Development of the amniote ventrolateral body wall

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Abstract
In vertebrates, the trunk consists of the musculoskeletal structures of the back and the ventrolateral body wall, which together enclose the internal organs of the circulatory, digestive, respiratory and urogenital systems. This review gives an overview on the development of the thoracic and abdominal wall during amniote embryogenesis. Specifically, I briefly summarize relevant historical concepts and the present knowledge on the early embryonic development of ribs, sternum, intercostal muscles and abdominal muscles with respect to anatomical bauplan, origin and specification of precursor cells, initial steps of pattern formation, and cellular and molecular regulation of morphogenesis.

KEYWORDS
embryo, muscle, rib, somites, sternum, vertebrate

1 | INTRODUCTION
While the embryonic development of the vertebral column and its associated musculature has been covered by a number of recent reviews,1-3 the growing knowledge on the development of the ventrolateral body wall has not yet been brought together in an overview comprising skeletal and muscular development of thorax and abdomen. In this review, I will highlight both classical embryological data and recent molecular data on ventral body wall development in a number of amniote model organisms, but focus will be on the chicken and the mouse embryo has these have been of prime importance in unraveling the embryology and molecular genetics of body wall development, respectively. Taxa with specialized bauplan like turtles and snakes will be mentioned only briefly. Understanding malformations resulting from defects in the development of the ventrolateral body wall in humans is of great clinical importance, but beyond the scope of this review. However, it is highly desirable that the research data summarized here will help preventing or treating the multitude of congenital disorders of thorax and abdomen.4

2 | DEVELOPMENT OF THE RIBS
2.1 | Anatomy and phylogeny of amniote ribs
2.1.1 | Evolution of the ribs
Ribs are segmental extensions of the vertebral column into the ventrolateral body wall. Presumably, they evolved as endoskeletal stabilizers of the trunk compensating the loss of the exoskeleton of primitive fishes and are therefore found in all vertebrates except in some taxa with ancestral traits like acrania, cyclostomata, holocephala and the crossopterygian Latimeria chalumnae.5 Ribs have evolved in the connective tissue septa which separate the muscle segments in fishes. In comparative anatomy, principally two different types of ribs are discriminated: dorsal ribs that develop in the junction of transverse myosepta and the horizontal myoseptum, which separates dorsal (back-) from ventral trunk muscles, and ventral ribs that develop in the junction of the transverse myosepta and the coelomic wall.6 The ribs of amniotes are thought to be a part of the dorsal...
type of ribs, even though their position in the thoracic wall is reminiscent of the ventral ribs that are found in fishes.\(^5\)

Archetypically, ribs are formed in every segment from the head to the tip of the tail, including occipital ribs which persist, for example, in lungfishes. In those parts of the body wall which enclose the body cavities the ribs are of capital importance regarding the stability of the trunk, and therefore the ribs prevail at thoracic level in all vertebrates, whereas at cervical, lumbal and caudal levels ribs have become rudimentary in most amniotes.\(^5\) In this review, the focus will be on the ribs of amniotes, and the numerous variations of ribs in anamniotes will not be elaborated further.

### 2.1.2 The bauplan of the ribs

In archetypical reptilians like *Sphenodon punctatus*, ribs are formally organized in four articulating elements: (a) the vertebral part of the rib that articulates directly with the vertebral column via two articular processes, the capitulum of the costal head and the tuberculum of the costal neck; (b) the uncinate process that is a caudal protrusion of the shaft of the vertebral rib; (c) the cartilaginous intermediate rib; and (d) the ventral or sternal part of the rib which articulates with the sternum\(^7\) (Figure 1A). This pattern is likely to represent the basic bauplan of the amniote ribs. In birds, based on descriptive anatomy, the vertebral and the sternal part of the ribs are thought to persist, whereas the intermediate element is lost. The uncinate process forms a long protrusion of the vertebral rib and articulates to the caudally adjacent vertebral rib by syndesmosis, thus stabilizing the rib cage to optimize ventilation. While the vertebral rib is projecting ventrocaudally, the sternal part, which is linked to the vertebral rib by a joint, extends cranially before attaching to the sternum, so that the rib in total forms a chevron-shaped structure pointing in caudal direction (Figure 1B,D). The sternal rib has also been described as a separate *Os sternocostale* in the literature and can be linked to the sternum by a diarthrotic joint, as is the case, for example, in chicken.\(^8\) In mammals, the vertebral and sternal rib elements are less evident but are likely to correspond to the bony proximal part and the cartilaginous distal part of a typical mammalian rib, even though the variety of rib morphology in mammals is large (Figure 1C,E).

With regard to their connection to the sternum, three different rib types are found: True ribs (*Costae verae*) and false ribs (*Costae spuriae*) with direct or indirect attachment to the sternum, respectively, and floating ribs (*Costae fluctuantes*) that are not attached to the sternum but end freely in the muscular body wall. The variability between taxa is large—birds and mammals have both true and floating ribs, and snakes have only floating ribs.

### 2.2 Developmental origin of the ribs

#### 2.2.1 Sclerotomal origin of the ribs

Insight into the embryological origin of the ribs has been mainly gained from the chicken embryo. The ribs form as lateral projections of the vertebral anlagen, called costal processes, which arise from the sclerotomal compartment of the somites. The costal processes are first evident

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**Figure 1** Rib morphology in amniote vertebrates. A, Schematic of an archetypical amniote rib, as it is still found in *Sphenodon* (after Reference 7). The rib consists of four elements, the vertebral rib, the uncinate process, the cartilaginous intermediate rib and the sternal rib which articulates to the sternum. B, Schematic of an avian rib. The uncinate process persists, whereas the intermediate rib is missing. C, Schematic of a mammalian rib, only the vertebral and the cartilaginous sternal rib elements persist. D, Avian thorax (Great hornbill, *Buceros bicornis*) showing long uncinate processes overlapping the caudally adjacent ribs, and the bony sternal ribs in a sharp angle to the vertebral ribs. E, Mammalian thorax (European wildcat, *Felis silvestris*) showing the long vertebral ribs and the short, bent, cartilaginous sternal part of the rib. Scale bar 1 cm. Cranial to the left. ir, intermediate rib; sr, sternal rib; up, uncinate process; vr, vertebral rib. Skeletal specimens are part of the collection of the Institute of Anatomy, University of Cologne, Germany.
as laterally located mesenchymal subpopulations of the sclerotomes and appear as such in all segments. During later development, however, they remain rudimentary at cervical, lumbar and sacral levels, whereas at thoracic levels, the mesenchymal costal processes grow out ventrolaterally in between two neighboring myotomes, along the dorsoventral extent of the body wall, and eventually fuse with the sternal bars (reviewed in Reference 9).

### 2.2.2 | Evolving concepts on rib origin during embryonic development

Sclerotomal origin of the ribs has already been postulated by classical descriptive studies based on the observation that the costal mesenchyme is continuous with the vertebral mesenchyme (reviewed in Reference 10). Nevertheless, for decades, other authors still assumed that the ribs develop independent of the vertebrae as separate skeletal elements. These were thought to secondarily connect to the vertebrae, either to form costovertebral joints with the vertebrae as in the thorax or to synostotically fuse with the vertebral processes forming rib rudiments as in the cervical, lumbal, and caudal spine. This erroneous view might have arisen due to the correct observation that the costal processes form independent centers of chondrification and ossification, which subsequently fuse with those of the vertebral centra. As a combination of both hypotheses, in 1953, Straus and Rawles inferred from their experiments based on tissue labeling by carbon particles that only the vertebral part of the rib is derived of the somites, whereas the distal, sternal part originates from the lateral plate mesoderm. In contrast, later studies by Seno using a similar technique and by Pinot inserting physical barriers in the lateral plate mesoderm, argued for the somitic origin of the entire rib including the sternal part. This discrepancy could finally be solved with the advent of the quail-chick chimerization technique, which allowed to conclusively demonstrate that all parts of the rib including the sternal part are derivatives of the somites.

With the somites having been identified as the sole source of rib precursor cells, there remained some controversies as to from which somite compartment the ribs arise. Somites can be divided into a dorsal half which consists of the dermomyotome and its progeny, the muscle-forming myotome, and a ventral half that consists of the sclerotome, which gives rise to the vertebral column (Figure 2). As detailed above, the classic view is that the ribs are formed by the sclerotome and that ribs can thus be considered as lateral elements of the vertebrae. This consensus had been temporarily challenged by tissue ablation and quail-chick chimerization experiments by Kato and Aoyama who suggested that the distal parts of the ribs are of dermomyotomal origin. However, Huang et al revisited this question in great detail by transplantations of isolated dermomyotomes and sclerotomes, which lead to the confirmation of the classical sclerotomal origin of the ribs without contribution from the dermomyotome. Detailed LacZ labeling experiments by Evans in which somitic subcompartments were differentially labeled, further substantiated the finding by Huang et al that the entire rib is of sclerotomal origin. Moreover, this study allowed to identify two different subpopulations of rib precursor cells within the sclerotome. In confirmation of earlier quail-chick chimerization data by Olivera-Martinez et al the cell lineage experiments by Evans suggested that the proximal part of the rib, which is defined as costal head, neck, and tubercle, is a derivative of the medial half of the sclerotome, and all more distal parts of the rib shaft including the vertebral and the sternal part is a derivative of the lateral half (Figure 3). Thus, with regard to sclerotomal subcompartments, head and neck regions of the ribs together with the pedicles and transverse processes of the vertebrae arise from the central sclerotome, and the rib shaft arises from the lateral sclerotome. The medial and dorsal sclerotomal subcompartments give rise to the vertebral bodies and laminae, respectively, but do not contribute to the ribs. Moreover, knockout studies in mouse embryos showed that caudal and cranial sclerotome halves differentially contribute to the ribs. Deletion of the homeodomain transcription factor Uncx4.1, which is expressed in the caudal sclerotome and is required for mesenchymal condensation, leads to loss of the proximal rib but not the distal part. A similar phenotype is observed after forced expression of the cranial somite determinant Tbx18 in the caudal somite halves, resulting in the loss of caudal traits.

A cellular contribution of the sternal plates to the distal ribs, as postulated by some earlier researchers (see above), has been positively ruled out by Chevallier using quail-chick chimerization and later by Evans who infected lateral plate mesoderm with LacZ encoding retrovirus and did not find labeled cells in the ribs, neither in the sternal part.

An interesting aspect concerning the origin of ribs comes from experiments in chicken embryos by Sweeney and Watterson which have been confirmed by Sudo et al. If a physical barrier consisting of metallic foil is inserted between somites and somatopleura immediately lateral to the intermediate mesoderm, so that the somatopleura cannot be invaded any more by somitic tissue, the vertebral part of the ribs forms normally, but the sternal part of the ribs is missing. This means that the
The vertebral part of the rib is formed by somite cells which do not enter the somatopleura and can thus be designated as primaxial structures in the terminology proposed by Burke and coworkers, and only the sternal part of the ribs has an abaxial location. This is further corroborated by the observation of these authors that the foil barriers implanted into the medialmost somatopleura were relocated to the distal tip of the vertebral part of the ribs at E11 in operated embryos, which indicates that the somite-derived, or primaxial, part of the body wall spans over the entire extent of the vertebral part of the ribs. The morphogenetic processes underlying this are unknown.

2.2.3 Resegmentation of ribs

The resegmentation hypothesis originally published by Remak in 1850 states that a single somite gives rise to a motion segment, including two adjacent vertebral halves, and the ligaments and muscles combining them. Vice versa, this means that a single vertebra is a derivative of two adjacent half-sclerotomes, thus offsetting the segmentation ratio of the somites (formerly called "Ursegmente," ancestral segments) to the definitive vertebral segments by one half. In accordance with the concept of resegmentation, quail-chick chimerization experiments by Bagnall et al. and the group of Bodo Christ revealed that also the ribs, being vertebral extensions, are subject to resegmentation and are derived from two adjacent sclerotomes. By labeling cranial and caudal somite halves, Evans revisited the issue of resegmentation in great detail. According to these results, the ribs have three different regions of origin along their proximodistal extent: The proximal part of the vertebral rib with head, neck and tubercle is solely derived from the caudal half of a single somite and thus does not undergo resegmentation. The distal portion, that is, the shaft, of the rib...
vertebral part of the rib is derived in its cranial rim from the caudal half of the same somite as the head and neck region, but in its caudal rim derives from the cranial part of the caudally abutting somite, and is thus subject to resegmentation like the vertebrae. The sternal part of the rib is also derived from two adjacent somites, but the cells do not maintain their segmental arrangement and mix within the rib, so that no resegmentation in the proper sense takes place (Figure 3). It is yet unknown how this complex origin of the different sections of a rib is achieved by cell migration processes during development, possibly *scleraxis* expression is involved here.33 Intriguingly, neither after deletion of Tbx18 in mouse embryos, which leads to loss of cranial identity of the somites, nor after deletion of Uncx4.1, which leads to the loss of caudal identity, any gross anomalies in the distal ribs have been reported.22-24 This could either mean that resegmentation occurs independent of cranial and caudal marker gene expression or that the lack of cells of one somite half is compensated by proliferation of the remaining cells in the distal rib anlagen.

2.2.4 | A model for the origin of the ribs

In summary of these data on the origin of the ribs, and incorporating the anatomical and developmental differences within the rib anlagen, Aoyama et al34 have proposed to discriminate three parts of the avian ribs along their proximo-distal extent: the proximal rib, the vertebro-distal rib, and the sterno-distal rib (Figure 3). This terminology will be used in this review henceforth. Thus, integrating the cell lineage data detailed above, the three parts of a single rib arise from three different quadrants of two neighboring somites: The caudal-medial quadrant of the more cranial somite gives rise to the entire proximal rib, and the caudal-lateral quadrant of the more cranial somite and the cranial-lateral quadrant of the more caudal somite together form the vertebro-distal and sterno-distal rib. The cranial-medial somite quadrant does not participate in the rib formation (Figure 3).

2.3 | Cellular and molecular regulation of rib development

2.3.1 | Specification of the rib-carrying thoracic region

Using heterotopic transplantation techniques, Kieny et al35 and Jacob et al36 found that the commitment of the somites to form ribs is already laid down in the segmental plate prior to somitogenesis: If thoracic segmental plate or thoracic somites were transplanted to cervical levels, ectopic ribs were formed, whereas vice versa cervical segmental plate or cervical somites transplanted to thoracic levels did not give rise to ribs. These experiments demonstrated that only the thoracic paraxial mesoderm has the intrinsic property to form ribs. The regionalization of the vertebral column along the proximodistal axis is regulated by *Hox* gene activity in the paraxial mesoderm.37-39 *Hox* group 6 genes are crucial for the specification of thoracic regional identity in the vertebral column. Forced expression of *Hoxb6* in the entire paraxial mesoderm induces rib formation in all vertebral regions from cervical to sacral levels,40 thus phenocopying the ancestral state of ribs in all segments. Contrariwise, non-thoracic *Hox* gene activity like *Hox10* expressed at lumbar levels represses rib formation in the somites.39 Surprisingly, the rib-inducing activity of *Hox6* genes is not acting directly on sclerotomal cells, but on the myotome by regulating *Myf5* and *Myf6* expression which in turn induces rib development in the sclerotome.40 In summary, based on their genetic studies in the mouse, Mallo and coworkers40 propose the following model of vertebral regionalization: the thoracic domain is specified by the rib-promoting activity of *Hox6* via *Myf5* and *Myf6* activation, the lumbar domain does not form ribs because the rib-inhibiting influence of *Hox10* overrides *Hox6* activity, and the cervical domain does not form proper ribs due to
the absence of Hox6 activity cranial to the Hox6 expression domain (Figure 4). Interestingly, in snakes with their elongated rib cage, the rib inhibiting Hox10 activity is partially suppressed (see section 2.6 for details). The molecular regulation of the species-specific variations of rib morphology within the rib cage in different segments, like the formation of true ribs vs floating ribs, is not well understood. In total knockouts of the Hox9 paralogous group in mice, not only more ribs are formed, but the ribs in segments bearing floating ribs in wild-type mice develop as true ribs attaching to the sternum, indicating a role of Hox9 in segment-specific rib patterning.41

2.3.2 Proximodistal patterning of the ribs: Regulation of proximal rib development

Formation of the proximal part of the ribs, that is, costal head and neck, which Olivera-Martinez et al21 and Evans20 have shown to originate from the central sclerotome in the medial half of the somite (Figure 3), depends on signals from the notochord. After implantation of a physical barrier between the axial organs and the thoracic somites in chicken embryos, the proximal ribs do not form, while the vertebro-distal and sterno-distal rib elements, that is, the rib shaft, persist34. Loss of the proximal part of the ribs is also observed in Shh null mutants42 and in mouse mutants depleted of the Shh-dependent medial and central sclerotomal marker gene Pax141,44 (Figure 5). Interestingly, when a barrier is implanted between axial organs and segmental plate before somites have formed, loss of the entire rib including the distal part is observed.34 This could be explained by increased somite cell death in early absence of Shh.45 Proximal rib formation can be rescued by both, implantation of notochord and Shh-expressing cells,34 indicating that proximal rib formation requires Pax1-mediated Shh signaling (Figure 2B). These findings show that the proximal part of the ribs on the one hand and the vertebro-distal and sterno-distal parts of the ribs on the other hand are not only derivatives of different sclerotomal subpopulations but are formed in development by different regulatory mechanisms. In a recent study, the analysis of mouse embryos mutant for Shh and the apoptosis factors Apaf1 and Casp3 has shown that the fate decision between proximal and distal rib fate depends on graded Shh concentrations along the mediolateral extent of the sclerotome. This early specification is maintained during mediolateral expansion of the rib anlagen due to sclerotomal cell proliferation.46 Interestingly, while in single Shh-KO mice, the distal rib cartilage is at least partly maintained,32 Shh-Apaf1 and Shh-Casp3 double KO mice show complete loss of ribs including the distal rib cartilage. From this study, Fogel et al46 concluded that rib outgrowth depends on a fine balance of Shh-induced sclerotomal cell proliferation and apoptosis, which the authors were able to confirm in a simulation model in silico.

The different regulation of proximal and distal rib elements is also illustrated by experiments in which ectopic limbs have been experimentally induced in the lateral plate mesoderm at thoracic levels. The ectopic limbs reprogrammed myogenic cells of the thoracic somites to form limb muscles and to populate the ectopic limbs, instead of forming intercostal muscles. Remarkably, this only affected the precursors of the distalmost intercostal muscles, which were missing in these embryos, what moreover lead to loss of the sterno-distal rib components. In contrast, proximal intercostal muscles and proximal and vertebro-distal ribs developed normally.47
2.3.3 | Proximodistal patterning of the ribs: Regulation of distal rib development

Tissue ablation studies have revealed that rib formation depends on signals from surrounding embryonic structures. Whereas the proximal part of the ribs depends on axial signals as detailed above, the distal part of the ribs has been shown to be related to ectodermal and myotomal signaling.

Ectoderm ablation and barrier insertion experiments in the chicken embryo showed that the ectoderm overlying the somites is not only required for formation of the dermomyotome and its derivatives, but also required for proper formation of the vertebro-distal and sterno-distal ribs. If, in contrast, the ectoderm overlying the somatopleura is removed, only the sterno-distal part of the ribs is affected, but not the vertebral part, which confirms that the vertebral part of the ribs develops independent of signals from the somatopleural ectoderm. Even though a direct influence of ectodermal signals on the sclerotome cannot be excluded, it is more likely that the ectoderm acts indirectly, via dermomyotomal or myotomal signaling, on rib development. This is in line with the phenotype of mice lacking the dermomyotomal marker gene Pax3 or the myotomal muscle regulatory gene Myf5, in both of which only the distal parts of the ribs are misshaped or missing, and with the finding that rib-inducing Hox6 acts on a Myf5/Myf6 enhancer.

FGF signaling has been shown to be an important regulator of rib development. FGF8 is expressed in the myotome including the hypaxial myotomal compartment, which is adjacent to the rib anlagen (Figure 6). Interestingly, myotomal FGF8 expression, like the sclerotome, depends on Shh signaling from the quite distant notochord-floor plate complex. The FGF receptor FGFR-4 is expressed in the myotome and generally in skeletal muscle, but also in endochondral skeleton including rib cartilage. Excess FGF8 administered to the paraxial mesoderm by beads soaked in FGF8 leads to thickened, partly duplicated, and morphologically irregular ribs, vice versa blockage of FGF8 signaling by implantation of beads soaked in the chemical FGF inhibitor SU5402 effectuates that ribs are partially missing. This shows that FGF8 induced by Shh from the notochord...
and secreted by the myotome is an important growth factor regulating chondrogenic proliferation of the ribs. The downstream effectors of the rib-inducing activity of FGF are still unclear. Experiments in chicken embryos suggest that the bHLH transcription factor Scleraxis might be involved. Scleraxis has originally been described as a marker gene of the tendon-forming sclerotomal sub-compartment, the syndetome,\(^5\) but is also expressed in early rib precursor cells in the lateral sclerotome.\(^4,5\) FGF signals from the myotome have been shown to induce scleraxis expression on the one hand through the Ets domain transcription factors Pea3 and Erm,\(^5\) and on the other hand through the MAPK signaling pathway via ERK1/2, with the ERK antagonist MKP3 as negative regulator.\(^3\) Indeed, experimental manipulation of the MAPK pathway leads to loss of scleraxis expression and loss of the distal rib parts. This suggests that, in the chicken embryo, temporal expression of scleraxis in rib precursor cells prior to chondrogenesis could play a role in vertebro-distal and sterno-distal rib specification. In this model, the negative ERK regulator MKP3 is required to localize scleraxis expression in adjacent domains in neighboring somites which will unite to form the rib shaft during resegmentation.\(^3\) The proximalmost part of the ribs, which does not undergo resegmentation, is not affected in these experiments and thus seems to develop independent of FGF-induced scleraxis. However, in conflict with this model gained from experiments in the chicken embryo, scleraxis knockout studies in the mouse showed a severe tendon phenotype, but normal rib formation.\(^5\) Thus, the signaling mechanisms downstream of FGF and the potential role of scleraxis in rib formation need further investigation.

In addition to FGF signaling, PDGF signaling is involved in rib formation. PDGF\(\alpha\) is expressed in the myotome, and its receptor PDGFR\(\alpha\) is expressed in sclerotomal cells. Mice lacking PDGF signaling show a phenotype which is reminiscent of the Pax3 mutant phenotype in Sploch mice.\(^5,6\)

The transcription factors of the *Sine oculi* (Six) gene family are regulators of myogenesis and expressed in the vertebrate myotome. Mice lacking the homeodomain transcription factor Six1, and even more severely Six1/Six4 double knockouts, are not only affected in muscle development but also show severe rib phenotypes. The distal, cartilaginous portion of the ribs of Six mutants show rib duplications as well as rib fusions, and lack of distal rib insertions to the sternum.\(^5^1,6^2\) These defects are necessarily indirect effects, as Six1/4 are expressed in the myotome but not in the rib anlagen. The phenotypes are reminiscent of distal rib defects observed in Pax3 mutant mice.\(^4^9\) As Six1 and Six1/4 mutants also show severe defects in hypaxial muscle development, namely intercostal muscle hypoplasia, the rib phenotype is thought to result from defective FGF signals from the musculature.\(^6^2\)

Recent studies using transgenic mice have brought more light into the role of myotomal factors in rib induction. Vinagre et al\(^4\) have found that mouse embryos showing ectopic ribs due to ectopic expression of *Hox6* also show ectopic expression of myotomal *Myf5* and *Myf6*, and vice versa in *Hox10* mutants lacking ribs these genes were lacking, too (see also chapter 2.3.1). Likewise, the presumed downstream effectors of the rib inducing activity of *Myf5* and *Myf6*, FGF4 and PDGFA, were upregulated by *Hox6* and downregulated by *Hox10*, respectively. These authors therefore suggest a model according to which *Hox6* expressed in the paraxial mesoderm at thoracic levels induces *Myf5/Myf6* expression in the hypaxial myotome, perhaps indirectly via Pax3 and Six1/4 induction, and myotomal *Myf5/Myf6* activity in turn induces rib formation in the underlying lateral sclerotome through FGF and PDGF signaling. For a graphical summary of the molecular data on early rib development, see Figure 2B. A summarizing synopsis of selected key findings in rib development in chicken and mouse embryos is given in Table 1.

### 2.3.4 Ventrolateral outgrowth of the rib anlagen

The invasion of somitic cells into the somatopleura of the lateral plate mesoderm is first seen in E.4 chicken embryos, as shown by quail-chick-chimerization and the expression patterns of the sclerotomal marker gene *Mfh1* (see also Figure 5C,D showing the chondrogenic marker

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**FIGURE 6** In situ hybridization showing *FGF8* expression in a HH-stage 22 chicken embryo. *FGF8* is expressed in the myotomes (arrow). Note the marked additional expression domains in the apical ectodermal ridge of the limb buds, the branchial arches and the metanephric kidney. Scale bar 500 \(\mu m\)
Key findings on rib development in the mouse embryo
Origin in Independent

Table 1

Synopsis of selected key findings in rib development in the chicken and mouse embryo

| Schematic | Rib | Origin in | Key findings on rib development in the chicken embryo | Key findings on rib development in the mouse embryo |
|------------|-----|----------|---------------------------------------------------|---------------------------------------------------|
| vertebrae rib | Aoyama et al. (2000); Hirao et al. (2001); Liem et al. (2001) | Independent | Induction of Erk-MAPK signaling | Induction of BMP signaling |
| | | | Regulation of Myf5 expression | Regulation of BMP signaling |
| | | | Induction of Sox9 and other ossification markers | Induction of BMP signaling |
| | | | Formation of bony segments | Formation of bony segments |

Note: In the above image, the proximal rib area is indicated by maroon, vertebro-distal rib is indicated by red, and Sterno-distal rib is indicated by blue. Question mark (?) indicates that it is not clear from the original data whether this rib part is affected or not.

2.4 | Chondrification and ossification of the ribs

The later skeletogenesis of ribs can only briefly be exemplified here for the case of the chicken. In the chicken embryo, overt chondrification of the sclerome-derived costal processes starts at E7-E7.5, while markers of the chondrogenic lineage like Pax3 and Sox9 are expressed much earlier (Figure 5). Whereas the cervical rib rudiments form from a single chondrification center, the proper ribs of the thoracic region form from three separate chondrification centers giving rise to the vertebral (i.e., proximal and vertebro-distal) and sternal parts of the ribs and the uncinate process, respectively. The chondrification centers of the vertebral and sternal parts
are inter-connected by a band of mesenchyme and remain separate, whereas at the distal end the sternal parts of the ribs appear to be continuous with the sternum cartilage. The cartilage of the uncinate process fuses with the adjacent rib already at E8. Ossification of the different parts of the ribs occurs separately, starting in the vertebral part at about E10-E11 and in the sternal part with considerable delay at E16. The remaining mesenchymal cleft between both parts forms a syndesmotic intracostal joint, as it is also formed between the sternal part of the ribs and the sternum at E11. The uncinate processes are thought to ossify much later than the main parts of the ribs, probably only after hatching. The costovertebral, intracostal, and costosternal joints are forming concurrently about E11 (reviewed in References 9,12).

2.5 | Special case I: Rib development in turtles

In turtles, the basic body plan of the amniote thorax is fundamentally modified, in that the ribs are located dorsolateral to the limb girdles in the dermis where they fuse with dermal bones and the overlying epidermal scales to form the turtle carapax, whereas the shoulder and hip bones are situated inside the shell. (Figure 7A,B). Morphologically this has already been recognized by 19th century embryologist like Rathke, Owen, and Goette (reviewed in Reference 65). The costal processes are induced to grow this aberrant way by cues from a signaling center, which is uniquely found in turtles, the carapacial ridge (CR). The CR consists of an elevated ectodermal rim outlining the developing carapacial margin, and a strip of underlying dense mesenchyme originating in the dermomyotome. The CR plays a decisive role in orchestrating the unusual rib development in turtles. This probably implies Wnt and FGF signaling, directing the ribs to grow from the sclerotome into the dermis, rather than to grow ventromedially between the limb anlagen and the coelomic cavity as in non-turtle amniotes.67,68 Thus, the turtle ribs never invade the somatopleura like the distal rib elements in other amniotes but stay entirely in the axial domain as primaxial structures, a phenomenon that has been called axial arrest.69 Within the dermis, BMP signals from the ribs lead to membranous ossification of adjacent tissue.67 While it was classically assumed that this membranous ossification is induced in dermal tissue, more recent data suggest that, at least in soft-shelled turtles, periosteal cells of the primarily enchondral ribs undergo intramembranous ossification during carapax closure, meaning that the entire bony carapax is of endoskeletal origin.70 In hard-shelled turtles, the carapax is eventually covered by epidermal scales. In the frame of this review, the fascinating but still controversial issue of turtle carapax formation shall not be discussed further, the interested reader is referred to detailed reviews by experts in the field.65,71,72

2.6 | Special case II: Rib development in snakes

A common feature of snakes is, next to limblessness, their extremely elongated body axis resulting from a very large number of vertebrae73 (Figure 7C). This increase in segments is thought to result from accelerated somitogenesis relative to axial growth74 and is restricted...
to segments of thoracic identity. Pythons, for instance, have roughly 300 post-cranial segments carrying ribs, except the rib-less atlas.\textsuperscript{75} In other species like the corn snake (\textit{Pantherophis guttatus}), rib-less atlas, axis and three cervical vertebrae are followed by more than 200 thoracic vertebrae with ribs.\textsuperscript{76} Caudal to the thorax, at cloacal level, Pythons maintain rudimentary hindlimb elements, whereas more derived snakes like the corn snake are totally devoid of hindlimb elements. At this level, snakes have, instead of lumbosacral segments, a series of four to five “cloacal” vertebrae with forked ribs, followed by a variable number of rib-less caudal vertebrae (Figure 7C). The specification of the axial domains in snakes is, like in all amniotes, regulated by Hox-genes, though with some snake-specific peculiarities. The shift from rib-bearing thoracic vertebrae to rib-less cervical vertebrae is conserved in snakes as it is marked by \textit{Hoxa}6 expression boundary like in typical tetrapods.\textsuperscript{76} Importantly, however, the expression pattern of \textit{Hox10} genes, which in other tetrapods suppress rib formation and mark the thoraco-lumbal transition, is different in snakes. While the anterior expression boundary of the \textit{Hox10} parologue \textit{Hoxd10} is duely found at the level of the first rib-less caudal vertebra, the anterior expression boundaries of paralogues \textit{HoxA}10 and \textit{HoxC}10 extend far into the rib-bearing thoracic region without an overt morphological correlate. This indicates that in snakes, in order to enable their unique elongated morphology, some downstream responses to Hox activity are different, namely the rib-suppressing activity of Hox10\textsuperscript{38,39} has at least partially been lost.\textsuperscript{76,77} This loss of function of Hox10 has been shown to be due to a single base pair change in a Hox-responsive \textit{Myf5}-enhancer, which prevents the binding of Hox proteins.\textsuperscript{78} Nevertheless, colinearity of Hox genes is maintained in snakes.\textsuperscript{76} The cloacal level, which is marked by truncated, bifurcated ribs, is specified by Hox11,\textsuperscript{77} arguing that it corresponds to the sacral level in other taxa,\textsuperscript{49} whereas the lumbal level is lost. The evolutionary and developmental constraints leading to this cloacal rib phenotype in snakes are unknown.

3 | DEVELOPMENT OF THE STERNUM

With the conquest of land, the ribs of terrestrial animals gained further importance in not only protecting the thoracic organs from external force, but to provide a framework to support the body weight and to enable thoracic movements in order to facilitate ventilation. This required the ventral interconnection of the bilateral ribs by the anatomically unpaired sternum.

3.1 | Developmental origin of the sternum

The initially paired sternal anlagen, the sternal bars, with which the sternal parts of the ribs eventually fuse (Figure 8A), are derivatives of the lateral plate mesoderm which develop independent of the sternal ribs. The first to discover the bilateral origin of the sternum was Rathke in 1838,\textsuperscript{79} but he misinterpreted this observation by assuming that the sternum is a derivative of the distal ends of the ribs, a view that has been accepted by most researchers of the 19th century including Kölliker\textsuperscript{80} and Schauinsland,\textsuperscript{10} whereas Bruch\textsuperscript{81} held the heretic yet correct view that the sternal plates are independent of the ribs (reviewed in Reference 10). More than 80 years later, Bruch was experimentally confirmed by Fell\textsuperscript{82} who demonstrated using explant cultures that the sternum is of somatopleural origin. This was later confirmed by Chevallier\textsuperscript{17} using quail-chick chimeras, and Evans\textsuperscript{20} using LacZ encoding virus to label somite cells, both never detecting somite contributions to the sternum. In a more recent study Bickley and Logan\textsuperscript{83} corroborated this by labeling somatopleural cells ventral of the wing bud with Dil. These experiments indicated that, in chick, the sternal precursor cells are restricted to the ventralmost somatopleura at the level of somites 14 to 21, in close association with the wing anlage.

3.2 | Molecular regulation of sternal development

The molecular regulation of sternum formation is still not well understood. Barrier implantation experiments in chicken have shown that sternal development depends on the presence of the ectoderm covering the lateral somatopleural mesoderm.\textsuperscript{26} Loss of the BMP inhibitor Smad6 in mice leads to a delay in chondrogenesis and to defects in fusion of the sternal plates resulting in a bifid sternum.\textsuperscript{84} Double-knock outs of the two BMP-family members BMP5 and Gdf5 show quite variable phenotypes with loss of sternebrae (see section 3.3) and disturbed sternocostal joint formation.\textsuperscript{85} Moreover, loss of TGFBR2-mediated TGFß signaling leads to tissue defects in the sternocostal joints, as it does in intervertebral discs.\textsuperscript{86} In contrast to the ribs, Shh signaling is not required for sternum development, as the sternum forms and mineralizes regularly in Shh mutants even in the absence of the distal rib cartilage.\textsuperscript{82,46} Thus, it seems that the sternum develops independent of potential inductive influences of the ribs but dependent on BMP signaling in the lateral plate mesoderm.

Limbless amniotes like snakes do not have a sternum (Figure 7C), indicating that sternal development is linked
Tbx5, which in limbed vertebrates is expressed in the somatopleura at forelimb level, plays a decisive role in sternal development, as in the absence of Tbx5 in mice not only the forelimbs are absent but also the sternal bars are lacking and ventromedial thoracic closure is disrupted. The reason for this phenotype is thought to be disturbed cell migration, rather than disturbed sternal specification. Interestingly, embryos of the flightless Emu (Dromaius novaehollandiae), which has both smaller wings and sternum when compared to flying birds including the chicken, do not display a smaller Tbx5 expression domain as could have been expected, but show a later onset of Tbx5 expression, which might be causal for sternal reduction. Surprisingly, snakes that do not have a sternum at all still express Tbx5, but Tbx5 expression is not restricted to the virtual forelimb region as specified by Hox gene expression but extends caudally along the entire pre-cloacal trunk of the snake embryo, indicating an uncoupling of Hox gene activity and downstream signaling cascades leading to limb and sternal development in snakes.

In archetypical reptiles like Sphenodon, as well as in crocodiles, a series of ventral rib-like structures known as gastralia are found in the ventral abdominal wall caudal to the sternum. These gastralia, which are widely found in extinct reptiles including dinosaurs, form by desmal ossification in the subcutis and are not homologous to the true ribs, but are rather thought to be relics of the dermal bones of ancestral reptiles. In turtles, the bony elements of the ventral shell, the plastron, are derived from the gastralia, and in part from the clavicular bones.

### 3.3 | Ossification of the sternum

In the chicken embryo, the sternal bars are laid down as bilateral skeletal structures at about E8 and fuse in the ventral midline of the thorax when ventral fusion of the body wall is completed. Fusion proceeds in craniocaudal progression, in that the bilateral sternal plates first touch ventromedially in their cranial extremity, and in the following, they continue fusion caudally in a zipper-like fashion until they fuse completely at E10.79 Reptile sterna generally do not ossify, whereas avian sterna (Figure 8B) ossify to a large extent as an adaptation to the forces acting on the thorax during flight. In chicken, the sternal keel is formed subsequent to fusion, and ossification starts from several independent ossification centers but remains incomplete in the caudal extent of the sternum long after hatching. In many mammals including the mouse, the sternum shows a segmental structure consisting of the cranial manubrium, a number of serial skeletal elements within the sternal body which are called...

**Figure 8** Development and morphology of the sternum. A, Sternal anlage of a HH-stage 30 (E.7) chicken embryo visualized by Collagen 2a immunostaining. Medial view after removal of the left body half and viscera, ventral is to the left and cranial to the top. Note the vertebral and sternal rib components, the sternal rib is already connecting the sternum. B, Sternum of the Great Hornbill, Buceros bicornis, as an example of a typical avian sternum with a prominent keel serving as origin of the large flight musculature. Lateral view from the left, cranial to the top, with the sternum in the same position as the sternal anlage in A. C, Sternum of a kangaroo (Macropus sp.) showing sternal segments called sternebrae. Note that the sternal ribs insert at the intersegmental joints of the sternum. D, Human sternum, the sternal body is unsegmented. C and D, Ventral view, cranial to the top. Scale bar in A 500 μm, scale bars in B-D 1 cm. st, sternum; sr, sternal rib; vr, vertebral rib; sk, sternal keel; xp, yiphoid process; ma, manubrium. Skeletal specimens are part of the collection of the Institute of Anatomy, University of Cologne, Germany.
sternebrae (Figure 8C), and the caudal xiphoid process, all of which form independent ossification centers. In the human sternum, the metamery of the sternal body is lost (Figure 8D), even though it also ossifies from multiple serial ossification centers.

4 | DEVELOPMENT OF THE VENTROLATERAL TRUNK MUSCULATURE: INTERCOSTAL AND ABDOMINAL MUSCLES

4.1 | Developmental anatomy of intercostal and abdominal muscles

The musculature of the trunk can be divided in two major muscle groups, the back muscles and the muscles of the ventrolateral body wall, which comprise the intercostal and abdominal musculature (Figure 9). From an anatomical point of view, both muscle groups are sharply defined by their innervation. Whereas the back muscles are innervated by dorsal rami of the spinal nerves, the ventrolateral trunk muscles are innervated by ventral rami. This fundamental anatomical difference reflects the different embryonic origin of both muscle groups. The back muscles arise from the epaxial myotome, which is formed by muscle precursor cells supplied by the dorsomedial lip of the dermomyotome (DML), and the ventrolateral muscles arise from the hypaxial myotome, which receives cells from the ventrolateral lip of the dermomyotome (VLL) (Figure 2A). In addition, both myotomal domains receive muscle precursor cells from the cranial and caudal dermomyotomal margins88 and are subsequently supplemented by proliferative myogenic progenitor cells from the central dermomyotome.89 As both muscle groups, back and intercostal/abdominal muscles, emerge from the growing myotomes, they are called intrinsic (autochthonous) trunk muscles. In contrast, other muscle groups like the muscles of the pectoral and pelvic girdles, which are topographically located in the trunk but result from extensive migration of limb muscle precursor cells originating in the hypaxial dermomyotomes,90,91 are called extrinsic (allochthonous) trunk muscles. Whereas the development of the back muscles has received much attention in the last decades (for a recent review, see, eg, 92), the embryology of the ventrolateral trunk muscles, that is, the intercostal and abdominal muscles, is much less understood.

4.2 | Developmental origin of intercostal and abdominal muscles

4.2.1 | Principles of intercostal and abdominal muscle formation

Pioneering studies on the development of the musculature of the ventrolateral body wall in the chicken embryo were published by His in 1886,93 who suggested in the first instance that these muscles originate in the somatopleura. Later descriptive work by Fischel,94 Engert95 and other classical embryologists in the late 19th century, in contrast, strongly argued for somitic origin of abdominal and intercostal muscles. Nevertheless, more recent authors like Straus and Rawles13 and Seno14 still insisted on the somatopleural origin of at least part of the body wall muscles. Eventually, using the quail-chicken-chimerization technique, embryologists in the 1970s could unequivocally confirm that in the chicken thorax both the ribs and the intercostal muscles are somite derivatives,96-98 and that likewise all abdominal muscles are of somitic origin, whereas the associated connective tissue derives from the lateral plate mesoderm.99

As mentioned before, the somite compartment giving rise to the ventrolateral trunk muscles is the hypaxial myotome, which receives an ongoing supply of primary myotomal cells from the ventrolateral dermomyotomal lip (VLL) (Figure 2A), and additionally from the cranial and caudal dermomyotomal margins of the hypaxial dermomyotome. Within the early dermomyotome, the VLL is the last of the four dermomyotomal lips to contribute muscle cells to the primary myotome.88 The cellular movements occurring at the VLL have not been studied in detail but are likely to be similar to the mechanisms of myotomal cell recruitment at the DML.88,92,100 However, a fundamental functional difference between DML and VLL is that the DML is a stationary source of cells, which
remains in its position lateral to the neural tube throughout somite development, whereas the VLL is progressively translocating, at the front of the growing hypaxial dermomyotome and myotome, into the mesenchyme of the somatic lateral mesoderm (somatopleura) of the forming body wall.\textsuperscript{101} Thus, the VLL appears as a blastemalike, double-layered muscle bud heading toward the ventromedial midline of the embryo, leaving in its wake the anlagen of the intercostal and abdominal muscles (Figure 10). Indeed, the VLL is continuously mitotically active and Pax3-positive while the hypaxial myotome is already starting myogenic differentiation.\textsuperscript{102,103} Regarding the mode of hypaxial myotomal growth, Cinnamon et al\textsuperscript{100} postulated an intercalative mode of ventrolateral extension throughout the myotomal length, which is in line with more recent descriptive studies in human embryos suggesting differential growth as major force in ventral body wall development.\textsuperscript{104} On the other hand, VLL ablation studies rather suggested that the VLL, like the DML in the epaxial myotome, is a growth center essential for hypaxial myotomal elongation.\textsuperscript{105} This discrepancy awaits further investigations. At the sixth day of development, the VLL, which had been named “Muskelknospe, muscle bud” by the classical embryologists, dissolves into a dense mesenchyme which integrates into the forming abdominal muscles.\textsuperscript{94}

The somatopleural mesenchyme, which the hypaxial myotome invades during ventrolateral extension, does not form muscles and neither ribs, but contributes connective tissue, aponeuroses and the sternum to the ventral body wall, thus forming a matrix in which the ingrowing muscles are embedded.\textsuperscript{16,82,106,107}

### 4.2.2 Origin and formation of the intercostal muscles

Both emerging from the hypaxial myotomes, the intercostal and abdominal muscles show distinct developmental characteristics. The intercostal muscles maintain their

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**Figure 10** Myotomes and the forming intercostal muscles visualized by myosin heavy chain (MF20) immunostaining, lateral view, cranial to the left. In C and D, the limbs have been removed to expose the thoracic wall. A, HH-stage 23 (E.4) embryo showing segmental myotomes from occipital to caudal levels. The thoracic region in which intercostal muscles will form extends from somite 19 to somite 26, but myotomal extension has not yet overtly started. B, At HH-stage 26 (E.5) the hypaxial myotome has started to extend ventrolaterally into the somatopleura (arrow). C, At HH-stage 29 (E.6) the intercostal muscles span the thoracic wall. The asterisk indicates the transition from vertebral to sternal rib domain, which is visible as a slight kink in the intercostal muscles. D, At HH-stage 34 (E.8), the intercostal muscles in the sternal rib domain are covered by the anlage of the abdominal external oblique muscle (arrowhead). Scale bars 500 μm
segmental pattern until adulthood, in that they form thin mediolateral bands of muscle fibers connecting two adjacent ribs (Figures 10 and 12). Each intercostal muscle arises from one hypaxial dermomyotome, as has been shown by quail-chick chimerization, somite cell labeling experiments by DiI and retroviral markers. In the chick, the intercostal muscles arise from somites 19 to 26 (Figure 10A). As opposed to the ribs, the intercostal muscles are central to the motion segment and do not undergo resegmentation. The development of external and internal layers of the intercostal muscles, probably by invasion of resident connective tissue cells, is not yet understood (reviewed in Reference 110), and later steps of intercostal muscle development have not been studied. Turtles, which do not have intercostal muscles in adult anatomy, nevertheless form regular intercostal muscles in the early embryo. However, their intercostal muscles degenerate and disappear with the onset of carapax development and are temporarily replaced by connective tissue before the coalescence of the ribs to form the carapax. The mechanisms leading to intercostal muscle loss in turtles are unknown.

4.2.3 | Origin and formation of the abdominal muscles

The abdominal muscles of the chicken embryo arise, according to quail-chick chimerization data by Chevallier, from somites 27 to 29. Preliminary data by Liem and Aoyama, however, suggest that the majority of abdominal muscles emerges from somite 27. Engert has given a detailed account on the later steps of development of the hypaxial myotome at abdominal levels in chicken, which is based on the analysis of histological sections (see Figure 11). At about 3 days of development (HH-stage 18), the myotome extends laterally to the medial margin of the lateral plate mesoderm where somatopleura and splanchnopleura meet. Subsequently, the myotome grows into the somatopleural mesenchyme, while the central dermomyotome undergoes EMT and only the VLL remains epithelial. At 5 days (HH-stage 26), the myotome extends along about two thirds of the body wall. By this stage, the VLL disintegrates, and the distal part of the hypaxial myotome thickens considerably, without yet splitting into its muscular layers. At 5.5 days, around HH-stage 28, the first definitive muscle to split off the common muscle anlage is the external oblique muscle. At day 6 (HH-stage 29), also internal oblique and transverse abdominis separate, and finally, at day 7.5 (HH-stage 33), also the rectus abdominis muscle has formed from the distalmost remnant of the hypaxial myotome (see also Figure 10D for lateral view, Figure 12 for mediolateral views). This timing schedule of abdominal muscle formation has been experimentally confirmed by Christ and coworkers using the quail-chick-chimerization technique, with the exception that they could discern the rectus abdominis muscle slightly earlier than Engert, already at day 7 (HH-stage 30). They also report
that the external oblique muscle is not only the first to form anatomically but also the first to show myotubes, which are aligned according to their position in the adult muscles. At day 10, the distinct abdominal muscle anlagen have reached their definitive position and their connection to tendons and aponeuroses, thus finishing abdominal muscle patterning.

The data summarized in this section have been gained in the chicken embryo due to its amenability for lineage study experiments, but descriptive reports in human, rabbit and rat indicate that the principles of ventrolateral trunk muscle formation are similar, albeit more detailed studies in mammals are missing. In the special case of turtles, the abdominal muscles have evolved specialized functions in respiration, as costal respiration by movements of the rib cage is impossible due to the immobile integration of the ribs in the carapax. Specifically, the transversus musculature effectuates expiration by pressing the viscera dorsally against the lungs, while the oblique musculature acts as its antagonist and pulls the transversus muscle ventrally. To date, however, the developmental mechanisms in turtle abdominal and intercostal muscle formation are basically unknown.

4.3 Molecular regulation of the development of intercostal and abdominal muscles

Regarding the molecular regulation of ventrolateral body wall myogenesis, knowledge is very limited, as the process of myotome formation has been predominantly investigated in the epaxial domain. It is likely that most of the molecular and cellular events leading to hypaxial myotome formation by the VLL are similar, yet not identical, to those in the DML and the epaxial myotome (reviewed in Reference 117).

Prior to myotome formation, the hypaxial dermomyotomal identity is induced by BMP4 signaling from the lateral plate mesoderm. Hypaxial dermomyotome is marked by expression of Sim1, whereas epaxial dermomyotome is marked by expression of En1, the balance between both domains depends on Shh signaling from the notochord-floor plate complex. This epaxial/hypaxial subdivision in the dermomyotomal cells is inherited by their respective myotomal descendants. Huang et al have shown by surgical manipulations in the chicken embryo that Shh is not only required for epaxial myotome formation, but also for FGF8 expression in the hypaxial myotome, thus linking intercostal muscle development to rib development (see section 2.3.3).

Experiments in the mouse embryo have shown that the formation of the VLL in the hypaxial dermomyotome requires the basic helix-loop-helix transcription factor Pax3 and the homeobox transcription factors Six1 and Six4. Embryos mutant for Six1 and Pax3 show similar defects in hypaxial muscle development, which implicate severe muscle hypoplasia not only in limb muscles but also in the ventral thoracic and abdominal muscles. Accordingly, in Six1/Six4 double knockout mice, the ventral expansion of the hypaxial myotome is impaired and the intercostal and abdominal muscles do not form. This drastic phenotype is associated with defective rib development, which demonstrates the tight link between intercostal muscle and rib formation. While Pax3 is required for muscle migration, Six1 is thought to act at a later step of hypaxial myogenesis after myogenic cell migration is completed.

Within the lateral plate mesoderm into which the hypaxial myotomes expand, somatopleural Pitx2 is required for
correct myotomal extension. Accordingly, in Pitx2 mutant mice myotomal extension is disrupted.\textsuperscript{125}

Similar to the situation in the DML, ectodermal Wnt signaling is required for VLL maintenance in the dermatomyotome, but apparently via a non-canonical Wnt pathway.\textsuperscript{101} In line with this, in the mouse ectodermal Wnt signaling has been found to be necessary for abdominal muscle development and ventral closure of the body wall. Inhibition of Wntless-dependent ventral ectodermal Wnt secretion leads to loss of the ventralmost part of the abdominal musculature. This is likely due to disrupted myogenic differentiation, rather than to be caused by lack of myogenic migration into the body wall.\textsuperscript{126} The relevant Wnt in this process might be ectodermal Wnt6, but this still awaits experimental confirmation.

The molecular regulation of muscle splitting, that is, the formation of the anatomical muscle layers in the abdominal wall from a uniform ventrolateral muscle anlage (Figures 11 and 12), is basically unknown. Mutant phenotypes in the mouse indicate that the rectus abdominis muscle, which is the ventralmost and latest abdominal muscle to form, may not be simply formed by myotomal extension and splitting, but by additional cell migration. In Splotch mice mutant for Pax3, next to the migratory limb muscles, the rectus abdominis muscle is missing, and vice versa in Paraxis-Myf5-double-knockout mice, the non-migratory trunk muscles are affected, but not the rectus abdominis muscle.\textsuperscript{124,127,128} (reviewed in Reference 110).

The tendons connecting intercostal muscles to the ribs are formed by the scleraxis-expressing syndetomal subcompartment of the sclerotome, which develops in close association with the Sox9-expressing sclerotomal precursor cells\textsuperscript{20} (Figure 5). The mutual interactions between intercostal and abdominal muscles, resident connective tissue, and ribs and sternum during development of the ventrolateral body wall are still to a large extent unknown.

5 | CONCLUDING REMARKS

From the 19th century to today, research on the development of ribs, sternum, intercostal and abdominal muscles has seen a lot of progress. Thanks to cell lineage studies in the chicken embryo, the origin of rib and intercostal muscle precursor cells has been elucidated. Genetic studies in mouse embryos have shed light on the specification of rib-bearing vs rib-less vertebrae. Molecular studies both in chicken and mouse have helped to sketch a molecular network regulating rib and muscle development in the ventrolateral body wall. However, many questions are still open, for instance, on the precise molecular interaction of rib and muscle precursor cells, the developmental mechanisms leading to abdominal muscle splitting, the interactions between ribs and sternum, and the morphogenesis of different rib types in the thorax. Further research will bring light to these issues, and the increasing use of novel model organisms including “exotic” body plans like turtles and snakes will help to understand the wealth of variations in the body wall of amniotes.

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