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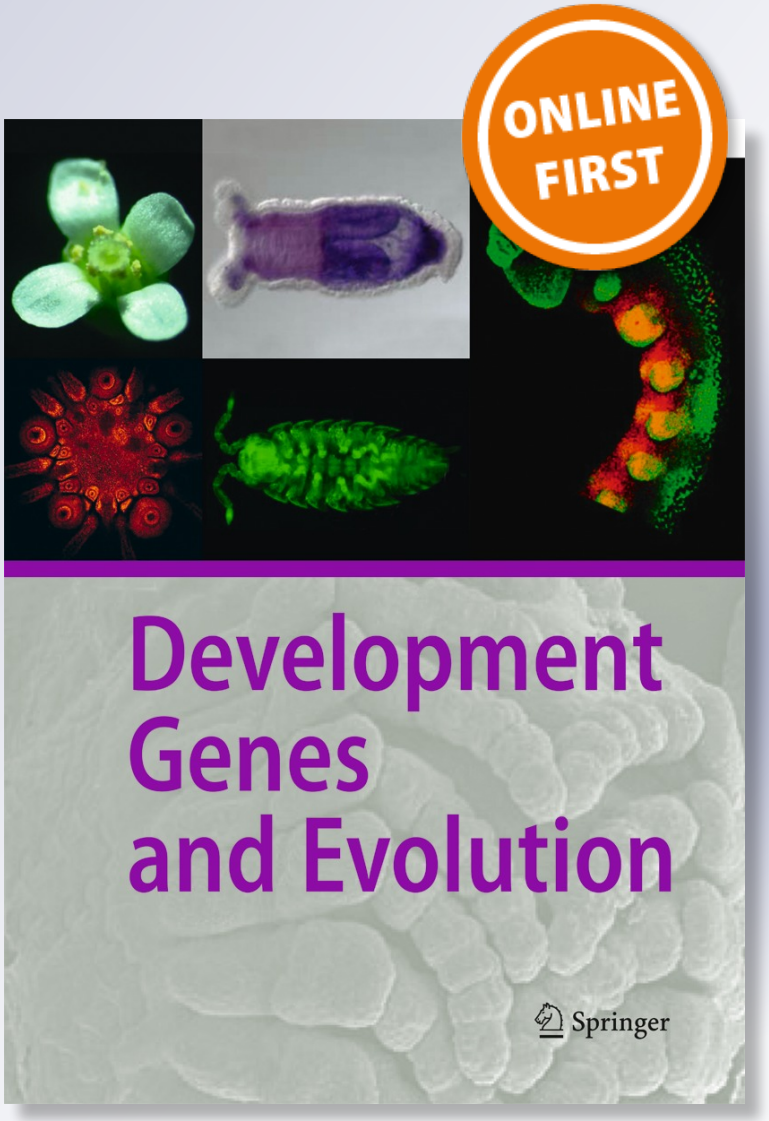

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ORIGINAL ARTICLE

The effect of RA on the chick Ebf1-3 genes expression in somites and pharyngeal arches

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Abstract Expression of chick early B cell factor 1-3 (*cEbf1-3*) genes in regions of high retinoic acid (RA) activity, such as somites and pharyngeal arches (PAs), and regulation of other EBF members by RA raise the possibility that the internal cue RA may regulate *cEbf1-3* expression in these tissues. To check this possibility, RA gain and loss of function experiments were conducted. Ectopic expression of RA led to up-regulation of *cEbf2*, *3* but did not change *cEbf1* expression in somites. Expectedly, inhibition of RA by disulfiram resulted in down-regulation of *cEbf2*, *3*, but did not change *cEbf1* expression in somites. The same RA gain and loss of function experiments did not change *cEbf1-3* expression in PAs. However, ectopic expression of RA in the cranial neural tube before migration of neural crest cells downregulated *cEbf1*, *3* and up-regulated *cEbf2* expression in the PAs. The same experiment, but with application of disulfiram, resulted in downregulation of *cEbf2*, but did not alter the expression of the other two genes. We conclude that the three *cEbf* genes act differently in response to RA signals in somitic mesoderm. *cEbf1* may be not RA dependant in somites; however, the other two *cEbf* genes positively respond to RA signalling in somites. Additionally, only the migratory *cEbf*-expressing cells into the PAs are affected by RA signals.

Keywords *cEbf1-3* · Retinoid acid · Somites · Pharyngeal arches · Chick embryo

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Introduction

The chick early B cell factor 1-3 (*cEbf1-3*) genes are members of a novel highly conserved EBF family of atypical HLH transcription factors (Dubois and Vincent 2001). The pioneer member of this family is mouse *Ebf1* which is expressed in pre-B lymphocytes (Hagman et al. 1991). Isolation of the *Ebf* homologue, *Collier* (*Col*) from *Drosophila*, provided a proof of principle for the existence of a new family of evolutionarily conserved proteins (Crozatier et al. 1996). Subsequently, several very related genes, *Ebf2*, *Ebf3* and *Ebf4*, have been isolated from mouse embryos (Garel et al. 1997; Wang et al. 2002). The conservation of functional domains in EBFs has aided in the identification of other family members in several species (Appendix). Isolation of the *Ebf* homologues in sea anemone, hydra and sponge together with the absence of evidence for any *Ebf* gene outside metazoans (as no related sequence could be found in sequence databases for prokaryotes, yeasts or plants) confirmed the conclusion that *Ebfs* are metazoan (animal kingdom)-specific genes (Daburon et al. 2008; Mazet et al. 2004; Pang et al. 2004; Simionato et al. 2007).

EBF was originally discovered in rodents as regulator of B lymphocyte and olfactory neuron differentiation (Hagman et al. 1993). Since then, the expression pattern and role of *Ebf* genes have been studied extensively in the immune (Lukin et al. 2008) and nervous tissues (Davis and Reed 1996; Garel et al. 1997). Expression of *Ebf* genes is not limited

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