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Induction of and by the AER

Recent experiments (Laufer et al., 1997; Rodriguez-Esteban et al., 1997; Tanaka et al., 1997) demonstrate that the apposition of dorsal and ventral ectoderm from the chick limb bud is necessary to cause the formation of an AER. When dorsal limb bud ectoderm was grafted into the ventral ectoderm of another limb bud, a new AER was formed in addition to the original one. It appears that at stage 15 (just prior to limb bud formation), the dorsal ectoderm is synthesizing a secreted protein called Radical fringe**.** As the limb buds emerge (at stage 17), there is a sharp demarcation between the dorsal cells that express the *radical fringe* gene and the ventral ectoderm cells that do not. As the bud continues to grow, the expression of *radical fringe* becomes restricted almost exclusively to those dorsal ectoderm cells at the dorsal/ventral limb bud border. These cells begin to express *Fgf8*, and they become the AER.

The importance of the radical fringe-expressing and non-expressing border is confirmed by studies wherein this gene is expressed ectopically on retroviruses. If ventral limb bud cells are infected with a retrovirus expressing *radical fringe*, a new boundary between cells expressing *radical fringe* and those not expressing it is created, and a new AER is generated there. Conversely, if the ectopic expression of *radical fringe* destroys the existing boundary between expressing and non-expressing cells, that region of the original AER does not form.

The formation of the AER may involve the interaction between the secretion of FGFs (such as FGF10) from the mesoderm and the boundary of *radical fringe* expression along the dorsal-ventral ectoderm border. The limited secretion of FGFs may be critical in localizing which cells along the dorsal-ventral flank of the embryo produce the limb buds. While it is still not known how the *radical fringe*

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In *Drosophila* limb discs, Fringe will interact with Notch by adding a carbohydrate moiety to it (Brückner et al., 2000). This lessens the activity of Notch. A similar system might also exist in the vertebrate limb. Serrate 2, a ligand for Notch, is found in the AER, and mutations of Serrate-2 in mice form an abnormal and enlarged AER (Sidow et al., 1997).

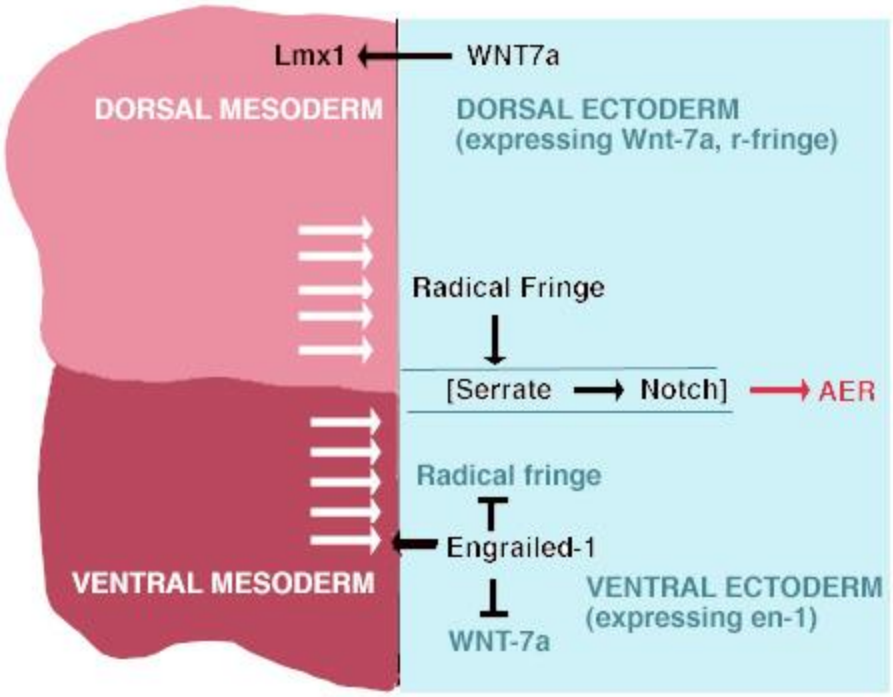

Figure 1 Model for the formation of the AER at the boundary between the dorsal and ventral regions of the chick limb. Expression of Engrailed represses radical fringe and Wnt-7a in the ventral ectoderm. Wnt-7a is expressed only in the dorsal ectoderm, and it induces Lmx-1 in the underlying dorsal ectoderm. Radical fringe is likewise expressed in the dorsal region, with some entering slightly into the ventral side. The AER forms at this junction between the dorsal and ventral sides. The Notch signaling pathway appears to be important in relaying the dorsal ventral signal into the cells that will form the AER. (After Laufer et al., 1997.)

Limbless and *legless* mutations

The need for a dorsal-ventral boundary to form the AER is shown in two mutants: *limbless* and *legless*. In *legless* mice, the ventral ectoderm is dorsalized and there is thus no dorsal-ventral boundary. Wnt7a is found throughout the entire ectoderm. Similarly, the mesoderm is dorsalized by the Wnt-7a induction of Lmx-1. The AER cannot form properly in these mutants (Bell et al., 1998).

Chick embryos homozygous for the *limbless* mutation initiate the limb bud formation, but the AER fails to form. Recombination experiments show that the *limbless* ectoderm is unable to form an AER, even when placed on wild-type limb mesoderm. A normal AER can form when normal ectoderm is grafted to the limb field in place of the mutant ectoderm (Carrington and Fallon, 1988).

Although the phenotype of the *legless* chick embryo is difficult to explain (see Grieshammer et al., 1996; Noramly et al., 1996; Ros et al., 1996), at least some of it has to do with boundary formation. The *limbless* limb bud ectoderm expresses neither FGF4 nor FGF8. However, the *limbless* mesoderm expresses the Hoxd-11 through Hoxd-13 genes in a posteriorly nested fashion, along with the asymmetrical expression of BMP4 and Wnt5a. It does this in the absence of detectable sonic hedgehog expression or an AER. This raises the possibility that the lateral plate mesoderm already has the ability to express the anterior-posterior and proximal-distal patterning genes, and that this pre-pattern is subsequently stabilized, maintained, or augmented by the AER and Sonic hedgehog.

The *limbless* buds *will* form AERs and limbs if given beads secreting FGFs. Moreover, such beads induce Sonic hedgehog in the posterior region, showing that there is a polarity to the limb bud, even in the absence of the AER. However, the limb formed is a "bi-dorsal" limb, expressing Wnt7a throughout the ectoderm. This confirms that a dorsoventral ectodermal interface is necessary for AER induction.

The limb is a complicated organ, and new research makes it seem even more complex. There are several other genes whose function is needed in the formation of the AER, but whose role is not yet established. The *limb deformity* gene and the NF-kB transcription factor are both needed in the progress zone mesenchyme during the early stages of limb development (Kuhlman and Niswander, 1997; Bushdid et al., 1998; Kanegae et al., 1998); however, it is not known how their actions relate to the formation of the AER in the ectoderm. The analysis of development of the tetrapod limb has provided biologists with some of its greatest successes in understanding development, but it also keeps posing some of our greatest challenges.

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