

### Figures and figure supplements

A shift in anterior–posterior positional information underlies the fin-to-limb evolution

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**Figure 1**. Anterior–posterior patterning in *Scyliorhinus canicula* pectoral fin buds. (**A**) Skeletal patterns of *S. canicula* pectoral fin and mouse limb. Blue colours, homologous elements. (**B**–**G**) In situ hybridisation for *Alx4* (**B**), *Pax9* (**C**), *Hand1* (**D**), *Zic3* (**E**), *Hand2* (**F**), and *Tbx2* (**G**) in *S. canicula* pectoral fin buds at stage 30 and mouse limb bud at E11.5 (left panel in **G**). Arrowheads in **G**, anterior boundary of posterior *Tbx2* expression. Dorsal view; anterior is to the top. Scale bars, 100 µm. (**H**) Schematic of the gene expression patterns. Arrowheads, *Hand2* expression boundary. Expressions of mouse limb buds at E11.5 are after EMBRYS database (*Yokoyama et al., 2009*; *Fernandez-Teran et al., 2003*).

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Figure 1—figure supplement 1. Molecular phylogenetic trees of relevant *S. canicula* genes. (A–E), Trees for Alx4 (A), Pax9 (B), Hand1 (C), Zic3 (D), Tbx2 (E), and Ptch1 (F) were generated from amino acid sequences of the homeodomain and neighbouring sequences (A), the paired box and C-terminal sequences (B), Helix-loop-helix domain and C-terminal sequences (C), N-terminal sequences (D), and C-terminal sequences (E and F). The neighbour-joining method was used for constructing the trees. The numbers at nodes indicate bootstrap probabilities with 1000 replicates. DOI: 10.7554/eLife.07048.004



Figure 1—figure supplement 2. Temporal expression analysis of Alx4, Pax9, Hand2, and Hoxa13 in S. canicula pectoral fins. (A–D) Expression of Alx4 (A), Pax9 (B), Hand2 (C), and Hoxa13 (D) at the indicated stages. Dorsal view of right pectoral fin buds of S. canicula embryos (anterior to the top). (A) Alx4 was initially expressed throughout the fin buds at stage 25, but the posterior expression of Alx4 was reduced by stage 27. Alx4 expression was then restricted to the anterior two-thirds of the fin bud at stage 29 and to the anterior half of the fin bud at stage 31. (B) Pax9 expression was not detected at stage 27 but was present in anterior distal fin buds at stage 29. This expression persisted until at least stage 31. (C) Hand2 was initially expressed throughout the fin bud, although slightly more-robust expression was observed at the posterior side. Subsequently, Hand2 expression became posteriorly restricted by stage 27. (D) Hoxa13, which is a marker for a late developmental stage in tetrapod limb buds, was detected since stage 28 (left) and late stage 29 (right). pg, pectoral girdle; ms, mesopterygium; mt, metapterygium. (F) Comparison of Alx4 (black), Pax9 (grey), Hand2 (blue), and Hoxa13 (light green) expression patterns between S. canicula fin buds and chick forelimb and hindlimb buds (see Figure 1—figure supplement 3 for detailed gene expression in chick limb buds). st, stage. Scale bars, 100 µm. DOI: 10.7554/eLife.07048.005



**Figure 1—figure supplement 3**. Temporal expression analysis of *Alx4*, *Pax9*, *Hand2*, and *Hoxa13* in chick limb buds. (**A**–**H**) Expression of *Alx4* (**A**, **B**), *Pax9* (**C**, **D**), *Hand2* (**E**, **F**), and *Hoxa13* (**G**, **H**) at the indicated stages. Dorsal views of chick forelimb (**A**, **C**, **E**, **G**) and hindlimb (**B**, **D**, **F**, **H**) buds (anterior to the top; distal to the right). (**A**, **B**) At stage 19, *Alx4* expression was broad but was slightly weaker in the posterior side of forelimb (**A**) and hindlimb (**B**) buds. At later stages, its expression became restricted to the anterior one-third of the limb buds, with subsequent further restriction to the anterior proximal region both in forelimb (**A**) and hindlimb (**B**) buds. *Alx4* expression also appeared in the posterior distinct region of chick forelimb (**A**) and hindlimb (**B**) buds at stage 25. (**C**, **D**) *Pax9* expression was first present in the anterior region at stage 24 in forelimb buds (**C**) and stage 23 in hindlimb buds (**D**) but was more distally restricted than *Alx4* expression. At stage 25, expression of *Pax9* was robust, but the extent of its overlap with *Alx4* expression was limited both in forelimb (**C**) and hindlimb (**D**) buds. (**E**, **F**) *Hand2* expression fully overlapped with the distal *Hand2* expression domain in forelimb (**G**) and hindlimb (**H**) buds. At later stages, co-expression of *Pax9* and *Hoxa13* was observed in the anterior distal portion of the limb buds, but this overlap was not as extensive as that between the *Hand2* and *Hoxa13* expression domains. st, stage. Scale bars, 100 µm. DOI: 10.7554/eLife.07048.006







Figure 2—figure supplement 1. Phylogenetic tree of Gli2 and Gli3, and PDD amino acid sequences. (A) Trees for Gli2 and Gli3 were generated from amino acid sequences of the zinc finger domain and PDD. The neighbour-joining method was used for constructing the trees. The numbers at nodes indicate bootstrap probabilities with 1000 replicates. (B) Human Gli3 (the upper diagram) is composed of an N-terminal repressor domain (Repressor), a DNA-binding domain (Zinc finger), and a C-terminal activator domain (Activator). The alignment shows the homologous PDD domains from both human and S. canicula Gli2 and Gli3.

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**Figure 3**. The *Gli3* limb-specific enhancer of *S. canicula* and *C. milii.* (**A**) VISTA plots of *Gli3* intron 3 from indicated animals. Blue vertical bars, exons of human *Gli3*; black rectangle, element 1586. Regions with >70% identity are indicated: blue, exon; pink, non-coding sequences. (**B**) The enhancer construct. (**C**) GFP expression in chick forelimb buds driven by chick element 1586 at stage 19 (top, n = 3/3), stage 23 (middle, n = 14/14), and empty vector (bottom, n = 0/7). pCAGGS-*RFP* (right). (**D**–**F**) GFP expression driven by element 1586 of *S. canicula* (**D**, n = 11/11), *C. milii* (**E**, n = 10/10) and Sc1586mt (**F**, n = 4/4). Scale bars, 100 µm. (**G**) Scheme of Sc1586mt, *S. canicula* enhancer (blue) partially replaced by chick sequence (green) and alignment. Boxes indicate tetrapod (i) and sarcopterygian (ii) specific sequences. DOI: 10.7554/eLife.07048.009



**Figure 3—figure supplement 1**. Detailed functional analyses of element 1586. (**A**) Alignment of element 1586 sequences. Points where chick and *S. cancicula* enhancers are recombined in (**B**) are indicated by arrows a and b. Box i and Box ii are sequences that are replaced with *S. canicula* sequence in (**C**), activities of element 1586 recombined at the indicated points. Note that the posterior repressive activity is not altered at arrow a, but partially destroyed at arrow b, suggesting that the repressive sequences are located between arrow a and b and after arrow b. (**C**) Activities of chick element 1586 that are partially recombined with *S. canicula* sequences at Box i and Box ii in (**A**). The sequences were chosen because of specific conservation among tetrapods (Box i) and sarcopterygians (tetrapod and coelacanth, Box ii). Each of replacement can slightly alter the posterior repression of GFP (n = 3/3). *Figure 3—figure supplement 1. continued on next page* 

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#### Figure 3—figure supplement 1. Continued

When the chick enhancer is replaced by both sequence (the most right panel), the distal GFP expression is significantly shifted to the posterior limb buds (n = 4/4, white arrow). And weak expressions are also detected in the posterior proximal limb buds (n = 2/4. arrow head in the bottom panel, magnified view of the above white box with enhanced contrast), suggesting that the sequences at Box i and Box ii are partially responsible for the posterior repression. Scale bars, 100  $\mu$ m.

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**Figure 4.** RA treatment causes ectopic activation of Shh signalling and loss of anterior skeletal elements. (**A**–**E**) In situ hybridisation of *S. canicula* pectoral fin buds for *Ptch1* (**A**; left fins flipped horizontally), *Hand2* (**B**), *Pax9* (**C**), *Alx4* (**D**), and *Hoxa13* (**E**) treated with 1% DMSO or 1 µg/ml retinoic acid (RA) (n = 2/2 for each for each except n = 4/4 for *Hand2*). Arrowheads in **C**, a weak expression of *Pax9*. White brackets in **E**, width of *Hoxa13* expression domain along the proximal-distal (PD) axis. (**F**) Pectoral fin skeletal patterns of 1% DMSO control (n = 4) and 1–2 µg/ml RA (n = 4). Right panels, schematics of interpretive skeletal patterns. \*, an anterior proximal radial; \*\*, a fused radial attached to the metapterygium; pg, pectoral girdle; ppr, pms and pmt, proximal propterygium, mesopterygium and metapterygium. Scale bars, 100 µm. (**G**) Comparison between *S. canicula* fin and mouse limb. Green and blue colours represent anterior–posterior (AP) positional information. ppr, pms, and pmt denote proximal propterygium, mesopterygium, and metapterygium, respectively.

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