

Dynamic Expression of Lef/Tcf Family Members and β -Catenin During Chick Gastrulation, Neurulation, and Early Limb Development

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Members of the Lef/Tcf family of HMG-box transcription factors mediate the response to Wnt as part of the canonical Wnt signaling cascade. Positive and negative cofactors, including β -catenin, CtBP, and Smad3, regulate the activity of Lef/Tcf transcription complexes. Interaction of Lef/Tcfs with β -catenin results in target gene activation or repression, depending on the context. Here, we report the cloning of a novel chick *Tcf-1* splice variant and of a partial cDNA for chick *Tcf-3*. We describe their expression patterns during early development and have compared them with the expression profiles of *Lef-1* and β -catenin. We found restricted patterns during gastrulation, neurulation, somitogenesis, and early limb development. β -catenin and Lef/Tcf expression did not always coincide, indicating developmental contexts in which Lef/Tcf proteins may interact with other cofactors and conversely, the areas in which β -catenin may interact with other coregulators, or be involved in regulating adhesive properties of cells. *Developmental Dynamics* 229:703–707, 2004. © 2004 Wiley-Liss, Inc.

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INTRODUCTION

The Wnt family of secreted glycoproteins consists of over 20 members, which act by binding to Frizzleds, seven-pass transmembrane receptors, on target cells. Frizzled mediated signaling can activate at least three different pathways. In the so-called classic pathway, binding of Wnt to its receptor results in activation of disheveled, which inactivates the cytoplasmic serine-threonine kinase GSK-3 β (Moon et al., 2002). One target of GSK-3 β is β -catenin, which is degraded when phosphorylated. In the presence of Wnt, β -catenin becomes stabilized

and translocates to the nucleus where it interacts with Lef/Tcf transcription factors and regulates the activity of target promoters (Clevers and Van de Wetering, 1997). In addition, Lef/Tcfs can interact with other positive and negative cofactors, such as groucho, Smad3, and CtBP (Cavallo et al., 1998; Roose et al., 1998; Waltzer and Bienz, 1998; Eastman and Grosschedl, 1999; Labbe et al., 2000; Nishita et al., 2000; Takemaru and Moon, 2000).

This signaling pathway has been shown to play important roles in developmental patterning and cell fate decisions during embryogene-

sis, as well as during stem cell differentiation. Our previous work has demonstrated that several Wnt family members in combination with Shh can activate skeletal muscle specific gene expression in cultured chick somite explants, most likely by means of a β -catenin-dependent pathway (Münsterberg et al., 1995; Schmidt et al., 2000). Of interest, this pathway is reactivated during skeletal muscle regeneration in the adult (Poleskaya et al., 2003; Snider and Tapscott, 2003). This recent work demonstrates the importance of understanding embryonic signaling networks for stem cell differentiation.

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Another important function of β -catenin is to regulate cell adhesion by means of its interaction with cadherin cell adhesion molecules at the plasma membrane (Peifer et al., 1993; Aberle et al., 1996). Here, we compare the developmental expression of β -catenin with *Lef-1*, *Tcf-1*, and *Tcf-3*, three of the Lef/Tcf transcription factors in early chick development. *Tcf-1* and *Tcf-3* expression has not been described previously in chick, and β -catenin expression has not been described in prestreak and gastrulation stage embryos. *Lef-1* expression has been documented in developing somites, limb buds, and in gastrulation stage chick embryos (Kengaku et al., 1998; Schmidt et al., 2000; Skromne and Stern, 2001); however, we found additional areas of *Lef-1* expression that have not been described before.

The comparative expression analysis revealed tissues where β -catenin transcripts are coexpressed with Lef/Tcf transcription factors, indicating where they might interact. In other regions, β -catenin could interact with other transcriptional regulators (see for example Zorn et al., 1999; Bauer et al., 2000; Tago et al., 2000; Takemaru et al. 2003; Wei et al., 2003) or play a role in cell adhesion processes.

RESULTS AND DISCUSSION

Cloning of Chick *Tcf-1* and *Tcf-3*

We used degenerate reverse transcriptase-polymerase chain reaction (RT-PCR) and cDNA library screening to isolate chick Lef/Tcf family members. Sequence alignment identified one cDNA as a new splice variant of chick *Tcf-1* (previously called chick *Tcf-1/4*, see Gastrop et al., 1992), which does not contain the predicted Groucho binding domain (Levanon et al., 1998) and possesses an alternative 3' end. The chick *Tcf-1* gene seems to follow the splicing pattern described for human *TCF-1* (Van de Wetering et al. 1996), suggesting that further splice variants may exist. We also identified a partial clone of chick *Tcf-3*, a novel homologue of

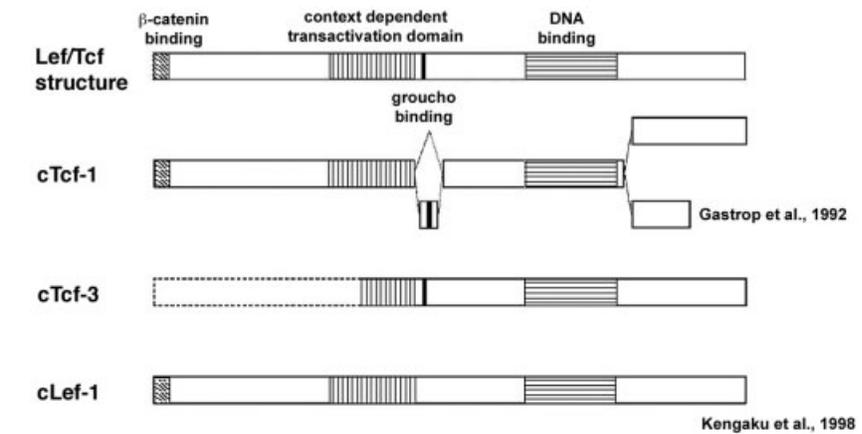


Fig. 1. Schematic comparison of chick Lef/Tcf family members. Domains shared between Lef/Tcf family members are indicated on top. Black and white patterns indicate interaction domains for β -catenin binding (angled stripes), the context-dependent transactivation domain (CTA, vertical stripes) with the potential Groucho binding domain depicted separately (solid black). The highly conserved HMG-box confers DNA binding (horizontal stripes). All chick Lef/Tcf family members possess the HMG-box and CTA-domain. However, chick *Lef-1* does not have the predicted Groucho binding domain, and in chick *Tcf-1*, this domain can be spliced out. For *Tcf-1*, the splice variant described in this study is shown on top. A dashed outline indicates the predicted N-terminus of chick *Tcf-3*.

this transcription factor family in chick. We subsequently searched the chick expressed sequence tag database (Boardman et al., 2002); however, *Tcf-3* was not found. A schematic comparison of the domain structure of chick Lef/Tcf is shown (Fig. 1).

Expression of *Tcf-1*, *Tcf-3*, β -catenin, and *Lef-1* During Gastrulation and Neurulation

Embryos from prestreak stages to mid-limb stages of development (Hamburger and Hamilton, 1951; Eyal-Giladi and Kochav, 1976) were examined by in situ hybridization (Figs. 2, 3). At prestreak stages (Eyal-Giladi and Kochav, EGK, stage XI), low levels of *Tcf-1* transcripts were detected in the marginal zone, with higher levels posteriorly and *Tcf-3* was expressed strongly in the area pellucida. Sections showed that *Tcf-1* and *Tcf-3* were expressed in epiblast cells (Fig. 2A,A',B,B'), β -catenin expression was found in both the area opaca and area pellucida with lower levels of expression in the latter (Fig. 2C,C'). Expression of *Lef-1* in prestreak and gastrulating embryos has been described elsewhere (Skromne and Stern, 2001), and here we show representative panels only (Fig. 2D,H,L,P). In pre-

streak embryos, β -catenin expression overlapped with *Tcf-1*, *Tcf-3*, and *Lef-1* in different regions.

At stage Hamburger and Hamilton (HH) 3 *Tcf-1*, β -catenin, and *Lef-1* were expressed in the primitive streak (Fig. 2E,G,H), suggesting that here β -catenin may be acting in conjunction with these transcription factors. β -catenin was still expressed in the area opaca at HH3 (Fig. 2G). Of interest, *Tcf-3* showed an almost complementary pattern to β -catenin, *Tcf-1*, and *Lef-1*, with strong expression in the epiblast and lower levels in the primitive streak (Fig. 2F), indicating that it may interact with other transcriptional coactivators or carry out its suggested function as a transcriptional repressor (Kim et al., 2000).

In HH4 embryos, *Tcf-1* was highly expressed in the posterior half of the primitive streak and in regions lateral to it. Sections showed that *Tcf-1* transcripts are localized in ectodermal and mesodermal cells and excluded from the endoderm (Fig. 2I,I'). *Tcf-3* was expressed strongly in prospective neural plate tissue, consistent with the observation that *Tcf-3* is essential for vertebrate head formation (Kim et al., 2000). Slightly lower levels of *Tcf-3* were found throughout the epiblast (Fig. 2J,J'). β -catenin was expressed throughout

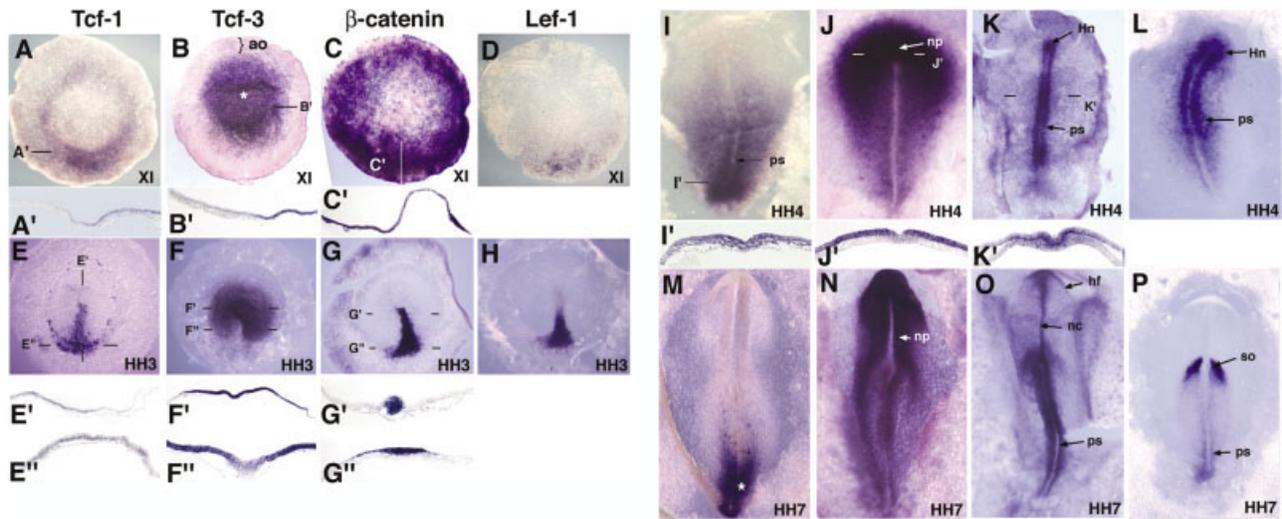


Fig. 2. *Tcf-1*, *Tcf-3*, β -*catenin*, and *Lef-1* in chick gastrulation and neurogenesis. Whole-mount in situ hybridization of EGK stage XI to stage Hamburger and Hamilton (HH) 7 embryos and sections are shown. A,E,I,M: *Tcf-1*. B,F,J,N: *Tcf-3*. C,G,K,O: β -*catenin*. D,H,L,P: *Lef-1*. Stages are indicated on each panel, anterior is to the top. A'-C', E'-G', I'-K': Sections are indicated by a line and shown beneath the relevant panels. A', B': Only half of the section is shown to allow for higher magnification. ao, area opaca; hf, head fold; Hn, Hensen's node; nc, notochord; np, neural plate; ps, primitive streak; so, somite; asterisk in B indicates area pellucida; asterisk in M indicates expression in and around primitive streak.

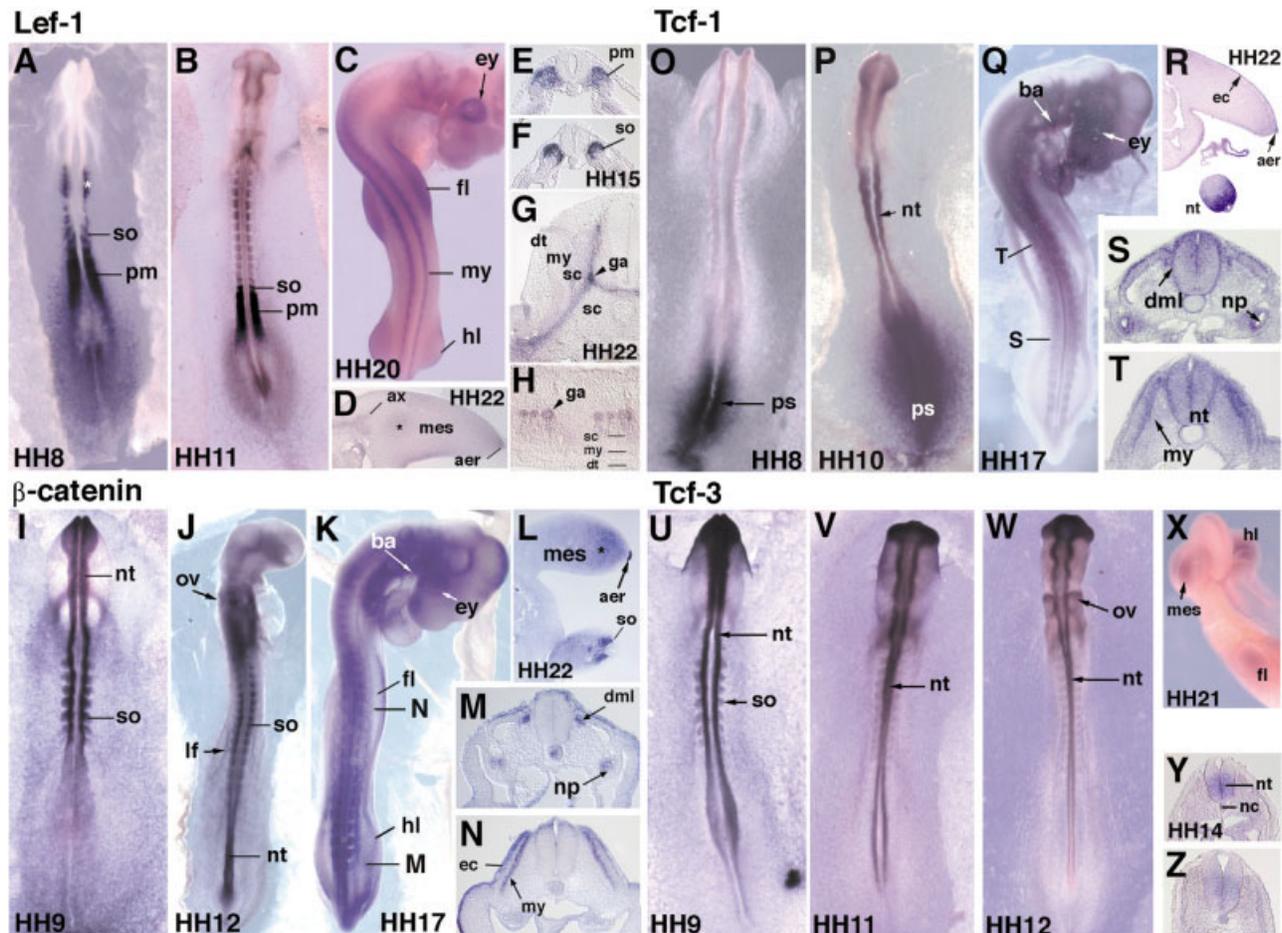


Fig. 3. Expression of *Lef-1*, β -*catenin*, *Tcf-1*, and *Tcf-3* during chick somite and limb stages. Representative embryos between stages Hamburger and Hamilton (HH) 8 and HH22 are shown as whole-mounts (A-C, I-K, O-Q, U-X) and sections (D-H, L-N, R-T, X-Z). A-H: *Lef-1*. I-N: β -*catenin*. O-T: *Tcf-1*. U-Z: *Tcf-3*. The HH stages are indicated. D,L,R: Transverse sections through limb buds of HH22 embryos. E,F: Transverse section through the trunk of a HH15 embryo. G: Transverse section through the trunk of a HH22 embryo. H: Longitudinal section through the trunk of a HH22 embryo. M,N: Transverse sections through the trunk of embryo in (K), levels are indicated. S,T: Transverse sections through the trunk of embryo in (Q), levels are indicated. Y,Z: Transverse section through the trunk of a HH14 embryo. ax, axons; aer, apical ectodermal ridge; ba, branchial arches; dt, dermatome; dml, dorsomedial lip; ec, ectoderm; ey, eye; fl, fore limb; ga, ganglia; hl, hind limb; lf, lateral fold; mes, mesenchyme; my, myotome; nc, notochord; np, nephric primordia; nt, neural tube; ov, otic vesicle; pm, presegmented mesoderm; sc, sclerotome; so, somite.

the embryo at low levels with high levels in the primitive streak (Fig. 2K), sections showed that transcripts were expressed in mesoderm overlapping with *Lef-1* (Fig. 2K'). *Lef-1* transcripts were detected at high levels in Hensen's node, primitive streak, and mesoderm emerging from the streak (Fig. 2L, see also Skromne and Stern, 2001).

At stage HH7, *Tcf-1* expression was restricted to the posterior primitive streak (Fig. 2M). *Tcf-3* transcripts were expressed throughout the embryo, with high levels in head fold and neural plate tissue (Fig. 2N). High levels of β -catenin were seen in primitive streak, notochord, and head fold (Fig. 2O). *Lef-1* transcripts were strongly expressed in the first somite (Fig. 2P) and could also be detected in primitive streak and mesoderm emanating from the streak. At stage HH7, β -catenin expression overlapped with that of various *Lef*/*Tcf*s in different regions. For example, β -catenin and *Tcf-3* transcripts were present in the headfold, while β -catenin, *Tcf-1*, and *Lef-1* were expressed in the posterior primitive streak. However, some tissues expressed only one of the four factors, or one factor at much higher levels, suggesting other binding partners or functions. For example, at HH7 *Lef-1* was strongly expressed in the first forming somite and *Tcf-3* in neural plate tissue, while β -catenin expression was detected at high levels in the anterior and middle primitive streak. At HH7, β -catenin expression was exclusive in the notochord, indicating that in this tissue its role may be in cell-cell adhesion or transcriptional regulation by means of a different member of the *Lef*/*Tcf* family (i.e., *Tcf-4*) or other cofactors.

Expression of *Lef-1*, β -catenin, *Tcf-1*, and *Tcf-3* During Somitogenesis and Early Limb Bud Stages

At stage HH8, *Lef-1* was strongly expressed in the region of otic vesicle formation (Ladher et al., 2000), somites, presegmented mesoderm, and primitive streak (Fig. 3A). At stage HH11, expression was found in the anterior half of presegmented mesoderm (somites -I to -VI) and the

anterior half of the first epithelial somite (somite I). In more mature somites, *Lef-1* was expressed in the myotome (Fig. 3B,C,E,F, see also Schmidt et al., 2000). In later stages, *Lef-1* was expressed in the apical ectodermal ridge of developing limb buds and limb bud mesenchyme (Fig. 3D, see also Kengaku et al., 1998). *Lef-1* also marked ganglia and projecting axons, which has not been reported previously (Fig. 3D,G,H).

At stage HH9, β -catenin remained highly expressed in epithelial somites and neural tube (Fig. 3I). At HH12, expression was found in neural tube and expression within the somites became restricted as they differentiated (Fig. 3J,M,N). Transcripts were also detected around the otic vesicle, eye, and brain and ectoderm of the developing lateral folds, which will grow out and form limb buds. In older stages, expression was found in the mesenchyme and apical ectodermal ridge of developing limb buds and in the dorsomedial lip and myotome of developing somites and in nephric primordia (Fig. 3K-N, see also Schmidt et al., 2000).

Until stage HH11, *Tcf-1* was expressed at high levels in the posterior primitive streak (Fig. 3O,P). From stage HH11, expression became apparent in the closing neural tube and in the neural folds in the brain. At limb bud stages, *Tcf-1* was expressed at low levels throughout the embryos. Slightly more prominent expression was detected in neural tube, nephric primordia, dermomyotome and myotome, apical ectodermal ridge, and dorsal limb bud ectoderm (Fig. 3Q-T).

Until stage HH11, *Tcf-3* was expressed in the developing brain, neural tube epithelial somites, and primitive streak (Fig. 3U,V). Subsequently, *Tcf-3* expression decreased in somites and remained high in neural tissue (Fig. 3W,Y,Z). At HH12, *Tcf-3* transcripts were detected in the otic vesicles (Fig. 3W). At later stages, higher transcript levels for *Tcf-3* were apparent in the ventral two thirds of the neural tube, the notochord, and the mesenchyme of developing limb buds (Fig. 3X-Z).

At early limb bud stages, β -catenin expression correlated with *Tcf-1*

and *Lef-1* in the developing myotome of somites while *Tcf-3* transcripts were no longer detected in somites after HH11. In contrast, β -catenin and *Tcf-3* were both expressed in the developing brain and neural tube until HH9 (Fig. 3I,U). At stage HH8, *Lef-1* transcripts were present in prospective otic vesicles, while at HH12, the otic vesicle expressed β -catenin and *Tcf-3* but *Lef-1* transcripts were no longer detected. In developing limb buds, β -catenin transcripts overlap with *Lef-1* and *Tcf-1* in the apical ectodermal ridge, and in mesenchyme, β -catenin is coexpressed with *Lef-1* and *Tcf-3*.

In summary, the expression of *Lef*/*Tcf* family members and β -catenin was very dynamic and only partially overlapping. Thus, similar to the findings in mice, *Xenopus*, and zebrafish (Oosterwegel et al., 1993; Cho and Dressler, 1998; Molenaar et al., 1998; Pelegri and Maischen, 1998; Dorsky et al., 1999), this study indicates the potential for complex interactions between these transcriptional regulators in the early stages of chick development.

EXPERIMENTAL PROCEDURES

An HMG-box containing probe, amplified by RT-PCR from 3.5 day embryonic cDNA, was used to screen a cDNA library (HH12-HH15, kindly provided by Angela Nieto and David Wilkinson) by using standard procedures. We used 5' RACE (Marathon, Clontech) to extend *Tcf-3*; however, we did not obtain a full-length clone, indicating the presence of a stable secondary structure in *Tcf-3* mRNA (Van de Wetering et al., 1996). The isolated sequences were submitted to GenBank database (accession nos. *Tcf-3* AF454503 and *Tcf-1* AF454504).

Embryos were staged according to Eyal-Giladi and Kochav (1976) and Hamburger and Hamilton (1951). In situ hybridisation, sections, and photography was performed as described (Schmidt et al., 2000). The *Lef*/*Tcf* probes used excluded highly conserved HMG box regions to avoid cross-hybridization; however, probes were not specific for splice variants.

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REFERENCES

- Aberle H, Schwartz H, Kemler R. 1996. Cadherin-catenin complex: protein interactions and their implications for cadherin function. *J Cell Biochem* 61: 514–523.
- Bauer A, Chauvet S, Huber O, Usseglio F, Rothbacher U, Aragnol D, Kemler R, Pradel J. 2000. Pontin52 and reptin52 function as antagonistic regulators of beta-catenin signalling activity. *EMBO J* 19:6121–6130.
- Boardman PE, Sanz-Ezquerro J, Overton IM, Burt DW, Bosch E, Fong WT, Tickle C, Brown WR, Wilson SA, Hubbard SJ. 2002. A comprehensive collection of chicken cDNAs. *Curr Biol* 12:1965–1969.
- Cavallo RA, Cox RT, Moline MM, Roose J, Polevoy GA, Clevers H, Peifer M, Bejsovec A. 1998. Drosophila Tcf and Groucho interact to repress Wingless signalling activity. *Nature* 395:604–608.
- Cho EA, Dressler GR. 1998. TCF-4 binds beta-catenin and is expressed in distinct regions of the embryonic brain and limbs. *Mech Dev* 77:9–18.
- Clevers H, Van de Wetering M. 1997. TCF/LEF factors earn their wings. *Trends Genet* 13:485–489.
- Dorsky RI, Snyder A, Cretekos CJ, Grunwald DJ, Geisler R, Haffter P, Moon RT, Raible DW. 1999. Maternal and embryonic expression of Zebrafish *lef1*. *Mech Dev* 86:147–150.
- Eastman Q, Grosschedl R. 1999. Regulation of LEF-1/TCF transcription factors by Wnt and other signals. *Curr Opin Cell Biol* 11:233–240.
- Eyal-Giladi H, Kochav S. 1976. From cleavage to primitive streak formation: a complementary normal table and a new look at the first stages of the development of the chick. I. General morphology. *Dev Biol* 49:321–337.
- Gastrop J, Hoevenagel R, Young JR, Clevers HC. 1992. A common ancestor of the mammalian transcription factors TCF-1 and TCF-1alpha/LEF-1 expressed in chicken T cells. *Eur J Immunol* 22:1327–1330.
- Hamburger V, Hamilton HL. 1951. A series of normal stages in the development of the chick embryo. *J Morphol* 88:49–92.
- Kengaku M, Capdevila J, Rodriguez-Esteban C, De La Peña J, Johnson RL, Belmonte JCI, Tabin CJ. 1998. Distinct WNT pathways regulating AER formation and dorsoventral polarity in the chick limb bud. *Science* 280:1274–1277.
- Kim CH, Oda T, Itoh M, Jiang D, Artinger KB, Chandrasekharappa SC, Driever W, Chitnis AB. 2000. Repressor activity of *Headless/Tcf3* is essential for vertebrate head formation. *Nature* 407:913–916.
- Labbe E, Letamendia A, Attisano L. 2000. Association of Smads with lymphoid enhancer binding factor 1/T cell-specific factor mediates cooperative signaling by the transforming growth factor-beta and wnt pathways. *Proc Natl Acad Sci U S A* 97:8358–8363.
- Ladher RK, Anakwe KU, Gurney AL, Schoenwolf GC, Francis-West PH. 2000. Identification of synergistic signals initiating inner ear development. *Science* 290:1965–1967.
- Levanon D, Goldstein RE, Bernstein Y, Tang H, Goldenberg D, Stifani S, Paroush Z, Groner Y. 1998. Transcriptional repression by AML1 and LEF-1 is mediated by the TLE/Groucho corepressors. *Proc Natl Acad Sci U S A* 95:11590–11595.
- Molenaar M, Roose J, Peterson J, Venanzi S, Clevers H, Destree O. 1998. Differential expression of the HMG box transcription factors XTcf-3 and XLeF-1 during early Xenopus development. *Mech Dev* 75:151–154.
- Moon RT, Bowerman B, Boutros M, Perrimon N. 2002. The promise and perils of Wnt signaling through beta-catenin. *Science* 296:1644–1646.
- Münsterberg AE, Kitajewski J, Bumcrot DA, McMahon AP, Lassar AB. 1995. Combinatorial signaling by Sonic hedgehog and Wnt family members induces myogenic bHLH gene expression in the somite. *Genes Dev* 9:2911–2922.
- Nishita M, Hashimoto MK, Ogata S, Laurent MN, Ueno N, Shibuya H, Cho KW. 2000. Interaction between Wnt and TGF-beta signalling pathways during formation of Spemann's organizer. *Nature* 403:781–785.
- Oosterwegel M, van de Wetering M, Timmerman J, Kruijsbeek A, Destree O, Meijlink F, Clevers H. 1993. Differential expression of the HMG box factors TCF-1 and LEF-1 during murine embryogenesis. *Development* 118:439–448.
- Peifer M, Orsulic S, Pai LM, Loureiro J. 1993. A model system for cell adhesion and signal transduction in Drosophila. *Dev Suppl* 163–176.
- Pelegri F, Maischein HM. 1998. Function of zebrafish beta-catenin and TCF-3 in dorsoventral patterning. *Mech Dev* 77:63–74.
- Polesskaya A, Seale P, Rudnicki MA. 2003. Wnt signaling induces the myogenic specification of resident CD45+ adult stem cells during muscle regeneration. *Cell* 113:841–852.
- Roose J, Molenaar M, Peterson J, Hurenkamp J, Brantjes H, Moerer P, van de Wetering M, Destree O, Clevers H. 1998. The Xenopus Wnt effector XTcf-3 interacts with Groucho-related transcriptional repressors. *Nature* 395:608–612.
- Schmidt M, Tanaka M, Münsterberg A. 2000. Expression of beta-catenin in the developing chick myotome is regulated by myogenic signals. *Development* 127: 4105–4113.
- Skromne I, Stern CD. 2001. Interactions between Wnt and Vg1 signalling pathways initiate primitive streak formation in the chick embryo. *Development* 128: 2915–2927.
- Snider L, Tapscott SJ. 2003. Emerging parallels in the generation and regeneration of skeletal muscle. *Cell* 113:811–812.
- Tago K, Nakamura T, Nishita M, Hyodo J, Nagai S, Murata Y, Adachi S, Ohwada S, Morishita Y, Shibuya H, Akiyama T. 2000. Inhibition of Wnt signaling by ICAT, a novel beta-catenin-interacting protein. *Genes Dev* 14:1741–1749.
- Takemaru KI, Moon RT. 2000. The transcriptional coactivator CBP interacts with beta-catenin to activate gene expression. *J Cell Biol* 149:249–254.
- Takemaru K, Yamaguchi S, Lee YS, Zhang Y, Carthew RW, Moon RT. 2003. Chibby, a nuclear beta-catenin-associated antagonist of the Wnt/Wingless pathway. *Nature* 422:905–909.
- Van de Wetering M, Castrop J, Korinek V, Clevers H. 1996. Extensive alternative splicing and dual promoter usage generate Tcf-1 protein isoforms with differential transcription control properties. *Mol Cell Biol* 16:745–752.
- Waltzer L, Bienz M. 1998. Drosophila CBP represses the transcription factor TCF to antagonize Wingless signalling. *Nature* 395:521–525.
- Wei Y, Renard CA, Labalette C, Wu Y, Levy L, Neuveut C, Prieur X, Flajolet M, Prigent S, Buendia MA. 2003. Identification of the LIM protein FHL2 as a coactivator of beta-catenin. *J Biol Chem* 278: 5188–5194.
- Zorn AM, Barish GD, Williams BO, Lavender P, Klymkowsky MW, Varmus HE. 1999. Regulation of Wnt signaling by sox proteins: XSox17 alpha/beta and XSox3 physically interact with beta-catenin. *Mol Cell* 4:487–498.