

# Development of the diaphragm – a skeletal muscle essential for mammalian respiration

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## Keywords

CDH; congenital diaphragmatic hernia; development; diaphragm; muscle; tendon

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The mammalian diaphragm muscle is essential for respiration, and thus is one of the most critical skeletal muscles in the human body. Defects in diaphragm development leading to congenital diaphragmatic hernias (CDH) are common birth defects and result in severe morbidity or mortality. Given its functional importance and the frequency of congenital defects, an understanding of diaphragm development, both normally and during herniation, is important. We review current knowledge of the embryological origins of the diaphragm, diaphragm development and morphogenesis, as well as the genetic and developmental aetiology of diaphragm birth defects.

## Introduction

Of all the skeletal muscles in the human body, the diaphragm is one of the most essential. It is a domed muscle separating the thoracic and abdominal cavities and is critical for respiration. Birth defects and diseases that affect diaphragm structure and function reveal its critical function. Congenital diaphragmatic hernias (CDH) result from defects in diaphragm development and are both common and frequently lethal [1]. In addition, the lethality associated with many myopathies, such as Duchenne muscular dystrophy, and is the result of a failure of the diaphragm and other respiratory muscles [2]. However, despite its critical function, knowledge of the molecular and cellular mechanisms regulating diaphragm development, both normally and during herniation, is limited. The present review describes current knowledge about diaphragm development, reviews the genetic and developmental aetiology of diaphragm birth defects and, compares

the developmental processes regulating limb and diaphragm muscle morphogenesis.

## Diaphragm structure, function and evolution

The diaphragm muscle is composed of two domains [3]. The costal diaphragm is a thin domed sheet of muscle composed of a radial array of myofibres extending laterally from the ribs and medially to a central tendon (Fig. 1). The crural diaphragm is thicker and located more posteriorly (dorsally), where it attaches to the vertebrae and surrounds the oesophagus and aorta (Fig. 1). Medially, the myofibres of both the costal and crural muscles insert into the central tendon. The central tendon is located at the apex of the domed diaphragm, holding the diaphragm muscle domains together. Caudally, it attaches to the liver via

## Abbreviations

CDH, congenital diaphragmatic hernia; E, embryonic day; HGF, hepatocyte growth factor; RA, retinoic acid.

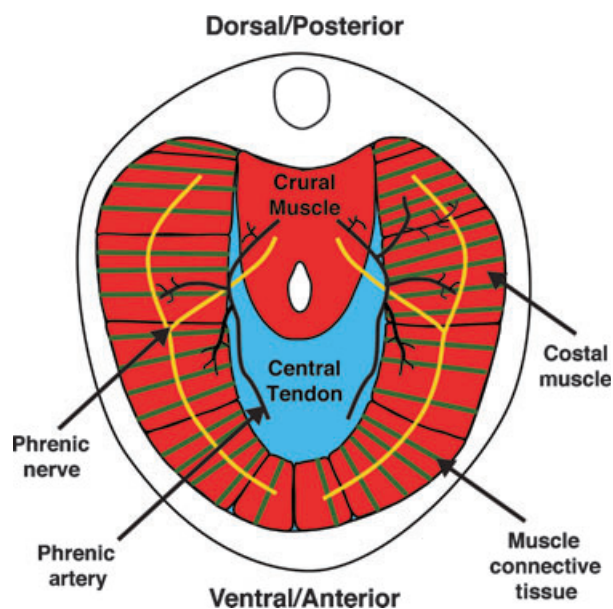


Fig. 1. Major components of the adult diaphragm.

the falciform and coronary ligaments. The tendon is a connective tissue sheet composed of extracellular matrix and the tendon cells that secrete it [3]. Although less visible in whole mount, each of the myofibres of the costal and crural muscles is surrounded by muscle connective tissue. The right and left halves of the diaphragm are innervated by the right and left phrenic motor nerves [4]. These nerves originate from the cervical nerves (C3–C5), descend along the interior of the vertebrae, pierce the left and right diaphragm, and spread posteriorly (dorsally) and anteriorly (ventrally) to innervate myofibres of the crural and costal muscles. The diaphragm is vascularized by the phrenic, internal thoracic and intercostal arteries [5].

The diaphragm has multiple functions, with its principle function being its critical role in respiration. During respiration, the actions of the diaphragm are central for inspiration. Contraction of the diaphragm muscle flattens the dome-shaped diaphragm and central tendon, which in turn expands the volume of the thoracic cavity, reduces thoracic pressure, and allows air to flow into the lungs [6]. Interestingly, the diaphragm is not strictly required for respiration when humans or animals are resting. However, analyses of rats, dogs and humans with bilateral diaphragmatic paralysis (in which the phrenic nerves are severed) demonstrate that the diaphragm is required for respiration and lung ventilation during supine posture (particularly during rapid-eye-movement sleep) and vigorous activity [7–9]. In addition to respiration, the diaphragm (particularly the crural domain) has functional roles in swallowing

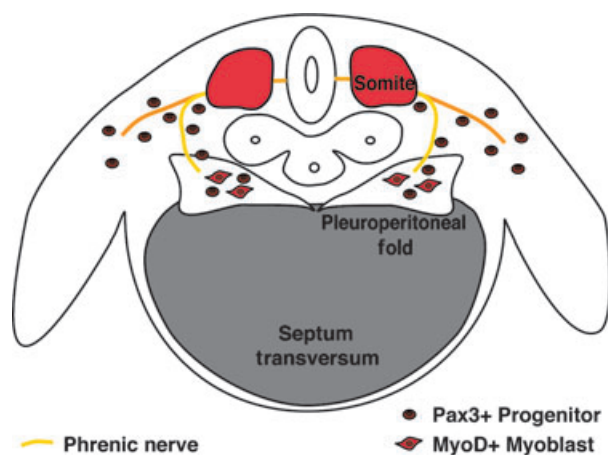
and emesis [10]. Finally, the diaphragm also has a passive functional role. The diaphragm serves as a barrier between the thoracic and abdominal cavities. The importance of this barrier function is dramatically apparent in newborns with CDH, whereby a weak or incompletely formed diaphragm allows abdominal contents to herniate into the thoracic cavity and impair lung development [1].

The presence of a muscularized diaphragm is a unique and defining characteristic of mammals [11,12]. Although a muscularized diaphragm is unique to mammals, the presence of a septum separating the lungs from the abdominal viscera is an ancient character, and some variant of this septum is present in reptiles and birds (but not in fish and amphibians) [12,13]. In mammals, this septum becomes muscularized to form the diaphragm. It has been proposed that the diaphragm evolved in mammals as a stabilizer of the abdominal viscera and an inspiratory muscle [12]. Together, these functions of the diaphragm allowed mammals to evolve as high-performance homeotherms, capable of concomitant respiration and locomotion. Thus, the question of how the mammalian diaphragm evolved is an important question. Presumably, developmental innovations were critical for the development of a muscularized diaphragm, although the molecular and cellular nature of these innovations is currently unknown.

## Embryological sources of the diaphragm

The diaphragm develops from multiple embryonic sources. The muscle and its associated connective tissue and central tendon develop from three sources: the septum transversum, the pleuroperitoneal folds and the somites.

The septum transversum is the first structure present in the developing diaphragm and serves as the initial barrier between the thoracic and abdominal cavities (Fig. 2). In all vertebrates, the septum transversum is a thin, mesodermal sheet of tissue that separates the heart from the liver [12]. In many reptiles and all birds and mammals, septa separate the heart and lungs from the liver and the rest of the abdominal contents [12]. Expression analysis of *Cited2* (originally called *Mrg1*), which is expressed in the mouse septum transversum, suggests that the septum originates as the rostral-most mesoderm in the embryo [14]. During the process of foregut invagination, folding causes the *Cited2*<sup>+</sup> tissue to lie in a position caudal to the heart and form the septum transversum, which can be detected by the 9–12 somite stage in mice. Other genes, such as  $\alpha 4$ -*integrin* [15] and *Pbx3* [16], are also expressed in the mouse



**Fig. 2.** Embryonic sources of the diaphragm. Mouse developing diaphragm at E12.5. The diaphragm develops from the septum transversum, pleuroperitoneal folds and the somites, and is innervated by the phrenic nerve.

septum transversum by embryonic day (E)9. It is unclear what the septum gives rise to (if anything) in the adult, although some studies have suggested that the septum gives rise to the non-muscle and central tendon components of the diaphragm [17]. However, without markers or genetic tools to follow the fate of the septum, it is still unclear whether or what the septum contributes to in the adult diaphragm. Although not explicitly tested, during development, the septum is likely to provide a scaffold for diaphragm morphogenesis.

The pleuroperitoneal folds (also referred to as the posthepatic mesenchymal plate) are the second important component of the developing diaphragm. The pleuroperitoneal folds are two transient, pyramidal-shaped structures lying on either side of the esophagus that protrude from the body wall between the pleural and peritoneal cavities (Fig. 2). The pleuroperitoneal folds have been identified both histologically in sections [4,17] and through scanning electron microscopy [18,19]. Molecularly, the cells comprising the pleuroperitoneal folds express the nuclear receptor *NR2F2* (also known as *Coup-TFII*) and the transcription factors *Wt1* and *Gata4* [20]. In mouse, the pleuroperitoneal folds are first present at E11 [17], proliferate (to be most prominent at E12.5), then appear to spread ventrally, and, eventually are assumed to fuse with the septum [4,17]. It is currently unclear whether the pleuroperitoneal folds are simply transient embryonic structures with no adult derivatives, or whether they give rise to cells or tissues of the adult diaphragm.

Similar to trunk and limb muscles, the somites are the source of the diaphragm's muscle cells (Fig. 2) [4,21,22]. Evidence that the diaphragm's muscle

originates from the somites comes from analysis of the transcription factor *Pax3* and the receptor tyrosine kinase *Met* (also known as *c-Met*). Both of these genes are strongly expressed in the muscle progenitors within the somites [23–25]. Furthermore, these genes are required for the muscle progenitors to migrate from the somites to form muscles in the limb and trunk. Strikingly, mice with null mutations in either *Pax3* or *Met* have no limb or diaphragm muscles (including both the costal and crural muscle domains) [21,26–28]. Thus, the diaphragm derives from migratory muscle progenitors originating from the somites. Expression studies [4,21,22] show that the diaphragm's muscle progenitors originate from the cervical somites, likely C3–C5.

### Migration of muscle and nerve to the developing diaphragm

The muscle progenitors and the phrenic nerve axons migrate from the somites and neural tube, respectively, to the developing diaphragm (Fig. 2). The target of their migration is the pleuroperitoneal folds [4,22]. In mice, the phrenic nerve axons and muscle progenitors migrate toward the folds by E10.5 [4,21,22]. The phrenic nerve migrates after the muscle progenitors and initially migrates with nerves of the brachial plexus before separating and targeting the folds [29]. When the nerves and muscle progenitors reach the folds, the folds are located at the level of cervical somites. However, gradually the folds and the developing diaphragm with its nerves and muscle descend caudally, ultimately to lie at the thoracic/lumbar boundary [4].

The pleuroperitoneal folds are likely to be an important source of signals guiding the migration of nerves and muscle to the developing diaphragm. Our knowledge of the molecular nature of these signals is limited. Neural cell adhesion molecule and low-affinity nerve growth factor receptor are expressed along the path from the neural tube to the pleuroperitoneal folds and may guide outgrowth of the phrenic nerve [4]. In addition, hepatocyte growth factor (HGF), the ligand for the *Met* receptor, is expressed along the migratory path for both the muscle progenitors and the nerves [21]. The finding that *HGF* null mice have no diaphragm muscle strongly suggests that *HGF* is important for guidance of the myogenic progenitors to the developing diaphragm [30]. *HGF* may also be critical for phrenic nerve development [31,32].

### Diaphragm morphogenesis

To produce a functional diaphragm, the morphogenesis of the muscle, muscle connective tissue and tendon

derived from these different embryonic sources must not only be coordinated with each other, but with the nerves and vasculature.

After reaching the pleuroperitoneal folds, the muscle progenitors undergo the processes of myogenesis and morphogenesis. During myogenesis, the muscle progenitors differentiate into multinucleate myofibres. Similar to trunk and limb muscle, diaphragm progenitors initially express the transcription factors Pax3 and Pax7. These progenitors become committed myoblasts expressing the transcription factors Myf5 and MyoD, differentiate into Myogenin<sup>+</sup> myocytes and, finally, fuse to form multinucleate myofibres [22,33]. Pax3/7<sup>+</sup> muscle progenitors and MyoD<sup>+</sup> myoblasts are present in the pleuroperitoneal folds by E12.5 in mice, as well as by E13.5 in rats [22]. Subsequently the progenitors differentiate into myoblasts and then fuse into myofibres, which are assembled into the costal and crural muscles. The morphogenesis of the costal diaphragm has been described in more detail [22]. Myofibres first begin differentiating in posterolateral regions of each left and right hemi-diaphragms. Between E12.5 and E15.5, a wave of differentiation expands both ventrally and dorsally, as well as medially (towards the central tendon) and laterally (towards the ribs) of each hemi-diaphragm. Morphogenesis of the costal diaphragm is largely complete by E15.5. It is still unclear what drives this morphogenetic process, what orients the myofibres to form a radial array, and why the central tendon region is devoid of muscle.

The morphogenesis of the diaphragm's muscle connective tissue and central tendon and their relationship to the transverse septum and the pleuroperitoneal folds remain poorly understood. Based on histological analysis of sections through developing mouse embryos, it has been proposed that the septum transversum, initially present on the cranial surface of the liver, remains in place and gives rise to the central tendon [17]. The pleuroperitoneal folds have a complicated morphogenesis. After E12.5, the folds, initially located adjacent to the cervical somites, descend caudally (along with the phrenic nerves and muscle progenitors) to reach the thoracic/lumbar boundary [4]. Using scanning electron micrographs of a series of developing embryos, the pleuroperitoneal folds then appear to spread to cover the cranial surface of the liver and fuse with the septum transversum [18,19]. The molecular and cellular nature of the interactions between the folds and the septum is not known. Also, it is unclear what the ultimate fate of the folds is: the folds may simply be transient embryonic structures or they may give rise to the non-muscular parts of the diaphragm.

Thus, it is currently unknown whether the muscle connective tissue and central tendon arise from the septum transversum, the pleuroperitoneal folds, or some other embryonic source.

The close proximity of the muscle and non-muscle cells, which presumably give rise to the muscle connective tissue and central tendon, suggest that cell-cell interactions between these tissues may be critical for proper diaphragm development. Interestingly, analysis of *Met* null mice (in which muscle progenitors do not migrate into the developing diaphragm) demonstrate that the connective tissue is present even in the absence of muscle [34]. Thus, the connective tissue develops, at least initially, independent of muscle, although it is unclear whether distinct tissues such as the central tendon still form. Whether the diaphragm's muscle requires signals from the developing muscle connective tissue and/or central tendon has not yet been tested.

Innervation of the diaphragm is critical for the development of a fully functional diaphragm. After reaching the pleuroperitoneal folds, the phrenic nerves must spread, branch and innervate the developing diaphragm by forming neuromuscular junctions with differentiated myofibres. After reaching the pleuroperitoneal folds, by E13.5 the phrenic nerve splits into three branches: sternocostal, dorsocostal and crural branches [35]. The sternal branches extend and cross past the midline of the diaphragm before retracting to reach their final positions [22]. Subsequently, the three major branches send out short secondary branches, arborize and then form neuromuscular junctions with the costal and crural muscle. The molecular signals and cellular interactions controlling axon outgrowth, branching and formation of neuromuscular junctions are beginning to be elucidated. The receptor protein tyrosine phosphatases  $\sigma$  and  $\delta$  are required for phrenic nerve branching as the phrenic nerves reach the pleuroperitoneal folds, but fail to extend and branch appropriately in mice mutant for these phosphatases [35]. Additionally, *Hoxa5* and *Hoxc5* are required for proper secondary branching of the phrenic nerve because the deletion of these genes in the motor neurons results in a severe reduction of branching, as well as limited synapse formation with the muscle [36]. Interactions between muscle and nerve are also critical for secondary branching and neuromuscular junction formation [37]. Conditional mutagenesis experiments in mice found that secondary branching and arborization of the phrenic nerve is regulated via  $\beta$ -catenin within the muscle, demonstrating that muscle-derived signals regulate phrenic nerve development [38,39]. In addition, a multitude of studies have shown that formation of phrenic nerve neuromuscular junctions

involves a complex interplay of muscle–nerve retrograde and anterograde signalling [40,41].

Finally, vascularization of the diaphragm is also critical for diaphragm development. This is an area of research that has received little attention. However, recent research using *XLacZ4* transgenic mice, which label the nuclei of vascular smooth muscle cells, shows the vascularization of the diaphragm by the phrenic, intercostal and internal thoracic arteries. The use of *XLacZ4* transgenic mice permits visualization of the complex branching structures of both the arteries and veins. This work provides the first detailed description of diaphragm vascularization and holds promise for future research [5].

### CDH

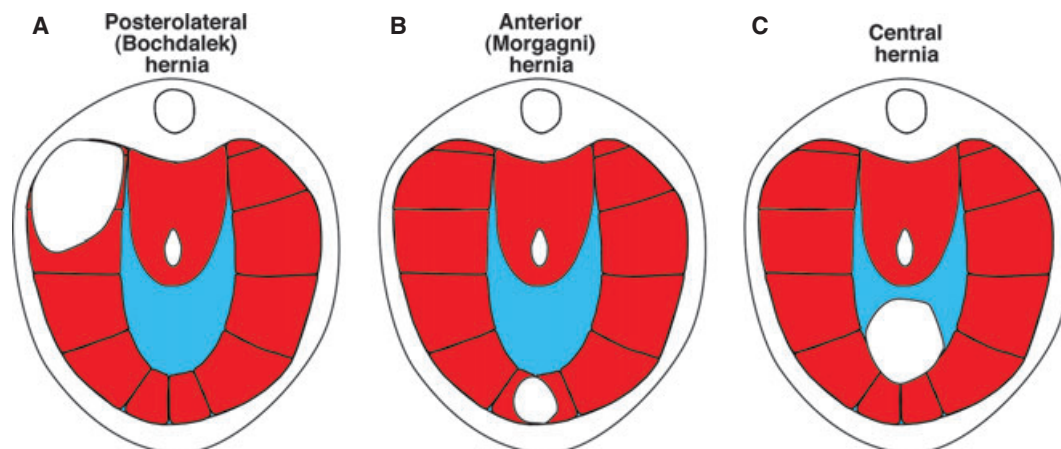
CDH is a common birth defect (1 : 3000) that often has severe medical consequences [1]. CDH occurs from a failure of the diaphragm to form properly, resulting in weak or incomplete regions of muscle. Through these weakened or incomplete regions, the abdominal contents herniate into the thoracic cavity. In turn, the herniated abdominal contents impede lung development, leading to hypoplastic lungs. Although it is generally considered that lung hypoplasia simply results from a physical impediment of lung growth by the herniated tissue, some genetic defects associated with CDH directly affect both diaphragm and lung development [42,43]. The lung hypoplasia accompanying CDH is the main cause of the high morbidity and mortality associated with CDH. Despite medical intervention, the mortality rate for CDH is 50% and results from respiratory failure [1,44–46]. For patients that survive,

chronic respiratory and neurodevelopmental problems are common [42].

Diaphragmatic hernias vary both in the region of the diaphragm in which they form and in size. In the majority of cases (90%), hernias form in the posterior lateral diaphragm, and these posterolateral hernias (termed Bochdalek hernias) develop predominantly on the left side of the diaphragm (Fig. 3A) [1,46,47]. Posterolateral hernias are the form of CDH most commonly associated with lung hypoplasia because the abdominal contents herniate into the posterior pleural cavity where the developing lungs are forming. Hernias also form in anterior regions (termed Morgagni hernias) or in the central tendon (termed central hernias) (Fig. 3B,C) [1]. However, hernias in these regions generally have less severe consequences [48]. Diaphragmatic hernias also vary in size [49], with larger hernias having more critical impacts on health, whereas smaller hernias may be asymptomatic.

Diaphragmatic hernias can develop in isolation or in association with other developmental abnormalities [1]. CDH that arises in association with other abnormalities is often associated with recognized syndromes, commonly including Fryns syndrome, Denys–Drash syndrome, Cornelia de Lange syndrome, Donnai–Barrow syndrome, Wolf–Hirschhorn syndrome, Ehlers–Danlos syndrome and focal dermal hypoplasia [50–56].

Diaphragm morphogenesis requires muscle progenitors to migrate to the developing diaphragm and differentiate into muscle. The deletion of genes required for the delamination and migration of muscle precursors from the somite, such as *HGF* and *Met*, results in a failure of muscle to migrate into the diaphragm from the somite and leads to an amuscularized diaphragm



**Fig. 3.** Major types of congenital diaphragmatic hernias: (A) posterolateral (Bochdalek) hernia; (B) anterior (Morgagni) hernia; and (C) central hernia.

[21,26]. Similarly, the deletion of genes required for myogenesis, such as *MyoD* or *Myogenin*, results in thinner or absent diaphragm muscle [57,58]. Interestingly, the complete loss of muscle in mouse *Met* mutants does not result in herniation of abdominal content through the diaphragm [34]. Thus, the lack of diaphragm muscle is not sufficient to allow herniation of abdominal content through the diaphragm.

Formation of the diaphragm also requires that the connective tissue forms with proper structural integrity, and mutations that inhibit the development of the extracellular matrix result in CDH. Mice with null mutations for *lysyl oxidase*, an enzyme responsible for cross-linking collagen, develop central tendon hernias [59]. The central tendon of these mice is unable to withstand pressure from the growing liver and allows herniation of abdominal content through the weakened connective tissue. Similarly, patients with mutations in *Collagen 3a1* develop CDH [54]. In these patients, the reoccurrence of herniation can occur because of the weakened state of the diaphragm. Together, the *lysyl oxidase* and *Collagen 3a1* mutations indicate that the loss of connective tissue integrity can be a cause of CDH.

The molecular pathway most frequently associated with CDH is the retinoic acid (RA) signalling pathway. Much of the initial work investigating the development of CDH in rodent models used the teratogen nitrofen, which inhibits RA signalling [17,60,61]. Administration of nitrofen, as well as other teratogens that inhibit RA signalling, to pregnant mice or rats induces the development of CDH in their fetuses [62]. Furthermore, simultaneous administration of RA with these teratogens is sufficient to rescue CDH [62]. This confirms that the production of RA is critical for the developing diaphragm. Further evidence for the importance of RA signalling comes from the finding that mutations in *Strat6*, which is involved in retinol uptake and the production of retinoic acid, are present in some humans with CDH [63,64]. Also, mutations in the retinoic acid receptors *RARA* and *RARB* result in CDH in a small percentage of mice [65]. Production of RA requires processing by retinal dehydrogenase. RALDH2, the only retinal dehydrogenase enzyme present in the diaphragm, has been localized by immunofluorescence to the non-muscle cells of the pleuroperitoneal folds [62]. This suggests that the non-muscle cells are the source of RA in the developing diaphragm. An intriguing hypothesis is that RA signals from the non-muscle cells to the muscle cells are important for proper diaphragm morphogenesis.

In addition to mutations associated with RA signalling, other genetic causes underlying CDH have been identified by examining chromosomal abnormalities in

CDH patients. Three of the frequently identified chromosomal regions are 8p23.1, 8q22-23 and 15q26.1-26.2, with the most commonly suggested candidate genes in these regions being *Gata4*, *Zfpm2* (also known as *Fog2*) and *Nr2f2* (also known as *Coup-TFII*), respectively. Significantly, these three genes can interact with one another and have also been proposed to interact with the RA signalling pathway [66,67]. Although *Gata4* has long been implicated in the formation of CDH, until recently, no coding variants had been identified within this gene in CDH patients. Whole exome sequencing of a familial case of CDH has identified a novel variant in a highly conserved arginine residue in the zinc finger domain of *Gata4* that is predicted to be pathogenic [68]. Additional enhancer and intronic variants in *Gata4* in CDH have also been identified [69,70]. Functional analysis of the role of *Gata4* in the formation of CDH in mice is limited because mice with homozygous null mutations of *Gata4* die before the formation of the diaphragm as a result of heart defects. However, in a study of mice heterozygous for a deletion of *Gata4*, 14% of the mice developed anterior congenital diaphragmatic hernias, and 29% of mice had some type of diaphragm defect [43], providing further evidence for *Gata4* being a candidate gene in the 8p23.1 region. *Zfpm2* has also been implicated as being important for CDH and is a binding partner of *Gata4* (*Fog2* is an abbreviation for Friend of *Gata 2*) [71]. Mice with null mutations of *Zfpm2* develop CDH [42]. Further evidence for a role of *Zfpm2* in CDH comes from the finding of a *de novo* mutation resulting in a premature stop in *Zfpm2* in a child with CDH [42]. Finally, *Nr2f2* is located in the 15q26.1-26.2 chromosomal region that is frequently deleted in CDH patients [72]. Although no causal variants within *Nr2f2* have been reported, mice with a conditional deletion of *Nr2f2* in the foregut mesentery develop CDH [73]. In addition to these genes, a multitude of other genes have been implicated by human genetic studies [1]. Also, a recent screen of genes expressed in the developing diaphragm in mouse has identified a list of 27 candidate CDH-causing genes [74].

Despite the frequency with which CDH occurs and extensive research identifying genes implicated in the formation of CDH, little is known about the molecular and cellular mechanisms by which these genetic mutations cause the diaphragm to form aberrantly. First, it is unclear in which tissues of the developing diaphragm mutations in the CDH genes are causative. Immunofluorescence of candidate CDH genes shows that these genes are predominantly expressed in non-muscle cells of the pleuroperitoneal folds [20]. This suggests that expression of CDH genes in these cells is critical. However, conditional deletion of these genes

in particular cells (e.g. the non-muscle cells of the folds) will be necessary to determine in which cells CDH genes are important. Second, although CDH is undoubtedly caused by defects in diaphragm morphogenesis, what particular cells or tissues behave aberrantly is not known. The incomplete development of the diaphragm could be a result of defects in the proliferation and/or survival of myogenic progenitors, aberrant migration of myogenic cells, or defects in muscle differentiation or morphogenesis. Alternatively, the incomplete development of the diaphragm could result from aberrant development of the septum transversum or defects in the formation or spread of the pleuroperitoneal folds. Finally, another unknown aspect of diaphragmatic herniation is the question of why hernias only form in local regions of the diaphragm, as opposed to throughout the entire diaphragm muscle. The local and variable nature of the defects suggests that local changes in levels, timing or the site of gene expression during diaphragm development may regulate the size and location of hernias.

### Comparison of diaphragm and limb muscle development

A comparison of diaphragm and limb muscle development reveals many similarities in their regulation, a few differences, and suggests avenues of future research. Both diaphragm and limb muscles derive from populations of migratory muscle progenitors. These progenitors delaminate from the somites and migrate into either the pleuroperitoneal folds or the limb buds. Migration of muscle precursors to both sites relies on the signalling of HGF to Met<sup>+</sup> muscle precursors to induce delamination and migration [26]. However, although *Lbx1* marks progenitors migrating either into the developing diaphragm or limb [21], unexpectedly, null mutations in *Lbx1* affect only the migration of limb progenitors and not diaphragm progenitors [75,76]. Once in the developing diaphragm or limb, myogenic progenitors go through a similar process of myogenesis whereby progenitors become committed myoblasts, differentiate into myocytes and fuse into myofibres. In the limb, the processes regulating muscle morphogenesis and patterning are beginning to be elucidated. Lateral plate-derived muscle connective tissue is critical for determining the pattern of individual limb muscles [77,78]. How the pattern of costal and crural diaphragm muscles is established is currently unknown. If diaphragm muscle morphogenesis is similar to that in the limb, the diaphragm's muscle connective tissue may be an important determinant of the diaphragm's muscle pattern.

### Conclusions

The diaphragm is a unique mammalian muscle, essential for respiration. Unfortunately, defects in diaphragm development, leading to congenital diaphragmatic hernias, are common birth defects and result in severe morbidity and mortality. Given its functional importance and the frequency of congenital defects, an understanding of the genetic, cellular and morphogenetic mechanisms regulating diaphragm development, both normally and during herniation, is critical. However, many fundamental questions about diaphragm development remain unanswered. Embryonically, the diaphragm derives from three sources: the septum transversum, the pleuroperitoneal folds and the somites. Currently, it is unclear whether the septum and folds are only transient embryonic structures or whether they contribute to tissues of the mature diaphragm. Also unknown is whether the muscle connective tissue and central tendon arise from these structures. Muscularization of the diaphragm requires muscle progenitors to migrate from the somites to the developing diaphragm, proliferate and differentiate, and form costal and crural muscles. Although a few molecular signals (e.g. HGF/Met) have been identified that regulate progenitor migration, the signals and cell-cell interactions regulating muscle differentiation and morphogenesis have not been established. Finally, although a multitude of genes are identified as likely being important in CDH, how mutations in these genes mechanistically lead to hernias has not been determined. Identifying the molecular, cellular and morphogenetic processes that CDH genes regulate should reveal both how the diaphragm develops normally and how defects in diaphragm development lead to these devastating birth defects.

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