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Isolated Dorsal Animal Blastomeres of *Xenopus laevis* Are Capable to Form Mesodermal Derivatives, while the Ventral Animal Blastomeres Differentiate into Ciliated Epidermis Only

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ABSTRACT—Isolated two dorsal animal blastomeres of 8-cell-stage *Xenopus embryos* differentiated in about 24% of the cases into mesodermal structures, while the two ventral animal ones formed exclusively atypical epidermis. Of special interest is the fact that most of the dorsal animal blastomeres without mesodermal structures, differentiated into atypical epidermis with large parts of cement gland. Cement glands could not be detected in the derivatives of the ventral animal blastomeres. In earlier concepts it has been suggested that the animal hemisphere of the amphibian egg is an uncommitted area, which receives its instructions for further determination and differentiation from the vegetal part of the embryo. However, the results, shown in this paper, support the view that the developmental determinants are distributed in distinct gradients already in the early cleavage stages. Although the highest concentrations of these putative determinants are located in the dorsal vegetal area (Melton, 1995) and the dorsal marginal zone, lower concentrations of these substances are thought to be present in the animal part, especially in the dorsal animal hemisphere of the egg and the early embryo. Our results indicate that dorsal animal blastomeres of early cleavage stage embryos possess the capacity to form mesodermal derivatives after their separation from the vegetal hemisphere.

INTRODUCTION

Fate maps of the early embryo are very important for the study and understanding of the mechanisms at the cellular and molecular level during early embryonic development. Many informations are now available about the formation of the animal /vegetal and dorsal/ventral polarity during early embryogenesis resulting in the establishment of a rather complex body axis in the larval stage (Grunz, 1993a; Dawid and Taira, 1994; Lamaire and Gurdon, 1994). Different methods were used to study these mechanisms and processes, i.e. dissection, cell lineage techniques with different tracers or UV-irradiation, etc. (Ancel and Vintemberger, 1948; Nieuwkoop, 1969a,b; Gimlich and Gerhart, 1984; Gimlich, 1986). The classical vital dye staining method of Vogt (1929) could recently be extended by powerful microdisection and cell lineage techniques (Nakamura and Kishiyama, 1971; Hirose and Jacobson, 1979; Jacobson and Hirose, 1981; Dale and Slack, 1987; Moody, 1987; Moody and Kline, 1990; Keller, 1991). It could be shown that rRNA is synthesized in differnt rates in cells derived from the dorsal and ventral blastomeres of 4-cell stage-embryos (Shiokawa and Yamana, 1979). Circumstantial evidence suggested that determinants for endoderm and mesoderm are distributed in animal-vegetal and dorsal-ventral gradients (Nakamura *et al.*, 1971; Tiedemann, 1975; Nakamura, 1978; Grunz and Tacke, 1986; Takasaki, 1987; Tannahill and Melton, 1989; Gallagher *et al.*, 1991; Köster *et al.*, 1991; Hainsky and Moody, 1992; Tiedemann *et al.*, 1993; Grunz, 1992, 1993a, b; Rebagliati and Dawid, 1993; Dohrmann *et al.*, 1993).

In earlier papers it could be shown that the animal blastomeres of eight cell stage embryos will differentiate in high percentages into dorsal mesodermal structures (Grunz, 1977, 1994). The results indicate that the early amphibian embryo contains maternal factors which are distributed in form of gradients rather than in form of distinct centers. Although there is no doubt that certain factors are mainly located in the vegetal half of the egg, it cannot be excluded that the same factors are also present in low concentrations in the marginal zone and the animal half. Recently it could be shown that Vg1 biological active processed from the inactive precursor into the mature protein. The mature molecule is presumably located in a restricted area only, i.e. the dorsal vegetal zone (Thomsen and Melton, 1993; Melton, 1995). Until now it is not clear in detail how the gradients are formed, how certain genes are expressed in a specific spatial/temporal manner and how the complicated body axis develops from the relatively simple organized egg. Two main mechanisms are most important: 1. the presence of maternal factors including transcription factors and growth factors (determination factors, embryonic inducers) and 2. complicated cell-to-cell interactions including embryonic induction during cleavage, gastrulation and neurulation. There exists a distinct animal/vegetal polarity of the egg

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before insemination. After fertilization correlated with cortical rotation certain factors will be shifted preferentially into the dorsal zone opposite to the sperm entry point suggesting that mesodermal determinants will also be included in the animal part of the egg (Vincent and Gerhart, 1987; Fujisue *et al.*, 1993). By this event a dorsal/ventral polarity will be established in addition to the animal/vegetal polarity, in some species indicated by the so-called grey crescent. The animal cap of later stages (after midblastula transition) differentiates into ciliated (so called atypical epidermis) only. However, there exist observations that the dorsal ectoderm is more susceptible to inducing signals than the ventral ectoderm (Sokol and Melton, 1991). This suggests that the dorsal ectoderm contains factors, which are not present in the ventral ectoderm. Recent experiments with *Rana* indicate that there exist no strict boundaries between the presumptive germ layers ectoderm, mesoderm and endoderm in the 32-cell stage. In contrast there exist overlapping zones in the area of the animal hemisphere and the marginal zone (Saint-Jeannet and Dawid, 1994).

By our earlier papers (Grunz, 1977, 1994) it could be shown that the four animal blastomeres of the 8-cell-stage are able to differentiate into mesodermal structures. However, it was not clear if the dorsal or the ventral animal blastomeres contributed preferentially to these mesodermal structures. Therefore in this article we present data, which show that the dorsal animal blastomeres are more potent to differentiate into mesodermal structures than the ventral ones.

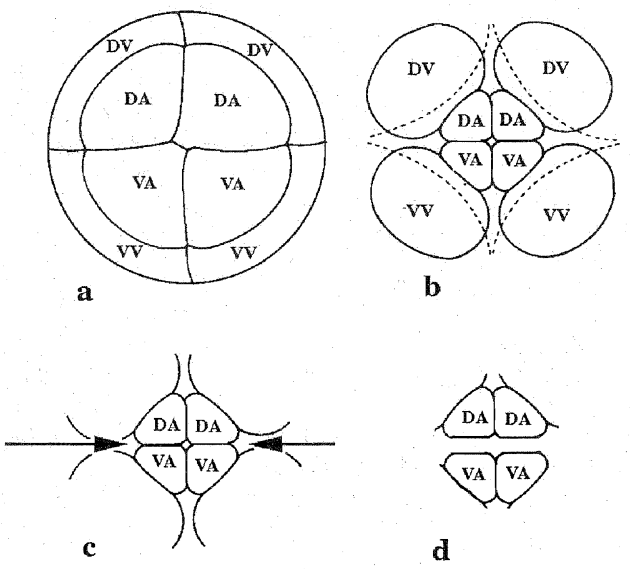

Fig. 1. Schematic demonstration of the isolation of the 2 dorsal animal and the two ventral animal blastomeres of the 8-cell-stage. After the removal of the vitelline membrane the embryo is turned up side down so that the 4 vegetal blastomeres can be disintegrated with glass needles with ball like melted tips, starting from the vegetal pole. They still remain in contact with the animal blastomeres. The result is shown in Fig.1b. Large parts of the vegetal blastomeres are cut away with glass needles (dotted line). In the next step (1c) the dorsal animal blastomeres (DA) were separated from the ventral animal blastomeres (VA). The two dorsal animal and two ventral animal blastomeres were transferred into a new agar-coated petri-dish for further culture up till 3 days at 20°C. a) view from the animal pole; b),c),d) view from the former vegetal pole. DA: dorsal vegetal blastomeres, VA: ventral animal blastomeres, DV: dorsal vegetal blastomeres, VV: ventral vegetal blastomeres.

MATERIALS AND METHODS

Embryos of *Xenopus laevis* were obtained by artificial insemination after injection of female frogs with 1000 IU human chorion gonatropin (HCG, Schering AG, Berlin). They were dejellied with 3.5% cysteinium chloride (pH 7.4) at the 8-cell stage, and carefully washed several times in Holtfreter solution. Strong variations in the cleavage pattern exist in different spawnings of *Xenopus*. The frequencies of "regular" cleavage (described in the textbooks) is sometimes very low. The results reported here are from those series of experiments in which the frequencies of the regular cleavage pattern was about 50% of the total number of embryos at 8-cell stage. Only embryos with stereotypic radial (regular) cleavage pattern were used for the experiments (see Grunz, 1977, 1994). The two-dorsal-animal-half (2DA) blastomeres of these embryos were easily distinguished from the two-ventral-animal-half (2VA) blastomeres by the smaller size and lighter pigmentation. These dejellied, "regular" embryos were transferred into 50% Leibovitz-Medium (L-15; pH 7.4) supplemented with 10% fetal calf serum (FCS) on petri dishes coated with 1% agar. In earlier reports it could be shown that FCS has no inducing capacity under our experimental conditions (Grunz, 1977, 1994). The vitelline membrane was manually removed with sharpened watch-makers forceps. First the four-animal blastomeres were isolated from the four-vegetal ones as described elsewhere (Grunz, 1977, 1994) and Figure 1a, b. Then the 2DA-blastomeres were separated from the 2VA-blastomeres (Fig. 1c,d) with fine glass needles with ball-like melted tips. These 2DA and 2VA-blastomeres were transferred into another agar-coated dish containing 50% L-15 medium (plus 10% FCS) and cultured at 20°C. The possible debris were discarded timely all along the incubation process. The L-15 was replaced stepwise by Holtfreter solution (pH 7.4) after 4 hours incubation when the blastomeres divided several times and formed round spheres. Explants were cultured for 3 days at 20°C prior to the fixation in Bouin's fixative, when normal embryos have reached stage 42-46 (Nieuwkoop and Faber, 1967). After gross staining in borax-carmine, the tissue was dehydrated and embedded in paraffin. Section (6 μm) were stained with aniline-blue-orange G.

RESULTS

During the first hours of culture, the 2 DA- and 2 VA-blastomeres divided quickly. After 3 days by macroscopic observation over 20% of the cases of the 2DA differed significantly from the 2VA explants. The 2DA explants had formed vesicles and protrusions, while all 2VA explants have differentiated into wrinkled atypical epidermis only.

Differentiation of the two ventral animal blastomeres (VA)

From a larger number (about 70) of isolated 2VA blastomeres 44 survived in good conditions for 3 days at 20°C. Most of them (93%) developed exclusively into atypical

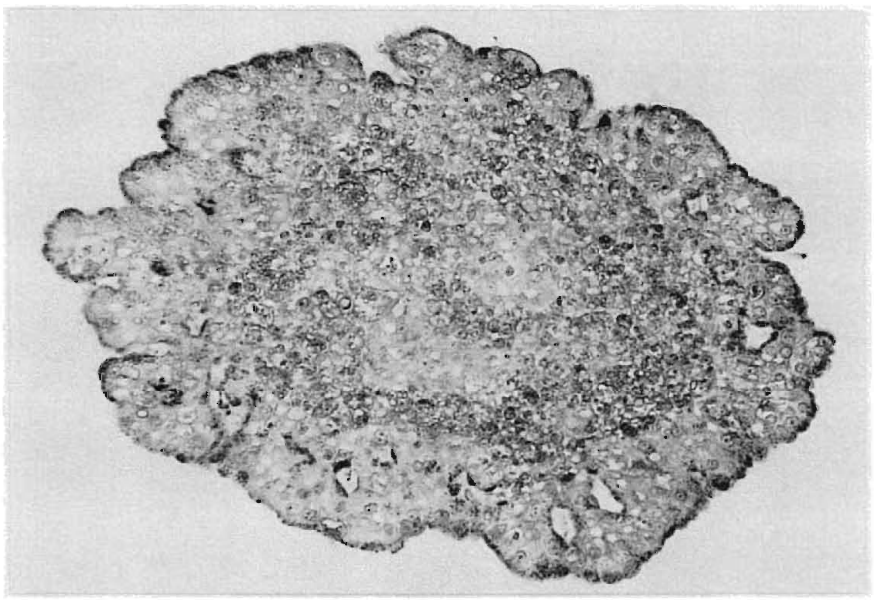

Fig. 2 . Atypical epidermis has differentiated from the isolated two ventral animal blastomeres.

Table 1. Differentiations of the two dorsal or two ventral animal blastomeres of eight-cell-stage embryos

Experimental conditions	Number of cases(n)	Atypical epidermis exclusively	Yolk-rich parts within the atypical epidermis	Epidermis +cement gland	Cement gland	Regular epidermis	Mesenchyme	Neural structures		Notochord	Somites	Coelomic epithelium
								brain	eye (tapetum)			
dorsal animal blastomeres	48	16 (33)	—	22(46)	30 (62)	8 (17)	8 (17)	5 (10)	3 (6)	5(10)	4 (8)	8 (17)
ventral animal blastomeres	44	41 (93)	2 (5)	—	—	—	1 (3)	—	—	—	—	1 (3)

percentages of the cases in parenthesis

epidermis (Fig. 2, Table 1). No dorsal mesoderm were found in the 2VA blastomeres derived explants. Only one case (3%) differentiated into ventral mesoderm (coelomic epithelium), mesenchyme and normal epidermis. In 5% the explants formed yolk-rich derivatives without any differentiations. Of special significance is the fact that in contrast to the DA-series no cement glands were found in the explants of this series.

Differentiation of the two dorsal animal blastomeres (DA)

Sixteen explants (33% of the cases) differentiated exclusively into atypical epidermis (Table 1). Twenty two (46%) differentiated into atypical epidermis with large cement glands. A typical representative is shown in Fig. 3a. In 17% of the cases the explants contained normal epidermis. Mesenchyme and coelomic epithelium (ventral mesoderm) was found in 17% of the cases. Four of these cases have also formed heart-like structures (Fig. 3b). Dorsal mesoderm (notochord) differentiated in 10%, muscle (somites) in 8% of the cases (Table 1, Fig. 4a). Neural tissue was found in 14% of the cases (eye [tapetum] 6%, brain 10%; Table 1, Fig. 4b). It must be pointed out that the cement glands were relatively large (Figs. 3a, 4a, b).

DISCUSSION

In this paper we could show that the two dorsal animal blastomeres of the 8-cell-stage differentiated in over 24% into mesodermal structures. On the other hand all explants, derivatives of the two ventral blastomeres, differentiated into atypical epidermis only. Traditionally the animal hemisphere of the amphibian egg has been considered as an uncommitted zone, which exclusively receives its signals for differentiation from the vegetal part of the egg. This view was supported by the fact that animal caps isolated from middle blastula or early gastrula stages differentiated into so called atypical (ciliated) epidermis only (Grunz *et al.*, 1975). Therefore the animal caps were used as an excellent test system for inducing factors, for example the vegetalizing factor, identical with activin (Grunz, 1983; Tiedemann, 1986; Knöchel *et al.*, 1987). However, it could be shown that in the blastula stage the animal cap is not an homogeneous entity (Sokol and Melton, 1991). The dorsal ectoderm reacts much more sensitive to inducing factors than the ventral ectoderm. In our earlier studies we could show that the animal blastomeres of the 8-cell-stage cannot be considered as an exclusively permissive part of the egg, receiving all its information from the vegetal part of the egg (Grunz, 1977,1994;

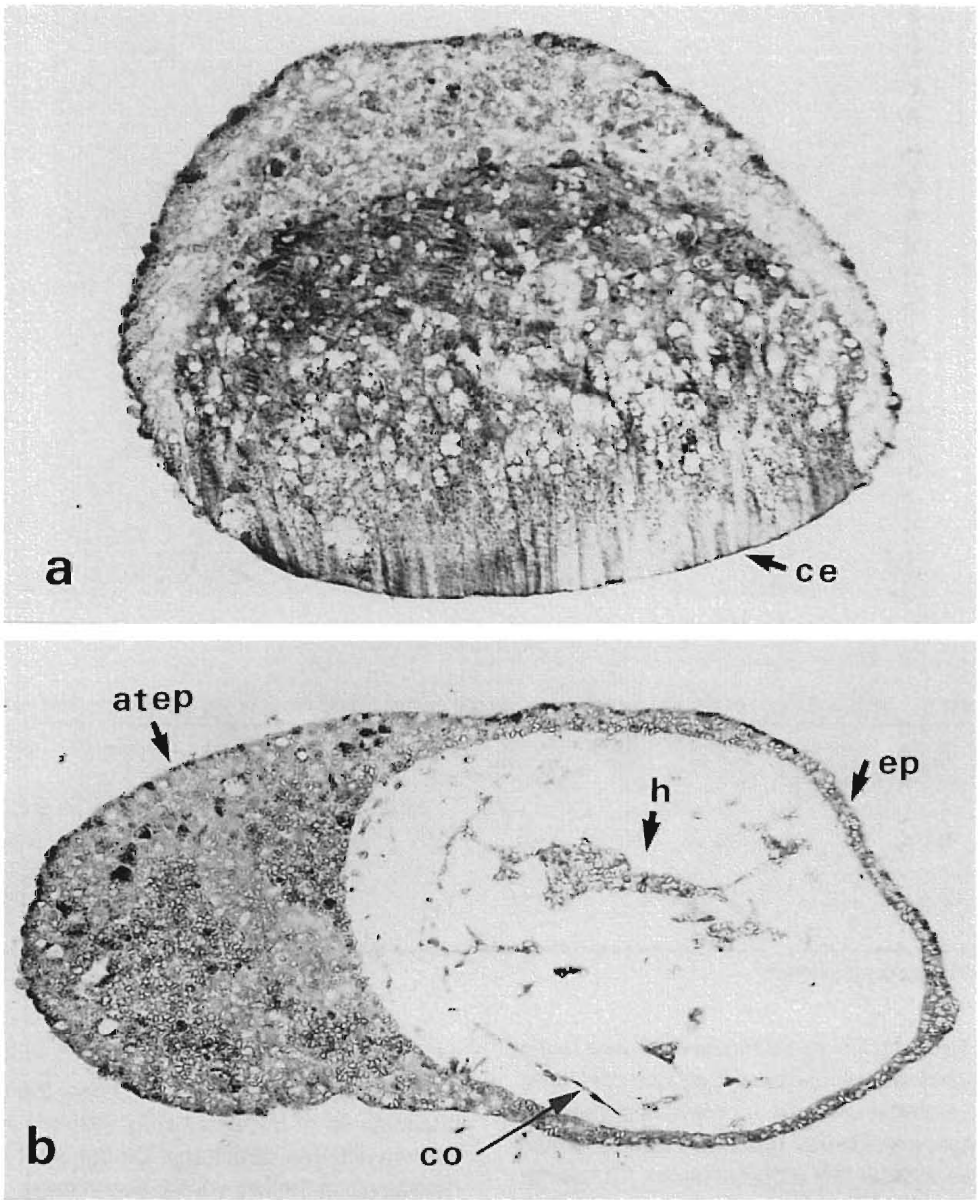

Fig. 3. a) Derivative of two isolated dorsal animal blastomeres of an eight-cell-stage embryo. The blastomeres have differentiated into atypical epidermis with a large cement gland (ce). b) Derivative of two isolated dorsal animal blastomeres of an eight-cell-stage embryo. The explant has differentiated into atypical epidermis (atep), epidermis (ep), heart-like structures (h), and coelomic epithelium (co).

Kageura and Yamana, 1983). In a large number of cases the four isolated animal blastomeres formed mesodermal structures instead of atypical epidermis. Recently it could be shown that in the 32-cell-stage of *Rana pipiens* there exist no deterministic boundaries between the blastomeres. Most of the blastomeres give rise to multiple tissues (Saint-Jeanett and Dawid, 1994). The data above show that at least the dorsal animal blastomeres are able to differentiate into mesodermal structures, although the whole animal hemisphere formerly has been considered as an uncommitted area, which will form atypical epidermis only without instructions from the marginal part of the egg. This indicates that dorsal blastomeres in contrast to their ventral counterparts contain after the third cleavage (8-cell-stage) components, which allow their differentiation into mesodermal structures. On the other hand Kinoshita *et al.* (1993) could show that single dorsal animal or single vegetal blastomeres do not autonomously differentiate into mesodermal structures. The absence of mesodermal structures (Kinoshita *et al.*, 1993) and the lower percentage of mesodermal derivatives than in the earlier papers (Grunz, 1977, 1994) can be explained by the smaller initial cell mass. In the earlier experiments (isolation and cultivation of all animal blastomeres of the 8-cell-stage) the two ventral animal blastomeres (VA) could support the mesodermal differentiation of the two dorsal animal blastomeres (DA). The community effect and the larger initial

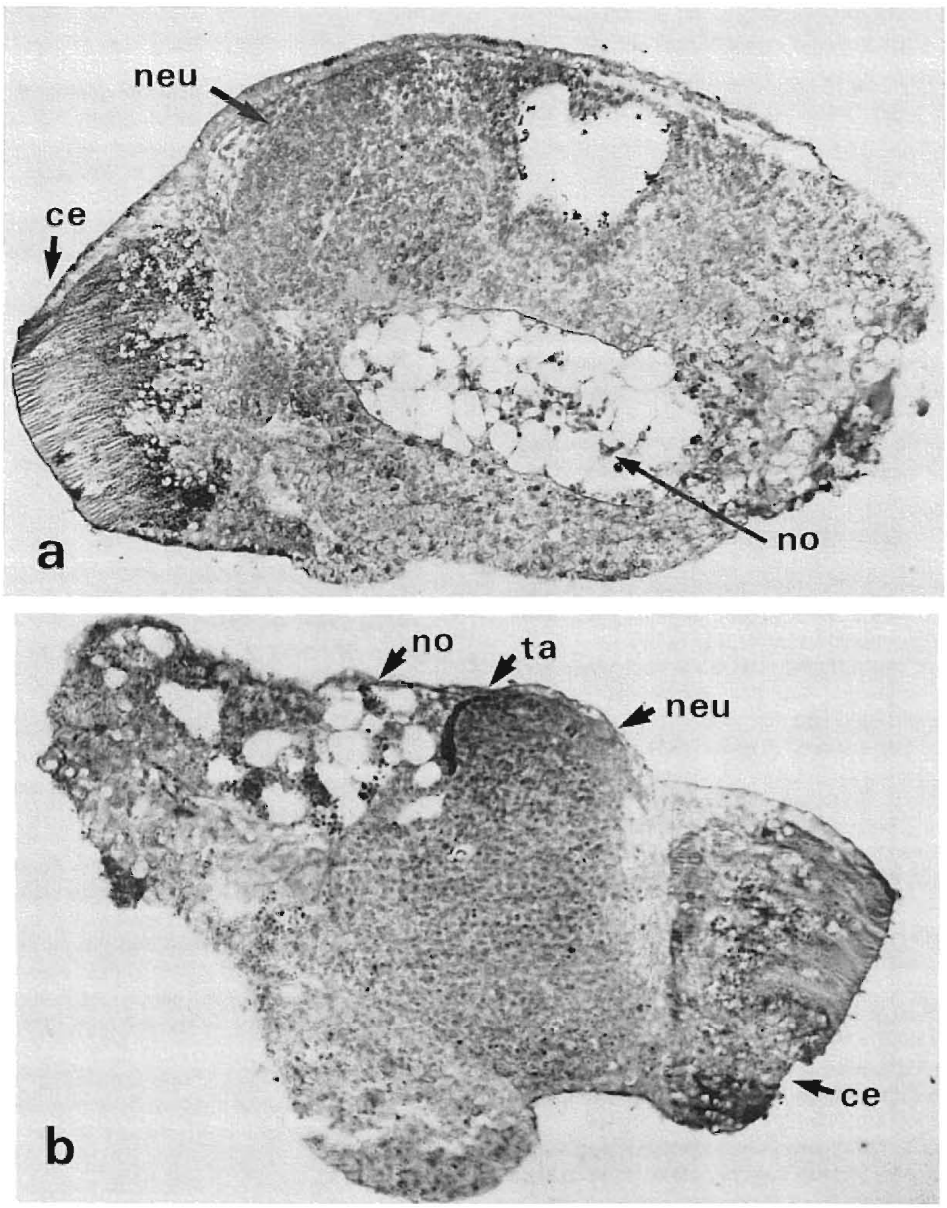

Fig. 4. a) Derivative of two isolated dorsal animal blastomeres of an eight-cell-stage embryo. The explant has differentiated into notochord (no), neural tissue (neu) and cement gland (ce). b) Derivative of two isolated dorsal animal blastomeres of an eight-cell-stage embryo. The explant has differentiated into notochord (no), neural tissue (neu) eye-like structure with tapetum (ta) and cement gland (ce).

cell mass could be favourable for the mesodermal differentiation (Grunz, 1979; Gurdon, 1988). In the two isolated VA-blastomeres alone or single DA- or DV-blastomeres the threshold concentrations are apparently too low to favour a mesodermal pathway of differentiation. This view is supported by the fact that all isolated two ventral animal blastomeres differentiated into atypical epidermis. Under *in vivo* conditions (normogenesis) mesodermalization of the animal part of the egg may be prevented by inhibitory molecules located in the ectoderm [presumptive epidermis and neural tissue] (Kato and Gurdon, 1994). Follistatin-like proteins could be good candidates for such mechanisms (Hemmati-Brivanlou *et al.*, 1994). After separation of the animal blastomeres from the rest of the embryo the activation of mesodermalizing factors may be enhanced in several cases resulting in the differentiation of mesodermal derivatives. On the other hand the dissociation of animal pole cells at blastula and gastrula stages causes the neuralization of the cells (Grunz and Tacke, 1989). The reason could be the dilution or elimination of mesodermalizing factors like BMPs from the single cells leading to the neuralization of the re-aggregated ectoderm material (Grunz and Tacke, 1990; Wilson and Hemmati-Brivanlou, 1995). These results indicate that mesodermalizing factors may be present in very low concentration or as unprocessed precursors, which prevent the ectoderm from its differentiation into neural tissue, the

postulated default state of ectoderm. In normogenesis neuralizing factors may act like competitive inhibitors by blocking BMP-receptors (Xu *et al.*, 1995) and signal transfer of BMP-like morphogens resulting in the shift of the presumptive epidermal state of ectoderm into the neural pathway of differentiation.

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