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Tamoxifen-dependent, inducible Hoxb6CreER^T recombinase function in lateral plate and limb mesoderm, CNS isthmic organizer, posterior trunk neural crest, hindgut and tailbud

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Abstract

The ability to generate conditional mutant alleles in mice using Cre-lox technology has facilitated analysis of genes playing critical roles in multiple developmental processes at different times. We used a transgenic *Hoxb6* promoter to drive tamoxifen-dependent Cre recombinase expression in several developing systems that serve as major models for elucidating inductive interactions and mechanisms of morphogenesis, including lateral plate mesoderm and descendant limb buds, neural crest progenitors of the neural tube, tailbud, and CNS isthmic organizer. The Hoxb6CreER^T line gives very rapid and complete recombination over a short time window after a single tamoxifen dose, allowing precise time requirements for gene function to be assessed accurately. Embryonic cells cultured from the Hoxb6CreER^T line also display rapid recombination *ex vivo* after tamoxifen exposure. Hence, the Hoxb6CreER^T line provides a valuable tool for analyzing gene function, as well as lineage tracing studies using genetic cell marking, in several developing systems.

Keywords

Tamoxifen-dependent Cre; Hoxb6 transgenic promoter; limb; lateral plate; neural crest; CNS isthmic organizer; tailbud; gut endoderm

Introduction

Tamoxifen-regulated CreER^T transgenic lines (Feil et al, 1996; Brocard et al, 1997; Vooijs et al, 2001; Hayashi and McMahon, 2002; Nakamura et al, 2006; rev. by Nagy, 2000; Lewandoski, 2001) offer an avenue to dissect gene function in specific tissues at selective times, and also provide a very powerful tool for lineage and fate mapping analysis in mice (eg. Kimmel et al, 2000; Harfe et al, 2004; Ahn et al, 2004; Arques et al, 2007; Ohtola et al, 2008). A transgenic *Hoxb6* promoter containing a lateral plate mesoderm (LPM)-limb enhancer has been extensively characterized previously (Schughart et al, 1991; Eid et al, 2003; Becker et al, 2006), and used to generate a versatile Cre-expressing line demonstrating robust recombination at sites of expression (Lowe et al, 2000), as well as efficiently driving other transgene expression in the early limb bud (Knezevic et al, 2007). Vertebrate limb

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development serves as a seminal model for elucidating how signaling cascades regulate pattern formation and morphogenesis. Yet very few conditional CreER^T deleter lines that enable selective gene removal in the limb bud mesoderm are currently available, and those that have been generated are either expressed in a specific subdomain of the early limb bud (eg. *Shh* promoter; Harfe et al, 2004), or are somewhat mosaic or relatively delayed in activity after appearance of the early limb bud (eg. *Prx* promoter; Hasson et al, 2007). We therefore generated a CreER^T line under the control of the *Hoxb6* promoter to achieve selective and temporally regulated recombination in limb mesoderm from the earliest limb bud stages.

Conditional expression of Cre recombinase driven by the robust *Hoxb6* promoter has enabled precise temporal delineation of Cre-mediated recombination enabling the time course of gene function to be analyzed (eg. Zhu et al, 2008. As we observed previously (Nakamura et al, 2006), effective tamoxifen levels for embryonic Cre activation following intraperitoneal (IP) administration were restricted to a fairly narrow temporal window of <12 hours and, during the stages of peak expression levels, a single IP dose also gave complete recombination within 12-18 hrs. In younger embryos (<E10.5), recombination was complete in as little as 8hrs. (this report, see also Zhu et al., 2008), facilitating a precise temporal analysis of gene function. This *Hoxb6*-driven conditional Cre line also has utility in lineage tracing experiments for progenitors that contribute to several multi-potent tissues, such as neural crest, tailbud or lateral plate (eg. Ohtola et al, 2008).

Results and Discussion

The 3.6 kbp *Hoxb6* promoter including limb/LPM enhancer was introduced into the CreER^T pSG5 expression vector upstream of the β-globin intron, replacing the CMV promoter (Fig1A; Schughart et al., 1991; Feil et al, 1996). Transgenic founders were evaluated in crosses with the R26R LacZ reporter line (Soriano, 1999) to generate embryos for analysis of Cre recombinase activity by assessing LacZ reporter activation. Two out of eight founders gave efficient and complete recombination at sites of transgenic *Hoxb6* promoter expression after tamoxifen treatment. One of these also displayed minimal Cre activity in the absence of tamoxifen (see Fig 1), and was bred to establish a Hoxb6CreER^T line, the characterization of which is presented in this report (Mouse Genome Informatics designation Tg(Hoxb6-cre/ESR1)1Smac).

In preliminary studies, the dose-response of Cre activity to tamoxifen was evaluated. Comparable to results previously reported for other tamoxifen-regulated Cre lines (eg. Hayashi and McMahon, 2002; Nakamura et al, 2006), injections of a minimum of 1.5 mg were required to achieve efficient recombination levels in 18-24 hrs (data not shown). We subsequently used doses that reproducibly gave efficient recombination with minimal side effects (1.5 mg IP /25 gm pregnant female; see also methods) to characterize the spatial distribution, kinetics and extent of recombination in the Hoxb6CreER^T line at different stages of embryogenesis ranging from E8 to E12, when the transgenic *Hoxb6* promoter is most active (see Schughart et al, 1991; Eid et al, 1993; Becker et al, 1996). We also compared tamoxifen routes of administration since some prior reports have employed gavage administration (eg. Ahn and Joyner, 2004; Hasson et al, 2007; Park et al, 2008). To evaluate the kinetics of tamoxifen action, embryos were harvested 8 hrs following a single tamoxifen dose given by either route. We found that LacZ reporter activation was much more rapid following IP than oral administration for this early time point (8 hrs after treatment; suppl. Fig. 1). In one previous study reporting higher efficacy of gavage over IP route (Park et al, 2008), tamoxifen was administered at a very early stage (~E6.75) prior to establishment of an effective placental-embryonic circulation and, although recombination was clearly higher after gavage treatment, the kinetics were somewhat delayed compared to

our results with IP administration at early stages (12-18 hrs vs. 8-12 hrs needed for efficient recombination, see below). The relative delay in peak activity for the oral route compared to IP may explain the better efficacy of gavage previously observed in very early embryos (ie. <E7.5).

A survey of the overall Cre activity during early embryogenesis (E8-12) is shown in Fig. 1B, with LacZ reporter activation assayed at 24 hrs after a single Tamoxifen dose. Some scattered LacZ positive cells were always observed in the previously reported sites of transgenic *Hoxb6* promoter expression (see Schughart et al, 1991; Lowe et al, 2000) even in the absence of tamoxifen injection, increasing in extent from E9.5 to E12.5. This background level of recombination probably reflects high level $CreER^T$ expression coupled with some proteolysis (see also Nakamura et al, 2006) to produce a non-conditional recombinase. However, the extent of this background recombination did not produce any overt mutant phenotypes in animals homozygous for one of several different floxed mutant alleles tested, that were bred to also carry the transgenic CreER allele (Zhu et al, 2008; and MTN and SM unpublished observations).

As previously reported for a non-conditional Hoxb6Cre line (Lowe et al, 2000), Cre activity was present in early ventrolateral body wall, including the lateral plate, and the yolk sac (Fig. 1B, E8-8.5, and not shown). Activity persisted through E12 in the posterior lateral plate mesoderm beginning at the forelimb level, with the anterior-most part of the forelimb negative. From the hindlimb region and caudal, the dorsal neural tube and neural crest derived dorsal root ganglia, as well as the entire tail bud, were also positive for LacZ activity (Fig. 1B, see also Figs. 2, 5). Strong Cre activity was also evident in the CNS isthmic organizer (midbrain-hindbrain junction; see review by Liu and Joyner, 2001; Wurst and Bally-Cuif, 2001) beginning from E8.5 onward. After E11.5, recombinase activity declined at all sites of *Hoxb6*-driven expression, but waned more rapidly in the ventrolateral body wall mesoderm than the limbs. By E12.5, activity at all sites was weak and mosaic, consistent with the declining expression from this transgenic promoter. Notably, recombination in the early mammary buds was also apparent by this stage (Fig. 1B, E12.5 arrow).

Spatiotemporal characteristics of Hoxb6CerER activity in developing limb

Because very few conditional Cre drivers that span the course of early limb development beginning from early pre-limb field stages have been developed to date, the kinetics of recombination were extensively characterized in the limb mesoderm from E8.5-11.5 (Figs. 2-4). Tamoxifen administration at E8.5 already gave substantial recombination within 12 hrs (Fig. 2A). Sectioning at 18 hrs after treatment showed complete recombination throughout lateral plate mesoderm from posterior forelimb field to tailbud (Fig. 2B-D). The ectoderm showed no reporter activation, including the AER evaluated at 48 hrs after tamoxifen was given (Fig. 2I). The anterior-most part of the pre-forelimb field and early forelimb bud did not display any Cre activity and analysis of forelimbs collected at E13.5 following tamoxifen treatment at E8.5 (or later) revealed that only digit 1 precursors arose from this anterior Cre-negative zone (Fig. 2E-G, I, J; Fig. 4). Single dose tamoxifen treatment at E9.5, or at E10.5 also gave complete recombination in limb bud mesoderm within 12-24 hrs (Fig. 3). However, somite-derived myoblasts and vascular endothelial cells that had entered the limb bud (Chevalier et al, 1977; Christ et al, 1977; Pardanaud et al, 1996) did not display any Cre activity (Suppl. Figure 2). By E10.5, recombination was not as robust in kinetics in the ventral body wall-flank mesoderm and tail region compared to limb, although recombination in these domains was also complete within 24 hrs (see below). In a previous kinetic analysis of recombination efficiency of the $Hoxb6CreER^T$ line after single tamoxifen doses (ranging from E9.5 to E10), the loss of *Shh* function in early $Shh^{flox/flox}$ limb buds was assayed by monitoring expression of *Ptch1*, a direct Shh target (Zhu et al, 2008).

Complete recombination was also achieved rapidly in that kinetic study; *Shh* transcripts were absent within 6hrs of treatment and *Ptch1* transcripts were absent within 12hrs of treatment. This functional assay for residual signaling activity is arguably far more sensitive in determining completeness of recombination than evaluation of LacZ reporter activation in embryos, and confirms the efficacy predicted from the LacZ activation analyses.

After E11, recombination in limbs became restricted to mesenchyme because, as previously noted (Schughart et al, 1991), the *Hoxb6* transgenic promoter activity is down-regulated in limb mesoderm undergoing chondrogenic differentiation (Fig 4). Evaluation of LacZ reporter activity at late limb stages when digits condensations have appeared (Fig 4), revealed that tamoxifen administration as late as E11 in the hindlimb resulted in digit condensations that were entirely LacZ positive, whereas tamoxifen injection at E11.5 produced complete recombination mainly in the mesenchyme surrounding the differentiating chondrogenic condensations, with mosaic LacZ activity within the differentiating digit rays. In the forelimb, this loss of recombination in digit rays was shifted about 12 hrs earlier and recombination was already partly mosaic in the anterior digit 2 condensation by E10.5, and became reduced in all of the differentiating condensation cores (digit 2-5) by E11.5 tamoxifen administration. Since digit condensations are already beginning to form by E11.5-E12 (Nakamura et al, 2006; Zhu et al, 2008), the observed loss of LacZ reporter activity within condensations after tamoxifen treatment at ~E11 in forelimb and E11.5 in hindlimb is consistent with shut-off of the *Hoxb6* transgenic promoter in cells undergoing chondrogenic differentiation.

Tamoxifen treatment as late as E11.5 still gave complete recombination (assayed at E13.5) in mesenchymal interdigit domains where the *Hoxb6* promoter remained active, but by E12 administration, subsequent recombination was somewhat mosaic and non-uniform across the digital plate. Previous analysis of the transgenic *Hoxb6* promoter driving a LacZ reporter or other transgenes indicated a loss of promoter activity in the midzone of the hindlimb bud by about E11.5, but the timing and spatial extent of this loss of hindlimb mid-zone activity was somewhat variable for different transgenic lines (Schughart et al, 1991; Eid et al, 1993; Knezevic et al, 1997). In the *Hoxb6*-driven CreER^T line described here, a similar mid-zone loss of expression encompassing the digit 3-4 region was observed by E12 (Fig. 4), which is somewhat delayed relative to previous reports, perhaps reflecting variation of transgenic promoter activity, or possibly the high specific activity of Cre recombinase, even when expressed at reduced levels.

Characteristics of Hoxb6CreER^T-driven recombination in other developing organs and cultured embryonic cells

Cre activity was also evaluated at other sites of *Hoxb6* transgenic promoter expression. In the cranial CNS, strong activity and efficient recombination was seen in the midbrain-hindbrain junction after a single dose tamoxifen given at times ranging from E8.5 through E10.5 (Fig. 5A-E and not shown), and complete recombination was seen within 24 hrs following a single dose. Notably, activity was ventrally restricted and the dorsal midline of the midbrain-hindbrain region was negative for LacZ reporter activity, whereas the isthmic organizer region showed complete and robust recombination. We also noticed that, in early embryos treated with a single dose tamoxifen ranging from E8.5 to E10.5, the dorsal neural tube, which gives rise to the neural crest, was positive for LacZ reporter activity in the posterior trunk (Fig. 2D, H) and at later stages, recombination was also evident in the dorsal root ganglia (DRG) descendant from neural crest (Fig. 5F). Recombination was also noted in the mid- and hindgut endoderm and immediately subjacent mesenchyme (Figs 2C, D, H; 5E, F). The foregut-midgut transition occurs at the forelimb bud level (see Wells and Melton, 1999) and recombinase activity was never detected anterior to this axial level. Following tamoxifen treatment at E10.5, sagital whole mount sections of LacZ stained embryos

revealed Cre activity in the gut, beginning near the foregut-midgut transition, just at the point where the midgut exits the umbilicus and initiates rotation (reviewed by Kluth et al, 2003), and extending caudally through the hindgut (Fig. 5E, F). In the tailbud, tissues of all germ layers, excluding the surface ectoderm, showed uniform and complete recombination after tamoxifen injections ranging from E8.5 to E10.5 and, at later stages, the posterior-most epithelial somites and neural tube that had recently arisen from tailbud progenitors also showed strong LacZ reporter activity (Fig 5F).

It is often useful to isolate cells from embryos and activate recombination *ex vivo* in tissue culture for mechanistic analyses (eg. MEFs, micromass cultures). Since the early hindlimb bud expresses *Hoxb6* promoter-driven Cre activity very strongly and uniformly, E10.25 hindlimb buds were isolated, dissociated and plated at low-density in culture to evaluate the kinetics and efficiency of tamoxifen-induced recombination in culture. In contrast to a previous report using a ubiquitous promoter to drive transgenic CreER^T expression in which recombination in cultured cells required 2 days to near completion (Hayashi and McMahon, 2002), we found that recombination of the LacZ reporter in Hoxb6CreER^T positive cells was already extensive after 12 hrs (>50%) and was essentially complete after about 24 hrs in culture with 4-OH-tamoxifen (Fig. 6). A few LacZ negative cells always persisted in culture (<2%), even after a few days of tamoxifen treatment, perhaps reflecting persistence of some cell types not expressing the transgenic *Hoxb6* promoter (eg. surface ectoderm; somite-derived myoblasts, vasculature).

Conclusions

The Hoxb6CreER^T line characterized in this report gives efficient recombination in early stage embryos at sites of expression after a single tamoxifen dose (1.5 mg/25 gm animal) that occurs over a fairly narrow time window of less than 12 hours, as tested both by reporter activity (this paper) and functionally, by removing a conditional allele for a signaling factor (Zhu et al, 2008). This line will prove valuable as a tool for molecular genetic manipulations of genes functionally important in the early limb bud and other lateral plate derivatives, as well as the CNS isthmic organizer, the posterior trunk neural crest, mid/hindgut and in tailbud development. The conditional, tamoxifen-regulated activation of Cre recombinase in combination with appropriate reporters will also be useful for lineage tracing studies in sites of embryonic expression, such as the trunk neural crest, and has already been used to assess the contribution of lateral plate to ventral body wall dermal tissues (Ohtola et al, 2008).

Experimental Procedures

Transgenic constructs and generation, screening and breeding of transgenic mice

The 3.6kb *Hoxb6* promoter containing the limb enhancer (Schughart et al, 1991) was inserted upstream of the β-globin intron in the pSG5 derivative containing the CreER^T (T1) coding region (Feil et al, 1996) using SfiI to replace the SV40 promoter in that plasmid. A 3.6kbp SalI-EcoRV fragment containing the *Hoxb6* promoter was isolated and ligated to an SfiI (blunted)-SalI fragment of the CreER^T containing pSG5 vector to generate the Hoxb6CreER^T expression construct, which was injected into FVB/N zygotes to create transgenic lines, as described by Hogan et al. (1994). Transgenic founders were identified by DNA extraction from tails and Southern blot analysis using a CreER coding probe. Subsequent genotyping of animals in an established line was done using PCR primers from the Cre (5′-GAAAATGCTTCTGTCCGTTTGC-3′ and 5′-ATTGCTGTCACTTGGTCGTGGC-3′; 207bp amplicon) or the ER ligand-binding domain (5′- CCTGGCTAGAGATCCTG -3′ and 5′- GCCCTCTACACATTTTCCCT -3′; 110bp amplicon) coding region. The transgenic line selected as giving the strongest recombination

with minimal Cre recombinase activity in the absence of tamoxifen, was subsequently bred and maintained to give homozygous animals without any affects on viability or fertility. The Rosa26LacZ reporter line (Soriano, 1999) was used to assess recombinase activity by crossing to the Cre transgenic mice and analyzing LacZ activation in CreER^T; Rosa26LacZ compound heterozygous embryos (hemizygous for CreER^T transgene). Control progeny were harvested either following injection with vehicle alone, or without IP injection for analysis of LacZ activity levels in the absence of Tamoxifen.

Tamoxifen injections and embryo analyses

For determination of embryonic ages, noon on the day of the post-coital plug was taken to be E0.5. Tamoxifen (Sigma, T-5648) was administered by IP injection as previously described (Nakamura et al, 2006) with inclusion of half the dosage of progesterone to reduce abortion rates after tamoxifen. In our strains, pregnant mice at about 10-11 days post-coitus averaged 25 gms body weight. Unless otherwise noted, a 1.5 mg dose of tamoxifen was administered. For analysis of LacZ activity, embryos were fixed with 2% paraformaldehyde-0.2% glutaraldehyde in PBST as previously described, and staining for LacZ activity was performed using X-Gal substrate, for 2 to 4 hours in whole mount embryos (Nakamura et al, 2006). For sectioning, embryos stained in whole mount were post-fixed in 4% paraformaldehyde in PBS overnight, followed either by paraffin embedding, or by cryo-protection in 30% sucrose overnight at 4°C and OCT embedding. Unstained embryos (fixed for 30 min-2 hrs) were embedded in OCT and cryo-sectioned, and sections were then stained for LacZ activity using X-Gal. Myoblasts were identified by immunohistochemical staining with the pan-myosin marker MF20 (Developmental Studies Hybridoma Bank, Iowa) at a 1:200 dilution of primary IgG concentrate, using conditions described by Dong et al. (2006). Bound primary was detected with anti-mouse secondary antibody conjugated to Alexa 594 (1:500). Endothelial cells were identified morphologically based on their intravascular location.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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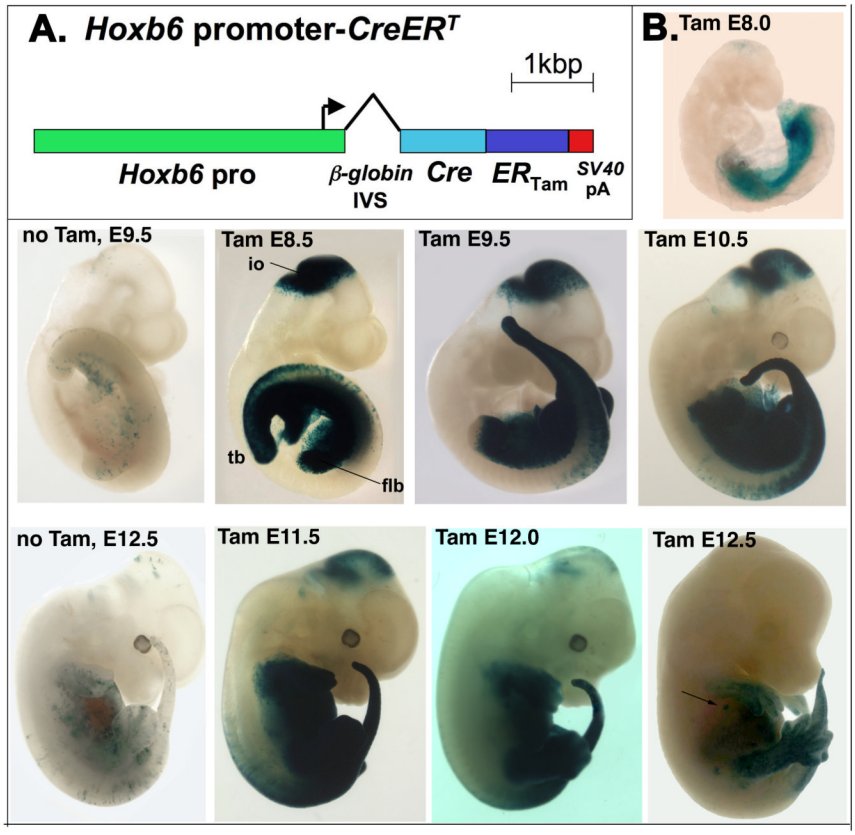

Figure 1.

Generation of a Hoxb6CreER^T line and activity in early embryos at 24 hrs after administration of a single tamoxifen dose.

A. Schematic diagram of transgenic Hoxb6CreER^T expression construct used to generate transgenic mice, which includes 3.6kbp of the *Hoxb6* promoter with limb-lateral plate enhancer, the β-globin intron (IVS), and the SV40 polyA additional signal. B. Overview of tamoxifen-dependent recombination in CNS, lateral plate and tailbud during early development (E8.0-12.5). A single dose tamoxifen was injected IP at the times indicated in each panel, and embryos were harvested and stained for LacZ activity at 24 hours post-injection. The far left panels (top and bottom) show representative embryos at E9.5 and at E12.5 stained for LacZ reporter activity without any tamoxifen treatment, demonstrating level of background Cre activity. Cre activity in lateral plate and ventral body wall is already evident after tamoxifen treatment at E8, and does not decline appreciably until about E12. Activity in midbrain-hindbrain neuroectoderm including the junctional isthmic organizer (io) and in tailbud (tb) is detected after tamoxifen treatment at E8.5 and remains robust until E11.5-12, as does activity in the forelimb (flb) and hindlimb (hb) buds. Arrow in E12.5 treated panel shows mammary bud staining.

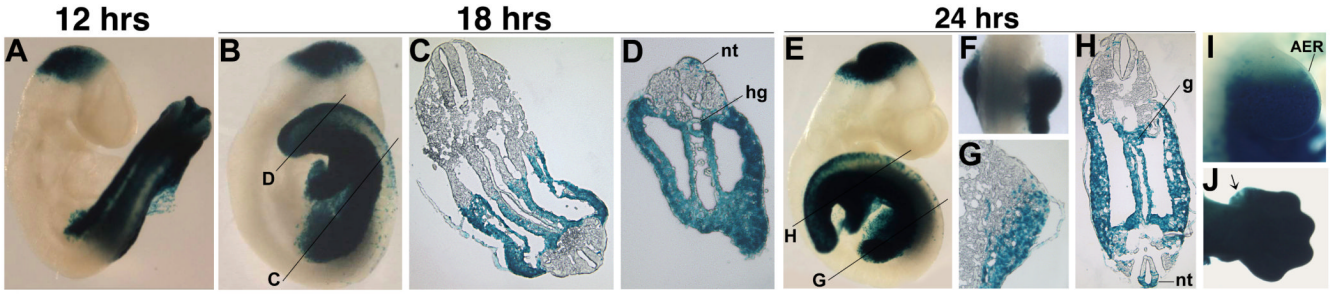

Figure. 2.

Time course and recombination efficiency of Hoxb6CreER^{T} in developing limb buds following single dose Tamoxifen injection at E8.5.

(A-D) Single dose tamoxifen resulted in strong LacZ reporter activity throughout both lateral plate mesoderm and midbrain-hindbrain junctional region within 12 hrs after injection (A), as well as within very early tailbud, apparent at 18 hrs (B, D). Sectioning at 18 hrs after treatment (levels of sections C, D are indicated in B) showed complete recombination in both somatic/parietal and splanchnic/visceral lateral plate mesoderm, as well as caudal midgut and hindgut (hg), caudal dorsal neural tube and incipient tailbud (C, D; see also Fig. 5E). (E-H) At 24 hrs, when the forelimb bud has emerged, both wholemount embryos (E), and sections (F-H; levels of sections are indicated in E) reveal strong activity extending rostrally up to the anterior 1/4 forelimb bud mesoderm (F,G). (I) The ectoderm, including mature AER shown at E10.5, is completely negative for *Hoxb6* promoter expression and Cre recombinase activity. (J) At E13.5, the anterior-most mesoderm of the forelimb remains negative for LacZ reporter activity and this persistently negative region has given rise to digit one (arrow; see also Fig. 4). All panels are oriented with rostral at the top.

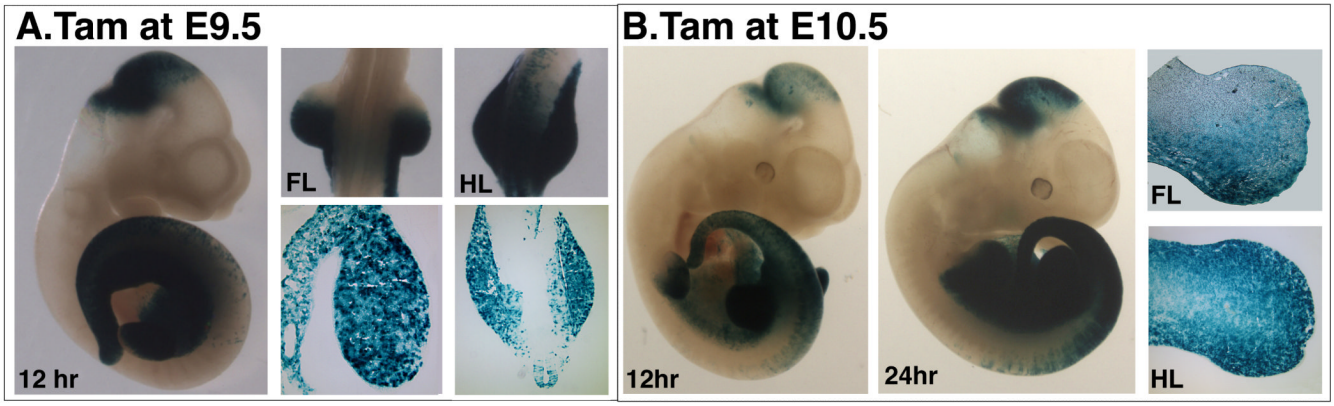

Figure 3.

Efficiency of Tamoxifen-regulated recombination by Hoxb6CreER^{T} following single Tamoxifen injection at E9.5 or E10.5.

A single tamoxifen dose at either E9.5 (A) or at E10.5 (B) gave strong recombination, as judged by LacZ reporter activity, in the posterior 3/4 of forelimb (FL) and throughout hindlimb (HL) buds by 12 hrs after administration. However, for E10.5, reporter recombination in the flank and ventral body wall region and the tailbud required 24 hrs to appear complete. Sections at 12 hrs (A-lower FL and HL panels) or at 18hrs (B- FL and HL panels) showed complete recombination within domains of expression in the limb bud mesoderm. All panels are oriented with rostral at the top.

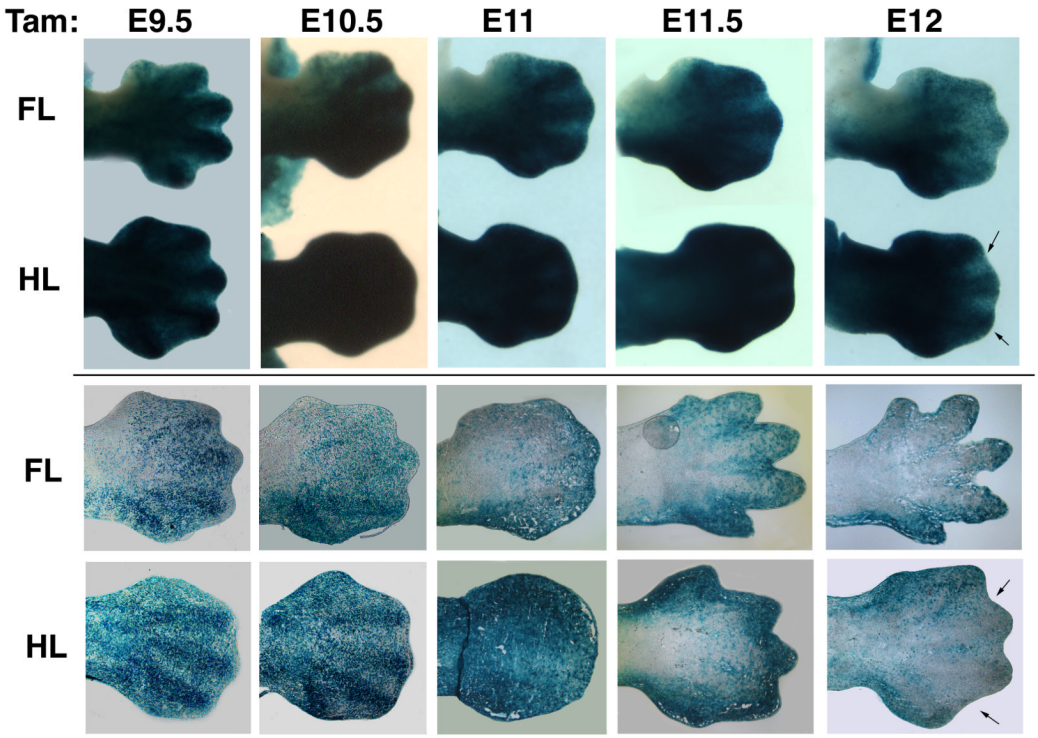

Figure 4.

Extent of recombination assayed in later-stage limb buds and decline of Hoxb6CreER^{T} activity in cells undergoing chondrogenic differentiation.

Embryos received a single dose tamoxifen administered at the stages indicated at the top (ranging from E9.5 to E12), and were collected at between 24-48 hrs after treatment (E12-E14) and sectioned to assess recombination in mesodermal tissues. Whole mount panels (upper panels) show examples of forelimbs (FL) and hindlimbs (HL) from embryos collected between E12.5-E13.5. Note that the digit 1 region of the FL is always negative for LacZ activity. The HL LacZ activity is uniform along the limb A-P axis until about E12, when the mid-region shows weaker activity than the anterior and posterior borders (arrows). The sections of limbs shown in lower panels were from embryos collected at E13 (for E9.5 and E10.5 injections), E12 (for E11 injection), E13.5 (for E11.5), and at E14 (for E12 tamoxifen IP injection). As previously reported (Schughart et al, 1991), the *Hoxb6* promoter activity driving Cre expression is restricted mainly to undifferentiated mesenchyme, and declines as chondrogenic differentiation proceeds. Reduced LacZ activity is evident in digit condensations compared to interdigital mesenchyme on sections from embryos that were treated with tamoxifen at E11 or later for FL, and E11.5 or later for HL. All panels are oriented with rostral at the top.

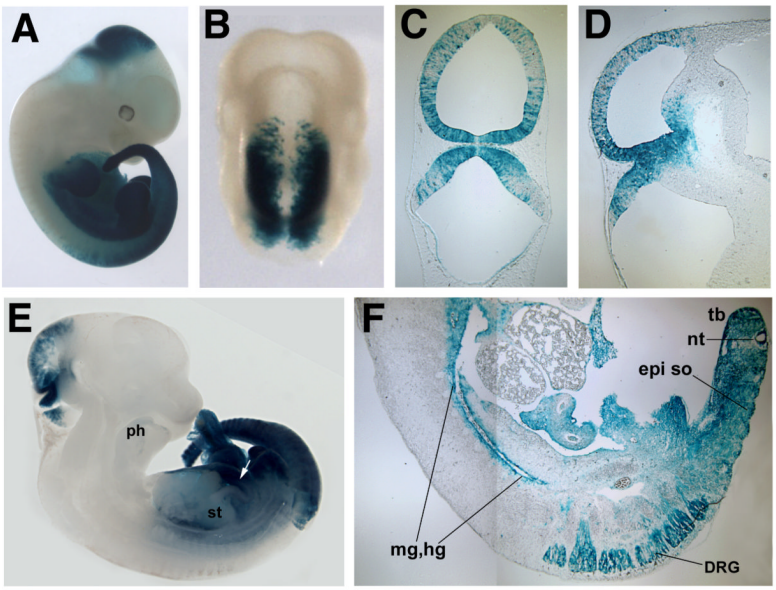

Figure 5.

Hoxb6CreER^{T} activity in non-limb sites: CNS isthmic organizer, caudal neural crest, mid/hindgut, and tailbud.

(A-D) Strong Cre activity in the midbrain-hindbrain junction was evident on wholemount staining for LacZ reporter activity beginning at E8.5 (panel B, tamoxifen injected at E8.5; collected after 24 hrs and stained for LacZ activity). Embryos exposed to tamoxifen at E10.5 and collected at 24 hrs. (wholemount shown in panel A) were also sectioned through the head and the body to evaluate recombination within non-limb tissues in which the *Hoxb6* transgenic promoter is active. Cre activity in the head CNS was highly restricted to the midbrain-hindbrain junctional region (C-frontal section, D-sagital section) and complete recombination was seen within the isthmic organizer zone. (E) Sagitally bisected whole mount embryo collected 24 hrs after tamoxifen treatment at E10.5 revealed gut LacZ staining beginning at the entry of midgut through the umbilical hernia (arrow), whereas the foregut (pharynx, ph; stomach, st) showed no LacZ activity. (F) In sagital histologic sections, the caudal midgut and entire hindgut endoderm (mg, hg), along with immediately subjacent mesenchymal cells, showed complete recombination; similar to earlier stages (see Fig. 2C, D, H). Sagital sections also showed recombination in caudal dorsal root ganglia (DRG), consistent with the earlier observed LacZ activity in the dorsal neural tube (from which neural crest arises) in the caudal embryo (see also Fig. 2D, H; 3A). The tail region (tb) showed expression in tissues of all germ layers including the entire neural tube (nt) and epithelial somites (epi so) descendent from tailbud mesoderm.

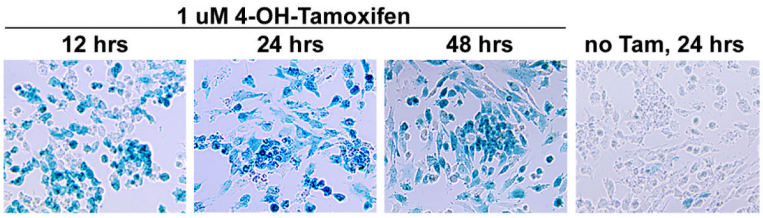

Figure 6.

Efficiency of Hoxb6CreER^T activity *ex vivo* in cultured limb bud cells.

Dissociated cells from E10.25 hindlimb buds of Hoxb6CreER^T; RosaLacZ embryos were cultured as described previously (Knezevic et al, 1997). Cultures were exposed to 1 uM 4-OH-tamoxifen or to vehicle alone (control) immediately after plating, harvested at the times indicated and stained for LacZ activity. Untreated control cultures showed a few scattered cells with LacZ activity (~4%). Cultures exposed to 4-OH-tamoxifen showed substantial, but not complete recombination after 12 hrs (~ 50%). By 24 hrs, recombination was essentially complete (~95%) and comparable to 48 hrs. Notably, there were always a few cells (~2%) remaining negative for LacZ activity, possibly reflecting cell types not expressing the *Hoxb6* promoter.