WNT signals control FGF-dependent limb initiation and AER induction in the chick embryo

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The establishment and consolidation of specific interactions between instructive signaling pathways is a key characteristic of embryonic development. During the development of any given structure or organ, several signaling mechanisms cooperate in providing positional information to cells in the corresponding developmental fields. In many cases, this «cross-talk» of signaling pathways involves interactions between different tissues of the developing embryo, with sequential (and often directional) transfer of positional information from one tissue to another in a very stereotyped way. If development is to proceed smoothly, the cross-talk between signals must be tightly regulated in space and time, so that the flux of positional information between tissues has the direction and intensity required at any specific time during development of each organ or structure.

One of the best examples of signaling molecules involved in the complex cross-talk mechanisms that pattern developing embryos is the Fibroblast Growth Factor (FGF) superfamily of secreted factors. FGFs play important roles in multiple aspects of embryonic development in a variety of organisms. One of their most interesting activities is related to their involvement in limb

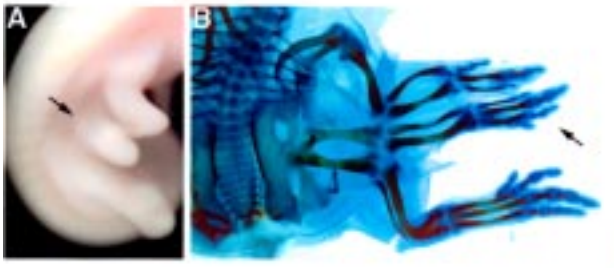

Fig. 1. *Wnt-2b* has limb inducing activity. (A) *RCAS-Wnt-2b infected CEFs were implanted into the LPM of stage 13/14 embryos. Three days after implantation, development of an ectopic limb bud (arrow) is observed in the flank.* **(B)** *When embryos were allowed to develop longer, a complete limb developed.*

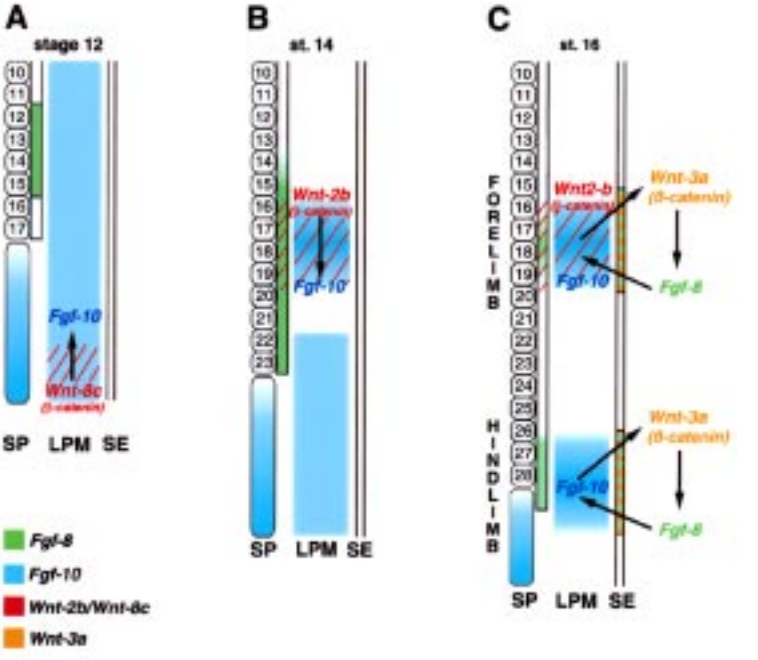

Fig. 2. An expanded model of early limb determination in the chick embryo. (A) *Prior to limb initiation (stage 12), Fgf-8 (in green) is expressed in the IM adjacent to the presumptive forelimb area. Fgf-10 (in blue) is expressed at the same level in the LPM, and also very strongly in the segmental plate (SP) and diffusely in the caudal portion of the LPM, where it co-localizes with Wnt-8c (in red). Wnt-8c may be regulating Fgf-10 in this area, as suggested by the ectopic induction of Fgf-10 by Wnt-8c-expressing cells implanted in the flank. Somites are indicated with their corresponding numbers.* **(B)** *During limb induction (stage 14 is shown here), both Fgf-8 (in green) and Wnt-2b (in red) are expressed in the IM. Wnt-2b is also expressed in the LPM of the presumptive forelimb area. WNT-2B signals through ß-catenin to induce and/or maintain Fgf-10 specifically in the prospective forelimb. Fgf-10 is still present in the caudal LPM in a diffuse pattern. Nonetheless, a gap of Fgf-10 expression between the prospective fore and hindlimb bud starts to be observed.* **(C)** *At stage 16, expression of Wnt-2b and Fgf-10 is confined to the presumptive forelimb bud field in the LPM. In the posterior region of the embryo, expression of Fgf-10 is now resolved and confined to the presumptive hindlimb bud field. We speculate that prior expression of Wnt-8c in the caudal LPM contributes to this restriction of Fgf-10. This is consistent with Wnt-8c-expressing cells inducing Fgf-10 expression 8 hours after being implanted in the flank, so that WNT-8C, present in the caudal part of the embryo at stage 12, may conceivably restrict Fgf-10 to the presumptive hindlimb region at stage 16 (approximately 8 hours later). In the LPM of the presumptive limb areas, FGF-10 signals to the surface ectoderm (SE) to induce Wnt-3a (in yellow) resulting in activation of Fgf-8. Not all expression patterns are shown, for simplicity. For instance, Fgf-8 and Wnt-2b are expressed in the somites, Fgf-8 is expressed in the segmental plate, and Fgf-10 is expressed in the IM. While we acknowledge that other gene interactions could be at play, we only show in this simplified model the expression patterns and events relevant to the problems addressed experimentally in the manuscript.*

Note: The color version of these Figures is available at http://www.ijdb.ehu.es/01suppcontents.htm

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development in vertebrates. Specifically, several FGFs have been shown to play key roles in the control of limb initiation, the induction of the apical ectodermal ridge (AER), and the activity of the AER itself. Due to the importance of the vertebrate limb bud as a model system for the study of pattern formation, the analysis of the mechanisms of action of FGFs, as well as the study of this experimental system, is of paramount importance if we are to understand the multiple instructive activities of FGFs during development.

The current models of limb initiation and AER induction in vertebrates stress the role of a regulatory loop between two members of the FGF superfamily: FGF-8 and FGF-10. Among the possible candidates for engaging in regulatory cross-talk with FGFs during limb initiation and AER induction, the WNT superfamily of secreted factors is particularly interesting. FGFs and WNTs have been shown to interact in a variety of developmental systems, including tracheal development in *Drosophila*, mesoderm induction and neural patterning in *Xenopus*, and brain, tooth and kidney development in other vertebrates. During vertebrate limb outgrowth, the *Wnt-3a* gene has been shown to act upstream of *Fgf-8* during AER induction in the chick limb. So far, however, no *Wnt* gene has been implicated in limb initiation, and the mechanisms by which *Wnt-3a* participate in AER induction and *Fgf-8* activation are not fully understood.

We show that specific instances of cross-talk between WNTs and FGFs control limb initiation and AER induction in the chick embryo. The *Wnt* genes, *Wnt-2b* (expressed in the intermediate mesoderm [IM] and the lateral plate mesoderm [LPM] at the forelimb level) and *Wnt-8c* (expressed in the LPM at the hindlimb level), in a ß-catenin-dependent process that results in activation of *Fgf-10,* are both capable of inducing ectopic limbs in the embryonic flanks. Moreover, a third *Wnt* gene, *Wnt-3a*, mediates the induction of *Fgf-8* in the limb ectoderm by FGF-10, in a process also mediated by ß-catenin. Thus, three *Wnt* genes that signal through ß-catenin mediate the FGF-8/FGF-10 loop that directs limb initiation and AER induction in the chick embryo.

The data to be presented provide a specific example of cross-talk between signaling pathways that results in a stereotyped flux of positional information between the tissues involved in the early control of limb development. This kind of specific interaction between FGF and WNT signals may be an efficient mechanism to control the timing and directionality of inductive signals in a variety of developmental processes. It will be interesting to investigate whether WNT pathways also interact with FGFs in other regions of the embryo where FGFs are also known to play an organizing role, such as the brain, lungs and other structures. Overall, our results underscore the importance of cross-regulation between signaling pathways, which ensures a fine-tuning of the activities of organizing factors that shape the developing embryo.