POLARISING ACTIVITY OF FGF-8 IN THE AVIAN MIDBRAIN.

Huma SHEIKH and Ivor MASON
MRC Brain Development Programme, Dept. Developmental Neurobiology, UMDS Guy's Hospital, London SE1 9RT, U.K.

Objectives

Fibroblast growth factors (FGFs) comprise a family of ten secreted protein cytokines; one of these, FGF8, is expressed at the isthmus, a constriction between the midbrain and the hindbrain of the neural tube. The isthmus exhibits polarising activity when grafted into anterior midbrain and midbrain-inducing activity when grafted into diencephalon (see e.g. 1,2). This is preceded by ectopic expression of genes which have been shown to be required for the normal development of posterior midbrain (e.g. *En-2*; 1,2,3). Histological analysis revealed that induced, ectopic midbrain tissue was in a mirror-image orientation to the host midbrain (1). Its spatial and temporal expression suggested that FGF8 is a good candidate for the organising activity of the isthmus responsible for patterning the posterior midbrain (Figure 1A). Here we report experiments which have examined (i) the spatial and temporal expression of FGF8 relative to a number of genes required for posterior midbrain development and (ii) the ability of FGF protein to mimic inducing activities associated with the isthmus.

Methods

i) Ectopic expression of FGF protein. FGF8 and FGF4 have similar receptor specificities and can be used interchangeably (see e.g. 13). FGF4-soaked heparin acrylic beads were grafted into stage 10-12 chicken embryos as previously described (4). The beads were washed in Leibowitz's 15 medium, crushed and then soaked in 0.1µg/µl recombinant FGF4 in PBS for 1 hour. Fragments were grafted into P2 of the diencephalon or the anterior of the midbrain vesicle. Embryos were incubated for in a humidified atmosphere at 38°C.

ii) *In situ* hybridisation. Whole mount *in situ* hybridisation to experimental and control 24 hr incubation embryos and 72 hr incubation midbrains was performed as previously described (14).

iii) Histological analysis. Heads and dissected midbrains were fixed and processed for wax or vibrotome sectioning. Wax sections were stained with cresyl violet.

Results

i) Temporal expression of genes associated with development of the posterior midbrain. A number of genes have been found to be essential for normal midbrain and cerebellar development these include *En-1* , *En-2*, *Wnt-1* and *Pax- 2* (5-11). To establish a temporal sequence for their expression, and therefore a possible relative sequence for their activities, we examined the onset and subsequent refinement of their expression relative to FGF8. We found that all were first expressed at about the 3-4 somite stage. *Wnt-1* expression was restricted dorsally in a broad domain including the midbrain and hindbrain as previously described (12) but the others were expressed in a broad domain of the neural tube roughly corresponding to the prospective midbrain territory. Subsequently, expression was further refined to produce overlapping domains of expression restricted to the posterior midbrain (data not shown).

ii) Morphological effects of ectopic FGF protein in midbrain and diencephalon. In experiments in which recombinant FGF4-soaked beads were grafted into the posterior diencephalon of the chicken neural tube it was found to induce midbrain structures, as judged by both morphology and gene expression, with reversed polarity in a similar manner to isthmic grafts. Similar results were recently reported by others using recombinant FGF-8 protein (4). The morphological effects of ectopic FGF4 fell into three classes: (i) expansion of the midbrain on the bead side, (ii) development of an ectopic vesicle with a clear constriction between itself and the normal midbrain or (iii) bilateral foliation of both the normal and induced midbrain tissues (data not shown).

The isthmus normally exerts its affects on posterior midbrain tissue. We therefore next examined the inductive capabilities of FGF on anterior midbrain tissue and were able to induce posterior midbrain characteristics in that region. In embryos incubated until embryonic day 9 (E9) the following morphologies were seen: (i) expansion of the bead side midbrain, (ii) development of an ectopic vesicle on the caudal end of the bead side midbrain, (iii) development of a folded ectopic vesicle on the bead side midbrain or (iv) bilateral folding of midbrain and development of a folded ectopic vesicle on the bead side midbrain (Figure 1B and data not shown). Overall, these were similar to the results obtained in experiments on the diencephalon.

iii) Induction of posterior midbrain markers by ectopic FGF protein. Ectopic expression of *Pax-2, En-1, En-2* and *Wnt-1* was induced by FGF in both midbrain and diencephalon within 24 hours of introducing the FGF-soaked bead (Figure 1C and data not shown). By contrast, FGF8 transcripts were only induced in midbrain tissue. In addition, while all regions of the midbrain seemed competent for induction of these genes, *En-1, En-2* and *Wnt-1* could only be induced in the posterior diencephalon. These data suggest that FGF8 might normally regulate the expression of these genes in the posterior midbrain or be part of a positive feedback loop maintaining their expression.

We next examined the expression of two genes implicated in regulation of the spatial ordering of the retinotectal projection. RAGS and ELF-1 are GPI-linked ligands for members of the eck/eph family of receptor tyrosine kinases and are implicated in the guidance of nasal retinal axons to the posterior tectum). Both genes have a posterior to anterior

decreasing gradient of expression in the developing midbrain (see 15 for references and discussion). Ectopic FGF protein was introduced into the midbrain or the diencephalon and the expression of these two genes was examined in 5 day-old embryos. In such grafts expression of Rags and ELF1 was extended to the level of the bead and the apparent gradient of expression was lost (data not shown). The effect of such manipulations on the retinotectal projection is being examined.

General Conclusions

FGF8 is expressed in the developing neural tube in a manner consistent with it being an isthmus-derived organising activity. FGF protein is capable of inducing ectopic midbrain tissue in the posterior diencephalon with a reversed anteroposterior polarity. These effects may be mediated by the induction of genes previously found to be required for normal development of the posterior midbrain. The foliations induced in the midbrains of certain embryos may be due to effects of FGF on proliferation and this may, in turn, be a reflection of the dose of FGF on the bead

References

1) Martinez et al. (1991) Neuron 6; 971-981
2) Bally-Cuif et al. (1994) Development 120; 3379-3394
3) Bally-Cuif et al. (1992) Development 115; 999-1009
4) Crossley et al. (1996) Nature 380; 66-68
5) Joyner, A. L. (1996) Trends Genet. 12; 15-20
6) Bally-Cuif et al. (1995) Mech. Dev. 53; 23-34
7)Puschel et al. (1992) Mech. Dev. 38; 197-208
8) Asano et al. (1992) Mech. Dev. 39; 29-39
9) Wurst et al. (1994) Development 129; 2065-2075
10) Millen et al. (1994) Development 120; 695-706
11) McMahon et al. (1992) Cell 62;581-595
12)Rowitch et al. (1995) Mech. Dev. 52; 3-8
13) Mahmood et al. (1995) Current Biology 5: 797-806
14) Mahmood et al. (1995) Development 121; 1399-1410
15) Itasaki et al. (1991) Development 113; 1133-1144

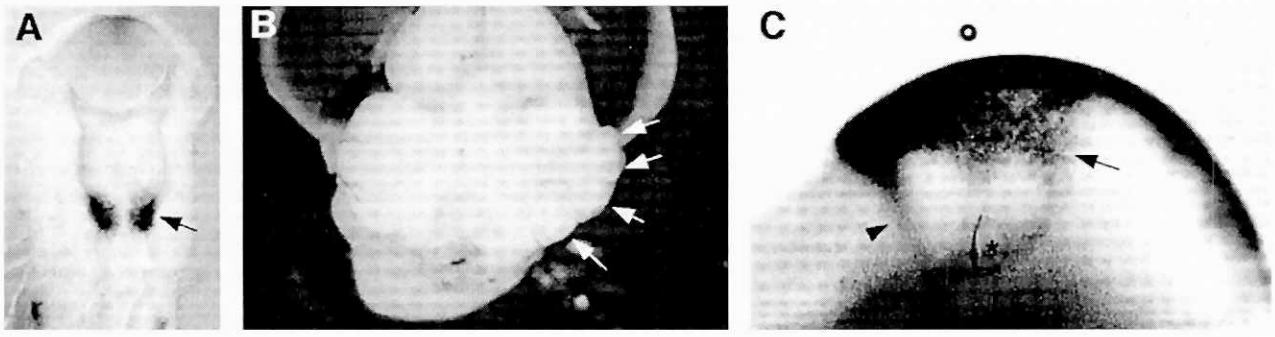

Figure 1.
A. Expression of FGF8 transcripts (arrow) at the isthmus of an 11 somite chick embryo. B. Foliations induced bilaterally in the midbrain following implantation of an FGF-soaked bead to the right side of the midbrain. C. Induction of ectopic *wnt-1* transcripts (arrow) in the midbrain 24 hr after FGF application. The normal domain of *wnt-1* expression is indicated by the arrowhead.