes restricted to cardiovascular lineages (Mossiman et al. 2015). *Drl* expression becomes undetectable after 24 hours post-fertilization (24hpf) by mRNA in situ hybridization (Mosimann et al. 2015).

In *drl:creERT2;ubi:Switch* hybrids, activating cre-recombinase triggers all cells currently expressing *drl* to switch from producing EGFP to producing mCherry.
This irreversible genetic change allows for the lineage tracing of the cells that expressed \textit{drl} when \textit{cre}-recombinase was induced. If \textit{drl} is expressed pan-LPM when \textit{cre}-recombinase is induced, then the majority of LPM-derived cells are expected to be mCherry-positive. Importantly, there are practical issues with the \textit{drl}:\textit{creERT2} \textit{ubi:Switch} system that lead to incomplete expression of mCherry in LPM-derived tissues as well as scattered mCherry-positive muscle fibers and gut endoderm. These will be addressed in the Results and Discussion sections.

\textit{Preparation of vibratome and cryo-sections}

Tamoxifen-induced \textit{drl}:\textit{creERT2};\textit{ubi:Switch} hybrids were raised to embryonic, larval, and juvenile stages (24-96hpf, 4-12mm), anesthetized with Tricane (3-amino benzoic acid ethylester solution) and fixed in 4\% paraformaldehyde (g/ml). After repeated washes in PBS-Tween, fish were embedded for vibratome and cryostat sectioning. For vibratome embedding, fish were embedded in 4\% agarose (g/ml) dissolved in ddH\textsubscript{2}O, and embedded blocks were stored in PBS at 4\°C until sectioning (within 1 week). For cryostat embedding, fish were embedded in 1.5\% agarose, 5\% sucrose (g/ml) dissolved in PBS, and embedded blocks were stored in 30\% (g/ml) sucrose at 4\°C until the blocks stopped floating (within 1 day). Vibratome and cryostat blocks were sectioned into 200-300\(\mu\)m and 12 \(\mu\)m sections respectively. Vibratome sections were put through immunostainings within 1 day of sectioning. Cryostat sections were stored at -20\°C until immunostaining.
**Immunostaining of vibratome and cryostat sections**

In both vibratome and cryostat section immunostainings, primary antibodies: Living Colors anti-dsRed (1:600, Invitrogen), pax7 (1:10, DSHB), MF20 (1:20, DSHB), and flt4 (1:100, donated by N. Lawson, Lawson and Weinstein 2002), were used in conjunction with appropriate Alexa Fluor secondary antibodies: goat-anti-rabbit-546 (anti-dsRed), goat-anti-mouse IgG1-647 (pax7) and IgG2b-488 (MF20, all Life Technologies, 1:800), as well as Phalloidin-488 (1:100, Life Technologies) and DAPI for counterstaining.

**Vibratome section immunostaining** Sections were placed in net wells in a 12-well plate. All washes and incubations were at room temperature in at least 2ml of liquid to ensure submersion of all sections. Due to the high volume of solutions used, primary and secondary antibody solutions were able to be re-used many times with no decrease in labeling. The following washing and incubation steps were followed:

1. 15 minutes in blocking solution (1% bovine serum albumin and 5% normal goat serum in PBS)
2. 2 hours in primary antibody diluted in blocking solution
3. 2 rinses PBS-Tween, 3 10-minute washes in PBS-Tween
4. 15 minutes blocking solution
5. 2 hours in secondary antibody diluted in blocking solution (in the dark from this point on)
6. Repeat #3
7. 20 minutes in 1 μg/ml DAPI solution
8. Repeat #3
9. Mount with 25% glycerol in PBS

_Cryosection immunostaining_ Sections were stained on slides using a PAP pen to separate different liquid treatments. The following washing and incubation steps were followed:

1. Rehydrate sections in PBT for 1 minute
2. 2 minutes in blocking solution (1% bovine serum albumin and 5% normal goat serum in PBS)
3. 45 minutes in primary antibody diluted in blocking solution
4. 3 rinses PBS-Tween, 2x3 minutes PBS-Tween
5. 2 minutes blocking solution
6. 30 minutes in secondary antibody diluted in blocking solution (in the dark from this point on)
7. Repeat #4
8. 20 minutes in 1 μg/ml DAPI solution
9. Repeat #4
10. Mount with 25% glycerol in PBS
Results

To map the LSF, we must determine the degree to which LPM-derived tissue contributes to body wall musculature. mCherry expression served as our proxy for LPM-derived tissue, although there was not a one-to-one correspondence between mCherry expression and LPM (see below and Discussion).

We first experimented with activating cre recombinase at different embryonic stages. We subsequently tracked mCherry expression through zebrafish body wall development. Primary body wall formation occurs as the LPM extends ventrally around the yolk. This stage lasts the first three days of development. Secondary body wall formation occurs as the muscular body wall begins to form and extend ventrally around the trunk. This stage begins around 3 d and continues into juvenile stages. By 12 mm, the last stage we looked at, the juvenile body wall has all the muscles of the adult.

1) Activating cre-recombinase at High stage leads to the greatest percentage of mCherry-positive cells over yolk

We first experimented with activating cre-recombinase at different early embryonic stages. Our goal was to determine which time-point for activation resulted in the largest proportion of mCherry-expressing cells over the yolk, the location of the presumptive lateral plate mesoderm (PLPM). Drl:cre;ubi:switch hybrid embryos were dosed with Tamoxifen at high, shield, and 90% epiboly stages. The embryos were then raised to 3 d to examine the distribution of mCherry-positive cells. All fish
had significant mCherry expression in the heart and pelvic fin bud regardless of when Tamoxifen was added. MCherry expression in the PLPM varied depending on when Tamoxifen was added (Fig. 3 A and B).

We found the most reliable activation of mCherry expression in PLPM when cre recombinase was activated at high stage, prior to the onset of gastrulation (Fig. 3 A and B). The strongest drl expression is observed in the ventral and ventro-lateral hypoblast during early gastrulation (Herbomel et al. 1999). Activating cre-recombinase immediately before this drl expression may lead to the most reliable DNA recombination and consequent mCherry expression in drl-expressing cells. Activating cre-recombinase this early, however, leads to DNA recombination not only in the presumptive lateral mesoderm, but also in some endoderm.

Regardless of whether cre-recombinase was activated at high, shield, or 90% epiboly, scattered muscle fibers and vasculature were observed to express mCherry (see below and Discussion). As we are interested in identifying the LPM connective tissue lineage, it was important to have a way of verifying which mCherry-expressing cells were connective tissue. MCherry-expressing muscle fibers are readily distinguishable from connective tissue based on morphology. MCherry-expressing capillary vessels, however, are roughly the same size as fibroblasts and occur between and around muscle fibers. We therefore used the vascular antibody flt4 to identify mCherry-expressing cells of vascular origin (Fig. 4, see below).

The flt4 and anti-mCherry antibodies used in this study are both rabbit polyclonal and could therefore not be used on the same tissue section. This impedes
our ability to identify cells co-expressing flt4 and mCherry. Endogenous mCherry protein, however, retains some fluorescence in the tissue. To identify vessels co-expressing mCherry and flt4, we labeled adjacent sections with the two antibodies and looked for flt4 labeling and endogenous mCherry in one section that corresponded to mCherry labeling in the adjacent section. Vessels are more likely than connective tissue cells to be present in multiple tissue sections, and can therefore be identified as expressing both flt4 and mCherry by labeling adjacent sections. Connective tissue fibroblasts, however, cannot be positively identified with this method. Positive identification of mCherry-expressing connective tissue between muscle fibers will require additional experiments using non-rabbit polyclonal vascular antibodies.

2) Primary body wall formation: mCherry-expressing PLPM expands ventrally around the yolk

To quantify the expansion of the mCherry-expressing domain around the yolk, the angle of the leading edge of mCherry-expressing cells was measured in yolk ball and yolk tube sections from fish around 24, 48, and 72 hpf. For both the dorsal-most and ventral-most mCherry-positive cell in each section, the angle between the cell and the notochord was measured, with the vertex at the center of the yolk (Fig. 6A).

Over the first three days of development, the mCherry-expressing region expanded ventrally around the yolk. At around 24 hpf, mCherry-expression was exclusively found on the dorsal surface of the yolk ball, deep to the ectoderm (Fig. 5
A and B and Fig. 6B). Farther posterior, around the yolk tube, mCherry-expressing cells were nearly to the ventral midline at 24 hpf (Fig. 6C).

Over the next two days of development, mCherry-expressing cells expanded their domain around the yolk ball. At 48 hpf, mCherry-expressing cells were nearly to the ventral midline in the yolk ball, and by 72 hpf, they had reached the ventral midline (Fig. 6 D and F). The mCherry-expressing domain in the yolk tube did not expand as visibly in the yolk tube during this time, but by 72 hpf, mCherry expression was detected consistently at the ventral midline in the yolk tube (Fig. 6G).

The total number of mCherry-positive cells did not appear to change significantly during this time, the population instead growing sparser as it spread around the yolk. The dorsal-most mCherry positive cell in each section examined did not vary significantly in its position during the ventral expansion of the mCherry-positive domain, instead remaining adjacent to the myotome and coming into contact with the expanding body wall muscle at about 72hpf (Fig. 6F).

3) Secondary body wall formation: the somatopleure is retained at the level of the posterior hypaxial muscle but is displaced ventrally in myotome extensions

The muscular body wall in zebrafish begins to form around 3 d during the early larval period. The early body wall consists of two muscle groups: the posterior hypaxial muscle (PHM, Fig. 2A black arrowheads) and direct extensions from the myotome (Fig. 2B red arrowheads).
The PHM attaches to the cleithrum and becomes continuous with the trunk myotome posteriorly (Winterbottom 1973, Windner 2011). Its first muscle fibers appear at 40hpf just lateral to somite 5, and it attaches to the cleithrum by 96 hpf (Haines et al. 2004). Posterior to the PHM, the body wall is formed via direct extension of the dermomyotome (Devoto et al. 2006). We observed that at 4 d, the myotome had barely begun to extend ventrally around the body (Fig 2A, red arrowheads, Fig. 7E).

*mCherry expression in the body wall at 4 d*

In early larval fish, the PHM had extended significantly farther around the body wall than the myotome (compare Fig. 2A black and red arrowheads). The anterior PHM was separate from the myotome, but became continuous with the myotome posteriorly. During early PHM formation at 4 d, cells expressing mCherry were found along the interior and exterior surface of the PHM. The mCherry-expressing cells exterior to the PHM were in contact with the overlying ectoderm, forming the somatopleure (Fig. 7B-B”). Farther posterior, mCherry expression along the exterior PHM was reduced as the PHM became continuous with the extending myotome (Fig. 7 C-C” and D-D”).

In the posterior body wall at 4 d, myotome extension replaced the PHM as the only source of body wall formation (Fig. 7E). mCherry-expressing cells were only found along the interior surface of the extending myotome. DAPI staining and bright field images confirmed that cells are present at the ventral tip of the extending
myotome, between the ectoderm and mCherry-expressing PLPM (Fig. 7D” dotted line). These cells did not contribute to muscle fibers, as evidenced by absence of phalloidin staining, and based on their proximity to differentiated muscle were likely muscle progenitors. We therefore concluded that formation of muscle via myotome extension occurs between the ectoderm and PLPM, resulting in the loss of a somatopleure.

*mCherry expression in the body wall at 8 mm*

At mid larval stages (8 mm), the muscular body wall had extended much farther ventrally, yet it remained a thin layer with little integrity (Fig. 8A). mCherry expressing cells were seen surrounding the ventral tips of the extending myotome (Fig. 8B). mCherry-expressing cells were also seen in the ventral body wall, on the interior of the ectoderm, surrounding the gut, neural tube, and dorsal aorta, and in the pectoral fin and liver. Scattered mCherry expression was also found in some ectoderm dorsally, but these cells did not appear to be in contact with muscle. Based on their location in the dermis and round morphology, we speculate these cells are vascular in origin.

*mCherry expression in the body wall at 9 mm*

At late larval stages (9 mm), the muscular body wall had thickened, but the ventral-most portion of the body wall remained devoid of muscle (Fig. 2B, 9A). The trunk myotome extended over the superficial surface of the posterior PHM to form
the obliquus superioris and obliquus inferioris respectively (Windner et al. 2011). The PHM contributed to the anterior two sections of the obliquus inferioris while the trunk myotome contributes to the posterior obliquus inferioris and the entire obliquus superioris. The obliquus superioris and inferioris are distinguished in cross section based on the direction of their muscle fibers. The obliquus superioris runs anterior-dorsal to posterior-ventral and the obliquus inferioris runs anterior-ventral to posterior-dorsal, forming an X along the body (Windner et al. 2011).

At the level of the anterior PHM, mCherry-expressing cells were retained along the exterior surface of the PHM and pectoral fin muscle (Fig. 9B arrowheads and open arrowheads). mCherry-positive cells were found between muscle fibers of both, but at a much higher density in the fin (Fig. 9B). mCherry was detected surrounding fin radials and in fin rays, and mCherry-expressing chondrocytes were found within the fin radials (Fig. 9B). mCherry expression was also found in liver cells and in the vasculature of the head kidney (Fig. 9A).

Farther posterior along the PHM, the myotome had extended ventrally over the exterior surface of the PHM (Fig. 10A and B). mCherry-expressing PLPM cells remained in contact with the superficial surface of the PHM up to the dorsal-most point, despite now being overlain with trunk myotome (Fig. 10B white arrowheads). This further supports the hypothesis that the dermomyotome disrupts the somatopleure, as its extension over the PHM appears to have come between the ectoderm and the mCherry-expressing PLPM. There were virtually no mCherry-expressing cells between muscle fibers in the posterior PHM (Fig. 10B).
mCherry expression was observed in cells along the exterior of the myotome overlaying the PHM (Fig. 10B red arrowheads). This region, however, consistently expressed the vascular marker flt4 (Fig. 10C, D, and D’, red arrowheads). We therefore concluded that mCherry expression along the exterior of the myotome immediately ventral to the horizontal septum is by vasculature and not connective tissue. This conclusion is supported by the fact that draculin-expressing cells give rise to both vasculature and connective tissue lineages (Mosimann et al. 2015).

Posterior to the PHM, the body wall is comprised exclusively of direct extensions from the myotome (Fig. 11A). A boundary of mCherry expression was found along the ventral tips of the trunk myotome (Fig. 11A and B, white arrows). This boundary was only visualized as an abrupt end of mCherry expression, with no corresponding change in morphology. DAPI staining identified a one cell-thick layer of cells along the exterior of the muscle (Fig. 11D, white arrowheads). mCherry expression in this layer, however, was only found at the ventral tips of the muscle, with a definite boundary of expression, roughly 80% of the way ventrally along the body wall muscle (Fig. 11D, white arrowhead). mCherry expressing cells with a vascular morphology were found within the dorsal myotome, particularly in the highly vascular slow muscle stripes along the horizontal myosepta (Fig. 11C).

Throughout this study, we observed two morphologies of mCherry expressing cells found between muscle fibers: an asymmetric, globular shape and a round, donut shape (Fig. 4A). Based on these morphologies, we speculated that the asymmetric cells were connective tissue and the donut-shaped cells were vascular in origin, but
we did not yet have any gene expression data to support this. In order to identify whether or not the mCherry-expressing cells between muscle fibers were of vascular origin, we compared mCherry expression with flt4 expression, a gene expressed by veins and lymphatic vessels (Lawson and Weinstein 2002). As mentioned earlier in Results, both the flt4 and mCherry antibodies used in this study were rabbit polyclonal, so they could not be used to label the same section. Endogenous mCherry protein, however, retains some fluorescence. Endogenous mCherry cells were seen to co-localize with flt4 throughout the myotome, especially in the slow muscle (Fig. 10B, white arrowheads in Fig. 4C and D).

In the ventral tips of the myotome, cells with non-vascular morphology were visualized with the mCherry antibody that were not seen in endogenous mCherry in adjacent sections (Fig. 4D, white arrow). There was no flt4 expression in the same region in adjacent sections (Fig. 4C, open arrow). These mCherry-expressing, flt4-negative cells were found within the myosepta between adjacent myotomes (Fig. 4C and D, dotted line) and were verified to be cellular with DAPI (Fig. 4D, white arrow).

*mCherry expression in the ventral body wall in juvenile zebrafish*

Beginning in late larval fish (7 mm) in the posterior body wall, bilateral bundles of muscle fibers began to form ventral to the leading edge of the myotome (Fig. 2B white arrows). We hypothesized that these portions of muscle are the beginnings of the anterior infracarinalis muscles (Winterbottom 1973). The
infracarinalis anterior attaches to the antero-ventral pelvic girdle and becomes continuous with the anterior hypaxial trunk muscle (Winterbottom 1973).

We observed that the presumptive infracarinalis muscles first appeared as small bundles of myofibers separate from the trunk myotome, roughly 75% of the way around the body wall (Fig. 2B white arrows). As these muscles develop, they acquired more myofibers, take on a round shape in cross-section, and were found just lateral to the ventral midline, inserting on the pelvic girdle posteriorly (Fig. 2C and 12A-E).

By early juvenile stages in zebrafish, the anterior infracarinalis muscles were well defined (Fig. 2C white arrows, Fig. 12 white arrowheads). These muscles were surrounded by mCherry-expressing cells, and mCherry positive cells were found between muscle fibers (Fig. 12D and E). mCherry-expressing cells were also found surrounding the small bones medial to the pelvic fin (Fig. 12D and E). As in the 9 mm fish, a boundary of mCherry expression was maintained in the ventral tips of trunk myotome (Fig. 12A-C, white arrows).


**Discussion of Zebrafish-Specific Results**

In this study, we aim to translate what is happening at the level of individual cells in one species to large-scale comparisons of how tissues organize across many taxa. The LPM population in zebrafish is very sparse, making it difficult to accurately characterize how the population as a whole is behaving. Drawing from work in other taxa, we were able to predict much about the behavior of zebrafish LPM before beginning this project. We expected LPM to be found lateral to the somites and migrate ventrally early in development. Visualization of LPM cells, however, is still a major challenge.

The *drl:cre-ubi:switch* system for fate-mapping lateral plate tissues is advantageous as it allows us to track cells and their derivatives throughout a fish’s lifetime. The disadvantage, however, was the labeling of non-connective tissue *draculin*-expressing populations, namely the vasculature and scattered muscle fibers. In the first section below, we go into detail about the nuances of our fate-mapping system and how we interpret the data we collect from *drl:cre;ubi:switch* hybrids.

In the second section, “Cellular mechanisms that determine the LSF” we discuss some of the insights that working with the zebrafish model organism have led to regarding how cellular behaviors during development may influence the position of the LSF. Finally, we discuss future experiments to build off the findings from this project.
1) Cells expressing mCherry are majority, but not entirely, lateral plate mesoderm derivatives

As mentioned in the Results, we observe scattered muscle fibers expressing mCherry. These are most likely somitic in origin as only one post-cranial muscle has been identified to be of lateral plate origin: the tetrapod cucullaris (Theis et al. 2010). The cucullaris is thought to be homologous to the zebrafish protractor pectoralis, which has attachments on the skull and the anterior side of the cleithrum. The embryonic origins of this muscle in zebrafish are not yet known (Greenwood and Lauder 1981, Ericsson et al. 2013).

We speculate that the scattered mCherry-expression in trunk muscle fibers is a result of the poorly defined border between presomitic and lateral plate mesoderm during gastrulation. In zebrafish, bmp2b and bmb7 are expressed throughout the blastoderm, but are restricted to lateral regions along the entire body axis following gastrulation. Mutations in bmp2b or bmp7 lead to an overabundance of somitic tissue in the embryo (Kishimoto et al. 1997, Dick et al. 2000). Bmp4 is seen to have a similar lateralizing effect in amniotes (Tonegawa et al. 1997, Winslow et al. 2007).

Bmp-mediated specification of lateral mesoderm, however, occurs after draculin expression has already become restricted to a subset of presumptive lateral plate (Herbomel et al. 1999). In order to ensure full representation of LPM-derived cells, we must activate cre-recombinase before the LPM has been fully specified. This likely results in mCherry expression in some cells that go on to a somitic fate. The mCherry-expressing muscle fibers we observe are irregularly distributed in the trunk
myotome, and their distribution varies significantly depending on the individual, indicating they do not reliably activate in a specific portion of trunk muscle. Furthermore, another laboratory using the same transgenic line found that when cre-recombinase is activated at the one-somite stage, significantly fewer muscle fibers are induced to express mCherry (Gays et al. 2017). These observations point to an incomplete specification of presomitic and lateral mesoderm prior to the onset of somitogenesis.

In addition to some cells of non-LPM origin expressing mCherry, a portion of drl-expressing cells fail to be induced to express mCherry. This is evidenced by tissues of known LPM origin only partially expressing mCherry (Gays et al. 2017). In this study, we routinely observed LPM-derived structures with only partial mCherry expression, particularly in chondrocytes within LPM-derived skeletal elements such as the pectoral girdle (data not shown).

One reason for this is that Trans-4-OHT (Tamoxifen), used to trigger the import of cre recombinase to the nucleus, likely precipitates in solution (Felker et al. 2016). This leads to lower concentrations of available Tamoxifen in solution over time, which in turn affects how efficiently cre-recombinase is imported to the nucleus. In this study, we addressed this issue by increasing the concentration of Tamoxifen added to the embryo medium from 5 to 10μM, but another successful strategy is to use a different Tamoxifen metabolite, Endoxifen, which is more stable in solution (Felker et al. 2016). Even when using Endoxifen, however, cre-mediated DNA recombination is observed to be around 60-70% effective (Gays et al. 2017).
It is therefore clear that mCherry-expressing cells do not directly correspond to cells of LPM origin. There is, however, evidence that the majority of LPM-derived cells are induced to express mCherry. The mCherry-expressing cells not of LPM origin, endoderm, prechordal plate, and somitic muscle fibers, are readily distinguished from LP-derived cells based on morphology. The notable exception is the somitic connective tissue lineage. We cannot rule out the possibility that somitic connective tissue expresses mCherry at some low frequency.

For a number of reasons, however, we expect the high majority of mCherry-expressing cells of connective tissue morphology are LP-derived. First of all, mCherry is expressed in a very small proportion of muscle fibers in the myotome, indicating that a similarly small proportion of somite-derived connective tissue would erroneously express mCherry (see Fig. 3B). Secondly, mCherry expression was never observed in somite-derived skeletal elements. Thirdly, the dense irregular connective tissue surrounding and within muscle is a sparse population. Because there is a potential for somitic connective tissue to express mCherry, however, we only conclude a tissue is of LPM origin when the majority of its cells express mCherry.

2) *Cellular Mechanisms that determine the LSF*

The tissue-level phenomenon of the LSF is the result of underlying cellular mechanisms. Why, at some seemingly arbitrary point, the somatopleure begins to persist along the body wall is determined by cellular mechanisms not yet elucidated. The zebrafish is an excellent model organism for investigating these cellular
mechanisms due to their short development time, transparency, and ability to accommodate genetic manipulation. In mapping the LSF during zebrafish body wall development, we observed behaviors of somitic and lateral plate cells that provide insights into mechanisms that may function in determining the LSF. Drawing on research from other labs, we present below some hypotheses for how lateral plate and somitic cells might be organizing during body wall development, and how the LSF may emerge from these underlying cellular interactions.

*Somatic LPM expansion around the yolk*

The somatic LPM comes into contact with the advancing secondary body wall, potentially contributing connective tissue to body wall muscle. As we are interested in to what extent LPM contributes to the zebrafish body wall muscle, it was of interest to determine how the somatic LPM population is established around the yolk.

In amniotes, mesoderm forms via ingression at the primitive streak and disperses laterally. Paraxial, lateral, or extraembryonic fate is determined by the distance from the primitive streak that mesoderm cells travel. The lateral body folds that lift the embryo off of the yolk form the primary body wall. The joining of the body folds at a ventral seam establishes the somatopleure and splanchnopleure, the coelom within the body, and the yolk sac external to it (Sadler et al. 2008).

In zebrafish embryos, who ultimately incorporate their yolk into the body cavity, the separation of somatic and splanchnic layers of LPM is not well described.
Following gastrulation, the LPM is established as bilateral stripes adjacent to the body axis. The LPM then separates into splanchnic and somatic layers as cells either migrate medially towards the developing gut (splanchnic) or ventrolaterally around the yolk (somatic) (Kaneko et al. 2014, Gays et al. 2017).

We observed that mCherry-positive presumptive somatic LPM reached the ventral midline earlier in the yolk tube than the yolk ball. The simplest explanation for this difference in timing is that cells travel a shorter distance to reach the ventral midline in the yolk tube versus the yolk ball, due to the yolk tube’s smaller diameter. Additionally, cell displacement (total distance traveled by each cell) has been found to be higher in posterior LPM, regardless of yolk shape. Fate-mapping experiments in zebrafish using DiI injection determined that LPM cells in the yolk tube are displaced further anterior than LPM cells in the yolk ball (Murata et al. 2010). A follow-up study found that a similar anterior displacement of the posterior-most LPM occurs in medaka, who lack a yolk tube. The authors hypothesize that anterior vectorial movement of LPM cells over the yolk was important for the anterior shift in pelvic fin position amongst the more derived teleosts (Kaneko et al. 2014).

Throughout the expansion of the mCherry-expressing presumptive LPM around the yolk, the dorsal-most cells of the presumptive LPM remained directly adjacent to the extending dermomyotome. This indicates that rather than the population moving a certain distance as a wave, the dorsal-most cells move the least, resulting in an expansion of the surface area occupied by LPM. The LPM appears to become sparser as the cells begin to occupy a larger percentage of surface area on the
yolk. Time-lapse videos depict hand2-expressing presumptive LPM moving ventrally between 24 and 72hpf with only a few hand2-expressing cells visibly proliferating during this time (time-lapse videos courtesy of Mosimann, Yin et al. 2010). This suggests that LPM is dispersing around the yolk rather than expanding via proliferation. A future experiment could investigate whether or not LPM cells are proliferating around the yolk by using a zebrafish cell cycle reporter line (Sakaue-Sawano et al. 2008, Sugiyama et al. 2009).

Although the LPM does not appear to be expanding via proliferation, the movement of presumptive LPM cells does not resemble that of non-proliferative, actively migrating populations, such as the neural crest. In time-lapse videos, hand2-positive presumptive LPM cells coordinate their movement with their neighbors, as if they are all part of the same fabric being stretched around the yolk (Gays et al. 2017). This contrasts the behavior of migrating neural crest cells, where each cell’s direction vector at any given time is independent of its neighbors (Matthews et al. 2008, supplementary movie 1). This suggests some extracellular factor is coordinating the expansion of the LPM.

The coordinated, fabric-like movement of the LPM is reminiscent of dorsal closure in Drosophila, where apoptosis of amnioserosa cells generates tension and shape changes in surrounding cells, facilitating the “zipping” of the dorsal seam (Toyama et al. 2008). Indeed, in chicken embryos, apoptosis along the lateral body wall occurs during body wall formation (Hirata and Hall 2000). A similar event could occur in zebrafish, where apoptosis of ectoderm around the ventral yolk generates...
tension to stretch the domain occupied by LPM ventrally, but further experiments are necessary to explore this. Alternatively, a similar epithelial-like movement of LPM is observed during the migration of intestinal smooth muscle cell progenitors towards the gut tube, and is hypothesized to be the result of a partial epithelial to mesenchymal transition induced by TGF-β signaling (Gays et al. 2017).

Partial epithelial-to-mesenchymal transition in the ventral body wall may result in a persistent somatopleure

Data from zebrafish and lamprey indicate that muscle formation via dermomyotome extension disrupts the connection between the ectoderm and LPM. As long as the dermomyotome remains closely associated with the ectoderm, new muscle fibers are added between the LPM and ectoderm (see Fig. 7D” in Results). In the zebrafish body wall, however, the dermomyotome eventually loses its association with the ectoderm, and LPM persists alongside the ventral body wall muscle.

We do not yet know what causes the dermomyotome to lose contact with the ectoderm and begin extending deep to the LPM. This behavior may be intrinsic to the extending dermomyotome, or it may be coordinated by the LPM or the ectoderm.

I hypothesize that a partial epithelial-to-mesenchymal transition (EMT) of the dermomyotome occurs as the dermomyotome begins to extend deep to the LPM. Partial EMT is characterized by an epithelium retaining its sheet-like organization yet adopting mesenchymal behaviors of migration and ECM remodeling (Gays et al. 2017).
Gays et al. observed what appears to be partial EMT in the splanchnic LPM during gut looping in zebrafish. For normal gut looping, bilateral portions of splanchnic LPM converge on and surround the gut tube, giving rise to intestinal smooth muscle cells. Gays et al. found this process to be dependent on TGF-β signaling. When TGF-β signaling was disrupted, splanchnic LPM failed to surround the gut tube, instead remaining as sheets of cells on either side of the gut tube (Gays et al. 2017). This organized phenotype may be the splanchnic LPM failing to undergo the partial EMT required for interaction with the gut tube and form the splanchnopleure and a functioning gut.

Partial EMT may be required for ventral body wall integrity, as the epithelia from both sides of the body converge on the ventral midline. Partial EMT may allow muscle progenitors to interact with their surroundings and remodel their ECM, allowing the body wall to “knit together” at a ventral seam. In the posterior body wall, this behavior may be further co-opted for the dissociation of muscle progenitors that give rise to the infracarinalis muscles.

A persistent somatopleure may be a result of partial EMT in the ventral body wall. Epithelial dermomyotome forming the ventral body wall may lose their connection to the ectoderm as a result of partial loss of epithelial organization and begin extending deep to the somatopleure. LPM may also play a role in inducing partial EMT in body wall epithelia. Future experiments may determine whether TGF-β signaling plays a role in this process.
Unlike fin MMPs, PHM progenitors retain epithelial characteristics

In this study, we observed the anterior PHM to retain a somatopleure throughout its development. This LPM investment was reduced in the posterior PHM, as the muscle became continuous with the primaxial trunk myotome. This is in contrast to Windner et al., who predicted the entire PHM to be abaxial (Windner et al. 2011). This prediction was based on PHM progenitors displaying molecular characteristics of MMPs that form abaxial pectoral fin muscles: expressing the MMP gene *lbx1* and forming the first muscle fibers at some distance to the trunk myotome.

Unlike the MMPs of pectoral fin muscles, however, PHM progenitors do not undergo a significant migration before differentiation into muscle. PHM progenitors differentiate into mature muscle fibers as the muscle continues to extend towards the cleithrum (Haines et al. 2004, Windner et al. 2011). This *in situ* maturation of muscle fibers during tissue elongation is also seen during muscularization of the trunk via the dermomyotome (Stellabotte and Devoto 2007). Additionally, time lapse imaging of the extending PHM has identified what appear to be alpha-actin-rich filopodia at the tip of the muscle extending towards the cleithrum (Haines et al. 2004).

Taken together, these data indicate an epithelial-like behavior of the extending PHM, distinct from what occurs in both the trunk and appendicular muscle formation. This may account for the partial LPM investment observed in this study. Initial de-epithelialization from the dermomyotome may disrupt the PHM’s association with the ectoderm, resulting in a persistent somatopleure in the anterior portion. Continued development of the muscle, however, may progress via a sheet-like expansion of
PHM progenitors towards the cleithrum. The posterior portion of the PHM, being the portion most closely associated with the trunk myotome, would lack the LPM investment seen in the anterior PHM. Further research into the mechanisms of PHM formation is required to verify this model.

3) Future Directions

Mapping the frontier in less derived teleosts and chondrichthyes

Zebrasfish are a relatively derived branch of ray-finned fish, having diverged relatively recently in evolutionary time. It is therefore important to understand how representative their body wall is of ray-finned fish in general. To accomplish this, data on the LSF from a more basal ray-finned fish can be compared to the LSF of zebrafish.

The paddlefish, Polyodon spathula, is one of the most basal extant ray-finned fish, and a possible candidate for LSF studies. As a non-model organism, however, lineage tracing experiments are significantly more difficult. Combining gene expression data in paddlefish, such as expression of lbx1, expressed by MMPs, and pax7, expressed by the dermomyotome, with DiI injection fate-mapping may provide enough information to confirm or refute similarity to the LSF in zebrafish.

In addition to investigating the LSF in other ray-finned fish, we can compare the LSF in ray-finned fish to the LSF of cartilaginous fish. Initial experiments have revealed a persistent somatopleure as part of the catshark body wall. Whether or not
an LPM investment within body wall muscles is present in shark remains to be seen. It will be interesting to learn if the LSF in shark resembles that of zebrafish, and if not, if differences in the shark LSF correspond to differences in body wall musculature.

*Manipulating the LSF in zebrafish*

Upon completing our map or the LSF in wild type zebrafish, we can experiment with how changes in gene expression and gene activity may affect the LSF. The zebrafish is an excellent model organism for gene expression manipulation, with many transgenic lines available. In particular, we are interested in manipulating the degree to which LPM contributes to somite-derived muscle and/or the persistence of a somatopleure. We can accomplish this by crossing our drl:cre;ubi:switch line with other transgenic fish lines that exhibit altered gene expression on one side of the LSF. This will allow us to compare distribution of LPM tissues in fish with altered somitic or LPM gene expression to fish with wild type gene expression.

On the somite side, we are interested in whether ectopic de-epithelialization of trunk somites might result in ectopic LPM-invested muscles. *C-met* and *lbx1* are both expressed in fin-level somites and are involved in the de-epithelialization and delayed maturation of MMPs respectively (Dietrich et al. 1999, Gross et al. 2000). Unfortunately, transgenic lines for inducing ectopic *c-met* expression have not yet been constructed. *C-met* mRNA injections in zebrafish embryos may provide us with preliminary information about phenotypic effects of *c-met* overexpression. A heat-
shock promoted \textit{lbx1/EGFP} line has been constructed (Guo et al. 2016). Using this line, we can experiment with timed and local overexpression of \textit{lbx1} using both whole embryo heat-shock activation and laser irradiation (Shoji and Sato-Maeda et al. 2008). Following \textit{lbx1} overexpression, we can investigate any defects present in muscle and any effects on LPM contribution to muscle.

Also on the somitic side, we are interested in investigating our hypothesis that partial de-epithelialization of the dermomyotome results in a persistent somatopleure. This would constitute a novel mode of muscle formation, distinct from both dermomyotome extension and MMP migration into the fins. TGF-\(\beta\)/Alk5 signaling is believed to induce partial de-epithelialization of splanchnic LPM, required for gut looping (Gays et al. 2017). It would be interesting to learn if similar signaling occurs in the ventral tips of the dermomyotome and/or PHM. If we find that partial de-epithelialization of the ventral myotome and/or PHM does occur, then further experiments may involve prematurely inducing or delaying the partial de-epithelialization to determine if there is an effect on the persistence of the somatopleure.

On the lateral plate side, we are interested in the relationship between pectoral fin bud formation and the persistent somatopleure. It has been hypothesized that a persistent somatopleure in the body wall preceded the thickening of the LPM at fin levels and formation of a fin bud (Tulenko et al. 2013). We are therefore interested in determining if fin bud formation is independent of somatopleure persistence. To test this, we will examine the LSF in zebrafish mutants with a finless phenotype. There
are several zebrafish lines with mutations in lateral plate genes resulting in reduced pectoral fin bud and absence of the pectoral fin (Yelon et al. 2000, Garrity et al. 2002, Fischer et al. 2003). We will investigate whether in these mutants the somatopleure is eliminated at fin level or if it persists as part of the finless body wall. We will also investigate whether these mutations affect LPM progression in the body wall posterior to the pectoral fin.
Discussion of Evolutionary Significance

Comparing the LSF between vertebrate lineages

Vertebrates represent a staggering amount of diversity, particularly in terms of their locomotor adaptations. Surprisingly, embryological studies have revealed their development to be highly conserved between taxa. This has led us to ask: how much developmental diversity is represented in the morphological diversity of vertebrates? Towards this question, we are interested in how cryptic changes to the LSF between taxa correlate with overt morphological changes.

To best understand how the LSF may have changed over evolutionary time, the Burke lab compares the position of the LSF across different vertebrate lineages. We hypothesize that changes to either the primaxial or abaxial domain frequently underlie major morphological changes, requiring only a plastic developmental response in the other domain.

Thus far the position of the LSF has been mapped in the lamprey, axolotl, chick, and mouse, and estimated in the shark. These species represent major phylogenetic branches in the vertebrate tree: the lamprey, a basal jawless vertebrate; the shark, a cartilaginous fish; the axolotl, an amphibious tetrapod; the mouse, a fully terrestrial tetrapod; and the chick, an avian tetrapod. The most specious branch of the vertebrates, the ray-finned fish, are notably absent from this list. The present study adds to our understanding of the LSF across vertebrates with data from the ray-finned fish, the zebrafish. Below, we summarize results from other vertebrate lineages and compare them to our findings in the zebrafish.
In the lamprey, the body wall forms entirely through extensions of the myotome. Migratory muscle precursors are only observed for formation of the hypobranchial muscle, homologous to the gnathostome tongue (Kusakabe and Kuratani 2005, Kusakabe et al. 2011). During body wall formation, the somatopleure is eliminated, and cells of the lateral plate mesoderm are displaced to the median fin and the interior of the muscular body wall (Tulenko et al. 2013). Thus in the basal limbless vertebrate, the lamprey, there is no lateral plate contribution to the muscular body wall and therefore no abaxial domain.

Data from the shark indicate that a persistent somatopleure is present in the catshark body wall (Tulenko et al. 2013). Initially, the extending dermomyotome maintains its connection with the ectoderm. At later stages, the dermomyotome loses contact with the ectoderm and extends within LPM to form the ventral body wall that includes a persistent somatopleure. Positive identification of lateral plate cells will be required for determining whether lateral plate connective tissue is found within the shark body wall muscles or only in the somatopleure adjacent to the body wall.

Until recently the shark was thought to form both fin and body wall muscle through direct dermomyotome extensions (Nyet et al. 2000). A recent study, however, found lbx1/pax3 expression, typical of migratory muscle precursors, in the pectoral fins as well as the hypobranchial muscle of the catshark (Okamoto et al., in press). Additionally, muscle precursors were seen to de-epithelialize prior to entering the fin bud, whereas in the body wall, direct dermomyotome extension was observed.
The tetrapods all have extensive trunk and abdominal musculature, important for maintaining body wall integrity in a terrestrial environment (Liem et al. 2001). These abdominal muscles are largely abaxial, where lateral plate connective tissue is found surrounding muscles as well as between muscle fibers in those species mapped (Tulenko et al. 2013, Nowicki et al. 2003, Durland et al. 2008). Some abdominal muscles, however, have only a partial investment in LPM. In the mouse latisimus dorsi, for example, the ventro-lateral portion of the muscle is abaxial and LPM investment ends midway towards the muscle’s attachment to the spine (Durland et al. 2008).

In the chick and mouse, the thoracic somites that give rise to the ribs and intercostals extend and deflect the LSF to the sternum. The intercostals remain primaxial until the ventral tips, which are invested in LPM. The somatopleure in these tetrapods is maintained quite dorsally on the body wall and ultimately hosts the abaxial abdominal muscles that zebrafish lack. Deflection of the LSF by the ribs and intercostals is seen to a much lesser extent in the axolotl, as ribs do not extend ventrally in the derived salamanders. Consequently, the LSF in the salamander is uniformly dorsal and un-deflected on the body wall.

The behavior of primaxial ribs and intercostals in these tetrapods are in direct contrast to the abaxial abdominal muscles. These are fully invested in LPM and attach to the abaxial sternum. Additional research into the mechanisms of abdominal muscle formation is needed to determine if the abaxial abdominal muscles in tetrapods form
via migratory muscle precursors, as in the limbs, myotomal extension, as in the lamprey and shark body wall, or a novel mechanism.

The musculoskeletal system of ray-finned fish possesses both basal and derived characteristics. Ray-finned fish have paired pectoral and pelvic fins homologous to tetrapod fore and hindlimbs. The ray-finned body wall is formed through myotome extension, as is seen in the lamprey, whose body wall is entirely primaxial. It was therefore of interest to determine whether the zebrafish body wall more closely resembled that of the lamprey or tetrapods in terms of investment in LPM.

We map the LSF based on the boundary of the mCherry-positive PLPM in body wall muscle. The position of the LSF differs in the three muscles of the zebrafish interlimb region: the PHM, trunk myotome, and anterior infracarinalis (summarized in Figure 13).

The anterior PHM, which attaches to the abaxial cleithrum, has a persistent somatopleure superficially and LPM connective tissue investing muscle fibers. The posterior PHM progressively loses this LPM contribution as it becomes continuous with the trunk myotome. Thus, in the PHM, the LSF bisects the muscle. This is similar to what is seen in the mouse latissimus dorsi, indicating that the PHM and latissimus dorsi are formed via a similar mechanism. Both muscles extend dorso-
laterally from the trunk to form attachments to components of the abaxial pectoral girdle, which results in the incorporation of LPM connective tissue.

Although we observed mCherry-positive PLPM between muscle fibers of the PHM, these cells are not at the same density observed in tetrapod abaxial muscles. The axolotl abaxial muscles serve as the best comparison, as Tulenko et al. used a similar technique as this study to visualize PLPM: the expression of a fluorescent protein. The density of PLPM in abaxial body wall muscles in axolotl is more similar to the mCherry-expressing PLPM density in the zebrafish pectoral fin (Tulenko et al. 2013, Fig. 9B). In both the axolotl body wall and zebrafish pectoral fin, the muscle appears to be marbled with PLPM. In the zebrafish body wall, in contrast, PLPM cells are irregularly distributed and appear as isolated cells rather than marbled throughout the muscle. This suggests that the mCherry-positive PLPM we observe in the zebrafish body wall is not as significant a population, and somitic connective tissue is also likely present within this muscle. Thus we interpret the more significant LPM contribution to the PHM to be the persistent somatopleure alongside the muscle.

In the trunk myotomes, the LSF runs through the ventral tips of the muscle, roughly 70% of the way ventrally. During trunk muscle formation, the extending dermomyotome encounters LPM early. This appears to be displaced (and the somatopleure eliminated) in advance of the extending dermomyotome. In all other species studied thus far, the somatopleure either persists much higher on the body wall (tetrapods) or is eliminated completely (lamprey). The somatopleure in sharks also begins to persist farther dorsally on the body wall than zebrafish, although data
from later stages in the shark may reveal the somatopleure to be displaced farther ventrally over developmental time. The more ventral persistent somatopleure in zebrafish may represent the basal condition that was expanded upon in the tetrapod lineages. Alternatively, the somatopleure may have originally persisted much farther dorsally on the body wall. The lower demand in the ray-finned fishes for extensive abdominal musculature may have depreciated the somatopleure to a smaller and smaller portion of the body wall over time.

The anterior infracarinalis muscles are continuous with the trunk myotome anteriorly and attach to the anterio-ventral pelvic girdle posteriorly. These muscles are not found in the lamprey body wall. Instead, in the lamprey, the dermomyotome extends uninterrupted to the ventral midline. Concurrent with this difference in body wall muscles between zebrafish and lamprey is the difference in LPM persistence in their ventral body walls. This has led to our hypothesis that an LPM population in the ventral body wall may be important for the formation of new muscle groups, an alternative to direct dermomyotome extension.

*Evolutionary origins of the abaxial domain*

The LSF in the vertebrate body wall delineates a cryptic change in the embryonic origin of body wall connective tissue. This change may have had no fitness benefits or consequences for the lineages who acquired it, yet it may have been a necessary first step for the evolution of the abaxial patterning domain. LPM
investment in body wall muscle may have occurred in a limbless ancestor of the gnathostomes (Tulenko et al. 2013).

In support of an LPM investment in body wall muscle being a common characteristic of the gnathostomes, muscularized fossils of the most basal known gnathostome group, the placoderms, reveal surprising abdominal musculature (Trinajstic et al. 2013). These fossils indicate that bilateral abdominal muscles, with fibers that run transverse along the ventral body wall were a basal condition in placoderms. These muscles are thought to be important in modulating shearing forces in the ventral skeletal armor and, judging from the LPM in the body walls of extant vertebrates, likely interacted with LPM tissue.

In descending lineages, a persistent somatopleure in the body wall would have provided a novel connective tissue environment to be invaded by muscle progenitors, leading to the development of abaxial paired appendages. In tetrapod lineages, who spend time on land, the abaxial domain expanded to include a sternum and extensive abdominal muscles.

Why are vertebrates so successful at musculoskeletal innovation? In evolution, as with many endeavors, success is the occasional result of trying different strategies. This experimentation, however, can be more than simply throwing things against a wall and seeing what sticks. If a novel tissue interaction during development were to evolve that allowed for more rapid divergence of new, viable body plans, then creatures utilizing that strategy would be overrepresented in the extant species.
Analogous to how an evolutionarily fit individual produces more offspring, a versatile developmental behavior may lead to the emergence of more new species.

Modularity in vertebrate development is one such behavior that has facilitated the rapid divergence of new species. We argue that the integration of axial and appendicular elements is a result of two independent patterning environments, the primaxial and abaxial domains. The ability to differentially modify the primaxial or abaxial domain without significantly affecting development in the other domain would increase the likelihood of generating viable and adaptive new forms.

Towards this hypothesis, we have observed a significant expansion of the abaxial domain to coincide with a rapid diversification of locomotory structures in the tetrapods (Figure 14). This same expansion is not present in the zebrafish, although LPM persists as part of its body wall. This paints the picture of the LSF in the ray-finned fish body wall (presumed to be the basal condition to what is seen in tetrapods) as a “foot-in-the-door” innovation. This is to say that the persistence of any amount of somatopleure was an important prerequisite for future innovation, without necessarily conferring any functional advantage initially. This basal abaxial domain may have subsequently expanded into a fully-fledged abaxial domain in the descending tetrapod lineages.
Conclusion

We have found a distinct LPM contribution to the zebrafish body wall, which indicates the position of the LSF. This LPM contribution is not seen in lamprey, despite the similarities of lamprey and teleost body wall closure via dermomyotome extension. The LSF in zebrafish, however, also appears distinct from the LSF of tetrapods. Unlike tetrapods, there are no fully abaxial muscles in the zebrafish body wall, and the persistent somatopleure begins roughly 70% of the way ventrally around the body. In tetrapods, the persistent somatopleure begins much farther dorsally on the body wall, and abdominal rectus and obliques are fully abaxial body wall muscles. These muscles in tetrapods attach to abaxial bones such as the sternum and pelvic girdle. In zebrafish, the anterior infracarinalis attaches to the pelvic girdle, but becomes continuous with the trunk myotome anteriorly, as teleosts do not have a sternum.

The LSF in the zebrafish body wall represents an intermediate between the lamprey and tetrapods. LPM invests zebrafish body wall muscle, but does not align with any morphological boundary. This led us to hypothesize that changes in cellular dynamics, namely partial de-epithelialization of the dermomyotome, during body wall formation occurred prior to the divergence of teleosts. De-epithelialization may have allowed for the persistence of LPM in body wall muscle.

We hypothesize that LPM presence in body wall muscle preceded the formation of a true abaxial domain. The LPM population in body wall muscle subsequently expanded to include skeletal elements, such as the sternum and
appendicular skeleton, and migratory muscle progenitors infiltrated the LPM to form fully abaxial muscles.

To investigate this hypothesis, we must first verify that the LSF in zebrafish appears representative of teleosts and possibly of all fish with paired appendages. We must also investigate whether perturbing the cellular dynamics involved in body wall formation can affect the degree to which LPM incorporates into body wall muscle.
Figure 1. The lateral somitic frontier. Cross-sections through a chicken embryo at early (A) and late (B and C) embryonic stages. (A) Early in development, the LPM is directly lateral to the somites, and their interface is the “incipient frontier.” (B) Later in development, at limb levels, cells from the somites cross the LSF as individual migrating muscle precursors to differentiate into limb muscle in an LPM connective tissue environment. (C) At the same time, in the trunk, somite cells remain as an epithelium and displace the LSF to differentiate into trunk muscles and ribs in a somitic connective tissue environment. The exception may be at the leading edge of the somitic epithelium, which may associate with LPM tissue. Modified from Shearman and Burke 2009.
Figure 2. Muscular body wall development in zebrafish. (A) At 4 d, the PHM (black arrowheads) has extended farther ventrally around the body than direct extension of the myotome (red arrowheads). (B) By 7 mm, the PHM is continuous with the myotome and relatively indistinguishable in whole mount. The rectus abdominalis/presumptive anterior infracarinalis muscles have begun to form ventral to the leading edge of the posterior myotome (arrows). (C) By 11 mm, the muscular body wall has nearly reached the ventral midline. The anterior infracarinalis muscles are more prominent and farther ventrally in the body wall (arrows). All scale bars 500μm. Dotted lines outline the pectoral fin. Images in B and C are used with permission, S. Devoto.
Figure 3. Tamoxifen addition at High results in the highest proportion of mCherry-expressing cells over the yolk. (A) Confocal z-stacks of live 3 d fish with Tamoxifen added at the indicated embryonic stage. (B) Tracings of the zstacks in A. GFP and mCherry-expressing cells over the yolk and in the pectoral fin bud (dotted black line) are colored green and red respectively. MCherry-expressing muscle fibers and myotome borders are also indicated. Scale bars are 200μm.
Figure 4. Differentiating between vascular and connective tissue using the flt4 vascular antibody. (A) Two morphologies of mCherry-expressing cells were observed associated with muscle. Based on these morphologies, we assume one to be connective tissue and one to be vasculature. (B-D) To verify our assumption in A, we assay for flt4 expression. (B) In the slow muscle and trunk myotome, flt4 staining labels all cells expressing mCherry in addition to some non-mCherry-expressing vasculature. (C, D) Adjacent sections through 9.1 mm fish, the first stained for flt4 and the second stained for mCherry (both antibodies are rabbit polyclonal). (C) Flt4 and DAPI staining identifies a blood vessel, which also has endogenous mCherry expression (arrowheads). (D) In an adjacent section, the same vessel is identified in mCherry and DAPI, but an additional mCherry-expressing cell is identified (white arrow), which is not visible in flt4 staining in the adjacent section (open arrowhead in C). This cell is found within the myoseptum (dotted line).
Figure 5. MCherry expression over the yolk at 24 hpf. (A) Confocal z-stack of a live 24 hpf *drl:cre;ubi:switch* embryo, anterior to the left, dorsal to the top. MCherry expression is seen over the dorsal surface of the yolk with a definite boundary of expression (arrowheads). Scattered muscle fibers in the myotomes also express mCherry. (B) Transverse section through the same z-stack. MCherry expression is lateral to the myotome and deep to the ectoderm, with a definite boundary of expression (arrowheads). NT = neural tube. MYT = myotome.
Figure 6. Expansion of MCherry-expressing region around the yolk between 24 hpf and 72 hpf. (A) Ventral-most and dorsal-most mCherry-expressing cells in each section were measured based on their angle of incidence to the line between the notochord and center of yolk. Arrowheads indicate the location of each mCherry-expressing cell. (B-G) Average ventral-most angle reached at 24, 48, and 72 hpf in the yolk ball and yolk tube with standard error. Dorsal-most angle did not differ significantly from directly adjacent to the myotome.
**Figure 7.** (previous page) The somatopleure persists in the PHM but not the trunk myotome at 4 d. (A) Body wall muscle at 4 d as evidenced by myosin heavy chain staining. (B-E) Transverse sections through regions indicated in A. (B’-E’) magnifications of boxed regions in B-E, with or without DAPI. (B”-E”) Further magnification of boxed regions in B’-E’. (B-B”) Section at the level of the anterior PHM. mCherry-expressing cells are found all along the superior surface of the PHM (arrowheads). (B”) mCherry-expressing cells are deep to a DAPI-positive presumptive ectoderm. (C-C”) Section through mid-PHM. Weak mCherry expression is seen along the superior surface of the PHM (arrowheads). (D-D”) Section through posterior PHM. mCherry expression is only seen along the interior surface of the PHM. (E-E”) Section through trunk myotome. mCherry expression is restricted to interior of muscle. (E”) Three DAPI-positive, phalloidin-negative cells are seen at the leading edge of the myotome, between the mCherry expressing cells and the presumptive ectoderm (dotted line).

![Image of Figure 7](image)

**Figure 8.** MCherry expression in the extending myotome at 8 mm. (A) Transverse section through trunk myotome. MCherry-expressing cells are associated with ventral body wall, surrounding the ventral tip of the extending myotome (magnified in B). MCherry expression is also found in the pectoral fins, surrounding the gut, neural tube, and dorsal aorta, in the liver, and scattered in the ectoderm.

![Image of Figure 8](image)
**Figure 9.** MCherry expression in anterior PHM and pectoral fin at 9.3 mm. (A) Transverse section at the level of the anterior PHM. (B) Magnification of boxed region in A. MCherry expression is seen along the exterior of the PHM (white arrowheads) and pectoral fin (open arrowheads). MCherry expression between muscle fibers is stronger in the pectoral fin than the PHM. MCherry expression is also seen in the fin radials, the liver, and the head kidney.
**Figure 10.** MCherry expression in posterior PHM at 9.6 mm. (A) transverse section at the level of the posterior PHM. Here, the trunk myotome has partially extended over the exterior of the PHM. (B) magnification of boxed region in A, with and without phalloidin. MCherry-expressing cells are associated with the exterior surface of the PHM (white arrowheads). MCherry expression is also seen along the exterior of the trunk myotome (red arrowheads), but this region is also labeled by the vascular marker flt4 (red arrowheads in C, D, and D').
**Figure 11.** MCherry expression in trunk myotome at 9 mm. (A) Transverse section at the level of the trunk myotome. (B, C) Magnifications of boxed regions in A, with and without phalloidin. MCherry-expressing cells are found along the ventral tips of the myotome continuously until a cryptic boundary (arrows in A and B). (C) In the slow muscle, significant mCherry expression is seen between muscle fibers. (D) Transverse section through a 9.6 mm fish with DAPI. At the ventral tip of the myotome, a thin mCherry and DAPI-positive layer of cells is visible along the
(Fig. 11 continued) exterior of the muscle. MCherry expression in this population ends ventrally, with no corresponding change in phalloidin or DAPI staining (arrowhead).

**Figure 12.** MCherry expression at 12.5 mm. (A) Transverse 200μm vibratome section at the level of the pelvic girdle. The anterior infracarinalis muscles just lateral to the ventral midline and pelvic girdle (“infr.”, arrowheads). In the ventral body wall, mCherry expression is strong, with a definite boundary of expression along the exterior of the trunk myotome (arrows). (B) Magnification of ventral body wall in A, with and without phalloidin. MCherry-expressing cells surround the ventral tips of the myotome and the infracarinalis, and are also found between muscle fibers in these muscles. MCherry-expressing cells are also found surrounding and within the bones of the pelvic girdle. Scale bars are 500μm in A and 100μm in B.
Figure 13. Summary of our findings on zebrafish body wall development. Body wall development is first visible at 48 hpf, when the first migratory muscle precursors delaminate from the somites to form the PHM. At 4 d, the PHMs flank the embryo, and the anterior portions of these muscles are abaxial. During larval development (6-9 mm), the somitic myotome extends over top of the PHM. In the juvenile (12 mm), the PHM and the myotome are only distinguishable in the anterior-most body wall. The ventral tips of the myotome are abaxial, and the abaxial infracarinalis muscles have formed on either side of the ventral midline (lower 12 mm image).
Figure 14. The abaxial domain has expanded in limbed vertebrates. Phylogeny spanning five vertebrate groups: agnathans, chondrycthes, teleosts, amphibians, and mammals with what is known about the abaxial domain in the species studied from each group, in cross-section and whole mount. All but the lamprey have an abaxial domain in the body wall, but this domain has expanded dorsally in the tetrapods axolotl and mouse. The cross-section of the catshark is grayed-out as more data is needed to determine the exact dorso-ventral position of the LSF in this species. Modified from Tulenko et al. 2013.
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