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Comparative analysis of the expression patterns of *Wnts* during chick limb development

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Abstract *Wnts* control a number of processes during limb development—from initiating outgrowth and controlling patterning, to regulating cell differentiation in a number of tissues. We analyzed the expression pattern of various *Wnts* (4, 5a, 5b, 6, 11, and 14) in whole mount *in situ* hybridization during chick wing development. From HH stage 26, expression of *Wnt 4* is observed in the central elbow region and wrist-forming regions, and during later stages, expression is seen in the joint-forming regions of the whole limb. *Wnt 5a* is expressed throughout the limb mesenchyme during early limb developmental stages, and later, at HH stage 23, it becomes predominantly confined to the distal tip, leaving low expression levels proximally. At HH stage 29, expression at the distal tip is restricted to the interdigital regions, and at day 8, expression is seen in the region surrounding the phalanges. *Wnt 5b* expression is first observed in the AER at HH stage 20 and later in the dorsal and ventral mesenchyme surrounding the cartilage elements of the limb. Expression of *Wnt 6* is observed from HH stage 17 until day 8 in the dorsal and ventral ectoderm and also in the dorsoventral limb boundaries. Expression of *Wnt 11* is observed in the proximal dorsal mesenchyme of the limb from HH stage 23 onward and later in the dorsal and ventral subectodermal mesenchyme and in the regions adjacent to the digits at day 8. Weak expression of *Wnt 14* is observed at the proximal mesenchyme of the limb at HH stage 23; later, it extends as a transverse strip surrounding the cartilage elements as well as in the interdigital mesenchyme.

Keywords Chick embryo · *Wnt 4* · *Wnt 5a* · *Wnt 5b* · *Wnt 6* · *Wnt 11* · *Wnt 14* · Limb

Introduction

The development of the vertebrate limbs is a complex, multistep process that involves specification of the early limb field, directed outgrowth, and patterning to establish the final three-dimensional structure of the limb. All of these processes have to be temporally and spatially coordinated and thus require communication between cells and cell layers, a function which is mediated by specific extracellular signals. Specialized regions of the developing limb bud, such as the zone of polarizing activity (ZPA), the apical ectodermal ridge (AER), and the nonridge ectoderm, direct and coordinate the development of the limb bud along the anterior–posterior (AP), dorsal–ventral (DV), and proximal–distal (PD) axes, giving rise to a stereotyped pattern of elements well conserved among tetrapods. The analysis of the specific gene function, which plays a role in mediating the patterning of limb bud axes, has revealed the existence of complex interactions between signaling pathways operated by secreted factors of the HH, TGF-beta/BMP, WNT, and FGF superfamilies, which interact with many other genetic networks to control limb positioning, outgrowth, and patterning (Capdevila and Izpisua Belmonte 2001). A number of Wnt molecules, in particular, have been implicated in playing specific roles during vertebrate limb development, including induction of the early limb bud, formation, and maintenance of the AER, outgrowth of the limb, and patterning of the limb bud axes (Dealy et al. 1993; Parr et al. 1993; Kengaku et al. 1998; Galceran et al. 1999; Kawakami et al. 2001). Components of this pathway have also been implicated in controlling differentiation, maturation, and segmentation of skeletal elements as well as bone homeostasis and are associated with diseases of the joint

This paper is dedicated to Professor Dr. W. Zenker on the occasion of his 80th birthday.

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such as rheumatoid arthritis (Kawakami et al. 1999; Sen et al. 2000; Hartmann and Tabin 2000, 2001; Guo et al. 2004).

The Wnt family of secreted glycosylated factors consists of 22 members in vertebrates, which have a range of functions during development from patterning of individual structures to fine-tuning at the cellular level, controlling cell differentiation, proliferation, and survival (Dickinson and McMahon 1992; Parr and McMahon 1994; Cadigan and Nusse 1997; Huelsken and Birchmeier 2001; Church and Francis-West 2002). Members of the *Wnt* family are differentially expressed either within the ectoderm or mesenchyme where they play a number of roles. Wnt signaling in the process of limb bud initiation in the chick involves the activity of two *Wnt* genes, in particular *Wnt 2b* and *Wnt 8c*, in mediating the expression of *FGF-8/FGF-10* (Kawakami et al. 2001). Previous studies have suggested that signaling by *Wnt 3a* is involved in the formation and maintenance of the avian AER (Kengaku et al. 1998). Strikingly, like the *Wnt* genes implicated in mediating limb bud induction in the chick, the expression of *Wnt 3a* in the AER is not conserved between chick and mouse, hence limb development is normal in *Wnt 3a* mutant mice (Yoshikawa et al. 1997; Galceran et al. 1999). Nevertheless, there is evidence that a β -catenin/LEF-mediated Wnt signal does play a role in establishing or maintaining the AER in the mouse as well.

Further outgrowth of the limb requires not only the presence of a functional AER but also continuous proliferation of the limb bud mesenchyme. Another member of the *Wnt* gene family, *Wnt 5a*, expressed in the distal mesenchyme underneath the AER in both chick and mouse, also plays a dominant role during limb outgrowth (Dealy et al. 1993; Parr et al. 1993; Yamaguchi et al. 1999). Patterning of the dorsoventral axis is also controlled by members of the *Wnt* family. In this regard, *Wnt 7a* expressed in the dorsal compartment of the ectodermal pocket in both mouse and chick (Dealy et al. 1993; Parr et al. 1993; Gavin et al. 1990) has been proved to be necessary for specifying fates of dorsal cells in the distal limb (Parr et al. 1995). Besides this function, *Wnt 7a* signaling is important for normal function of the ZPA (Parr et al. 1995). Another member of the *Wnt* family, *Wnt 6*, is expressed in the limb ectoderm including the dorsoventral limb boundary in both chick and mouse (Parr et al. 1993; Rodriguez-Niedenführ et al. 2003). The function of *Wnt 6* during limb development has not been reported. The expression of *Wnt 6* in the dorsal and ventral limb ectoderm indicates a role in limb muscle formation (Loganathan et al., unpublished data).

Members of the *Wnt* family are also shown to be involved in appendicular skeletogenesis. Gain-of-function experiments in the chick have suggested the activity of *Wnt 4* on chondrocyte maturation (Kawakami et al. 1999; Hartmann and Tabin 2000). These experiments also suggest that *Wnt 5a* and *Wnt 4* have opposing activities on chondrocyte maturation. Moreover, *Wnt 5a* has been reported to be involved in the regulation of the

expression of a closely related *Wnt* gene, *Wnt 5b* (Hartmann and Tabin 2000). Both *Wnt 5a* and *Wnt 5b* have been shown to delay chondrocyte maturation (Hartmann and Tabin 2000). *Wnt 11* expressed in the limb mesenchyme is involved in muscle and dermal development (Anakwe et al. 2003). *Wnt 14*, like *Wnt 4*, is expressed in the joint regions and has been reported to be a possible candidate involved in the joint-induction process (Hartmann and Tabin 2001). The early developmental role of *Wnt 14* in joint induction and its persistent expression in structures of the mature joint allude to a possible role for *Wnt 14* in maintaining joint integrity during postnatal development.

Thus, Wnts are key signals at various stages of limb development. However, because of the discrepancies in the expression patterns of certain *Wnts* observed between mouse and chick, it remains to be uncovered to what extent the different roles for the Wnt molecules in limb bud development are evolutionarily conserved. Although aspects of the limb expression of many of the *Wnt* genes have been reported in the literature, it is necessary to have a more comprehensive description of *Wnt* gene expression during limb development to fully understand experimental phenotypes and to understand the mechanisms controlling *Wnt* gene expression, thus patterning the limb. To this end, we present an overview of *Wnt* genes (*Wnt 4, 5a, 5b, 6, 11, and 14*) and report their expression pattern during chick limb development.

Materials and methods

Preparation of chick embryos

Fertilized chicken eggs were incubated at 38°C, and the embryos were staged according to Hamburger and Hamilton (1951).

In situ hybridization

Embryos from HH stage 17 to day 8 were fixed overnight at 4°C in 4% PFA. Embryos were washed twice in PBT, dehydrated in methanol, and stored at 4°C. Whole mount *in situ* hybridization was performed, as previously described (Nieto et al. 1996). Selected stained embryos were embedded in 4% agar and sectioned with a Leica Vibratome at 50 μ m. Chick *Wnt 4* (400 bp), *Wnt 5a* (400 bp), *Wnt 5b* (400 bp), and *Wnt 11* (1,000 bp) cDNAs were kindly provided by Professor Christophe Marcelle (Developmental Biology Institute, Marseille, France). For cWnt 6, we used the cloned *Wnt-6* 1,500-bp fragment (Rodriguez-Niedenführ et al. 2003) as a template and for *Wnt 14* we used a *Wnt 14* 600-bp (R&D systems, Germany) fragment as a template. Sense and antisense riboprobes were labeled with digoxigenin RNA labeling kit, as recommended (Boehringer, Mannheim, Germany).

Results

Expression of *Wnt* genes during chick limb development

To determine potential roles of Wnt signaling during limb development, we analyzed the expression of various *Wnts* by whole mount *in situ* hybridization and compared their expression, both temporally and spatially.

Expression of *Wnt 4*

Wnt 4 has been reported to be expressed in regions of developing joints in the chick. *Wnt 4* expression was reported to be first visible at HH stage 27 in the joint of the elbow region and from stage 30 onward in the joint regions of the digits (Kawakami et al. 1999); however, we detected *Wnt 4* expression in the central elbow region and in the joint interzones of the wrist-forming region as early as HH stage 26 (Fig. 1D). Sagittal sections revealed the expression in the joint interzone and in the mesenchyme surrounding the joints (Fig. 2A). At HH stage 29, *Wnt 4* expression was detectable in the autopod region as small spot-like signals and in the elbow and wrist-forming region (Fig. 1E). During later stages, localization of the Wnt 4 signals was observed in the joint-forming regions throughout the limb (Fig. 1F, G).

Expression of *Wnt 5a*

Wnt 5a is initially expressed throughout the mesenchyme from HH stages 17 to 19 (Fig. 1H, I), later becoming predominantly confined to the distal tip with lower expression levels proximally (Fig. 1J–N). At HH stage 23, *Wnt 5a* was intensely expressed in the distal mesenchyme as well as in the entire AER (Fig. 1J). At HH stage 29 (Fig. 1L), the expression at the distal tip is confined to the interdigital regions, which become prominent during day 7 (Fig. 1M) and is seen in the region surrounding the phalanges during day 8 (Fig. 1N). From HH stage 26, *Wnt 5a* expression is also found in the central core next to the developing cartilage elements and muscle mass (Fig. 1K), as reported previously (Dealy et al. 1993; Anakwe et al. 2003).

Expression of *Wnt 5b*

The highly related *Wnt* gene, *Wnt 5b*, is expressed in the AER of the early limb bud from HH stage 20 onward (Fig. 1P). At HH stage 23, *Wnt 5b* expression is seen in the proximal mesenchyme, and the expression in the AER remains the same (Fig. 1Q). At HH stage 26, expression was seen in the dorsal and ventral mesenchyme (Figs. 1R, 2c). The expression becomes stronger during later stages and is found to be located in the tendons surrounding the cartilage elements at day 8 (Fig. 1U).

Expression of *Wnt 6*

At HH stages 15–16, strong *Wnt 6* expression was observed at limb level in the ectoderm at the dorsoventral boundary, the prospective AER (data not shown, see Rodriguez-Niedenführ et al. 2003). *Wnt 6* is observed along the whole dorsoventral boundary of the limb bud at HH stages 17–23; expression was also observed in the ventral and dorsal limb ectoderm (Fig. 1V–X). At the base of the limb, stronger dorsal and ventral ectodermal expression domains were observed (Fig. 1X). During later stages, *Wnt 6* is strongly expressed in the dorsoventral limb boundaries and also in the dorsal and ventral ectoderm (Fig. 1Y, Z, a, b).

Expression of *Wnt 11*

Wnt 11 has been shown to be expressed in the dorsal mesenchyme of the limb bud at HH stages 24–28 (Tanda et al. 1995). We found that *Wnt 11* is first expressed in the proximal dorsal mesenchyme at HH stage 23 in the limb (Fig. 1e). From HH stage 25, *Wnt 11* transcripts are clearly detectable in the dorsal mesenchyme and also found in the ventral subectodermal mesenchyme; later, it extends distally and is slightly more advanced on the dorsal side (Figs. 1f–i, 2e). Localization of *Wnt 11* signals in the autopod is also detected in the region adjacent to the digits at days 7 and 8 (Fig. 1h, i) in addition to the expression in the dorsal and ventral mesenchyme.

Expression of *Wnt 14*

From HH stage 23 onward, *Wnt 14* expression is observed in the proximal mesenchyme at the connecting ridge between the limb and trunk (Fig. 1l). At HH stage 26, *Wnt 14* is expressed as a transverse stripe in the mesenchyme of the presumptive joint region (Figs. 1m, 2f). During later stages, *Wnt 14* continues to be expressed in the mesenchyme surrounding the cartilage elements as well as in a bipartite stripe within the joint region and also in the interdigital mesenchyme (Fig. 1n–p). At day 8, *Wnt 14* continues to be expressed in joints and in the mesenchyme surrounding the phalangeal elements (Fig. 1p).

Discussion

A number of *Wnt* molecules have been implicated in playing specific roles during vertebrate limb development, including induction of the early limb bud, formation and maintenance of a specific ectodermal structure known as the AER, outgrowth of the limb, and patterning of the limb bud axes (Dealy et al. 1993; Parr et al. 1993; Kengaku et al. 1998; Galceran et al. 1999; Kawakami et al. 2001). In this study, we analyzed the expression pattern of various *Wnts* (*Wnt 4, 5a, 5b, 6, 11*



Fig. 1 Expression of *Wnts* during normal chick wing development. Whole mount *in situ* hybridization of limbs for the genes *Wnt 4* (A–G), *Wnt 5a* (H–N), *Wnt 5b* (O–U), *Wnt 6* (V–b), *Wnt 11* (c–i), *Wnt 14* (j–p). Approximate stages are stage 17 (A, H, O, V, c, j), stage 20 (B, I, P, W, d, k), stage 23 (C, J, Q, X, e, l), stage 26 (D, K, R, Y, f, m), stage 29 (E, L, S, Z, g, n), day 7 (F, M, T, a, h, o), day 8 (G, N, U, b, i, p). A–C *Wnt 4* expression is not seen in the developing limb bud. D Expression of *Wnt 4* in the limb at HH stage 26 in the central elbow region and in the joint interzones of the wrist. E Limb of HH stage 29 embryo showing expression of *Wnt 4* in the autopod region, elbow, and wrist-forming region. F, G *Wnt 4* expression in the limb of day 7 and day 8 embryos showing expression in the joint-forming regions of the digits in addition to the expression in joints of the elbow and wrist. H, I Expression of *Wnt 5a* in the limb of HH stage 17 and 20 embryos; expression seen in the entire mesenchyme. J, K Limb of HH stage 23 embryo showing the restriction of *Wnt 5a* expression to the distal tip and to the entire AER, leaving lower expression levels proximally. L, M *Wnt 5a* expression in the limb of HH stage 29 and day 7 embryos; expression in the distal mesenchyme confined to the interdigital regions. N Expression of *Wnt 5a* in the limb of day 8 embryo showing expression around the phalanges. O No expression of *Wnt 5b* in the limb of HH stage 17 embryo. P *Wnt 5b* expression in the AER of stage 20 embryo. Q Limb of HH stage 23 embryo showing *Wnt 5b* expression in the proximal mesenchyme and in the AER. R

Expression of *Wnt 5b* in the dorsal and ventral mesenchyme of the limb at HH stage 26. S Expression of *Wnt 5b* in the dorsoventral mesenchyme is seen around the cartilage elements of the stage 29 limb. T, U Limb of day 7 and 8 embryos showing *Wnt 5b* expression in the tendons surrounding the cartilage elements. V, W, X, Y, Z, a, b *Wnt 6* is strongly expressed in the dorsoventral boundary and also in the dorsal and ventral limb ectoderm of HH stages 17, 20, 23, 26, 29, day 7, and day 8 embryos, respectively. c, d Limb bud of HH stage 17 and 20 embryos showing no *Wnt 11* expression. e *Wnt 11* expression in the proximal dorsal mesenchyme of the limb of HH stage 23 embryo. f *Wnt 11* transcripts are clearly seen in the dorsal and ventral mesenchyme of the limb of stage 26 embryo. g Extension of *Wnt 11* expression distally in the limb of stage 29 embryo. h, i Expression of *Wnt 11* in the regions adjacent to the digits in addition to the expression in the dorsal and ventral limb mesenchyme of day 7 and day 8 embryos. j, k No expression of *Wnt 14* in the developing limb of HH stage 17 and 20 embryos. l Weak expression of *Wnt 14* seen in the proximal region of the limb of stage 23 embryo. m Limb of HH stage 26 embryo showing *Wnt 14* expression as a transverse stripe in the mesenchyme of the presumptive joint region. n Expression in the presumptive joint region becomes stronger at stage 29. o, p *Wnt 14* expression is seen in the mesenchyme cells surrounding the phalangeal elements of day 7 and day 8 limbs. Scale bar: 1mm in A–p

and 14) during chick limb development. *Wnt 4* expression has not been observed in the chick limb bud until stage 24, as reported previously (Yoshioka et al. 1994). We found *Wnt 4* expression at restricted locations in the developing limb at HH stage 26 and later whereas in the mouse embryo, uniform expression of *Wnt 4* in the entire limb ectoderm has been observed (Parr et al. 1993). *Wnt 4* expression is seen in mesenchymal cells adjacent

to the joint region. This expression pattern suggests involvement of *Wnt 4* in joint and articular structure formation (Kawakami et al. 1999; Hartmann and Tabin 2000). Studies carried out in the chick have shown that ectopic *Wnt 4* expression promotes chondrocyte differentiation (Hartmann and Tabin 2000). In particular, gain-of-function experiments in the chick have suggested that *Wnt 4* and *Wnt 5a* have opposing activities on

chondrocyte maturation (Kawakami et al. 1999; Hartmann and Tabin 2000). In these studies, while *Wnt 5a* misexpression causes a delayed appearance of chondrocytes, *Wnt 4* misexpression results in an acceleration of chondrocyte maturation.

The expression of chick *Wnt 5a* from HH stage 19 to day 8 suggests that *Wnt 5a* may be involved in PD patterning of the limb bud. *Wnt 5a* is expressed in the entire limb mesenchyme until HH stage 20 but remains confined to the distal mesenchyme alone during the later stages. The expression pattern of *Wnt 5a* in the mouse limb is similar but not identical to that of the chick *Wnt 5a* presented here. Mouse *Wnt 5a* is initially expressed in the ventral ectoderm and distal mesenchyme (Gavin et al. 1990) whereas chick *Wnt 5a* shows graded expression along the AP axis at early stages. After the limb bud is formed, *Wnt 5a* is expressed in the AER and distal mesenchyme with a gradient along both the PD axis and the AP axis in the chick, as reported previously (Dealy et al. 1993; Kawakami et al. 1999; Anakwe et al. 2003). This expression pattern indicates that *Wnt 5a* is involved in the initiation and early patterning of the limb bud. Outgrowth of the limb requires not only the presence of a functional AER but also continuous proliferation of the limb bud mesenchyme. *Wnt 5a* is expressed in the distal mesenchyme underneath the AER in both chick and mouse (Dealy et al. 1993; Parr et al. 1993). The analysis of a *Wnt 5a* loss-of-function mutation in the mouse revealed that *Wnt 5a* acts predominantly as a mitogen in the distal regions of the limb bud, and loss of *Wnt 5a* activity results in a progressive shortening of the appendicular skeleton in a proximal-to-distal fashion and a total loss of the most distal structures, such as the digits (Yamaguchi et al. 1999). At later stages, the absence of the *Wnt 5a* signal in the proximal cartilage-forming region and the intense expression in the distal mesenchymal region surrounding the cartilage suggest a possible involvement of *Wnt 5a* in proliferation and differentiation of chondrocytes in the limb bud.

The highly related *Wnt* gene, *Wnt 5b*, is not expressed in the limb bud until HH stage 19. *Wnt 5b* is expressed in the AER of the early limb bud from HH stage 20 onward, as reported previously (Kengaku et al. 1997). *Wnt 3a* and *FGF 8* are expressed in the AER similar to *Wnt 5b* during limb development. Unlike *Wnt 3a*, *Wnt 5b* expression disappears from the AER after stage 23. This alludes to a possible involvement of *Wnt 5b* signaling during the early process of limb outgrowth. Apart from expression in the AER, an additional expression domain of *Wnt 5b* is seen in the proximal mesenchyme at HH stage 23 and during later stages in the mesenchyme around the cartilage elements. Hartmann and Tabin (2000) reported that *Wnt 5b* is expressed in perichondrial cells surrounding the entire cartilage elements. This expression pattern leads to the question of a possible role of *Wnt 5b* in the process of tendon formation, which will be the subject of a future study.

At HH stage 15–16, *Wnt 6* expression in the ectoderm became stronger in a subset of ectodermal cells located at

the dorsoventral boundary at limb level, the prospective AER (Rodriguez-Niedenführ et al. 2003). This restriction of *Wnt* proteins to the limb-forming region has also been observed for *Wnt 2b* and *Wnt 8c* in the chick intermediate and lateral plate mesoderm (Kawakami et al. 2001). Previously, expression of *Wnt 6* in the dorsoventral limb ectoderm was studied only until HH stage 26 (Rodriguez-Niedenführ et al. 2003). We observed the expression of *Wnt 6* in the dorsoventral boundary of the chick limb, in the AER (Fig. 2b), and also in the ventral and dorsal ectoderm until day 8. This is in contrast to a previous study, which reported *Wnt 6* expression solely in the AER (Schubert et al. 2002). The expression in the limb bud resembled the expression observed in the mouse, where *Wnt 6* was reported to be expressed rather uniformly throughout the limb ectoderm (Parr et al. 1993). In addition to the expression in the dorsal ectoderm, the ventral ectoderm also showed a strong expression domain at the limb level, as reported previously (Rodriguez-Niedenführ et al. 2003), which was not shown previously either in the chick (Schubert et al. 2002) or the mouse (Parr et al. 1993). The function of *Wnt 6* during limb development is still unknown but indicates a role in limb muscle formation (Loganathan et al., unpublished data).

Wnt 11 expression is observed from HH stage 23 in the proximal dorsal mesenchyme and later becomes clearly detectable in the dorsal and ventral subectodermal mesenchyme. The expression domain of *Wnt 11* transcript is always seen in the subectodermal mesenchyme overlying the developing myogenic cells (Anakwe et al. 2003). At later stages, *Wnt 11* expression extends distally over the developing myogenic cells and is slightly more advanced on the dorsal side in correlation with the advanced rate of myogenic differentiation (Fig. 2d). *Pax 3* is expressed in the dorsal and ventral mesenchyme adjacent to the ectoderm (Yang et al. 1996; Amthor et al. 1998) similar to *Wnt 11*. Thus, the expression pattern of *Wnt 11* suggests its participation in myoblast migration and patterning in the limb bud (Robson et al. 1994). The premyogenic cells are within the range of *Wnt* signals from the ectoderm and are in contact with or in close proximity to the mesenchymal *Wnt 11* signal. The different expression of *Wnt 5a* and *Wnt 11* between stages 23 and 27 correlate with the ultimate distribution of slow and fast fibers in the muscle anlage of the limb. Slow fibers are concentrated toward the center of the limb bud, where *Wnt 5a* transcripts are found, whereas fast fibers are found closer to the ectoderm, where *Wnt 11* transcripts can be seen (Anakwe et al. 2003). The expression pattern of *Wnt 11* in close association with the ectoderm reveals a possible interaction of *Wnt 11* with *Wnt 6* expressed in the dorsal and ventral ectoderm during limb muscle development.

Wnt 14 is expressed in the mesenchyme surrounding the cartilage elements as well as in a bipartite stripe within the joint region and also in the interdigital mesenchyme. At day 8, *Wnt 14* continues to be expressed in joints and in the mesenchyme surrounding the phalangeal elements. This pattern of *Wnt 14* expression in the mesenchyme

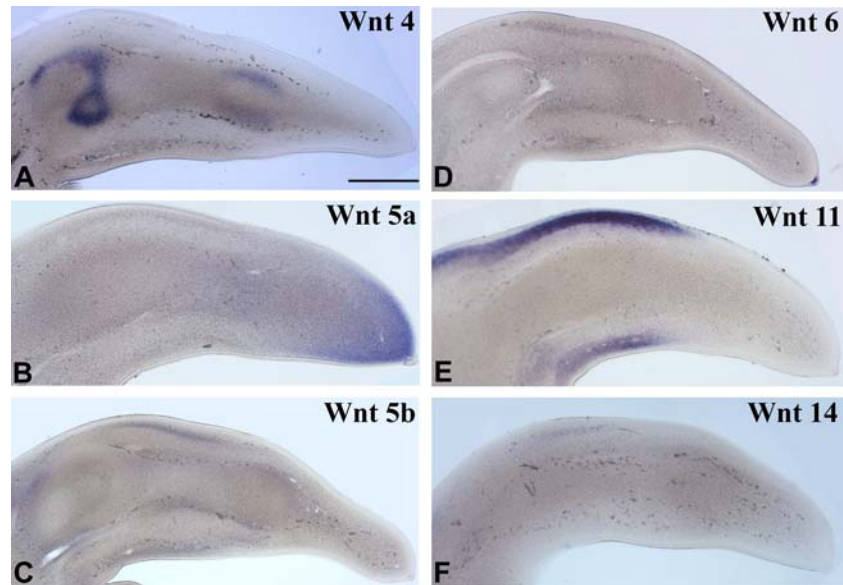


Fig. 2 Transverse sections showing the normal expression pattern of *Wnts* at HH stage 26 during chick limb development. **a** Transverse section of stage 26 limb showing *Wnt 4* expression in the elbow region and wrist-forming region; expression is seen in the joint interzone and in the mesenchyme surrounding the joints. **b** Transverse section of HH stage 26 limb showing *Wnt 5a* expression confined to the distal end and in the whole AER, leaving weaker expression proximally. **c** Transverse section of the limb of HH stage 26 embryo showing *Wnt 5b* expression in the dorsal and ventral mesenchyme of presumptive cartilage-forming region. **d** Expression of *Wnt 6* in the dorsal and ventral limb ectoderm of stage 26 embryo; strong expression in the AER. **e** *Wnt 11* expression in the dorsal and ventral subectodermal mesenchyme of HH stage 26 limb shown by transverse vibratome section; expression is slightly advanced on the dorsal side. **f** Transverse section of the limb of HH stage 26 embryo showing expression of *Wnt 14* in the dorsal mesenchyme of the presumptive joint-forming region. Scale bar: 500 μ m in a-f

around the developing cartilage elements and joint-forming regions suggests a possible influence on primary muscle fiber development (Hartmann and Tabin 2001). The possible role of *Wnt 14* in joint formation was proved by overexpression of *Wnt 14* in the chick limb leading to ectopic synovial joint formation (Hartmann and Tabin 2001). *Wnt 14* is expressed in the joint regions similar to *Wnt 4*, but the expression of *Wnt 4* is present at a high level in the joints whereas *Wnt 14* expression is located in the mesenchyme surrounding the cartilage elements. *Wnt 14* has been shown to be capable of arresting and reversing chondrogenic differentiation in vitro, which makes *Wnt 14* a good candidate to be involved in the joint induction process (Hartmann and Tabin 2001).

The expression pattern of different *Wnts* in a stage 26 limb is illustrated in Fig. 3. It can be concluded that *Wnts* expressed in distinct regions in the developing limb are involved in various processes of limb formation and cell differentiation.

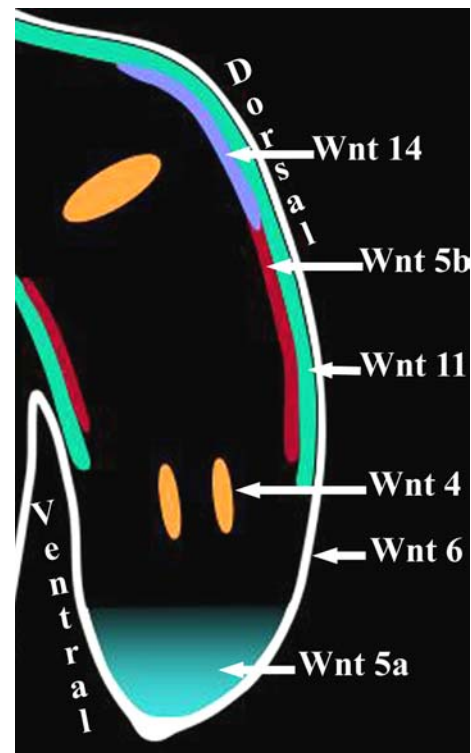


Fig. 3 A schematic representation of a chick wing bud through the dorsoventral axis of a stage 26 limb bud showing the expression of *Wnts*. Expression of *Wnt 4* (orange) is seen in the elbow and wrist-forming regions. *Wnt 5a* (blue) expression is located at the distal tip, having lesser expression level proximally. Expression of *Wnt 5b* (red) in the dorsal and ventral mesenchyme in close association with *Wnt 11* (green). Expression of *Wnt 6* (white) is seen throughout the dorsal and ventral limb ectoderm. *Wnt 11* (green) transcripts are located in the dorsal and ventral subectodermal mesenchyme. *Wnt 14* (indigo) expression is seen in the dorsal mesenchyme of the presumptive joint-forming region and located below the *Wnt 11* expression domain

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