Hydra constitutively expresses transcripts involved in vertebrate neural differentiation

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The diploblastic Hydra is among the most primitive multicellular organisms. Using cross-hybridization with *Xenopus* probes, *noggin*-like transcripts were detected in the hypostome and basal disc of adult Hydra (*Pelmatohydra oligactis*), regions with properties similar to that of the amphibian organizer. This points to the possibility of a close molecular similarity between the *Xenopus* and Hydra organizers. The constitutive expression of a *noggin*-like gene in Hydra may be responsible for its regenerative capacity.

1. Introduction

The fresh water coelentrate Hydra is one of the most primitive multicellular organisms to be endowed with a definite body plan. Its amazing regenerative ability has led it to be thought of as a potentially immortal creature (Gierer 1974; also see Wolpert *et al* 1998; Gilbert 2000). On account of these and other attributes, it has been used as a model system to gain insights into early embryonic development (Webster 1971; Gilbert 2000). With the advent of recombinant DNA technology, similarities in developmental phenomena in Hydra and vertebrate embryos are becoming apparent at the molecular level. Specifically, cognates of genes such as *Hnf3b* in *Xenopus laevis* (Martinez *et al* 1997), *Brachyury* in *X. laevis* (Technau and Bode 1999), *goosecoid* in *X. laevis* (Broun *et al* 1999) and several members of the *Wnt* pathway in *X. laevis* (Hobmayer *et al* 2000) have been detected in Hydra (*Hydra magnipapillata*, *H. vulgaris*). The expression patterns of these genes suggest that they participate in axis and head formation in Hydra too. In the present study we have employed cross-hybridization to detect *noggin*-like transcripts in *Pelmatohydra oligactis*. *Noggin* is crucial for nervous system development in *Xenopus* (Smith and Harland 1992), chick (Connolly *et al* 1997) and mammals (Bachiller *et al* 2000). We report the detection of *noggin*-like transcripts in regions of adult Hydra that functionally resemble to the Spemann organizer in amphibia [a comparison that actually ought to be made the other way round: the 'Organizer' was first discovered by Browne – in Hydra (Lenhoff 1991)].

2. Materials and methods

Brown Hydra (*P. oligactis*) collected from a local stream were cultured in the laboratory at room temperature (22–28°C) in glass bowls containing pond water. The animals were fed thrice a week with crustacea (*Cyclops* and *Daphnia*). Fully grown Hydra were starved for 24 h before use.

Xenopus noggin (*X-nog*, Smith and Harland 1992) and *Xenopus goosecoid* (*X-gsc*, Niehrs *et al* 1993) cDNAs cloned in pGEM5Zf(–) and pBS(KS) vectors, respectively, were a kind gift from Dr J C Smith, National Institute for Medical Research, London. The plasmid *pnoggin5*′*5* was linearized with *Eco*RI and transcribed using T7 RNA polymerase in the presence of DIG-labelled dUTPs to generate an antisense transcript of 463 bases. Likewise, DIG-labelled *X-gsc* (440 bases) was generated by using T3 RNA polymerase from the plasmid pBS(KS) linearized with *Xba*I. A rat GAPDH (glyceraldehyde 3-phosphate

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dehydrogenase) antisense transcript of 376 bases was prepared by using a commercially available clone (Ambion, Cat No. 7432) which contained a 316 bp fragment of the rat GAPDH gene derived from exons 5–8. Whole mount *in situ* hybridizations were carried out according to the method of Grens *et al* (1996). The animals were photographed in incident light under a Wild M3Z dissecting binocular microscope using a 35 mm 100 ASA colour film.

3. Results and discussion

The *X-nog* probe hybridized specifically to the hypostomal and basal regions of adult Hydra (figure 1a). During budding, *noggin*-like transcripts were initially detected all over the distal end of the bud but subsequently became localized around the mouth and at the base of tentacles, in the epithelial endodermal cells (figure 1b). The basal region of the bud attached to the parent did not show *noggin*-like expression (figure 1a). The *X-gsc* probe picked up transcripts in the hypostomal region, around the mouth and at the base of the tentacles and in the body column of the adult as well as in the forming bud (figure 1c). This expression pattern of *goosecoid* in the adult and in the bud is quite similar to the one reported recently (Broun *et al* 1999). A close comparison between the *goosecoid* staining pattern in *H. vulgaris* (Broun *et al* 1999) and *Pelmatohydra oligactis* (present study) shows that in both *goosecoid*-like transcripts are expressed in three domains – in the hypostome, at the base of the tentacles and in the endoderm of the body column. In the forming bud, the expression is once again seen in both the species. The only difference in the staining pattern is in the tentacles themselves; these do not express *goosecoid* in *H. vulgaris* (Broun *et al* 1999) but do so in *P. oligactis* (present study). This difference could be species specific. Transcripts that hybridized to a rat GAPDH antisense probe were uniformly present in all parts of the organism (figure 1d), confirming the specificity of staining. GAPDH was used as a positive control since it is a 'housekeeping' gene and an integral part of the highly conserved glycolytic pathway.

Noggin is expressed in the Spemann organizer in the *Xenopus* embryo and is required for neural induction

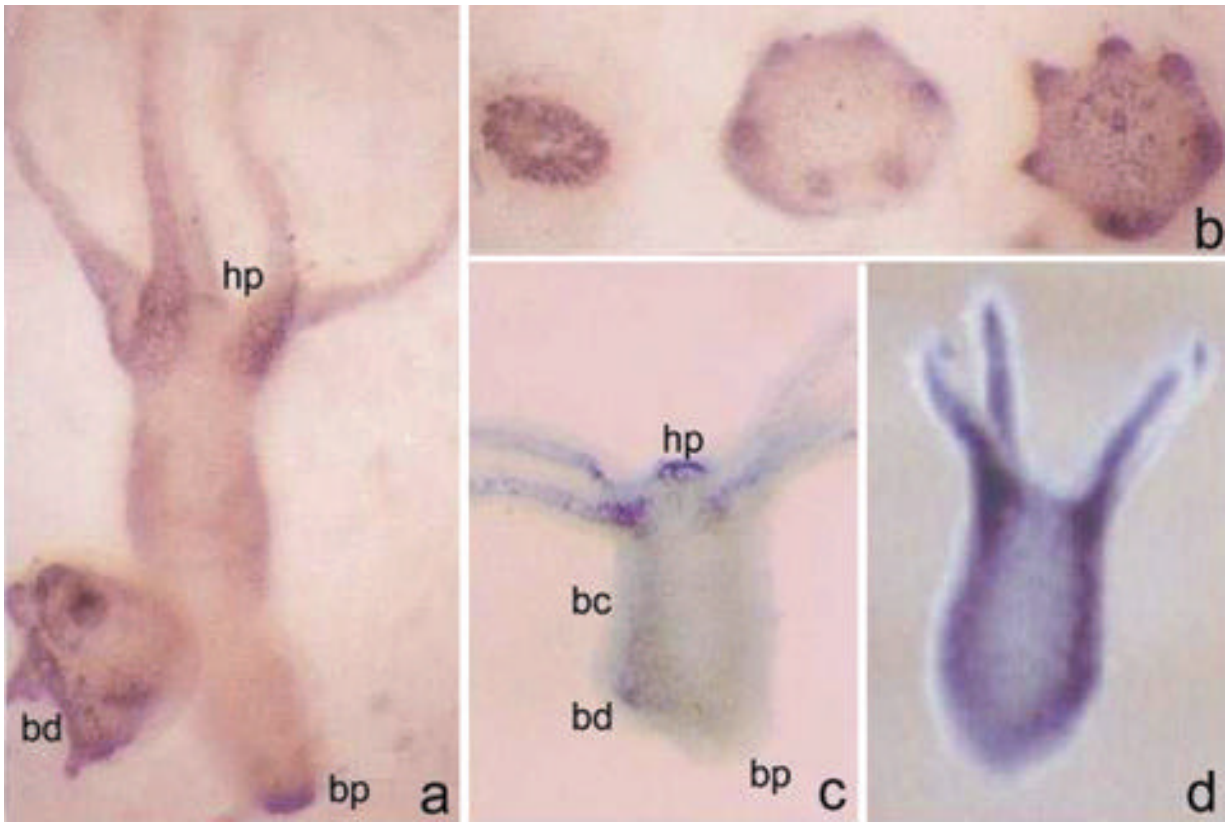

Figure 1. *Noggin*- and *goosecoid*-like transcripts in *P. oligactis*. (**a)** *Noggin*-like transcripts in the hypostomal region (hp) and basal disc (bp) in an adult hydra. (**b)** *Noggin*-like transcripts during budding. The bud on the left is youngest in developmental age. (**c**) *Goosecoid*-like transcripts in the hypostomal region (hp), around the mouth and at the base of tentacles and in the body column (bc). The newly forming bud (bd) also expresses *gsc*-like transcripts. (**d**) Transcripts that bind to an antisense rat GAPDH probe are uniformly distributed all over the body of the organism (control).

(Smith and Harland 1992). The expression of *noggin*-like transcripts in Hydra (figure 1a) is localized to regions that are equivalent to the *Xenopus* organizer: on transplantation both the hypostomal and the basal regions can induce new axis formation in a host Hydra (Browne 1909; Newman 1974). The present study provides further evidence in favour of the hypothesis that these two regions in Hydra are indeed homologous to the Spemann organizer.

Hydra is considered to be immortal as it can unendingly replace all its body cells (Gierer 1974; Müller 1996). Even nerve cell precursors, present in the body column, can give rise to differentiated neurons that reside in the hypostomal and basal regions (Müller 1996). These are precisely the regions where we found transcripts similar to *noggin*. Further, the expression of *noggin*-like transcripts that we detect during budding (figure 1b) overlaps the site of nerve cell differentiation which, in turn, is necessary for development of the bud (Berking 1980). It is tempting to speculate that these transcripts play a major role in the perpetual neuronal differentiation in Hydra.

Developmental events that occur in vertebrate embryos exist in Hydra, albeit in a primitive and simpler form. Homologues of several genes, important in the development of higher organisms, including *noggin*, have begun to be found in Hydra. The presence of *noggin*-like transcripts in Hydra organizing regions supports the assertion that "mere tradition rather than stringent logic has led to different concepts (of organizer function) in Hydra and *Xenopus*" (Müller 1996). Two other important implications of the present study are the following: (i) It is possible that the presence of such crucial 'developmental' transcripts in a fully developed Hydra confers on it the remarkable ability to regenerate and be immortalized. This suggestion assumes importance in the context of the elusive molecular explanation for regeneration in metazoans (Sànchez Alvarado 2000). (ii) The differences in the sites of expression of *noggin* and *goosecoid* may be one of the ways in which cells in Hydra sense their relative positions along the body axis.

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