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Differential Regulation of Neurogenesis by the Two *Xenopus GATA-1* Genes

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Previously, we have shown that the ventralizing factor bone morphogenetic protein 4 (BMP-4) can inhibit *Xenopus* neurogenesis. The erythroid transcription factor GATA-1 functions downstream of the BMP-4 signaling pathway and mediates BMP-4-induced erythropoiesis. We have found that similar to BMP-4, GATA-1b inhibits neuralization of *Xenopus* animal cap (AC) cells. The neural inhibition is not seen with GATA-1a, although both GATA-1a and GATA-1b RNAs are translated at the same efficiency and induce *globin* expression equally in AC cells. GATA-1b RNA injection into AC cells neither induces expression of *Xbra* (a general mesoderm marker) nor affects expression of *XK81* (epidermal keratin) or *BMP-4* and *Xvent-1* (two ventral markers). These data suggest that GATA-1b retains the epidermal fate of the AC. Intact GATA-1b protein is required for both inhibition of neurogenesis and induction of *globin* expression. Our findings indicate that GATA-1b can function in ectoderm to specifically regulate neural inducing mechanisms, apparently related to the expression of *chordin*, a neuralizing gene. Furthermore, tadpole stage embryos injected with GATA-1b are devoid of all dorsoanterior structures including neural tissue. This report provides evidence that the two transcription factors, derived from a recent genome duplication, share a common biological activity (stimulation of erythropoiesis) while also exhibiting a distinct function (inhibition of neurogenesis).

Bone morphogenetic protein 4 (BMP-4) has previously been identified as a ventralizing factor in *Xenopus* body patterning (1, 4, 7, 14, 21, 36, 40). We and others have recently demonstrated (30, 39, 41) that BMP-4 is also able to antagonize neurogenesis induced by noggin, chordin, or cell dispersion in cells derived from the presumptive ectoderm of the *Xenopus* animal cap (AC). Inhibition of BMP-4 signaling by injection of RNA encoding a dominant negative BMP-2/4 receptor (DN-BR), a mutated form of BMP-4, or BMP-4 antisense RNA causes neuralization in AC cells (8, 30, 41). These findings suggest that the ventralizing factor BMP-4, which antagonizes organizer-derived factors in the mesoderm, is also a neural inhibitor. In studies of BMP-4-induced blood programming, it has been found that ectopic expression of the erythroid transcription factor gene *GATA-2* was greatly elevated in BMP-4 RNA-injected AC cells (22, 44). Similarly, expression of *GATA-1* in cultured AC cells at the late neurula and tailbud stages was significantly enhanced by injection of BMP-4 RNA but attenuated by injection of DN-BR RNA (44). Therefore, *GATA-1* and *GATA-2* are likely downstream targets of BMP-4 signaling.

The *GATA* factor family of genes encodes proteins containing an extremely conserved central DNA-binding domain which recognizes the core sequence (A/T)GATA(A/G) or a closely related sequence (28). Six GATA factor family members have been identified and characterized in vertebrates (13, 15, 16, 25). By their expression profiles, the GATA proteins may be distinguished as hematopoietic (GATA-1 to -3) or nonhematopoietic (GATA-4 to -6), a functional grouping which mirrors their structural similarities. GATA-1 is restricted to cells of the erythroid, megakaryocytic, eosinophilic, and mast cell lineages (3, 23, 34, 45). GATA-2 is expressed in these lineages and in several nonhematopoietic cell types (2, 42), including the central nervous system at the tadpole stage (28). There is abundant evidence to implicate these two proteins as critical determinants of erythroid gene expression (38, 42). *Xenopus GATA-1* has been cloned as a pair of presumably duplicated genes named *GATA-1a* and *GATA-1b*, which share 87% nucleotide and 89% amino acid homology (43, 46). Both genes encode functional GATA-binding proteins with similar binding specificities and can activate a target *globin* promoter in transient transfection assays (43). During *Xenopus* development, both GATA-1 and GATA-2 transcripts are first detected in animal and marginal zone areas of the early gastrula (stage 11) and are subsequently localized to the ventral mesoderm, within the blood island region (16, 43, 46). Very similar patterns of transcription have been reported for the *BMP-4* gene (4), which may further indicate a functional relevance among GATA-1, GATA-2, and BMP-4 during embryogenesis.

Based on these findings, we sought to determine whether GATA-1 and/or GATA-2, in addition to mediating erythroid differentiation, might also affect neurogenesis. By using the AC culture system, we found that injection of RNA encoding GATA-1b (but not RNA encoding GATA-1a or GATA-2) inhibits neural induction, while all of these RNAs are able to induce globin expression in the AC. The antineurogenic activity is dependent on intact GATA-1b protein and correlates with the suppression of *chordin* expression. When embryos expressing GATA-1b ectopically are allowed to develop until

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the tadpole stage, they lack all dorsoanterior structures including neural tissue.

MATERIALS AND METHODS

DNA and RNA preparation. *Xenopus* GATA-1a, GATA-1b (43), and GATA-2 (42) cDNAs were cloned into the pBluescript SK+ vector, while DN-BR (7, 36), BMP-4 (35), noggin (20), chordin (31), 3m (a LIM domain mutant of the homeobox gene *Xlim-1*) (37), and β-galactosidase (β-gal) cDNAs were inserted into the pSP64T vector. To generate a defective GATA-1b derivative, a unique *Nde*I site was used to create a frameshift mutation by filling in the restriction site with a Klenow fragment. The frameshift results in truncation of the expressed protein just upstream of the zinc finger DNA-binding domain. All of the constructs were linearized and used for in vitro synthesis of capped mRNA by using a transcription kit in accordance with the manufacturer's (Ambion) instructions. The synthetic RNA was quantitated by ethidium bromide staining in comparison with a standard RNA.

Embryo injection and explant culture. *Xenopus laevis* embryos were obtained by in vitro fertilization (40). Developmental stages were designated in accordance with Nieuwkoop and Faber (26). Embryos were injected with synthetic RNA as described in the figure legends. ACs were dissected from the injected embryos at designated stages, cultured to various stages, and harvested for analysis by Western blotting or reverse transcription (RT)-PCR experiments. In some cases, the ACs were each sandwiched with a piece of dorsal marginal zone (DMZ) tissue excised from a stage 10 embryo.

RT-PCR. Total RNA was extracted from cultured explants with TRIzol reagent (GIBCO Bethesda Research Laboratories) in accordance with the manufacturer's instructions. RT-PCR was done with a Superscript Preamplification System (GIBCO Bethesda Research Laboratories). Primer sets and PCR conditions for *NCAM* (10), *BMP-4* (35), *Xvent-1* (6), *XK81* (39), *Xbra* (32), and *EF-1α* (19) have already been described. Primers for *Tα-globin* (44) were as follows: F, CAT GGC TCT GCT GAT CTT GCC AAC CAC; and R, CCC AGG CTG GTG AGC TGC CCT TGC TG. PCR conditions for *Tα-globin* (44) were as follows: 1 min at 94°C, 1 min at 54°C, and 1 min at 72°C for 30 cycles. Primers for *chordin* were designed as follows: F, TTA GAG AGG AGA GCA ACT CGG GCA AT; R, GTG CTC CTG TTG CGA AAC TCT ACA GA (nucleotides 3119 to 3144 and 3430 to 3455 for a product length of 337 bp). PCR conditions for *chordin* were as follows: 1 min at 94°C, 1 min at 56°C, and 1 min at 72°C for 25 cycles. Although data from individual experiments are shown, in all cases the results were confirmed independently.

Western blotting. Five or six explants per group were pooled, and lysates equivalent to two explants were electrophoresed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A blotted nitrocellulose filter was incubated with mouse monoclonal antibody 4d (Developmental Studies Hybridoma Bank) for NCAM or with rabbit polyclonal antibody xG1N-9804 for GATA-1, followed by the horseradish peroxidase-conjugated anti-mouse or rabbit immunoglobulin G antibody. The presence of NCAM or GATA-1 on the resulting blot was visualized by an ECL system (Amersham).

In vitro translation. Synthetic RNAs were translated in a reticulocyte lysate system (In Vitro Express translation kit [Stratagene]) in the presence of [^{35}S]methionine (Amersham). The product of the translation reaction was subjected to SDS-PAGE. Dried gels were analyzed by autoradiography.

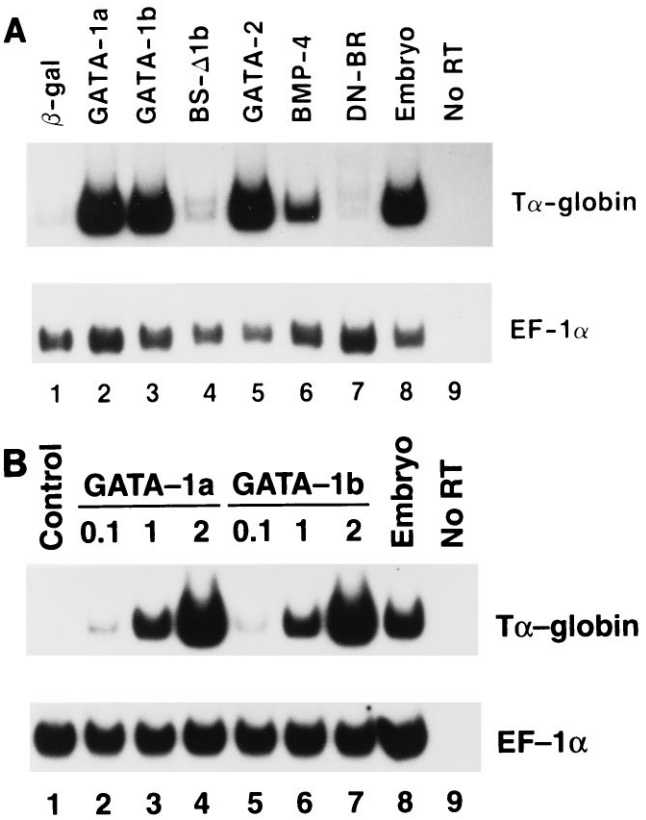

FIG. 1. (A) Both GATA-1a and GATA-1b, but not truncated GATA-1b, induce *globin* expression in cultured AC cells. Embryos were injected with RNAs encoding the following: lane 1, control β-gal (2 ng); lane 2, GATA-1a (2 ng); lane 3, GATA-1b (2 ng); lane 4, BS-Δ1b (2 ng); lane 5, GATA-2 (2 ng); lane 6, BMP-4 (1 ng); lane 7, DN-BR (1 ng). An embryo at the equivalent stage was used as a positive control (lane 8), while the same embryo sample processed for RT-PCR in the absence of RT was used as a negative control (lane 9). (B) GATA-1a and GATA-1b induce *globin* expression in a dose-dependent manner. Embryos were injected with water (lane 1) or RNA encoding GATA-1a or GATA-1b at 0.1, 1, or 2 ng/embryo (lanes 2 to 7). ACs were dissected from the injected embryos at stages 8.5 to 9, cultured until stage 24, and analyzed for levels of Tα-globin by RT-PCR. Expression of *EF-1α* was used as an internal control for equal RNA loading in all of our RT-PCR experiments.

RESULTS

Both GATA-1 and GATA-2 stimulate *globin* expression in AC cells. Expression of BMP-4 after the midblastula transition has been shown to be sufficient to induce erythropoiesis, including expression of *GATA-1* and *GATA-2*, in AC cells (44). Therefore, we tested the effect of directly expressing these GATA factors on *globin* expression in this system. With the RT-PCR assay, similar levels of Tα-globin transcripts were detected in cultured AC cells derived from embryos injected with RNA encoding GATA-1a, GATA-1b, GATA-2, or BMP-4 (Fig. 1A). This demonstrates that GATA-1, GATA-2, and BMP-4 can regulate erythropoietic activity in the *Xenopus* AC. Moreover, injection of various amounts of the two GATA-1 RNAs into the AC induced *Tα-globin* expression in a dose-dependent manner (Fig. 1B), further confirming that the synthetic RNAs had the same efficiency of globin induction.

Similar to BMP-4, GATA-1b inhibits neurogenesis. In addition to inducing erythropoiesis, BMP-4 exhibits antineurogenic activity in the presumptive ectoderm (8, 30, 39, 41). Therefore, we next assessed the effects of GATA-1a, GATA-1b, or GATA-2 expression on neurogenesis. With an AC-DMZ coculture system, neuroectoderm was induced by signaling molecules derived from invaginating dorsal mesoderm (27). We injected various RNAs into the animal pole of two-cell embryos and used the coculture system to analyze neural induction. At stages 8.5 to 9, the ACs were dissected and conjugated with a piece of DMZ tissue (the prospective dorsal mesoderm) excised from a stage 10 embryo. The combined tissue was cultured until the equivalent of stage 35 and harvested for analysis of NCAM levels by Western blotting. NCAM, a panneural marker (17), was expressed in explants from control β-gal, GATA-1a, or GATA-2 RNA-injected embryos but not in explants from GATA-1b RNA-injected embryos (Fig. 2A). This indicates that GATA-1b is able to suppress neurogenesis induced by signals derived from the dorsal mesoderm.

The GATA-1b-mediated suppression might result from modulation of the dorsal mesoderm by GATA-1b-expressing cells. To exclude this possibility, we expressed DN-BR in AC cells and cultured the explants alone. Under these conditions, neurogenesis occurs by inhibition of BMP-4 signaling in the AC without mesoderm involvement (39, 41). Coinjection of GATA-1b RNA totally inhibited the DN-BR-mediated neurogenesis, whereas AC cells coinjected with GATA-1a or

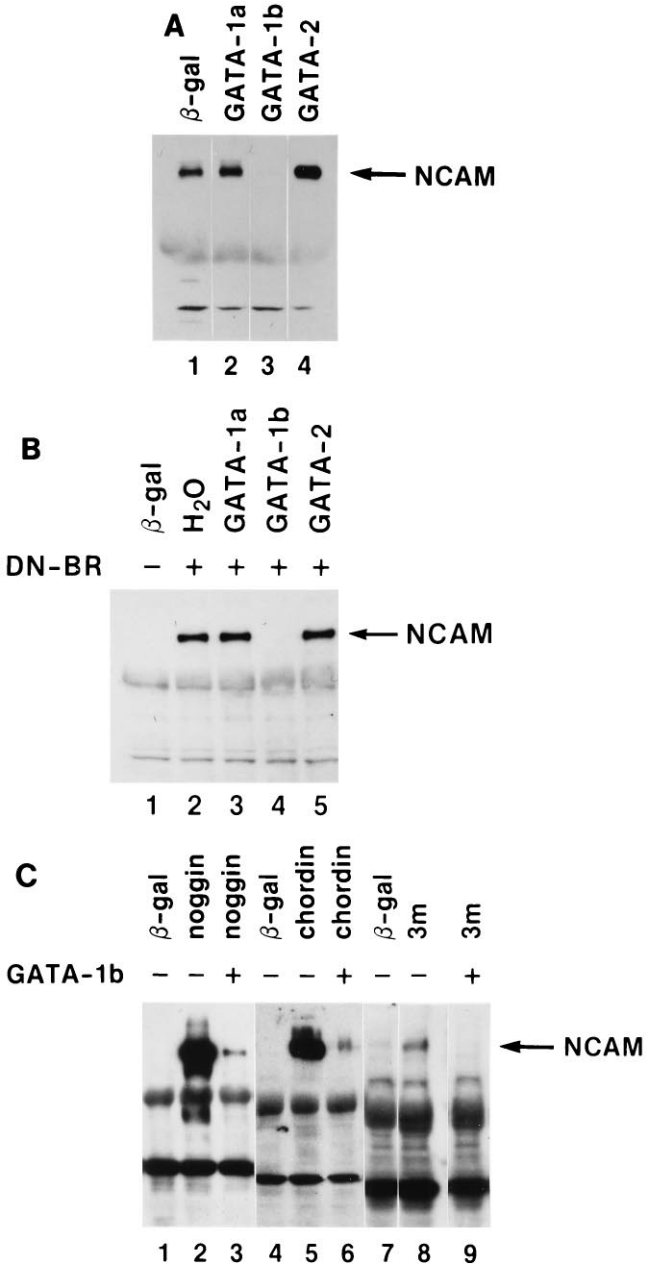

FIG. 2. (A) GATA-1b inhibits neurogenesis induced by contact with the dorsal mesoderm. DMZ samples were combined with the AC from embryos injected with 2 ng of RNAs encoding the following: lane 1, β-gal; lane 2, GATA-1a; lane 3, GATA-1b; lane 4, GATA-2. Note that GATA-1b, but not GATA-1a or GATA-2, inhibited NCAM synthesis. (B) Differential effects of GATA-1a, GATA-1b, and GATA-2 on neurogenesis elicited by DN-BR. AC cells were derived from embryos injected with RNAs encoding the following: lane 1, control β-gal (3 ng); lane 2, DN-BR (1 ng); lane 3, DN-BR (1 ng) plus GATA-1a (2 ng); lane 4, DN-BR (1 ng) plus GATA-1b (2 ng); lane 5, DN-BR (1 ng) plus GATA-2 (2 ng). (C) GATA-1b inhibits neurogenesis mediated by noggin, chordin, or 3m. AC cells were derived from embryos injected with RNAs encoding the following: lanes 1, 4, and 7, β-gal (3 ng); lane 2, noggin (0.5 ng); lane 3, noggin (0.5 ng) plus GATA-1b (2 ng); lane 5, chordin (1 ng); lane 6, chordin (1 ng) plus GATA-1b (2 ng); lane 8, 3m (0.5 ng); lane 9, 3m (0.5 ng) plus GATA-1b (2 ng). AC cells were isolated from the injected embryos at stages 8.5 to 9 and cultured alone (for panels B and C) or sandwiched with a piece of DMZ tissue from a donor stage 10 embryo (for panel A), cultured until the equivalent of stage 35, and analyzed for NCAM expression by Western blotting.

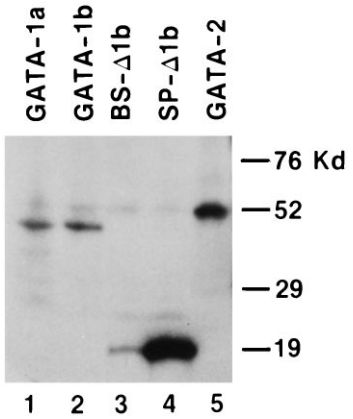

FIG. 3. Translation of GATA RNAs in vitro. Proteins with different molecular sizes were detected after translation of RNAs for GATA-1a (lane 1), GATA-1b (lane 2), BS-Δ1b (lane 3), SP-Δ1b (lane 4), and GATA-2 (lane 5). Synthetic RNAs were translated in a reticulocyte lysate translation system in the presence of [^{35}S]methionine (Amersham); products were analyzed by autoradiography on an SDS–4 to 20% polyacrylamide gel.

GATA-2 RNA expressed a neural program (Fig. 2B). These results indicate that GATA-1b acts directly on AC cells.

Noggin and chordin are two molecules secreted from the organizer (presumptive dorsal mesoderm) which also induce neuralization in AC cells (20, 30, 31, 33). Similarly, 3m, a mutated form of the homeobox gene *Xlim-1* (also expressed in the organizer), is able to neuralize the ectoderm (37). Coinjection of GATA-1b RNA inhibited neuralization induced by noggin, chordin, or 3m expression (Fig. 2C), whereas coinjection of GATA-1a or GATA-2 RNA failed to do so (data not shown). Therefore, GATA-1b, but not GATA-1a or GATA-2, is capable of suppressing neuralization induced either by DN-BR or the exogenous neuralization-inducing agents.

Functional GATA-1b protein is required for inhibition of neurogenesis. GATA-1b is a DNA-binding transcription factor that interacts with target genes via a well-characterized zinc finger domain. If the effect of GATA-1b RNA injection is due to expression of a functional protein, it is expected that an intact DNA-binding domain might be required. Therefore, a frameshift mutation in the GATA-1b cDNA was generated. The resulting cDNA was constructed either in pBluescript SK+ (named BS-Δ1b) or in pSP64T (named SP-Δ1b). Both constructs encode a truncated protein lacking the zinc finger domain. As shown in Fig. 3, transcripts derived from the BS-Δ1b and SP-Δ1b cDNAs were efficiently translated but generated a smaller-molecular-size protein relative to the wild-type GATA-1b RNA. BS-Δ1b failed to stimulate globin expression in cultured AC cells (Fig. 1, lane 4). Furthermore, both BS-Δ1b and SP-Δ1b failed to suppress *NCAM* expression in a coculture assay in which DMZ tissue was used to induce neurogenesis in AC cells (Fig. 4). This experiment rules out the possibility that the GATA-1b RNA itself nonspecifically inhibits neurogenesis, for example, by binding to and titrating out an embryonic factor. Therefore, an intact GATA-1b protein is required for both erythroid differentiation and inhibition of neurogenesis.

GATA-1a and GATA-1b have similar translational efficiencies. It does not appear that the difference between GATA-1a and GATA-1b in the inhibition of neurogenesis is due to translational efficiency. By using xG1N-9804 polyclonal antibodies that recognized both *Xenopus* GATA-1a and GATA-1b, the translation products of the two GATA-1 RNAs in the AC were analyzed by Western blotting and two bands were detected

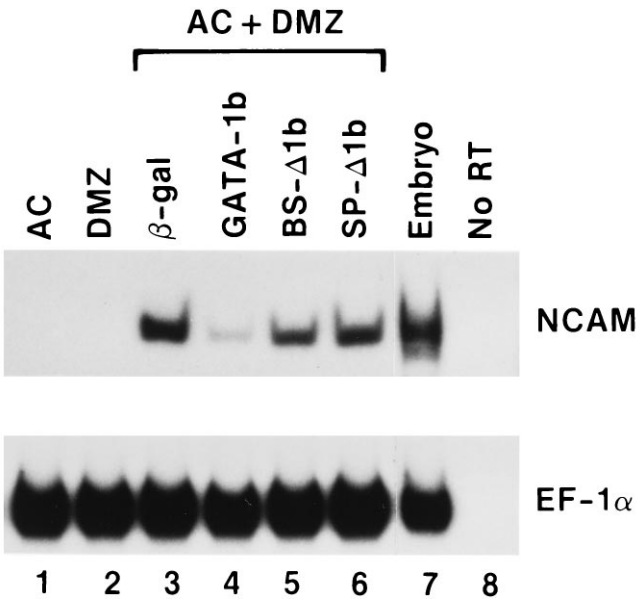

FIG. 4. GATA-1b RNA encoding a frameshift mutation fails to inhibit neurogenesis. Lanes: 1, AC alone; 2, DMZ tissue alone. DMZ samples were combined with an AC from embryos injected with 2 ng of RNA encoding the following: lane 3, β-gal; lane 4, GATA-1b; lane 5, BS-Δ1b; lane 6, SP-Δ1b. Sandwiches were prepared as described in the legend to Fig. 2A and cultured until the equivalent of stage 24. *NCAM* expression was analyzed by RT-PCR.

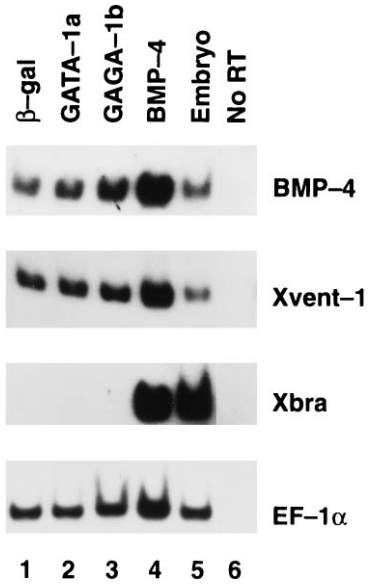

FIG. 6. GATA-1 neither affects *BMP-4* and *Xvent-1* expression nor induces *Xbra* expression in the AC. ACs were derived from embryos injected with RNAs encoding the following: lane 1, β-gal (2 ng); lane 2, GATA-1a (2 ng); lane 3, GATA-1b (2 ng); lane 4, BMP-4 (1 ng). ACs were excised from the injected embryos at stages 8.5 to 9, cultured until the equivalent of stage 11, and collected for RT-PCR analysis.

(Fig. 5). A protein detected by the antibodies with an apparent molecular mass of 39 kDa is present only in the injected ACs and indicates the specific presence of the GATA-1 proteins. According to the intensity of this band, GATA-1a RNA injected into the AC was translated with an efficiency similar to that of GATA-1b RNA. The translation was dose dependent in the range of 2 to 5 ng of RNA. Therefore, the difference in suppression of neurogenesis by GATA-1a and GATA-1b indeed reflects distinct biological activities.

GATA-1b does not change the epidermal fate of AC cells. Activin is another molecule able to counteract neurogenesis in the ectoderm (9, 10, 39). It has been reported (39) that the addition of activin to cultures of dispersed AC cells inhibits neuralization indirectly by inducing mesoderm, indicated by the expression of the early mesoderm marker *Xbra* (32). Injection into the AC of RNA encoding a truncated dominant-negative form of Xbra results in activation of anterior neural markers (29). To determine whether GATA-1b-mediated inhibition of neurogenesis is a secondary consequence of mesoderm induction, we analyzed levels of Xbra transcription at stage 11 in AC cells injected with GATA-1 RNA. GATA-1a or GATA-1b RNA-injected AC cells did not express Xbra (Fig. 6) unless in the presence of activin (data not shown), indicating the existence of nondetermined cells in the GATA-1-injected AC. In contrast, BMP-4 RNA-injected caps expressed Xbra, consistent with the report that BMP-4 exerts mesoderm-inducing activity in the intact AC (39). In addition, neither GATA-1a nor GATA-1b affected the expression of the ventralizing genes *BMP-4* and *Xvent-1* (Fig. 6). Xvent-1 is a transcription factor responsive to BMP-4 signaling (6). These data suggest that GATA-1b-mediated neural inhibition does not occur through transcriptional activation of the ventral program. Although GATA-1b induced expression of *globin* in epidermis-fated AC (Fig. 1), as well as in noggin-injected AC (data not shown), we do not think that *globin* expression necessarily leads to formation of the ventral mesoderm, since (i) many other molecules are required to form the blood island in the ventral mesoderm and (ii) there are GATA-1- and -2-binding sites on the promoters of the *globin* genes which can be directly activated by GATA-1 and GATA-2 (43). As shown in Fig. 7, GATA-1-injected AC cells remain epidermal, as the expression of the epidermal keratin gene *XK81* was not altered, and noggin-mediated inhibition of *XK81* expression was rescued by coinjection of GATA-1b. This is similar to the effect of BMP-4, which inhibits neural differentiation by retaining the epidermal fate (39). It has been shown that BMP-4 is able to activate *GATA-1* expression in cultured AC cells (44); therefore, GATA-1b may mimic the antineurogenic activity of BMP-4. Since BMP-4 is able to antagonize the function of neurogenic genes, we next tested the effect of GATA-1b on the expression of these genes.

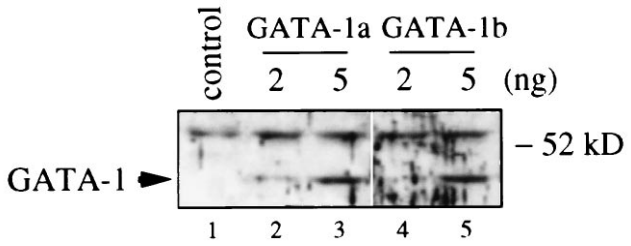

FIG. 5. In vivo translation of GATA-1a and GATA-1b RNAs. Two-cell stage embryos were injected with β-gal RNA (5 ng) or GATA-1a or GATA-1b RNA (2 or 5 ng). ACs were excised at stage 10 and cultured until stage 11 before being harvested for Western blotting with polyclonal antibody xG1N-9804, which recognizes both *Xenopus* GATA-1a and GATA-1b. The deduced molecular mass of GATA-1 is 39 kDa. The upper band appeared in lanes for all of the samples including uninjected AC, apparently due to cross-reactivity of the polyclonal antibodies with an endogenous AC protein.

GATA-1b downregulates *chordin* expression in neuralization-induced AC cells. Noggin, follistatin, and chordin have been shown to exhibit neuralization-inducing activity in vitro;

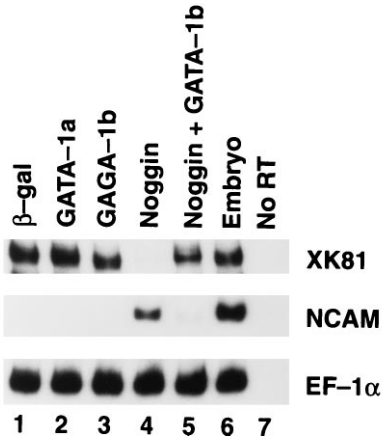

FIG. 7. GATA-1b does not affect *XK81* expression in cultured AC cells but rescues noggin-mediated inhibition of *XK81* expression. ACs were derived from embryos injected with RNAs encoding the following: lane 1, β-gal (2 ng); lane 2, GATA-1a (2 ng); lane 3, GATA-1b (2 ng); lane 4, noggin (0.5 ng); lane 5, noggin (0.5 ng) plus GATA-1b (2 ng). ACs were excised from the injected embryos at stages 8.5 to 9, cultured until the equivalent of stage 24, and collected for RT-PCR analysis.

each is normally expressed in the prechordal and notochordal mesoderm during gastrulation (9, 20, 30, 31, 33). Among them, *chordin*, a *Xenopus* homolog of *Drosophila short gastrulation*, encodes a novel secreted molecule antagonistic to the function of BMP-4 in the ectodermal patterning of both *Xenopus* and *Drosophila* (5, 11, 12, 30). We examined the transcription of these neurogenic genes in neuralization-fated AC cells. Both noggin and follistatin RNAs have not been detected in 3m or DN-BR RNA-injected caps (37, 41). In contrast, AC cells injected with 3m RNA express chordin mRNA (36a). We also observed relatively high chordin transcript levels in DN-BR- or 3m RNA-injected AC cells (Fig. 8, lanes 2 and 4). Coinjection of GATA-1b RNA with DN-BR or 3m RNA inhibited *chordin* transcription (Fig. 8, lanes 3 and 5). Noggin did not induce chordin (Fig. 8, lane 6), and vice versa (30). Injection of GATA-1a RNA did not inhibit DN-BR- or 3m-induced *chordin* expression (data not shown). These results indicate that (i) chordin may mediate or contribute to the neuralization elicited by DN-BR or 3m but not that elicited by noggin; (ii) *chordin* expression is under the negative control of BMP-4, consistent

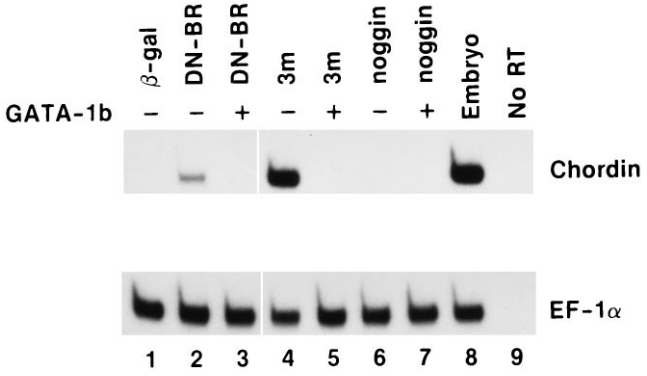

FIG. 8. GATA-1b inhibits *chordin* expression elicited by DN-BR and 3m. ACs were derived from embryos injected with RNAs encoding the following: lane 1, control β-gal (3 ng); lane 2, DN-BR (1 ng); lane 3, DN-BR (1 ng) plus GATA-1b (2 ng); lane 4, 3m (0.5 ng); lane 5, 3m (0.5 ng) plus GATA-1b (2 ng); lane 6, noggin (0.5 ng); lane 7, noggin (0.5 ng) plus GATA-1b (2 ng). The experimental method was as described in the legend to Fig. 6.

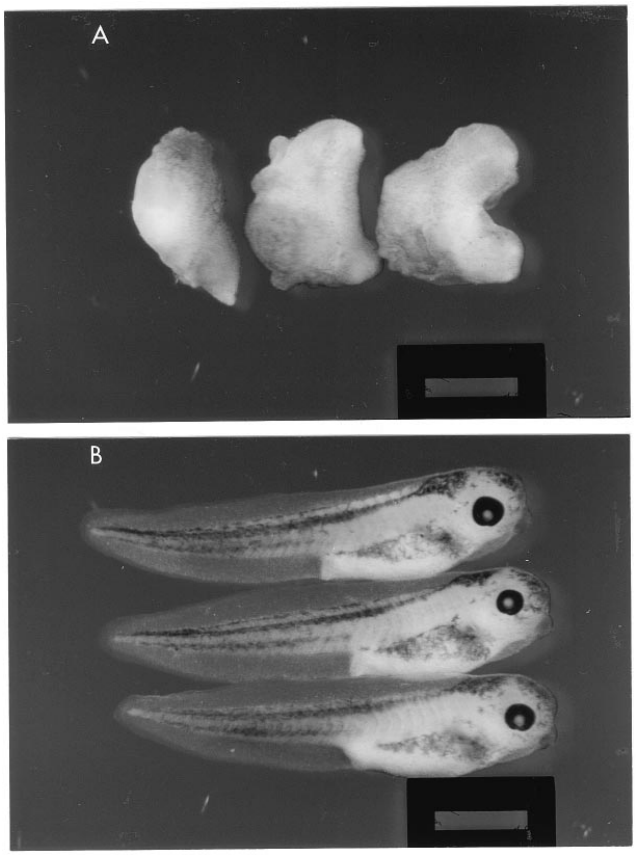

FIG. 9. GATA-1b inhibits dorsoanterior structures in whole embryos. GATA-1a or GATA-1b RNA (2 ng) was injected into the animal pole of two-cell stage embryos. The injected embryos were allowed to develop until stage 40. GATA-1b RNA-injected embryos were morphologically abnormal and totally devoid of a dorsoanterior axis (A). In contrast, GATA-1a RNA-injected embryos remained identical to uninjected embryos (B). Histological analysis showed that dorsoanterior structures, e.g., the notochord (n), muscle somites (m), brain (b), and spinal cord (s), were absent in GATA-1b-expressing embryos (C) but present in GATA-1a-expressing embryos (D).

with the mechanism involving antagonism between chordin and BMP-4 (5, 11, 12, 30); and (iii) GATA-1b may inhibit neuralization elicited by DN-BR or 3m by repression of *chordin* or genes that regulate *chordin*. However, neural tissue induced by chordin and noggin (it does not induce chordin) was also inhibited by GATA-1b (Fig. 2C). Therefore, repression of *chordin* expression probably accounts only in part for the GATA-1b effects; other mechanisms may also be involved.

GATA-1b inhibits neurogenesis in vivo. To extrapolate the results from the explant system to embryogenesis, two-cell stage embryos injected with GATA-1 RNAs were allowed to develop until the tadpole stage. Embryos expressing GATA-1b were totally devoid of a dorsoanterior axis (Fig. 9A), in contrast to embryos expressing GATA-1a (Fig. 9B). Histological analysis demonstrated that dorsal structures (brain, spinal cord, notochord, and muscle somites) were absent in GATA-1b-expressing embryos (Fig. 9C) but present in those expressing GATA-1a (Fig. 9D).

Finally, the nature of the whole-embryo defect caused by ectopic GATA-1b was investigated in explant sandwich assays. The AC or DMZ tissue was isolated from embryos injected with either GATA-1a or GATA-1b RNA. The outer layer of the DMZ tissue was removed to avoid contamination of the

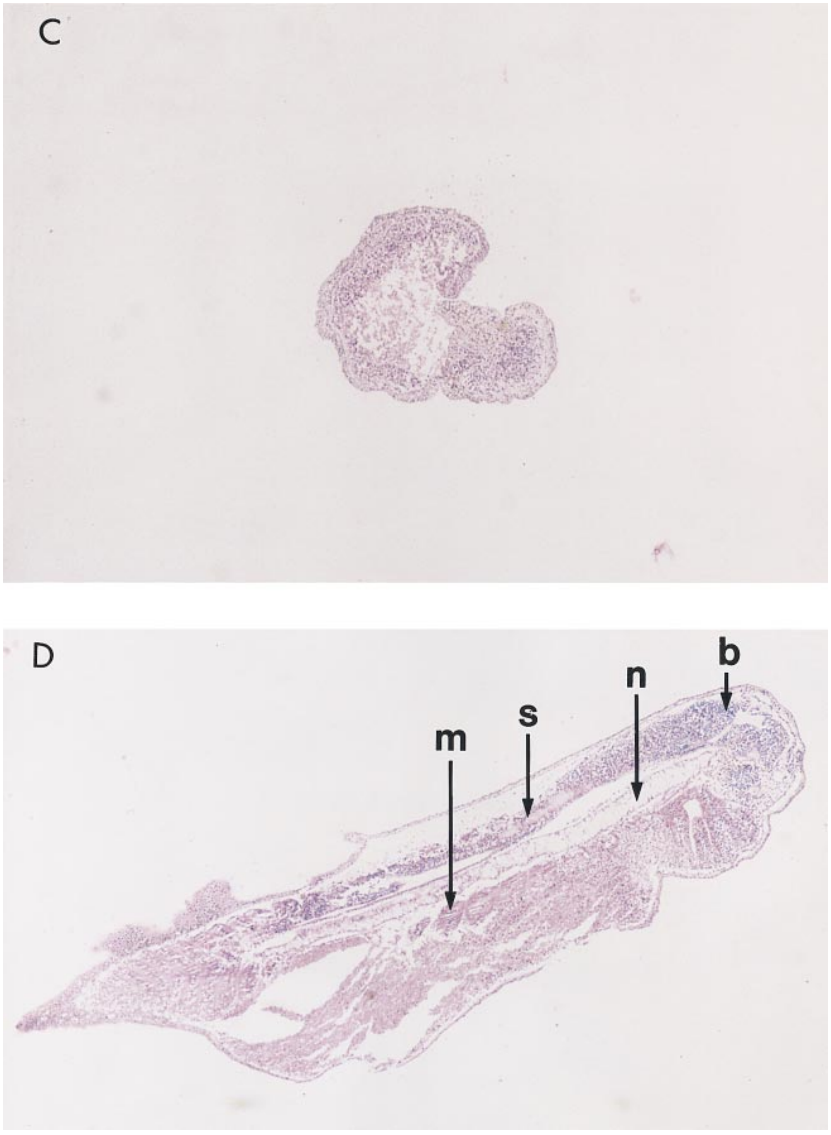

FIG. 9—*Continued.*

animal pole tissue. Conjugates were made by combining the following explants: (i) AC injected with GATA-1a plus DMZ injected with GATA-1a, (ii) AC injected with GATA-1a plus uninjected DMZ, (iii) AC injected with GATA-1b plus DMZ injected with GATA-1b, and (iv) AC injected with GATA-1b plus uninjected DMZ (Fig. 10, lanes 1 to 4). Conjugates were cultured until stage 11 or 24 prior to harvesting for RT-PCR analyses. As shown by NCAM RNA levels at stage 24, GATA-1b expression in the AC is necessary and sufficient to inhibit neurogenesis (Fig. 10, lane 4). However, this cannot explain the lack of dorsal mesodermal structures in the GATA-1b-expressing embryos (Fig. 9). As shown in Fig. 10 (upper panel), *Xbra* was transcribed equally in all explants at stage 11, which indicates that GATA-1b does not inhibit general mesoderm induction. However, *chordin* expression was inhibited only when GATA-1b was expressed in the DMZ (Fig. 10, lane 3). Similar results were found for actin at stage 24 (Fig. 10, lane 3 of the lower panel). These results suggest that GATA-1b exerts a dual effect by inhibiting neuralization in AC cells and the dorsal mesoderm in the DMZ. Both of these effects may be mediated by GATA-1b through inhibition of *chordin* expression, since chordin possesses both neuralizing and dorsalizing activities.

DISCUSSION

Transcription factors GATA-1a, GATA-1b, and GATA-2 are each able to regulate erythropoiesis but differ in neuralization-suppressing activity. Although the precise mechanism by which GATA-1b functions to inhibit neurogenesis is not known, we have determined the following. First, a functional DNA-binding form of the protein must be expressed for inhibition to occur (Fig. 4). Therefore, the protein is likely to function by activation or repression of GATA-specific target genes. Second, the chordin message induced by DN-BR or 3m in stage 11 AC cells (Fig. 8) or expressed in an AC-DMZ-conjugated explant (Fig. 10) or whole embryos (data not shown) was inhibited by GATA-1b but not by GATA-1a. This appears to correlate with the distinct effects these gene products have on neural differentiation. Third, GATA-1b mimics

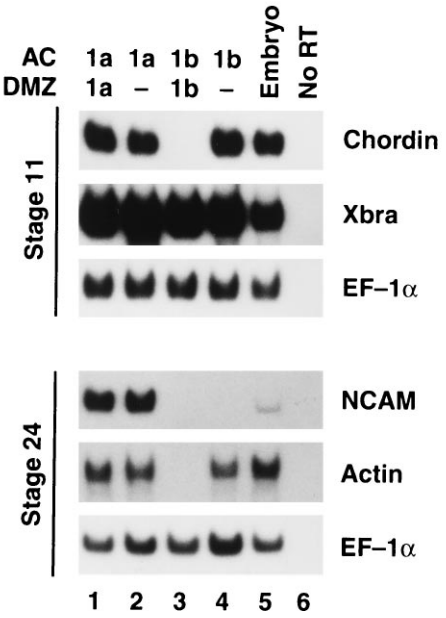

FIG. 10. GATA-1b inhibits neurogenesis independently of a dorsal mesodermal defect. AC cells or DMZ samples were isolated from embryos injected with either GATA-1a or GATA-1b RNA (2 ng/embryo). The outer layer of the DMZ tissue was removed. Conjugates were made by combining the following explants: lane 1, AC injected with GATA-1a plus DMZ tissue injected with GATA-1a; lane 2, AC injected with GATA-1a plus uninjected DMZ tissue; lane 3, AC injected with GATA-1b plus DMZ injected with GATA-1b; lane 4, AC injected with GATA-1b plus uninjected DMZ tissue. The conjugates were cultured until stage 11 or 24 prior to being harvested for RT-PCR analyses.

the action of BMP-4 on neural inhibition in the AC (Fig. 2) and whole embryos (Fig. 9) but does not appear to directly activate transcription of the *BMP-4* gene (Fig. 6). Of course, it is possible that the transcription factor regulates neurogenesis by altering some other component of the BMP signaling pathway; it has been proposed that GATA-1 might function similarly in a positive feedback mechanism with BMP signaling during erythropoiesis (44). Our data are consistent with an ability of GATA-1b, but not GATA-1a, to regulate *chordin* transcription either directly or indirectly.

The ability of GATA-1b, but not GATA-1a, to regulate neurogenesis could conceivably result from different DNA-binding specificities. By using a binding selection and PCR amplification scheme, GATA-1, -2, and -3 were each shown to bind an AGATAA erythroid consensus motif with high affinity but to display various specificities in binding other DNA sequences related to the consensus (18, 24). *GATA-1a* and *GATA-1b* are a pair of highly related genes, presumably generated by duplication in *Xenopus* cells at the *GATA-1* locus, and have very similar, but not identical, protein sequences. For example, the GATA-1b protein contains an insertion of two amino acids, Ser-168 and His-169, just upstream from the conserved DNA-binding domain and an additional three amino acids, Glu-362, Leu-363, and Ala-364, not present in GATA-1a at the carboxyl terminus. Of the remaining 359 amino acids of GATA-1a, the protein differs from GATA-1b at 38 residues. Four of these changes are in the DNA-binding domain: Met-195 to Leu, Val-224 to Ile, Ser-248 to Gly, and Arg-286 to Lys. Therefore, it is possible that these minor changes allow the two proteins to behave differently. They may recognize the same binding site in the globin promoter, whereas GATA-1b may interact more efficiently with the binding site(s) present in some genes which regulate neurogenic genes or in neurogenic genes themselves. Alternatively, these amino acid changes could influence the ability of GATA-1b to interact specifically and cooperate with other regulatory factors. It will be of interest to test mutations of GATA-1b or various types of chimeric derivatives of the two GATA-1 genes in a neuralization inhibition assay. In this manner, it may be possible to identify specific sequences that contribute to the ability of GATA-1b to regulate *chordin* expression and inhibit neurogenesis.

It may be of significance that the *GATA-1a* and *GATA-1b* genes are differentially regulated during embryogenesis (43), which is not commonly found for duplicated *Xenopus* genes. Therefore, the acquisition of a new biological function for the GATA-1b gene may have provided a selective pressure for changes in regulatory elements, or vice versa. Regardless of the normal functions of the GATA-1b gene during development, the system may provide insight into the evolutionary mechanisms that influence the diversion of members within a multigene regulatory family.

This study has demonstrated, for the first time, the possible involvement of a GATA factor in neural induction. GATA-1 and GATA-2 RNAs are first detectable at stage 11, which is concomitant with the initiation of neurogenesis but earlier than the appearance of globin RNA at stage 15 (46). The early expression of GATA-1 and GATA-2 prior to hematopoiesis has been considered to signal an early commitment of the mesoderm to form the blood island. Our present studies provide evidence for a new biological function of GATA factors in the ectoderm.

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