The diaphragm serves two very important functions in mammals. First, it is the primary muscle of respiration, and second, it forms a physical barrier between the thoracic and abdominal cavities. In the context of congenital diaphragmatic hernia (CDH), it is perhaps this latter function which is most important to the pediatric surgeon, for if the diaphragm does not form properly, the development of the lungs can be severely impeded. In this overview, we will discuss the current understanding of normal diaphragm development and insights into the pathogenesis of CDH arising from the use of animal models and examination of postmortem tissue. Current concepts regarding the etiology of CDH are discussed elsewhere.1,2

Normal embryology of the diaphragm

The basic structure of the diaphragm is established early in gestation and is intimately linked with the formation of the body cavities. The process can be broken down into several steps which are outlined below. All of the gestational ages referred to are given for rat development unless otherwise specified.

Development of the septum transversum

At embryonic day (E)8 the rat embryo begins gastrulation, forming an essentially flat trilaminar disc (3rd to 4th week in human gestation). Subsequent rapid growth of the cranial neural fold and invagination of the foregut radically changes the shape of the embryo. The presumptive septum transversum, which was located at the anterior aspect of the embryo (where the visceral yolk sac and amnion meet), is displaced such that its position relative to the heart changes from rostral to caudal. In its final resting place, the septum transversum lies caudal to the heart and rostral to the umbilicus.

KEYWORDS
Bochdalek CDH; Pleuro-peritoneal fold; Nitrofen; Vitamin A; WT1
Dunwoodie and coworkers provide a detailed description of this process in the developing rodent using the transcription factor *Mrg1* as a marker for the septum transversum.

**Separation of the body cavities**

As a result of embryonic folding, the septum transversum lies in a position that partially divides the intraembryonic cavity into the pleuro-pericardial cavity and the peritoneal cavity. In the following days, an intricate network of folds develops which separate the pleuro-pericardial cavity into distinct pleural and pericardial cavities. At this stage, the pleural cavity still communicates with the peritoneal cavity via the pleuro-peritoneal canals (PPCs). The separation of these two cavities occurs between E14 and E16 and represents the final stage in the formation of the basic foundation of the diaphragm. A series of scanning electron microscope images published by Kluth and coworkers excellently illustrate the closure of the PPCs.

The networks of folds that separate the body cavities are interrelated. In this article, we use the term pleuro-peritoneal fold (PPF) to describe the transient structure formed at the union of the pleuro-pericardial folds and the septum transversum. As shown in Figure 1, the PPFs are paired, pyramidal-shaped structures that project from the lateral body wall, fusing medially with the esophageal mesentery. The PPF is fully formed by E13.5 of rat gestation; in humans, this structure is clearly visible from weeks 4 to 6 of embryogenesis. Although the PPF is really a structure which is bounded by the pericardium as well as the pleural and peritoneal cavity, we prefer the term PPF for its relative simplicity. Myogenic cells and phrenic axons destined to form the neuro-musculature of the diaphragm migrate to the PPF (Figure 1), and it is their proliferation and distribution that lead to the formation of the mature diaphragm.

**Diaphragm muscularization**

This is the stage of diaphragm development that is perhaps best understood and has undergone considerable revision from earlier attempts to describe diaphragm development. It was previously thought that the musculature of the dia-
phragm was derived from the muscular layers of the body wall, and this view still permeates the literature today. However, with the recent ability to immunologically stain developmentally controlled proteins and the widespread use of transgenic mice, it has become possible to closely follow the process of diaphragm muscularization and elucidate its true origin. It initially became clear that the musculature of the diaphragm had a distinct origin than that of the body wall when the tyrosine kinase receptor \(c-met\) was inactivated in mice. The receptor-protein encoded by \(c-met\) is essential for the delamination and migration of muscle precursor cells (MPCs) from the somites (reviewed by Birchmeier and Brohman); \(c-met\) null-mutant mice notably have an amuscular diaphragm, yet the muscles of the body wall are normal. The phenotype of \(c-met\) null-mutant mice suggested that the diaphragm was populated by a distinct, migratory, population of MPCs. This conclusion was further substantiated when it was found that diaphragm MPCs express \(Lbx1\), a transcription factor only expressed in migratory MPCs. Thus, rather than being a derivative of the ventrally projecting part of the hypaxial myotome, which forms the body wall musculature, the diaphragm is formed by a migratory population of MPCs originating from the lateral dermomyotomal lip, analogous to the MPCs which populate the limb. Further, immunological analysis of diaphragm muscularization in the rat revealed no contribution of MPCs from the body wall. From this study, it is apparent that muscle precursors cells which have migrated to the PPF, proliferate and radiate out from the relative position of the PPF within the diaphragm to populate the entire structure. This process takes place between E15 and E17 of rat gestation and is illustrated in Figure 1. In parallel to the muscularization of the diaphragm, the phrenic nerve also projects to the PPF, from which point it trifurcates and its collateral branches innervate the entire diaphragm.

In summary, during the process of embryonic folding and separation of the body cavities, the basic structure of the diaphragm is formed. It is subsequently populated by migratory muscle precursor cells and the phrenic nerve which are targeted to the PPF; it is from this relative position in the diaphragm that these components spread out to populate the entire tissue. This process is complete by E17 of rat gestation and is shortly followed by the commencement of fetal breathing movements. This corresponds approximately to week 10 of human embryogenesis.

Abnormal embryology of the diaphragm

CDH can be phenotypically characterized into several subtypes depending on the location of the defect or its nature. The most common type of CDH, and the primary focus of this review, is the posterolateral diaphragm defect. Clinically referred to as a Bochdalek hernia, it accounts for greater than 95% of cases and is typically synonymous with the diagnosis of CDH. We will also briefly discuss three rarer types of CDH, including evagination of the diaphragm, defects of the central tendon, and Morgagni hernias. In all of these scenarios, the integrity of the diaphragm as a barrier between the abdomen and thoracic cavity is diminished, allowing the abdominal viscera to protrude into the thorax, forming a space-occupying lesion in this cavity and impairing fetal breathing movements, which together impedes lung development. Although this review focuses on the pathogenesis of the diaphragm defect in CDH, there is an extensive literature discussing lung abnormalities associated with CDH.

Animal models of Bochdalek CDH

In the 1970s, toxicological studies of the herbicide nitrofen (2,4-dichloro-phenyl-p-nitrophenyl ether) showed that, although relatively harmless to adult rodents, nitrofen induced developmental anomalies in the lungs, hearts, diaphragms, and skeletal tissues of fetuses exposed in utero. Further study showed that diaphragm defects could be induced by administering a single 100-mg dose of nitrofen to pregnant rats, typically between E8 and 11; and most significantly, the defects produced were remarkably similar to those documented in human Bochdalek CDH with respect to their size, location of the defect, and accompanying intrusion of the abdominal viscera into the thoracic cavity (Figure 2). In addition to nitrofen, three other CDH-inducing teratogens have been recently identified: 4-biphenyl carboxylic acid (BPCA), bisdiamine [N,N’-octamethylenebis (dichloroacetamide)], and SB-210661. BPCA is a breakdown product of a thromboxane-A\(_2\) receptor antagonist, bisdiamine is a spermatogenesis inhibitor, and SB-210661 is a benzofuranyl urea derivative developed for inhibiting 5-lipoxygenase. Although these drugs were designed for very different purposes, they have chemical structures similar to each other, and to nitrofen—hinting at a common mechanism of action. Importantly, each of the four teratogens have their effect in the same critical period of diaphragm development and produce diaphragm defects in developing rats that are similar to those in infants with CDH. With regards to the etiology of CDH, it has also been shown that all of these compounds can inhibit the retinoic acid synthesizing enzyme, retinol dehydrogenase-2 (RALDH2).

The nitrofen model of CDH has been most effectively used to discern at what stage of diaphragm formation the defect that leads to Bochdalek CDH arises. Initial studies into the pathogenesis of the defect demonstrated that abnormal phrenic nerve innervation or myotube formation were not responsible for the diaphragm defect and that the often cited theory that the defect is due to abnormal closure of the PPC was refuted. Subsequent studies combining the nitrofen model and transgenic mice were also inconsistent with the hypothesis that the primary defect in CDH is pulmonary, such that abnormal lung development somehow
induces the diaphragm defect. This hypothesis implied that primordial diaphragm embryogenesis is regulated or influenced directly by the development of the adjacent lung tissue.\textsuperscript{29} Transgenic mice with \textit{Fgf10} inactivated do not develop lung tissue and therefore provided an excellent tool to address this issue.\textsuperscript{30} Despite having essentially no lungs, \textit{Fgf10} null-mutant mice have normal diaphragms; defects in the diaphragm can be induced by teratogen exposure in the absence of lung tissue. Thus, it was concluded that diaphragm development is independent from lung organogenesis and that diaphragm defects in CDH appear to be a primary defect and are not a secondary result of lung hypoplasia.\textsuperscript{28,31} This conclusion is supported by so-called \textit{experiments of nature}; the literature contains numerous cases reports of lung agenesis in humans with normal diaphragm development.\textsuperscript{32-34}

In addition to refuting several historical hypotheses on the origin of CDH, data from the nitrofen model provided a foundation for the alternate hypothesis that it is a malformation of the primordial diaphragm tissue, the PPF, which underlies the pathogenesis of CDH. The PPF is best visualized at E13.5 of rat gestation. In nitrofen-exposed fetuses examined at this age, it is clear that the postero-lateral portion of the PPF is malformed (Figure 2). Notably, it is the postero-lateral area of the diaphragm that is missing in older rodent fetuses, and in humans with CDH. Importantly, in addition to nitrofen-exposed animals, PPF defects have been observed in rats bred on a Vitamin A-deficient diet and mice with a functionally inactivated \textit{wnt1} gene—both of which also have Bochdalek CDH.\textsuperscript{35} Further, a PPF defect has been hypothesized to be associated with Bochdalek CDH described in the recent mouse model engineered to

\begin{figure}[h]
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\caption{The nitrofen model of Bochdalek CDH. (A) Plan view of an E16.5 rat diaphragm exposed to nitrofen with a typical left-sided diaphragm defect (defective area bound by dashed line). (B) Transverse section of an E13.5 rat embryo exposed to nitrofen; the dashed line represents the extent of tissue missing from the left PPF. (C and D) Three-dimensional reconstructions of the PPFs from control (C) and nitrofen-exposed (D) E13.5 rat embryos, highlighting the location and extent of the abnormality (*).}
\end{figure}
have a conditional inactivation of the COUP-TFII gene.\textsuperscript{36} Thus, abnormal PPF development is a common pathogenic feature in these very different animal models of CDH.

The identification of an abnormal PPF in nitrofen-exposed fetuses meant that the embryogenesis of this structure became a major focal point toward elucidating the pathogenesis of CDH. Studies examining muscle precursor migration to the PPF and subsequent proliferation and differentiation of myoblasts in nitrofen-exposed rodents did not reveal any obvious abnormalities in myogenesis, suggesting that it was the nonmuscular substratum of the PPF that was abnormal.\textsuperscript{25} This has led to our current hypothesis that the mesenchymal component of the PPF is defective and does not provide a complete foundation for the formation of the diaphragmatic musculature. This hypothesis was tested using \textit{c-met} null-mutant mice (as described above) which have a diaphragm with no muscle, and is merely comprised of a connective tissue sheet, offering the opportunity to clearly visualize the formation of the amuscular component of the diaphragm. Examination of teratogen-exposed \textit{c-met} null-mutant mice demonstrated that diaphragm defects can be produced independently of myogenesis.\textsuperscript{28} As such, we believe that muscle precursor cells migrating to a malformed PPF accumulate in the remaining normal tissue and that this manifests as a thickening of the diaphragm around the defect. Correspondingly, it has been shown that thickening of the diaphragm associated with Bochdalek CDH is a common feature of animal models, and that examination of postmortem tissue from humans shows a similar gross pattern of muscularization, consistent with the hypothesis that the defect arises from a malformation of the PPF.\textsuperscript{35}

This hypothesis is in contrast to other theories proposed to explain the pathogenesis of CDH, specifically that nonclosure of the PPCs or a defect in muscularization gives rise to the hole in the diaphragm.\textsuperscript{37} The PPF mesenchyme forms between the 5th and 7th weeks of gestation in humans, before closure of the PPC and muscularization of the diaphragm; thus, a reconsideration of the developmental stage at which the anomaly occurs is warranted. Further, future research should focus on gaining a better understanding of the nonmuscular component of the diaphragm and how it is malformed in CDH.

### Eventration of the diaphragm

Diaphragmatic eventration is a relatively uncommon class of CDH. It is characterized by incomplete muscularization of the diaphragm, allowing the abdominal contents to protrude into the thoracic cavity in the areas where no muscle has formed, and the diaphragm is subsequently weaker. The pathogenesis has not been thoroughly studied; however, the recent characterization of mice expressing a mutant form of \textit{Fog2}, which have a phenotype consistent with diaphragmatic eventration as seen in humans, provides an excellent animal model to further study this rare subtype of CDH.\textsuperscript{38}

### Central tendon defects of the diaphragm

Central tendon defects are characterized by congenital herniation of abdominal contents through the central tendon of the diaphragm. The embryogenesis of this defect is poorly understood; failure to form, rupture, or stretching of the central tendon due to an underlying weakness have all been suggested to explain this defect.\textsuperscript{8} Mice with a null mutation in the \textit{Slit3} gene have central tendon defects

![Figure 3](image.png)

**Figure 3** A schematic representation of a transverse section through the cervical region of an E13.5 rat embryo, illustrating a hypothetical model for the development of Bochdalek CDH.
similar to those seen in humans, shedding light on the etiology of this defect and providing a novel tool for further study.39,40

**Morgagni hernia**

This rare anterior defect of the diaphragm is variably referred to as Morgagni, retrosternal, or parasternal hernia. It accounts for only ~5% of all CDH cases. Interestingly, the incidence among children with Down’s syndrome may be as high as 1:1000.41 It is characterized by herniation of abdominal contents through the foramen of Morgagni; small triangular areas of the diaphragm adjacent to the lower end of the sternum. Embryologically, this area is considered congenitally weak and that this is the source of herniation when it occurs.8 There is no mutant mouse model with a clear Morgagni-type phenotype. However, the golden lion tamarin (Leontopithecus rosalia), a small endangered primate of the Atlantic coastal rainforest of Brazil, may provide a very useful model of this specific type of CDH. The incidence of diaphragmatic Morgagni defects among newborn tamarins is ~9%, with a definite heritable basis.42,43 Although the precise mechanism has not been determined, the pattern is suggestive of a simple autosomal recessive mode of inheritance. Clearly, the genetic mutation(s) that underlies the Morgagni-type diaphragmatic defect has penetrated the tamarin genome to a striking degree and merits further investigation.

**Conclusion**

The improved understanding of diaphragm formation in normal and pathological circumstances has allowed us to construct a hypothetical model describing the origin of Bochdalek CDH (Figure 3). Central to this hypothesis is the PPF, a transient structure which forms early in diaphragm development and is the target for migratory muscle precursor cells and the phrenic nerve. Evidence from animal models suggests that it is a malformation of the nonmuscular component of the PPF that underlies the defect in the mature diaphragm. Further, there is indirect evidence to suggest that this may also be the pathogenic origin in human cases of Bochdalek CDH. The recent identification of mutant mice with phenotypes consistent with rarer subtypes of CDH has started to provide important new models to better understand these specific malformations.

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**References**


