

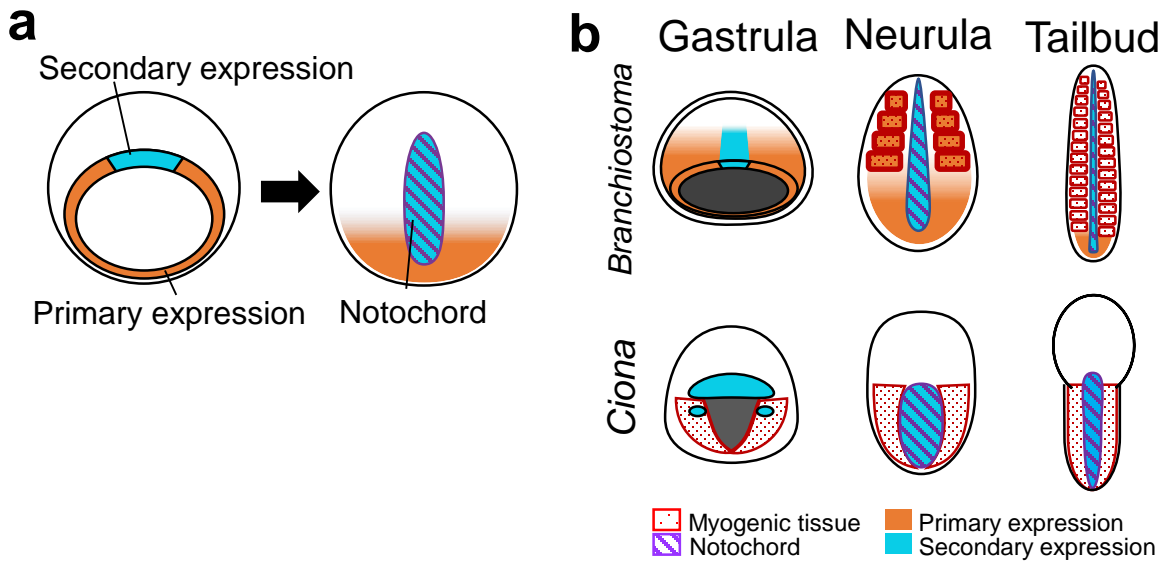


## Enhancer activities of amphioxus Brachyury genes in embryos of the ascidian, *Ciona intestinalis*

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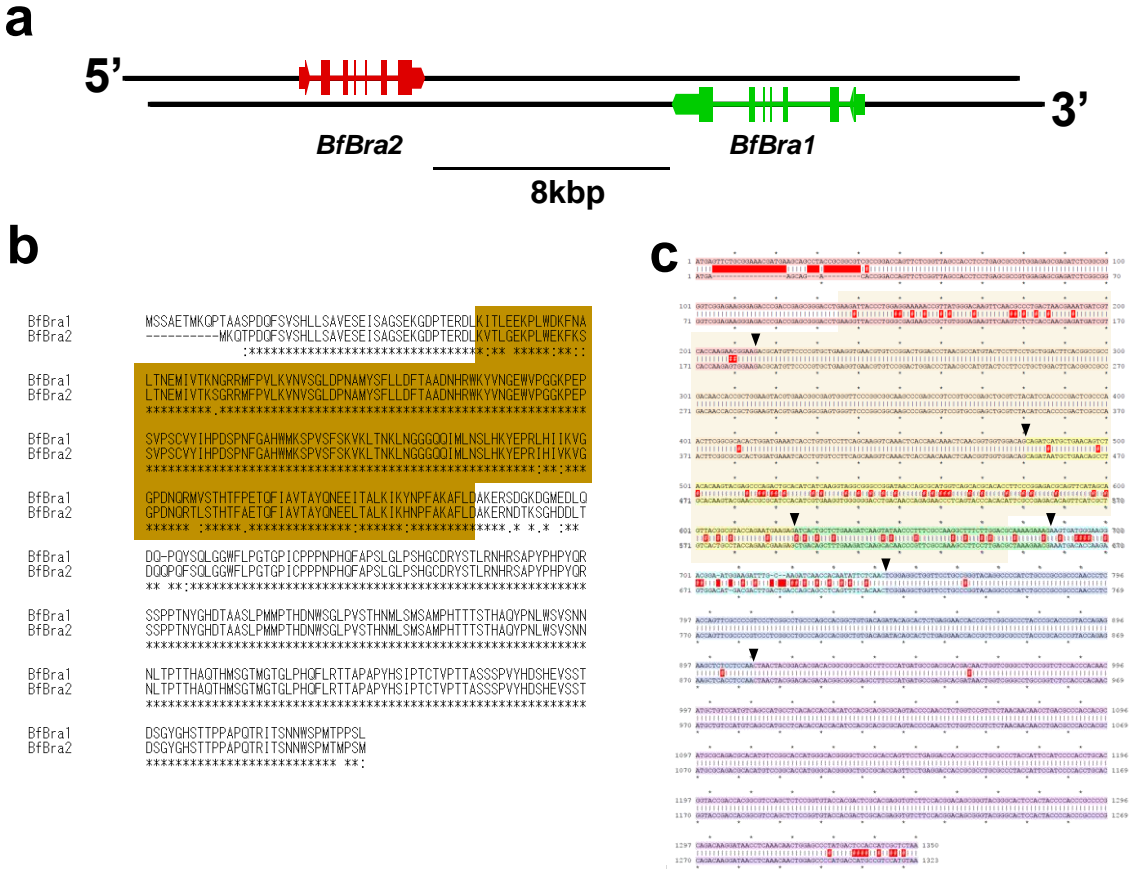
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# FIGURE 1



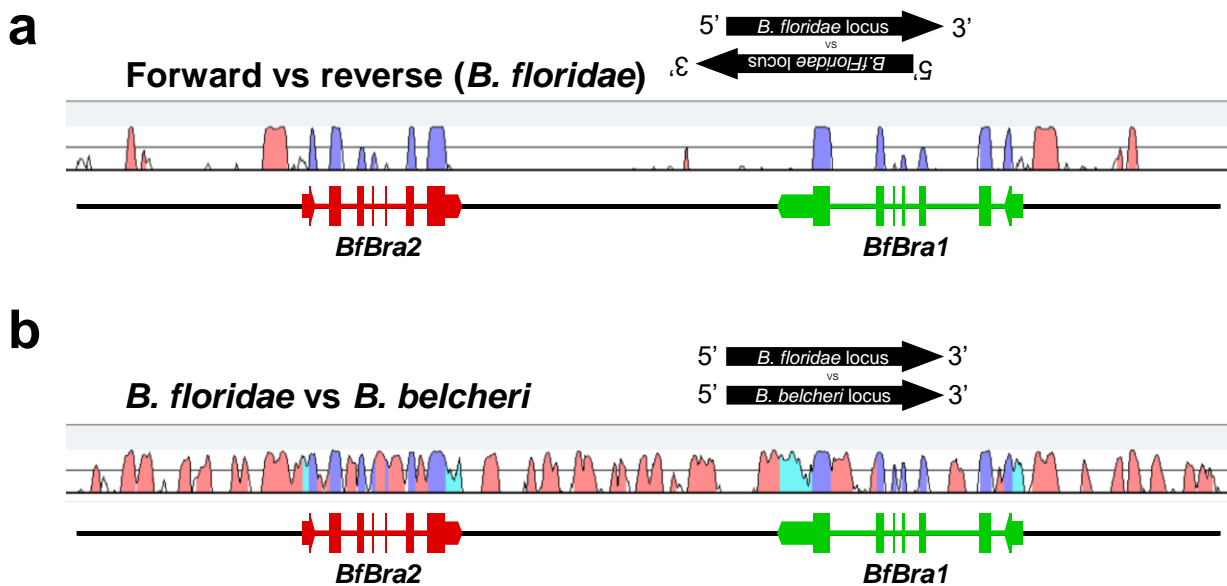
**FIGURE 1 Schematic drawing of *Brachyury (Bra)* expression in chordates.** (a) *Bra* is expressed in blastopore (primary expression, shown in brown) and in dorsal-midline from which notochord is formed (secondary expression, shown in blue). (b) A cephalochordate (*Branchiostoma*) *Bra* is expressed both in notochord (blue) and myogenic somites (red) whereas a urochordate (*Ciona*) *Bra* is expressed in notochord and its expression is suppressed in myogenic tissue (red dots area).

# FIGURE 2



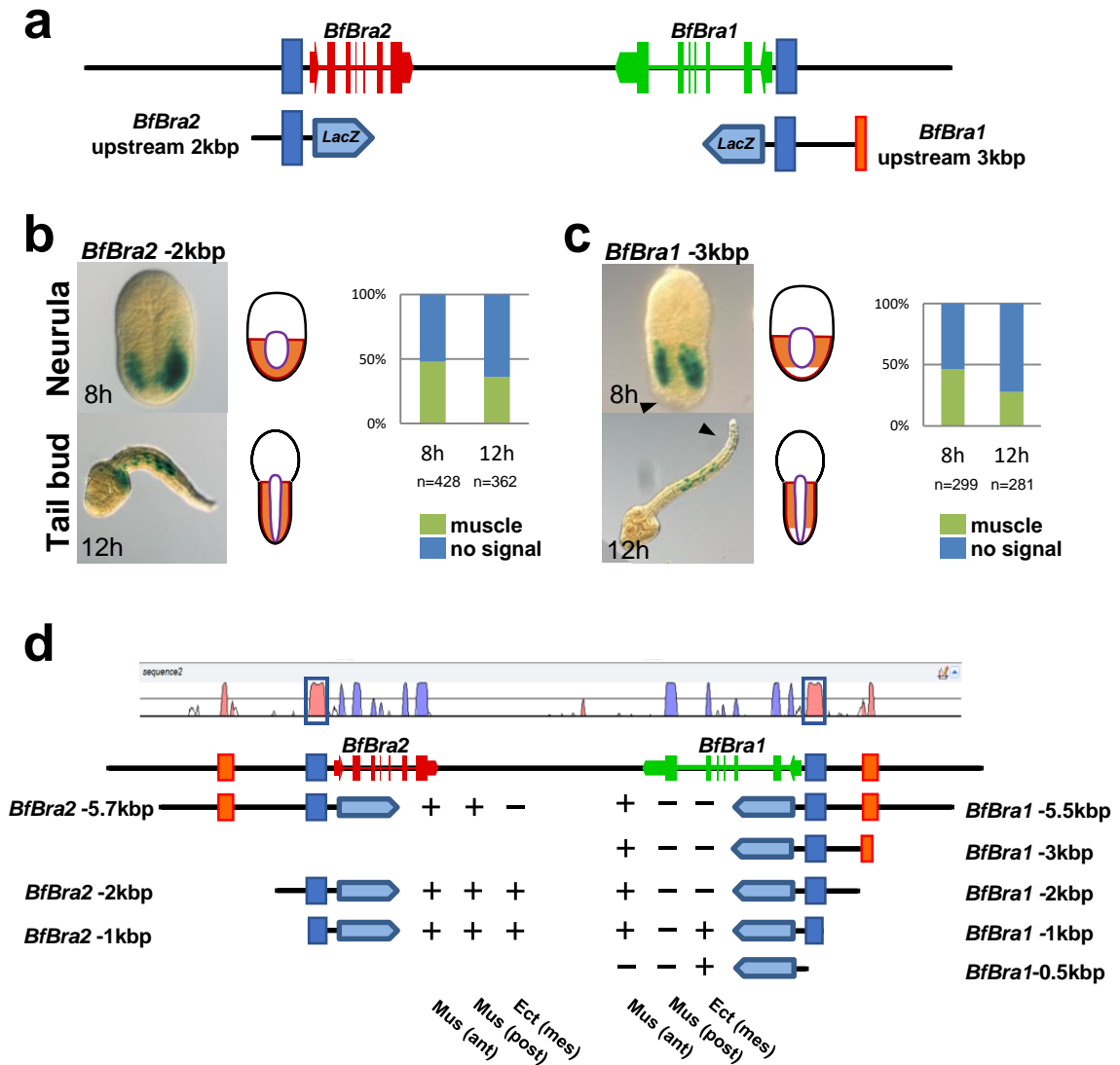
**FIGURE 2 Sequence similarity of *BfBra1* and *BfBra2*.** (a) An alignment of *BfBra1* (green) and *BfBra2* (red) in the *B. floridae* genome. Vertical lines represent exons, and thick bars show 5'- and 3'-non-coding sequences. (b) Amino-acid and (c) nucleotide sequences of *BfBra1* and *BfBra2* highly resemble each other. (b) Sequence identity is shown by asterisks and T-box is shown by dark yellow box. (c) Sequence identity is shown by vertical lines while difference by red. Black arrowheads show sites of intron insertion.

## FIGURE 3



**FIGURE 3 Sequence comparison of amphioxus *Bra* genes.** (a) Vista alignment of *BfBra2* with query of *BfBra1* nucleotide sequences. The Y axis shows percent conservation over a sliding of 100 bp window (solid line is 50% identity). Blue areas show genomic regions with conserved coding sequences (CNS) and red those with similar non-coding sequences. There are two peaks of CNS in 5'-upstream sequence of *BfBra2* and *BfBra1*. (b) Vista alignment of *B. belcheri* *Bra* with query of *B. floridae* *Bra* nucleotide sequences. CNSs are seen not only in 5' upstream but also in 3'-downstream and intron sequences. Right blues indicate 5' and 3' sequences of the genes.

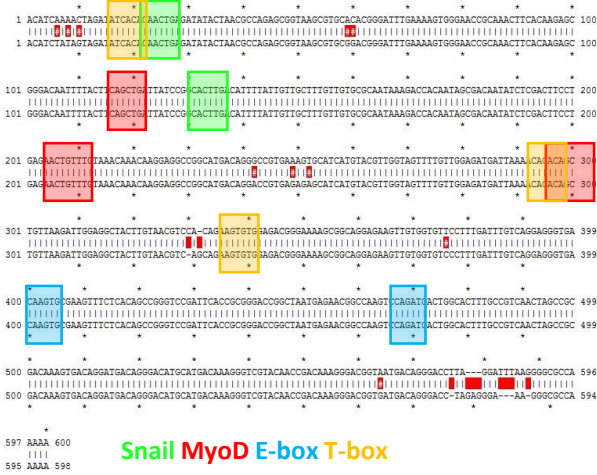
# FIGURE 4



**FIGURE 4 Enhancer activity of 5'-upstream regions of *BfBra1* and *BfBra2*.** (a) *LacZ*-reporters (arrows) were constructed with 3kb and 2kb upstream sequences of *BfBra1* and *BfBra2*, respectively, which include two non-coding sequences (CNSs) shared by two genes (see D). (b) The reporter expression of *BfBra2*-2kbp in *Ciona* embryos at neurula (8 h; upper) and tailbud stages (12 h; lower), and their schematic drawings are shown left. The numbers of embryos examined (n=428 at 8 h and in n=362 at 12 h) and percentages of embryos with reporter expression shown right. Green, expression in muscle; blue, no expression. (c) The reporter expression of *BfBra1*-3kbp. *LacZ* is expressed in muscle. (d) VISTA analysis shows two CNSs (proximal in blue and distal in red). Expression of reporter constructs of -5.7, -2 and -1 kbp of *BfBra2*, first including proximal (blue) and distal CNS (red) while second two with proximal only. *LacZ* expression of -5.5, -3, -2, -1.2 and -0.5 kbp of *BfBra1*. First two include proximal (blue) and distal CNS (red) while second two with proximal only and third with no CNS. +, expression; -, no expression.

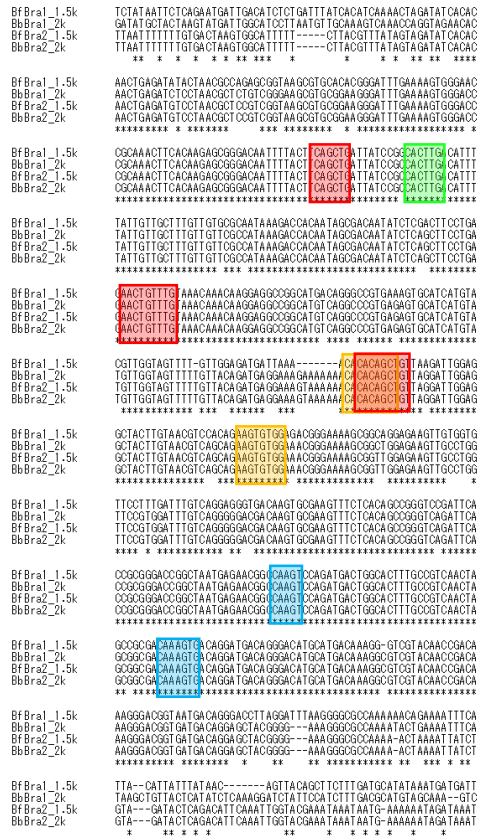
# FIGURE 5

**a**

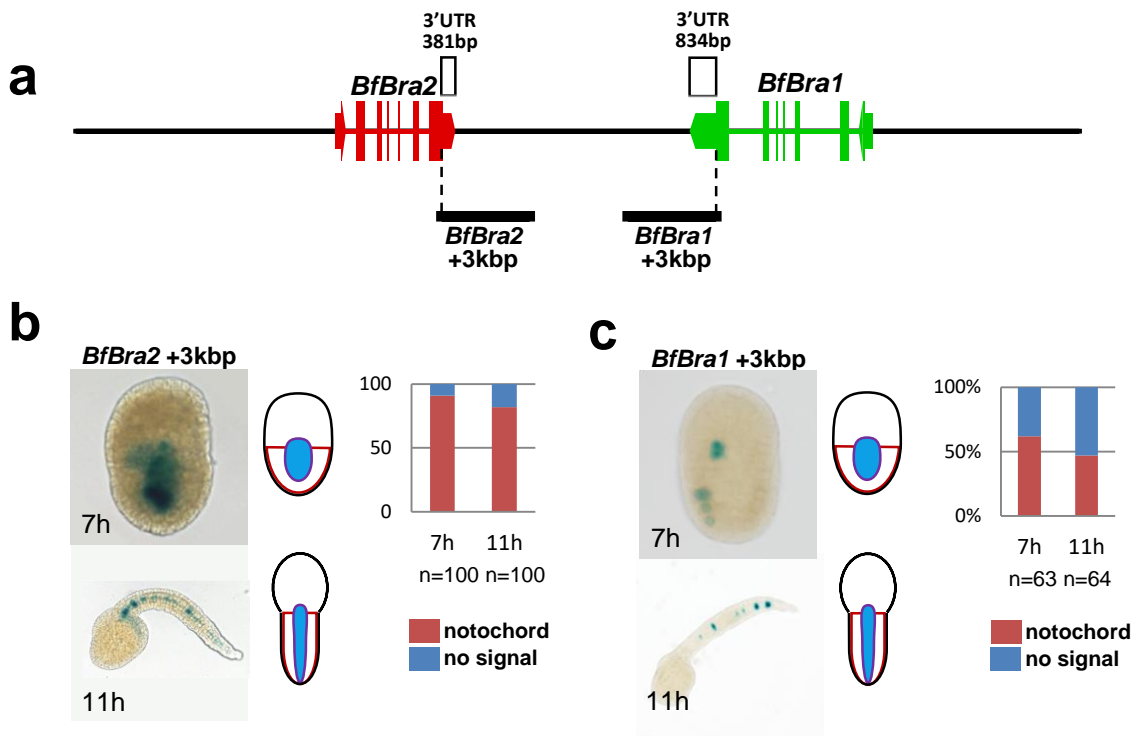


**FIGURE 5 Possible sequence-specific DNA binding motifs noticed in the 5'-upstream sequence of *Bra2* and *Bra1*.** (a) *BfBra1* and *BfBra2* and (b) *BfBra1*, *BfBra2*, *BbBra1* and *BbBra2*. Those include Snail (green), MyoD (red), E-box (blue), and T-box (yellow), respectively. Sequence differences between the two genes are shown by red (a, b).

**b**

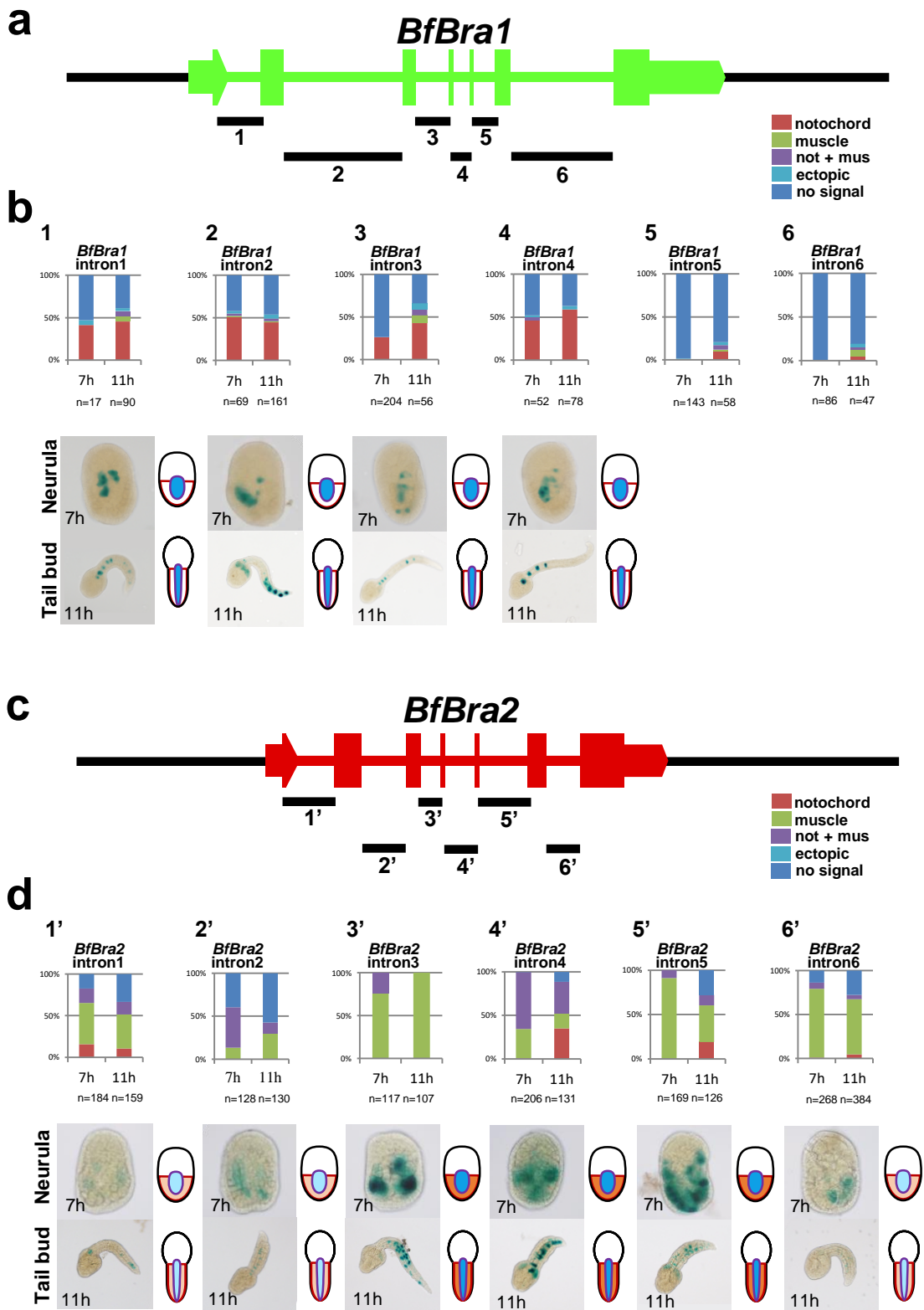


# FIGURE 6



**FIGURE 6** Enhancer activity of 3' downstream regions of *BfBra1* and *BfBra2*. (a) *LacZ*-reporters were constructed with 3kb downstream sequence of *BfBra1* and *BfBra2*, respectively, which include several CNSs shared by two lancelets (see Fig. S2B). (b, c) The reporter expression of *BfBra2*+3kbp (b) and *BfBra1*+3kbp (c) in *Ciona* embryos at neurula (7 h; upper) and tailbud stages (11 h; lower), and their schematic drawings are shown left. The numbers of embryos examined and percentages of embryos with expression shown right. Red, expression in notochord; blue, no expression.

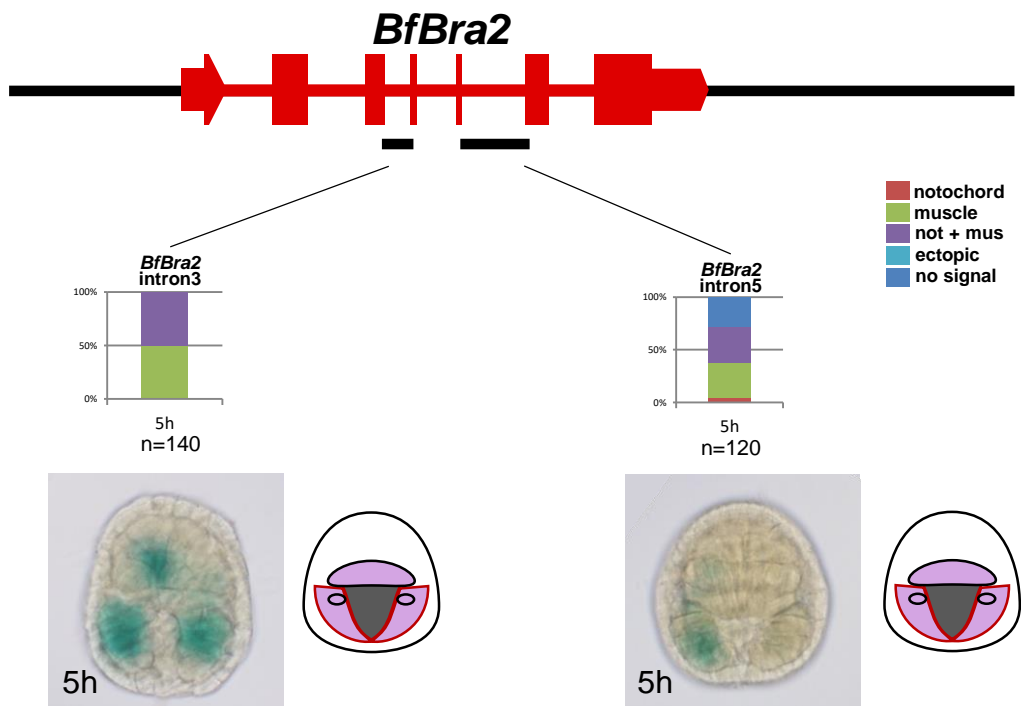
# FIGURE 7



**FIGURE 7** Enhancer activity of intronic regions of *BfBra1* (a,b) and *BfBra2* (c,d). Both genes are intervened by six introns each (1-6 in *BfBra1* and 1'-6' in *BfBra2*). Enhancer activity was examined at each of the intron sequences by mentioned in previous figures. The expression of reporter constructs in *Ciona* embryos was also the same as previously described. All intronic regions showed the activity except for intron-5 and intron-6 of *BfBra1*. Interestingly, *BfBra1* introns have enhancer activity primarily in *Ciona* notochord cells (b), whereas *BfBra2* introns have activity primarily in muscle cells (d). In addition, it is noticed a stronger activity of *BfBra2* than *BfBra1*.

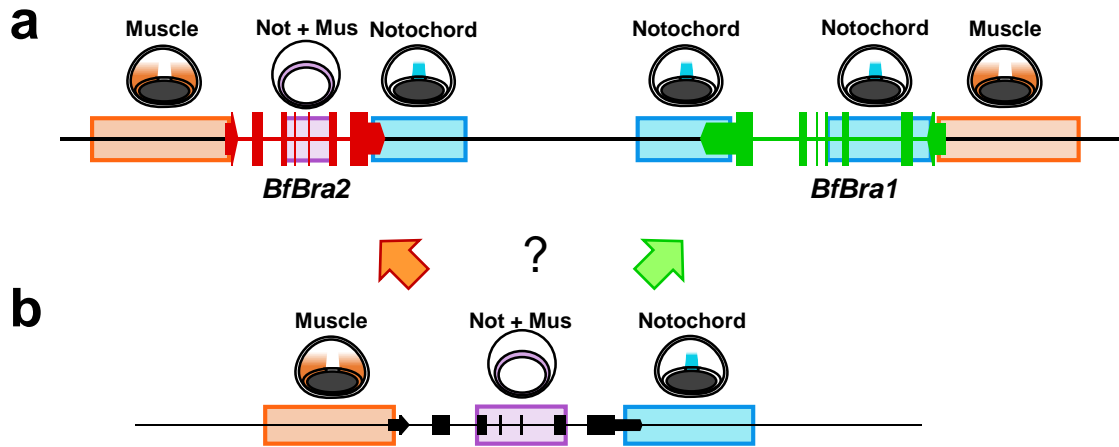


**FIGURE 8**



**FIGURE 8 Reporter activity of intron-3 and intron-5 of *BfBra2* is detected at early gastrula stage, 5h after fertilization. The expression is detected in primordium cells of both notochord and muscle.**

# FIGURE 9



**FIGURE 9** A summary of results of the present reporter assay (a) and possible interpretation of the results (b). Enhancer activity of 5' upstream sequences in muscle is shown in brown and that of 3' downstream in notochord in blue. The activity *BfBra2* introns to promote both in muscle and notochord is shown in purple.