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Original Article

Overexpression of the *Xenopus* Xl-*fli* gene during early embryogenesis leads to anomalies in head and heart development and erythroid differentiation

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ABSTRACT The product of the Xl-*fli* gene, a *Xenopus laevis* transcription factor of the *ets* family, specifically expressed in several lineage of migratory cells during *Xenopus* development (Meyer *et al., Int. J. Dev. Biol. 39:* 909-919, 1995) was overproduced during *Xenopus* embryogenesis, upon microinjection of a synthetic transcript in the fertilized egg or in the early embryo. This results in anomalies of the antero-posterior and dorso-ventral polarities, and in tissue differentiation, particularly in the eye- and head cartilage development, as well as erythroid differentiation (absence of erythrocyte differentiation in the circulating blood, often accompanied by ectopic localization of mature erythrocytes, leading to important hemangiomas). Cytological examination reveals at gastrulation the existence of abnormal cells separating the different embryonic layers, suggesting modifications of the cellular adhesion properties. The possible involvement of the *fli* gene in controlling the dissemination of migratory cells is discussed.

KEYWORDS: *transcription factor, ets gene family, overexpression, ectopic expression, malformations*

Introduction

The *fli*-1 gene was first characterized in the mouse as being overexpressed upon integration of the Friend leukemia virus proviral DNA in the host cell DNA (Ben-David *et al.*, 1990; Ben-David and Bernstein, 1991) leading to erythroleukemia. More recently, the *fli* gene was associated to a human malignant disease, Ewing's sarcoma (EWS) (Delattre *et al.*, 1992; Sorensen *et al.*, 1993). In this disease, a translocation between chromosomes 11 and 22 leads in more than 80% of the patients to a hybrid protein containing the DNA-binding domain of the FLI protein and the N-terminal region (NTR) of the EWS protein, described as a RNA binding protein. This translocation therefore results in three consequences: i) placing the hybrid gene under the control of the EWS promoter, which appears to be quite efficient (Delattre *et al.*, 1992); ii) making the expression more ubiquitous (Delattre *et al.*, 1992); and iii) replacing the transactivation domain of the FLI protein by the EWS-NTR. Bailly *et al.* (1994) showed that the EWS-NTR indeed behaves as a very efficient transactivation domain. Interestingly, another hybrid protein composed of the EWS-NTR and the DNA-binding domain of the *erg* gene (Rao *et al.*, 1987; Reddy *et al.*, 1987; Reddy and Rao, 1991) has been characterized in Ewing's sarcoma (Zucman *et al.*, 1993). This might be particularly significant as the *erg* gene is very similar to the *fli* gene (Watson *et al.*, 1992; Meyer *et al.*, 1993). We cloned a Xl-*fli* cDNA from *Xenopus* neurula library and showed that the gene is expressed during embryogenesis in a restricted pattern evocative of neural crest cell distribution (Meyer *et al.*, 1993). Recent results of whole-mount *in situ* hybridization showed that *fli* transcripts are detected in different lineage of migratory cells (Meyer *et al.*, 1995). It was therefore of interest to overexpress this gene in early embryogenesis, looking for clues to the gene function. This paper reports a teratogenic effect of Xl-*fli* overexpression upon microinjection of a synthetic mRNA in the fertilized egg or in the early embryo. The effects observed can be interpreted in terms of interference with the cellular adhesion properties. The possible contribution of the *fli* gene to the control of cell migration is discussed.

Results

Injection of the synthetic fli mRNA results in accumulation of a functional translation product in the embryo

Xenopus embryos were injected with the synthetic mRNA from 45 min after fertilization up to the 8-cell stage and embry-

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Abbreviations used in this paper: A-P, antero-posterior; D-V, dorso-ventral; ECM, extracellular matrix; EWS, Ewing's sarcoma; LTR, long terminal repeat; MSV, Moloney sarcoma virus; NC, neural crest; NTR, N-terminal region.

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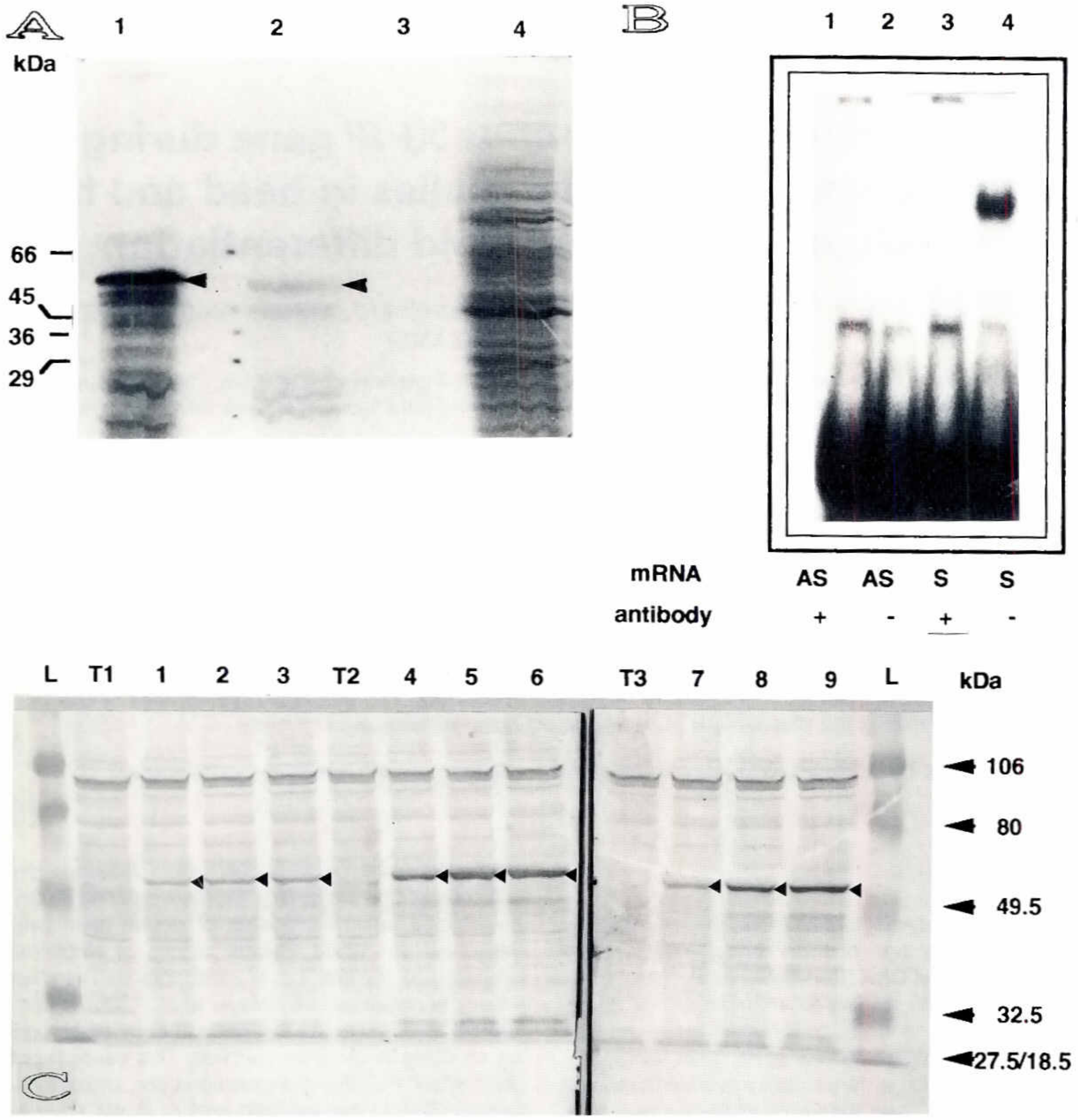

Fig. 1. Analysis of Xl-*fli* overexpression in injected embryos. (A) *Polyacrylamide gel electrophoresis of the ^{35}S-labeled translation products encoded by the Xl-fli synthetic transcript, in a rabbit reticulocyte system (lanes 1) and in a Xenopus oocyte system (lane 2, 3 and 4). Lane 1 shows the migration profile of the polypeptides recovered from the rabbit reticulocyte translation mixture. Lane 2 shows the polypeptides recovered from the oocyte extract upon immunoprecipitation with anti-FLI antibodies. In lane 3, the anti-FLI antibody was preincubated with the antigen prior to the immunoprecipitation reaction. Lane 4 shows the complete oocyte translation mixture, in the absence of immunoprecipitation.* **(B)** *Bandshifting activity of the overproduced FLI protein in extracts of Xenopus embryos at neurula stage towards the ETS-target sequence present in MSV-LTR. The embryos have been injected at the one-cell stage, either with the sense- (lanes 3 and 4) or antisense fli-synthetic transcript (lanes 1 and 2). The specificity of the complex formed with extracts of embryos injected with the sense transcript is assessed by the disappearance of the retarded band upon preincubation of the protein extract with an anti-FLI antibody (lane 3). No bandshifting activity is observed in control embryos, probably because endogenous FLI protein is present at a too low concentration. mRNA: S, injection with the sense transcript; AS, injection with the antisense transcript. antibody: (+), incubation in the presence of the anti-FLI antibody; (-), incubation in the absence of the antibody.* **(C)** *Immunocharacterization of the FLI protein overproduced in Xenopus embryos. Fertilized eggs were injected with increasing amounts of synthetic fli mRNA and allowed to develop for 2, 16 and 24 h. Embryos were then homogenized and the proteins were separated by SDS-PAGE and blotted to a nitrocellulose membrane. Proteins of control embryos of the same developmental stages were also analyzed. The Western blot was then probed with a rabbit polyclonal anti-FLI antibody. L, MW ladder; T1,T2,T3, control embryos analyzed at 2, 16 and 24 h, respectively. 1,2,3: proteins recovered 2 h after injection of the fertilized eggs with 0.55, 1.1 and 2.2 ng of mRNA, respectively. 4,5 and 6, proteins recovered after a 16 h incubation. 7,8 and 9, proteins recovered after a 24 h incubation. The bands corresponding to the Xl-fli protein present in the injected samples and absent from the control samples are indicated by an arrowhead. With the exception of a minor polypeptide of MW $\approx$30 kDa (probably corresponding to a degradation product), all other bands are non specific since they are observed both in the control and injected samples.*