

Evolutionary plasticity of developmental gene regulatory network architecture

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Supporting Information

Files in this Data Supplement:

[SI Figure 6](#)

[SI Figure 7](#)

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[SI Figure 9](#)

[SI Text](#)

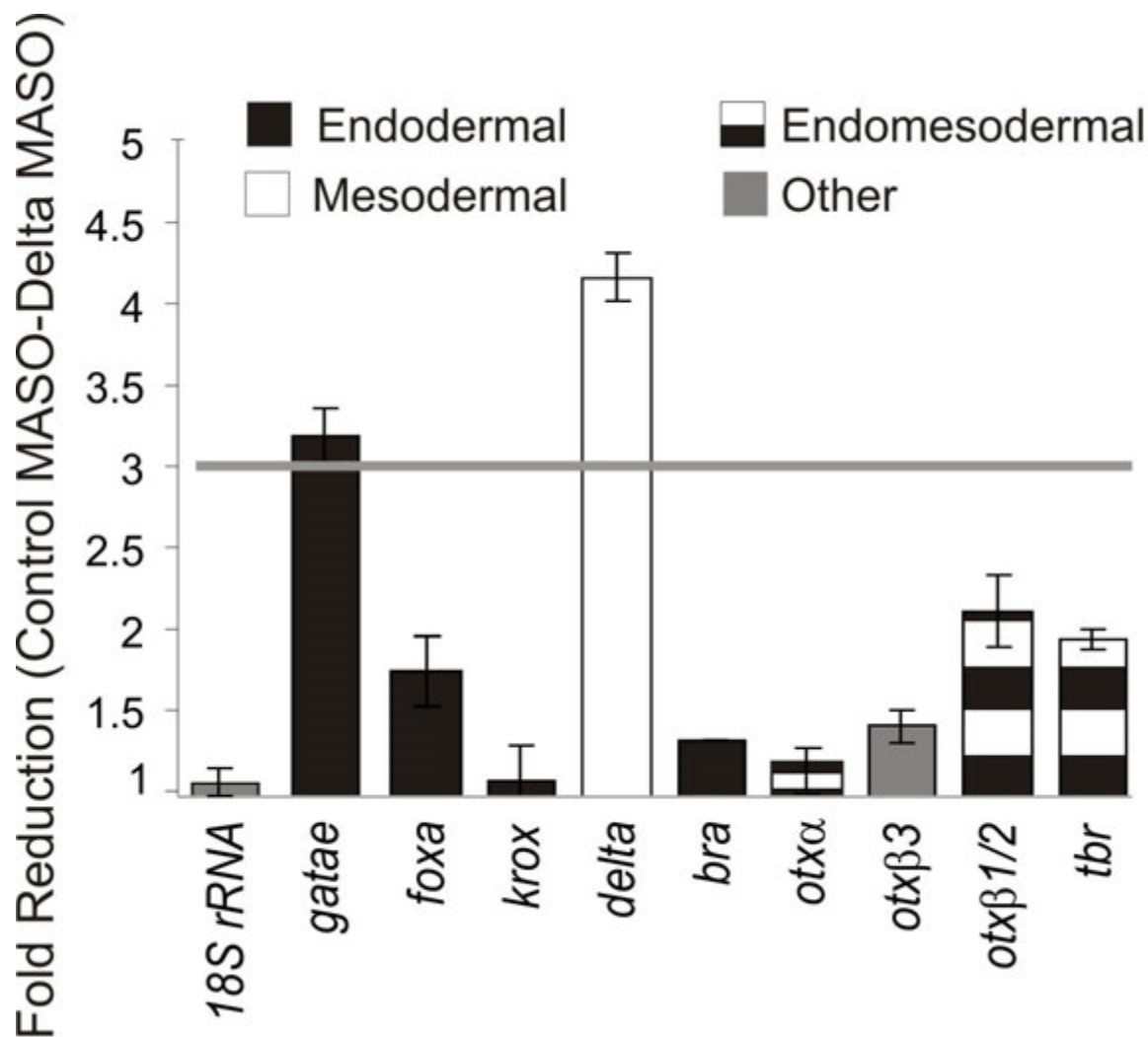


Fig. 6. Transcript abundance in Delta-deficient embryos. qPCR was performed on cDNA from $\gg 50$ embryos that had been injected with either a MASO targeted against Delta or a control MASO. Fold difference in cycle threshold (Ct) values between control and Delta MASO is plotted for each transcript assayed. All values were normalized to ubiquitin. Bars are ± 1 SD based on three replicates.

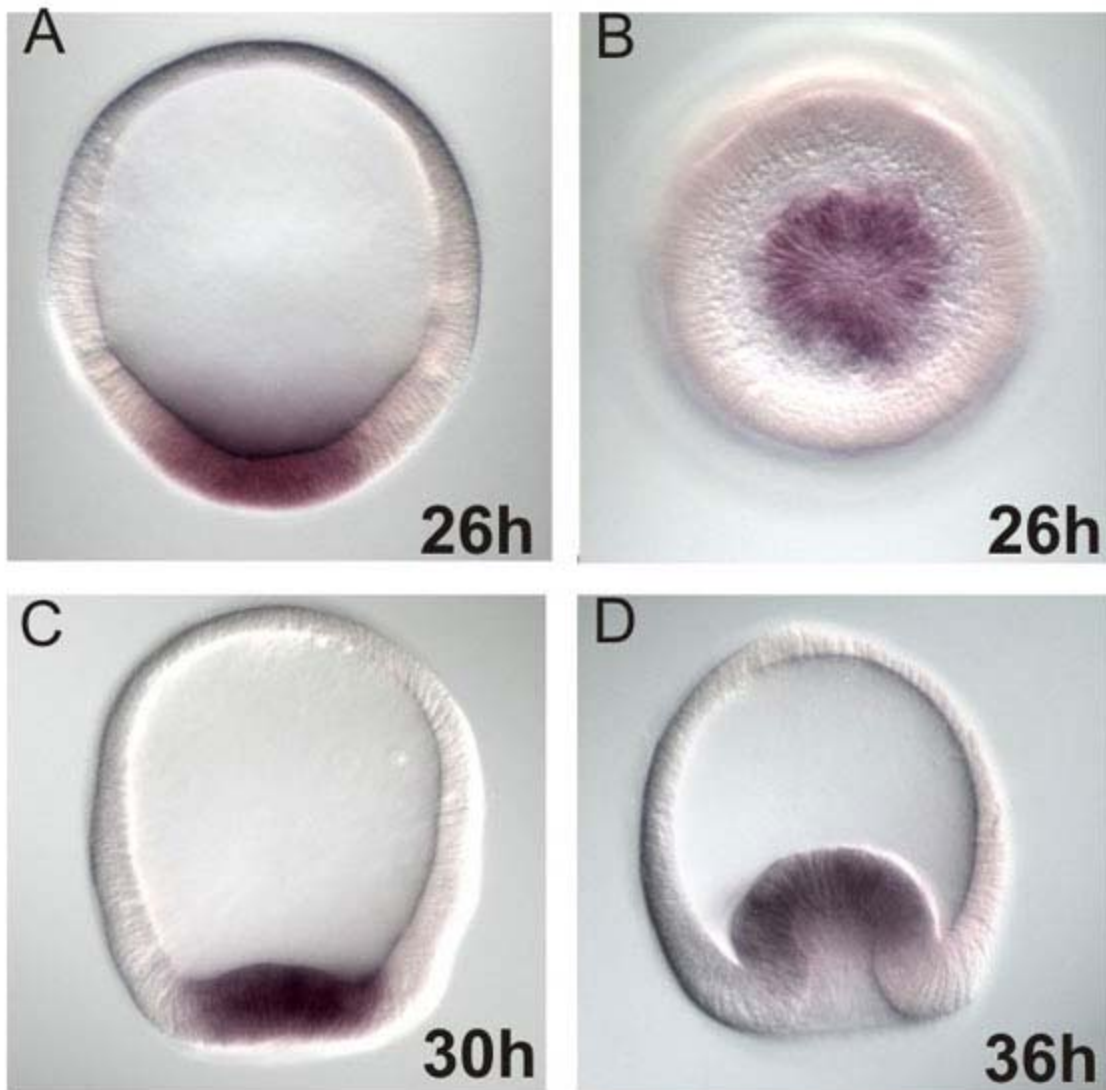


Fig. 7. Normal expression of *AmGataC* visualized by using WMISH. (A and B) Blastula stages shown laterally (A) or from the vegetal pole (B). (C and D) Expression remains in the mesodermal progenitors during early gastrulation.

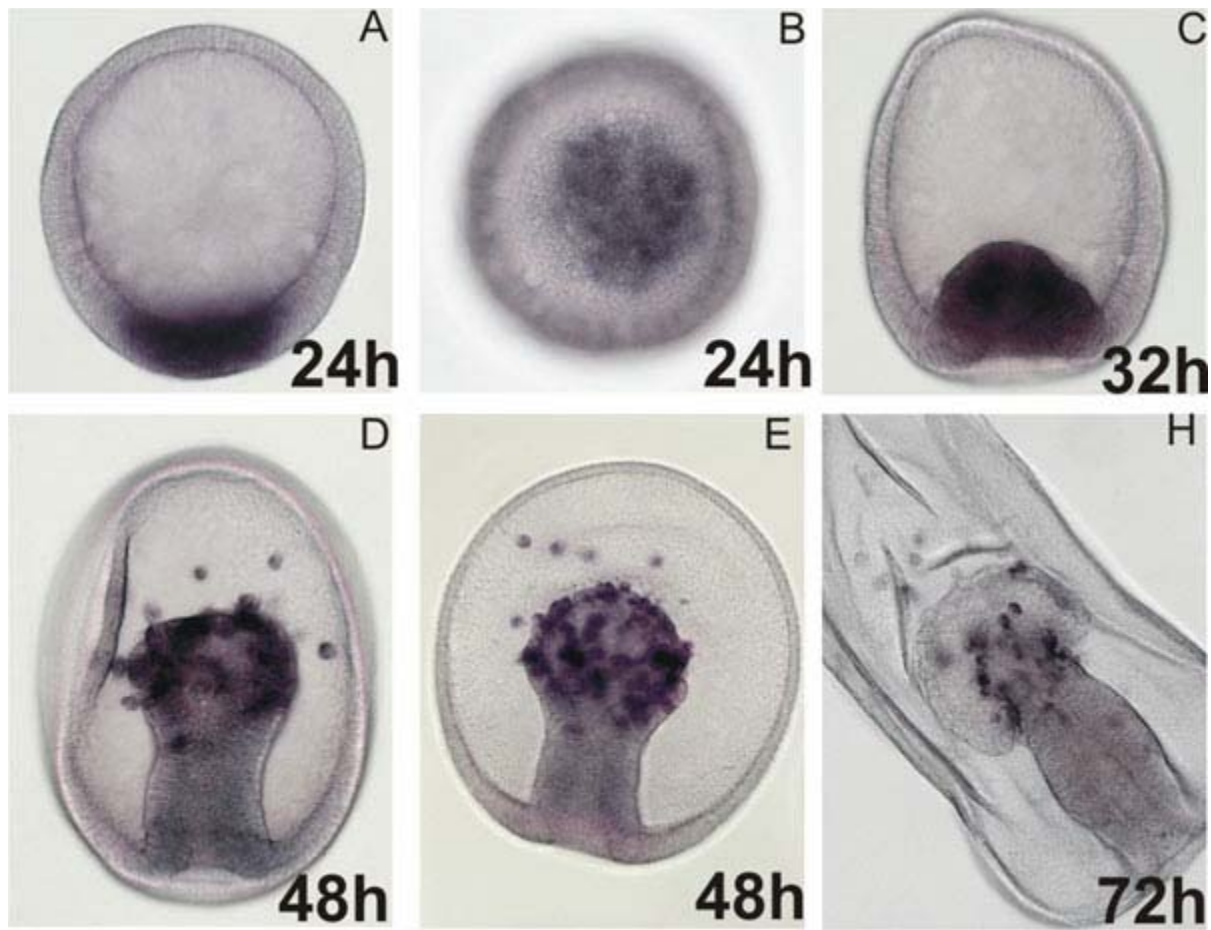


Fig. 8. Normal expression of *AmEts1/2* visualized by using WMISH. (A and B) Blastula stages shown laterally (A) or from the vegetal pole (B). (C-F) Expression remains in mesodermal progenitors during early gastrulation (C) and is localized to cells migrating from the tip of the archenteron in late gastrula (D and E) and larval (F) stages.

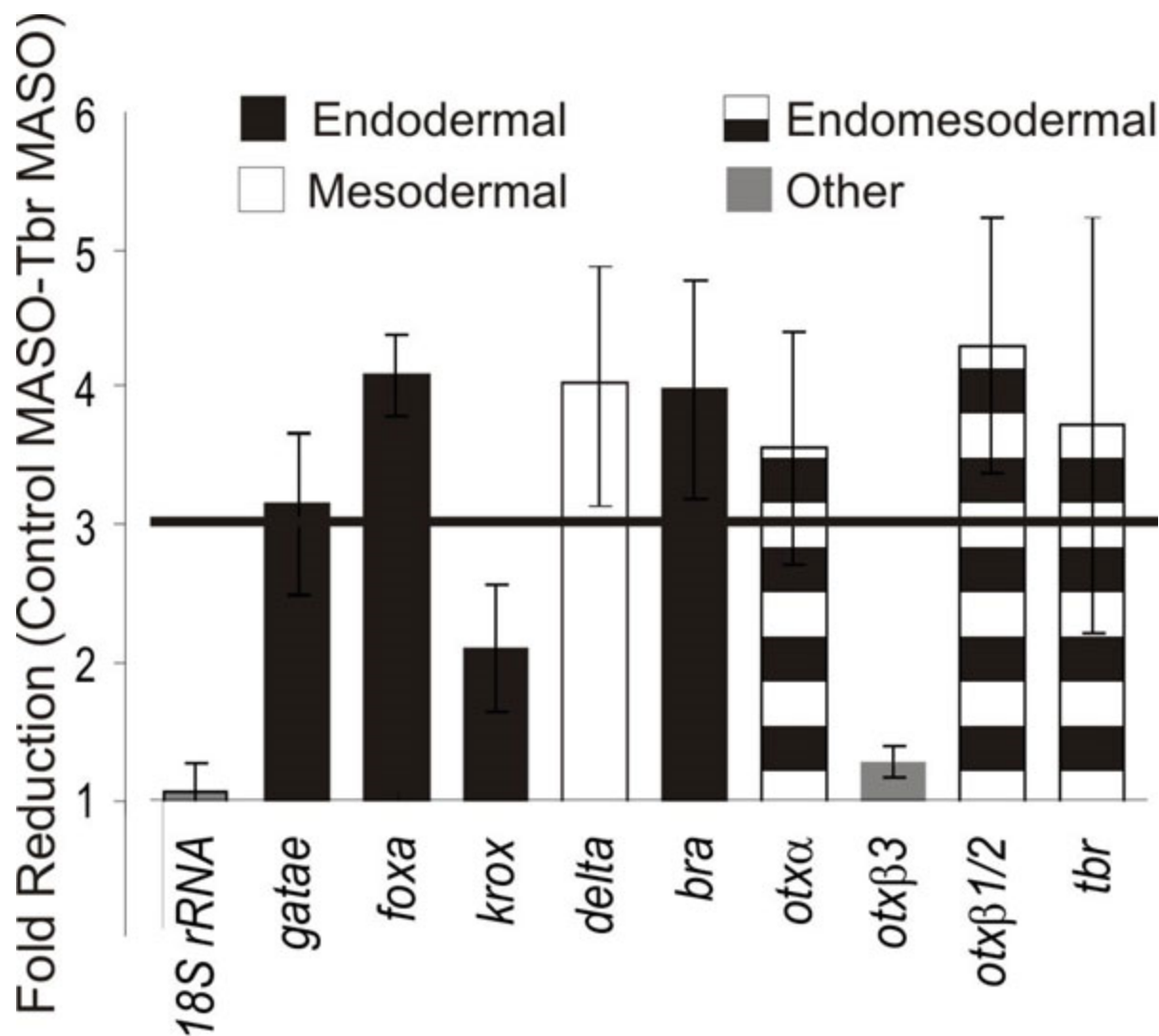


Fig. 9. Quantitative effects on transcript abundance in *Tbr*-deficient embryos. qPCR was performed on cDNA from »50 embryos that had been injected with either a MASO targeted against *Tbr* or a control MASO. Fold difference in cycle threshold (Ct) between control and Delta MASO is plotted for each transcript assayed. All values were normalized to ubiquitin. Bars are ± 1 SD.

SI Text

Evidence for Direct and Indirect Regulatory Inputs for GRN Nodes in the Sea Urchin and Sea Star

Many of the regulatory inputs into each of the nodes in the sea urchin network have been directly confirmed by using *cis*-regulatory analysis (see below). Most data from the sea star are based on perturbation experiments. The details of what is known of the direct or indirect regulation of each node are outlined below.

otx. All regulatory interactions have been demonstrated to be direct in both sea urchin and sea star by mutation of binding sites within their *cis*-regulatory modules (1, 2).

blimp1. All inputs in sea urchin are confirmed to be direct by *cis*-regulatory analysis (3s). Inputs have been demonstrated in the sea star by perturbation of *Otx* function (4).

gatae. *Cis*-regulatory analyses in sea urchin confirm the input from *Otx* to be direct but show that the *Delta* input is indirect as the *cis* regulatory module controlling *gatae* expression contains consensus sites for Su(H) (which is the effector of *Delta* N signaling) but mutation of these sites only reduce *gatae* expression by 50% (P. Y. Lee and E.H.D., unpublished work). We assume the same is the case for sea star.

foxa. Inputs in both species are indicated from MASO perturbation experiments against *Foxa*, *Gatae* and *Otx* (4).

bra. Inputs into sea urchin *bra* are confirmed to be direct via *cis*-regulatory analyses (R. A. Cameron and E.H.D., unpublished data).

tbrain. *Cis*-regulatory analyses have shown that inputs into sea urchin *tbrain* are direct (P. Oliveri and E.H.D., unpublished data). Input from sea star is shown through perturbation of *Otx* (4).

delta. In sea urchin all inputs are confirmed by *cis* regulatory analysis (5). In sea star there is a dramatic drop in expression after *Tbr* MASO perturbation (SI Fig. 9).

gcm. All inputs in sea urchin are confirmed to be direct (6).

gatac. Inputs into *gatac* in both taxa are indicated from perturbation experiments.

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