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Perspective Article

Hylonome

Wnt-signaling and skeletogenesis

C. Hartmann

Department of Genetics, Harvard Medical School, Boston, MA, USA

Abstract

Members of the Wnt gene family, encoding secreted cystein-rich glycoproteins, have been isolated from a variety of organisms. They serve as important developmental signaling molecules and have been implicated to play crucial roles in such diverse processes as cancer, organogenesis and pattern formation. Experiments by Zakany and Duboule[10], and Rudnicki and Brown[11] have suggested a role for Wnt molecules in negatively regulating chondrogenesis. However, neither of the two Wnt genes used in these studies is endogenously expressed in chondrogenic regions. We and others have found that in the chick limb at least four members of the Wnt gene family, Wnt-4, Wnt-5a, Wnt-5b, and Wnt-14, are expressed in defined regions of the developing chondrogenic elements. With the exception of Wnt-5b, which is expressed in perichondrial cells and prehypertrophic chondrocytes, the expression of the three other Wnt genes is restricted to the perichondrium surrounding the cartilage element. Viral misexpression studies in the chick suggested that Wnt-4 acts as a positive signal originating from the joint region and when misexpressed accelerates chondrocyte maturation, while Wnt-5a and Wnt-5b both negatively regulate chondrocyte maturation. We have further shown that they utilize different signaling pathways; while Wnt-4 signals through the canonical Wnt-pathway, Wnt-5a and Wnt-5b do not. Interestingly, the delay in chondrocyte maturation due to Wnt-5a misexpression is associated with an up regulation of Wnt-5b expression in the prehypertropic chondrocytes. Concomitantly, Wnt-5b misexpression also delays chondrocyte maturation. However, preliminary studies suggest that the two Wnt genes affect different steps in the maturation process. Wnt signaling, however, is not only regulating chondrogenesis but is also involved in the segmentation process of the appendicular skeleton. Localized misexpression of the fourth Wnt gene, Wnt-14, which is expressed early in the presumptive joint region, induces morphological and molecular changes indicative of an early joint interzone, suggesting that Wnt-14 plays a pivotal role in the induction of the joint interzone.

Keywords: Wnt, Chicken, β-catenin, Chondrogenesis, Synovial Joint

Wnt-proteins, secreted glycoproteins, serve as important signaling molecules during development of invertebrates and vertebrates[1-3]. They have been shown to play crucial roles in such diverse processes as cancer, organogenesis and pattern formation. To date, nineteen Wnt genes have been isolated in higher vertebrates, seven have been found in the genome of *Drosophila*, and five in the C. elegans genome (for more details see Wnt-homepage: http://www.stanford.edu/~rnusse/wntwindow.html). Wnt genes are defined by their sequence similarity to the founding members Wnt-1 in the mouse (originally called int-1[4,5]) and wingless (wg) in *Drosophila*[6,7]. The genetic analysis of the wg signaling pathway in *Drosophila* has led to the identification of many downstream components, which have been shown to be functionally conserved in other organisms. Wg/Wnt-proteins are thought to signal through seven-transmembrane receptors encoded by the frizzled (Fz) gene family to regulate the stability of an effector protein known as armadillo (arm) in flies or β-catenin (βcat) in vertebrates which eventually leads to the activation of target genes through a complex of arm/β-cat with DNA-binding transcription factors of the TCF/LEF family[2,4]. This pathway is now referred to as the canonical Wnt-pathway. In addition to the canonical pathway, there is evidence from other systems for the existence of two alternative Wnt signaling pathways, the non-canonical Ca^{2+} pathway[8] and the planar polarity pathway[9].

In recent years we and others have provided evidence that Wnt signaling in the chick is involved in a variety of processes associated with skeletogenesis such as chondrogenesis[10-14] and joint development[15]. We have previously shown that there are at least three Wnt genes, Wnt-4, Wnt-

Corresponding author: Christine Hartmann, Institute for Molecular Pathology, Dr. Bohr-Gasse 7, 1030 Vienna, Austria
E-mail: hartmann@imp.univie.ac.at

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5a, and Wnt-5b, as well as components of the canonical Wnt signaling pathway expressed in chondrogenic regions[14] and that there is a fourth Wnt gene, Wnt-14, which is expressed early in the joint forming region[15] (Fig. 1d). Wnt-4 is also expressed in regions of the joint, however, its expression is restricted to cells in the periphery of the joint interzone (Fig. 1c). Wnt-5a expression is restricted to cells in a region of the perichondrium which will develop into the periosteum (Fig. 1a), while the closely related Wnt-5b gene is expressed in a subpopulation of prehypertrophic chondrocytes as well as cells of the outer layer of the perichondrium (Fig. 1b).

The observation that there are multiple Wnt genes expressed in adjacent regions of the chondrogenic element raises the question whether they all have similar or different effects on chondrogenesis. We have previously addressed this question using a gain-of-function approach in the chick. Although, retroviral mediated overexpression of all three Wnt genes, Wnt-4, Wnt-5a, and Wnt-5b, resulted phenotypically in a shortening of the affected long bones, the underlying mechanism seems to be quite different in each case[14] (C. Hartmann and C. Tabin, unpublished observation). In particular, shortening of the long bones in the case of Wnt-4 misexpression is due to an acceleration in the chondrocyte maturation process, resulting in an accumulation of more terminal differentiated hypertrophic chondrocytes at the expense of the immature round chondrocytes at the ends of the cartilage element. In addition, we observe an increase in the bone collar thickness in response to Wnt-4 misexpression, which suggests that Wnt-4 could also have a direct effect on bone collar maturation. In contrast, misexpression of both Wnt-5a and Wnt-5b delay chondrocyte maturation. In cartilage elements where we overexpress either Wnt-5a or Wnt-5b we observe a temporally delayed appearance of mature, hypertrophic chondrocytes. This delay is more dramatic in the Wnt-5b infected samples, where we not only see, similar to Wnt-5a, a delayed appearance of hypertrophic, ColX expressing chondrocytes, but where we also see an effect on prehypertrophic chondrocytes, which is reflected in a reduced expression of Ihh, a marker for prehypertrophic chondrocytes (unpublished observation). We are currently favoring a model where Wnt-5a signals from the periosteum to the prehypertrophic chondrocytes negatively regulating their differentiation into hypertrophic chondrocytes. We further think that this negative effect of Wnt-5a on the chondrocyte maturation process is mediated at least in part through Wnt-5b, which is normally expressed in a subpopulation of prehypertrophic chondrocytes and is up regulated in response to Wnt-5a misexpression.

The fourth Wnt gene, Wnt-14, plays a different role during skeletogenesis[15]. Wnt-14, like Wnt-4, is expressed in the joint regions separating the individual skeletal elements (Fig. 1c, d). However, unlike Wnt-4, it does not control the chondrocyte maturation process within the adjacent cartilage elements, instead we think that it plays a crucial role in the initiation of the joint interzone formation. Wnt-14, similar to the early joint marker Gdf-5, is expressed in the presumptive joint region. Furthermore, localized, ectopic expression of Wnt-14 in the prechondrogenic region prevents the infected chondroblasts from differentiating into chondrocytes, instead these cells start to express markers characteristic for the early joint interzone and down regulate the expression of chondrogenic markers, such as Sox9 and Collagen II. The latter are also down regulated during normal joint development. The morphological, histochemical, and molecular changes induced by the ectopic expression of Wnt-14 are characteristic of an early endogenous joint interzone, this together with the endogenous Wnt-14 expression suggests that Wnt-14 is playing a role in the normal segmentation process of the appendicular skeleton. Wnt-14 continues to be expressed in structures of the mature joint, such as synoviocytes and cells of the joint capsule. This late expression is particularly interesting in light of degenerative diseases affecting joint integrity, such as RA and OA.

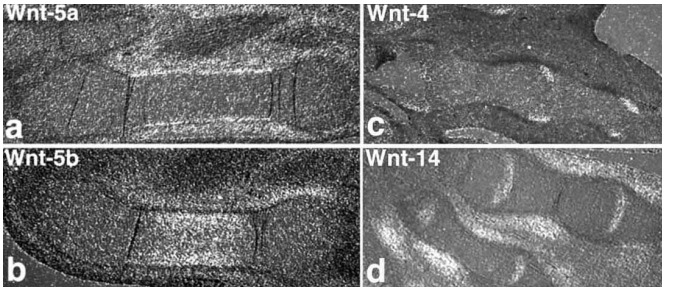

Figure 1. Radioactive section *in situ* hybridizations showing expression of the four Wnt genes, Wnt-5a (a), Wnt-5b (b), Wnt-4 (c) and Wnt-14 (d), in chondrogenic regions of the developing appendicular skeleton. The white signal illustrates gene expression. (a, b) ulna region at day 7.5. (c, d) digit regions of the foot at day 7.5.

The phenomenon that various Wnts regulate chondrocyte differentiation and maturation raises the question whether all of these Wnt molecules signal through the same intracellular pathway, but activate different target genes, or whether they use divergent signaling pathways. We are very interested in this question and attempt to address it by using dominant-positive and dominant-negative versions of the different intracellular signaling components. Preliminary results from these experiments provided the first insights showing that the two Wnts, Wnt-4 and Wnt-5a, signal through distinct pathways. Specifically, misexpression of a stabilized form of β-catenin, which acts as a dominant-activated molecule and mimics the intra-cellular activation of the canonical Wnt pathway, results in a phenotype, which on the morphological and molecular level is similar to the one observed after Wnt-4 misexpression. This observation suggests that Wnt-4 is acting through the canonical pathway. The phenotypic consequences observed after misexpressing the activated β-catenin virus, however, do not resemble the Wnt-5a phenotype, especially since we do not see an up regulation of the Wnt-5b expression. We therefore think that Wnt-5a is likely to signal through a different pathway than

the canonical pathway. We are currently investigating whether downstream effector molecules of the other two Wnt signaling pathways, the non-canonical Ca^{2+} or the planar polarity pathway, are involved in mediating the Wnt-5a effect.

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