

Gene expression pattern

Nested expression and sequential downregulation of the *Xenopus caudal* genes along the anterior-posterior axis

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Abstract

Expression of the *Xenopus Xcad-1* and *Xcad-2* genes initiates during early gastrulation exhibiting a dorsoventral asymmetry in their domains of transcription. At mid-gastrulation the ventral preference becomes stronger and the *caudal* genes take up a posterior localization in their expression, which they will maintain until their downregulation along the dorsal midline. Comparison of the three *Xenopus caudal* genes revealed a temporal and spatial nested set of expression patterns. The transcription of the *caudal* genes is sequentially downregulated with the one expressed most caudally (*Xcad-2*) being shut down first, this sequence is most evident along the dorsal midline. This pattern of expression suggests a role for the *caudal* genes as posterior determinants along the anteroposterior axis. In chicken, mouse, man and *Xenopus* three members of the *caudal* family have been identified in the genome. Even though in *Xenopus* the *Xcad-3* gene has been previously described, in order to obtain a better insight on the role of the *caudal* genes a comparative study of all three frog genes was performed. © 1998 Elsevier Science Ireland Ltd.

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1. The *Xcad-1* and *Xcad-2* pattern of expression

The spatial and temporal pattern of expression the *Xenopus caudal* homeobox genes *Xcad-1* and *Xcad-2* was studied from gastrulation (stage 10) throughout embryogenesis. Expression of both *Xcad* genes is detectable from the onset of gastrulation. *Xcad-2* and *Xcad-1* are expressed

around the blastopore as a narrow ring (Fig. 1A,J). The dorsal aspect of the *Xcad-2* ring exhibits very low transcript levels while the *Xcad-1* ring is more uniform (Fig. 1A,J). As gastrulation proceeds, the expression of both *Xcad* genes intensifies and the signal around the blastopore widens preferentially on the ventral side thus creating an uneven ring of expression (Fig. 1B,K). At stage 11–11.5 *Xcad-2*, unlike *Xcad-1*, still exhibits a dorsal gap (Fig. 1B,K). As gastrulation reaches its final stages, the *Xcad* genes form a cap of expression at the posterior end of the embryo around the blastopore (Fig. 1C,D,L,M). By this stage the circumblastoporal ring of *Xcad-2* expression has become continuous, but expression is absent from the midline which will include the notochord (Fig. 1L,M). During neurulation expression of both *Xcad* genes remains localized to the posterior end of

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the embryo, mainly the dorsal aspect of the posterior spinal cord (Fig. 1D–F,M–P) with *Xcad-2* expression being the first to decrease gradually. By about stage 31, *Xcad-2* expression is almost undetectable (Fig. 1Q), while *Xcad-1* is still visible in the posterior spinal cord region avoiding the chordoneural hinge and the notochord (Fig. 1G), (Gont et al., 1993). Expression of *Xcad-1* can still be detected by stage 33 (Fig. 1H). Later in development (stage 40/41) expression of both *Xcad* genes localizes to the endodermal lining of the gut (Fig. 1I,R).

2. Comparison and anterior boundaries of the *Xenopus* caudal genes

All three *caudal* genes are expressed during gastrulation, while *Xcad-1* always exhibits expression in the dorsal lip region, *Xcad-2* always exhibits a gap in this region (Fig. 2). *Xcad-3* appears to be more complicated, it is expressed throughout the marginal zone with no apparent dorsoventral localization except for a variable dorsal thinning of stain in some embryos (Fig. 2), (Northrop and Kimelman, 1994). As

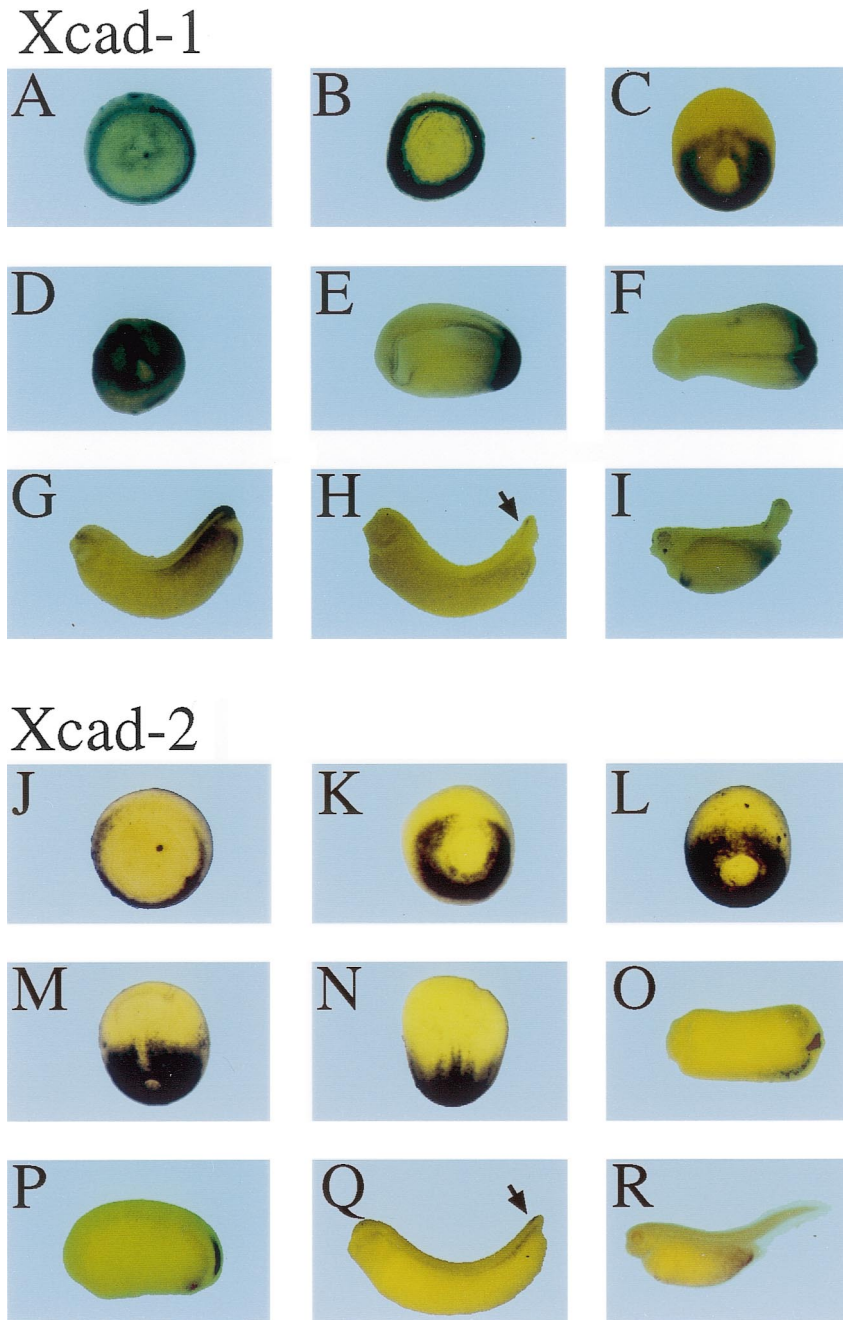


Fig. 1. Pattern of expression of *Xcad-1* and *Xcad-2*. *Xenopus* embryos were hybridized with probes specific for the *Xcad-1* (A–I) and *Xcad-2* (J–R) genes. The stages of the embryos are 11 (A,J), 11.5 (B,K), 12 (C,L), 13 (D,M), 14 (N), 21 (E,F,O,P), 29 (G), 31 (Q), 33 (H), 40 (R) and 41 (I). The arrows mark the last expression along the anteroposterior axis. Views: vegetal (A–C,J–L); posterior (D); dorsal (F, M–O) and lateral (E,G–I,P–R).

gastrulation proceeds all three genes are expressed as an uneven thick band around the blastopore where the ventral side is wider. By stage 13, as the blastopore closes, this band becomes a cap of expression which remains localized to the posterior end of the embryo (Fig. 2). During neurulation all three genes will be transcribed along the forming neural tube from the posterior end to different anterior boundaries of expression (Fig. 2). Using *MyoD* expression as an aid to

count the somites (Frank and Harland, 1991) the anterior boundaries were determined by probing together for one of the *caudal* genes and for *MyoD*. At stage 24 (14–15 somites), (Nieuwkoop and Faber, 1967), the anterior boundaries of *Xcad-3*, *Xcad-1* and *Xcad-2* were mapped to somites 2–3, 5–6 and 7–8, respectively (Fig. 2). These genes continue to move posteriorly so that the boundaries of *Xcad-3*, *Xcad-1* and *Xcad-2* by stage 27 (18–19 somites) are at

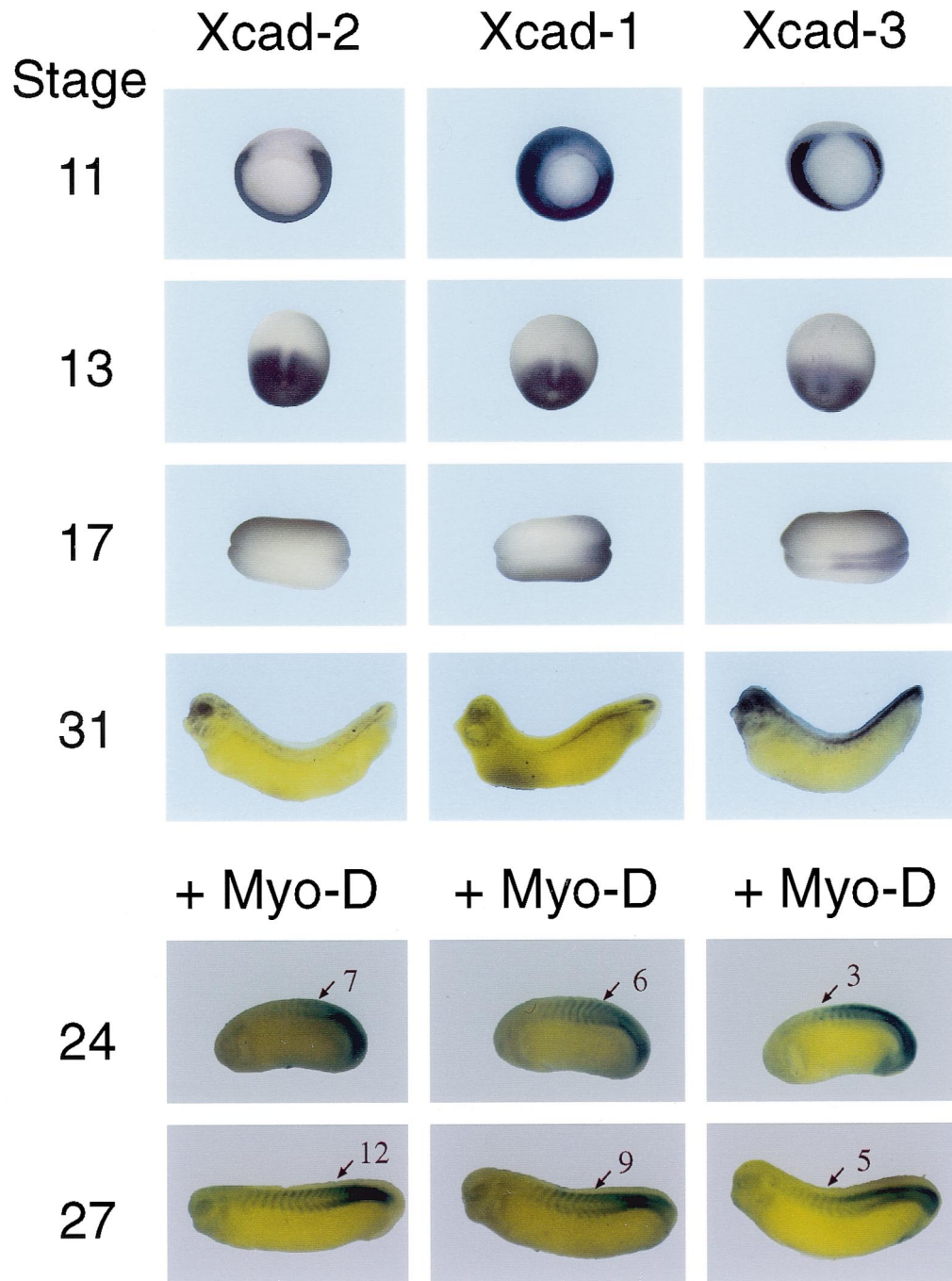


Fig. 2. Comparative analysis of the expression of the *Xenopus caudal* genes. *Xenopus* embryos from mid-gastrulation were hybridized with probes for *Xcad-1*, *Xcad-2* and *Xcad-3*. The probes and the stages of the embryos are shown. The embryos at stages 24 and 27 were hybridized with the *caudal* specific probe and a *Myo-D* probe to facilitate the visualization of the somites. The arrows mark the position of the anterior boundary of *Xcad* expression, the number is the somite number. Views: stage 11, vegetal; stages 13 and 17, dorsal; stages 24, 27 and 31, lateral. The embryos at stages 24, 27 and 31 were cleared.

somites 4–5, 8–9 and 11–12 (Fig. 2) and by stage 30 (24–25 somites) at somites 8–9, 12–13 and 15–16, respectively. In the region between the tailbud and the proctodeum all three *caudal* genes also exhibit a nested pattern (stage 24; Fig. 2). Later, sequential downregulation of the genes takes place, *Xcad-3* is the last gene to be downregulated and *Xcad-2* the first. *Xcad-1* is the intermediate gene in this sequence (Figs. 1 and 2), (Northrop and Kimelman, 1994).

Sequence comparisons have shown homology between the frog *Xcad-2*, *Xcad-1* and *Xcad-3* and chicken *Cdx-A*, *Cdx-C* and *Cdx-B* genes, respectively, and evolutionary conservation in the sequence of downregulation (Marom et al., 1997). This similarity strongly supports the proposal that the vertebrate *caudal* genes establish a nested system of gene expression at the posterior end of the embryo forming a transient gradient of *caudal* expression which plays a role in the patterning of the anteroposterior axis (Epstein et al., 1997). This model is not based solely on their pattern of expression (Frumkin et al., 1993; Marom et al., 1997), but also on mutations (Subramanian et al., 1995; Chawengsak-sophak et al., 1997) and experimental manipulation of expression levels (Pownall et al., 1996; Epstein et al., 1997). This anteroposterior patterning role appears to take place via interactions with other homeobox genes such as *Otx-2* and the *Hox* family members.

3. Materials and methods

Xenopus embryos were staged according to Nieuwkoop and Faber (1967) and processed according to Fainsod et al. (1994). The probes used were: *Xcad-2*, clone #73 (Blumberg et al., 1991); *Xcad-1*, clone #72 (Blumberg et al., 1991); *Xcad-3*, clone Xcad-50 (Northrop and Kimelman, 1994). In situ hybridizations were performed as described in Fainsod et al. (1994) according to the published modifications (Epstein et al., 1997).

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