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Analysis of the Role of Dystroglycan in Early Postimplantation Mouse Development[a]

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Dystroglycan was first identified in skeletal muscle as a component of the dystrophin-glycoprotein complex (DGC).[1] It is composed of two noncovalently linked α- and β-subunits that are posttranslationally derived from a single gene (*DAG1*).[2] In skeletal muscle, α-dystroglycan, is an extracellular 156-kD glycoprotein that binds to laminin-2 in the muscle cell basement membrane,[2] and β-dystroglycan is a transmembrane 43-kD glycoprotein that binds intracellularly to the carboxy-terminal region of dystrophin.[3] Thus dystroglycan spans the sarcolemma to provide a direct connection between the extracellular matrix and cytoskeleton.[4] Mutations in a number of genes encoding DGC components disrupt this dystroglycan-mediated linkage and lead to various forms of muscular dystrophy.[5] To date, however, no form of muscular dystrophy has been linked to *DAG1*. Dystroglycan is also expressed in a wide variety of nonmuscle tissues and in diverse cell types. Indeed, other studies point toward developmental roles for dystroglycan in the formation of the neuromuscular junction[6] and in epithelial morphogenesis.[7]

We have investigated the expression and function of dystroglycan during early embryonic development of the mouse. Immunohistochemical analyses of early mouse embryos reveal that prior to gastrulation, dystroglycan expression is localized predominantly to extraembryonic tissues, including Reichert's membrane (FIG. 1(A)). After gastrulation, dystroglycan is strongly expressed in the developing myocardium and continues to be expressed in extraembryonic tissues. This early expression of dystroglycan precedes the development of skeletal muscle. In order to understand better the involvement of dystroglycan in development and/or disease, we have generated a null allele of dystroglycan in mice.[8] *Dag1*-null embryos do not gastrulate and fail to progress beyond the egg cylinder stage. In *Dag1*-null embryos, we describe an early defect in the structure and function of Reichert's membrane — an extraembryonic basement-membrane structure containing laminin and collagen IV (FIGS. 1 and 2). This defect is not due to failed differentiation or migration of parietal endoderm cells that secrete the extracellular matrix components of Reichert's

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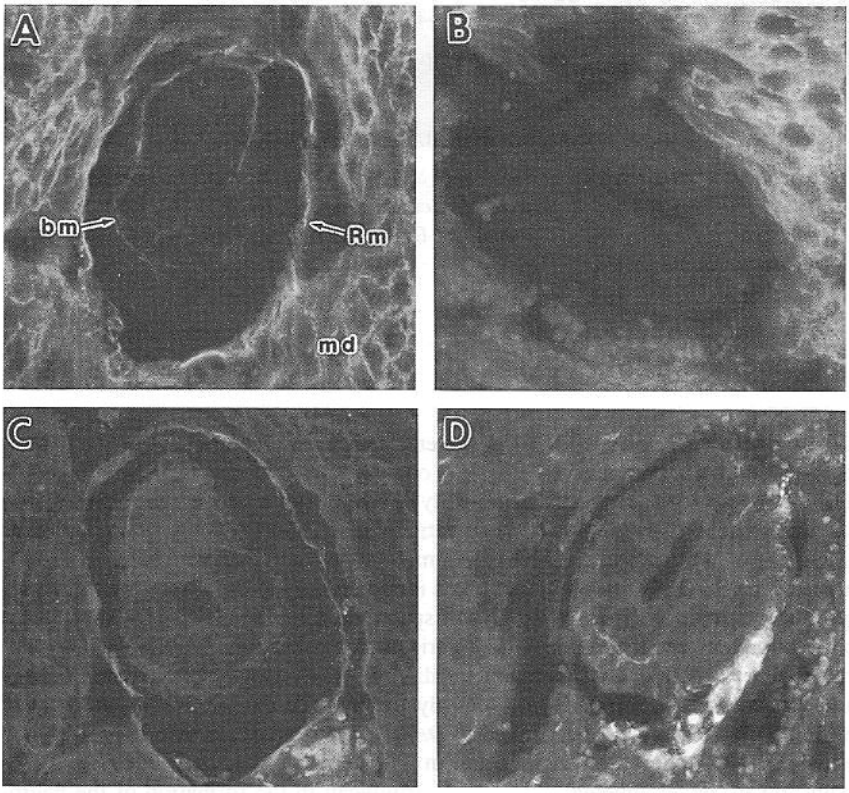

FIGURE 1. Immunofluorescence analysis of control **(A)**, **(C)** and dystroglycan-null **(B)**, **(D)** littermates at E5.5. Panels (A) and (B) show dystroglycan staining. Dystroglycan is normally localized in apposition to Reichert's membrane (Rm) and a basement membrane (bm) between the visceral endoderm and ectodermal layers in the egg-cylinder-stage embryo (A). Staining is also evident in the maternal decidual tissue (md) surrounding the implantation site. In dystroglycan-null embryos (B) staining is absent in the conceptus but persists in the maternal tissue. Panels (C) and (D) show laminin staining in serial sections of the same embryos shown in (A) and (B), respectively. Laminin is colocalized with dystroglycan in basement-membrane structures of the conceptus in control embryos (C), but this pattern is severely disrupted in dystroglycan-null embryos. Instead of continuous staining throughout Reichert's membrane, the staining associated with this structure is discontinuous and patchy. However, laminin staining in the basement membrane between the visceral endoderm and ectodermal layers remains continuous (it runs out of the plane of section in this example). This phenotype is further diagrammed in FIGURE 2.

membrane. Rather, it appears that dystroglycan function is required for the organization or assembly of extracellular matrix components into a basement-membrane structure. These data indicate that dystroglycan plays a critical role in tissue morphogenesis.

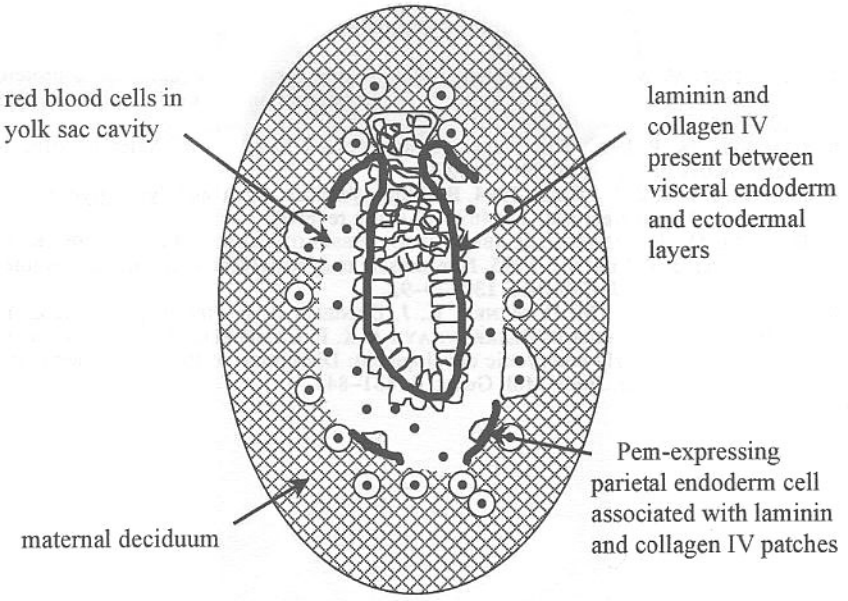

FIGURE 2. Schematic diagram of *Dag1*-null phenotype. The salient features of the *Dag1*-null phenotype in egg-cylinder-stage embryos are depicted. Laminin and collagen IV staining is disrupted in Reichert's membrane. However, parietal endoderm cells (marked by expression of Pem protein) that secrete the extracellular matrix molecules differentiate and migrate in the *Dag1*-null embryos, indicating that the disruption of Reichert's membrane is not a secondary consequence of a loss of these cells. Since laminin and collagen IV are the major structural proteins making up Reichert's membrane, the barrier to maternal blood normally presented by Reichert's membrane is contained in *Dag1*-null embryos, leading to the influx of maternal blood into the yolk sac cavity.

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