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Original Article

The four animal blastomeres of the eight-cell stage of *Xenopus laevis* are intrinsically capable of differentiating into dorsal mesodermal derivatives[1]

HORST GRUNZ*

Department of Zoophysiology, University GH Essen, Essen, Germany

ABSTRACT **Mesoderm formation in the amphibian embryo is thought to be induced in the ectoderm of the animal region by signals emanating from the endoderm of the vegetal region after cleavage up to the mid-blastula. During this process the dorsal vegetal zone is thought to stimulate the dorsal animal zone to establish the Spemann organizer, which will in turn trigger the overlaying neuroectoderm during gastrulation resulting in the development of the central nervous system. In this concept it is assumed that the animal hemisphere is an uncommitted area, which receives its instructions from the vegetal region of the embryo. However, the experiments of this paper show that the 4 animal blastomeres of the eight-cell stage will form dorsal mesodermal structures in over 50% of the cases. The results support the view that developmental determinants are distributed in distinct gradients already in the early cleavage stages and that in the embryo the mesoderm is determined by factors prelocalized in the marginal zone. The spatial and temporal activation of certain genes in a distinct pattern is not simply emanating from certain areas or centers, but is a result of complex interactions between the vegetal and animal hemisphere and vice versa.**

KEY WORDS: *mesoderm, gradients, developmental determinants, early cleavage stages, pattern formation, cell interactions*

Introduction

A central question in embryology is the establishment of the body plan during the early steps of normogenesis (Dawid, 1992; Tiedemann *et al.*, 1993; Grunz, 1993b). Two main mechanisms are responsible for the development from the oocyte to the early larvae, i.e. a specific prelocation of maternal factors in the cytoplasm of the egg and cellular interactions including embryonic induction processes. Spemann and Mangold (1924) transplanted the upper blastoporal lip of a *Triturus* gastrula into the ventral ectoderm of another gastrula. The transplant developed into muscle and notochord and induced a second neural system. Part of the somites and notochord were also induced in the host ectoderm, so that chimerical somites and notochord developed. The problem of at which stage the so called organizer is formed was briefly considered by Spemann (1936). In 1962 Nakamura discovered that the organizer region (the presumptive mesoderm of the marginal zone) did not differentiate into mesodermal tissue when isolated in very early developmental stages. Ôgi (1967, 1969) combined presumptive endoderm and presumptive ectoderm isolated from morula and blastula stages and obtained mesodermal tissues. The formation of mesodermal tissues after the combination of presumptive endoderm and ectoderm was investigated in detail by Nieuwkoop and collaborators (Nieuwkoop, 1969a,b, 1973) in the middle blastula stage and by Nakamura *et al.* (1971) in morula to blastula stages. Asashima (1975) found that the inducing activity for somites and notochord begins at the 64 cell stage. Our transfilter experiments (Grunz and Tacke, 1986) did show that the mesoderm inducing factors are diffusible.

From his experiments, Nieuwkoop concluded that mesoderm is induced in the presumptive ectoderm by the presumptive endoderm (Nieuwkoop, 1969a,b, 1973). In the embryo mesodermal tissues do not, however, arise in the presumptive ectoderm, but in the marginal zone located in between the presumptive endoderm and the presumptive ectoderm. Circumstantial evidence suggested that determinants for endoderm and mesoderm are distributed in an animal-vegetal gradient (Nakamura *et al.*, 1971; Tiedemann, 1975, 1978, 1990; Nakamura, 1978). The observation that all components required for the muscle-specific actin gene activation are already localized in the subequatorial region of uncleaved *Xenopus* eggs (Gurdon *et al.*, 1985) does not exclude the presence of mesoderm programming factors in the animal hemisphere. It

Abbreviations used in this paper: FCS, fetal calf serum; TGF, transforming growth factor; FGF, fibroblast growth factor; bFGF, basic FGF.

***Address for reprints**: Department of Zoophysiology, University GH Essen, 45117 Essen, Germany. FAX: 49-201.183.3173.

[1]*This article is dedicated to Prof. Dr. Dr. Heinz Tiedemann on the occasion of his 71st birthday*

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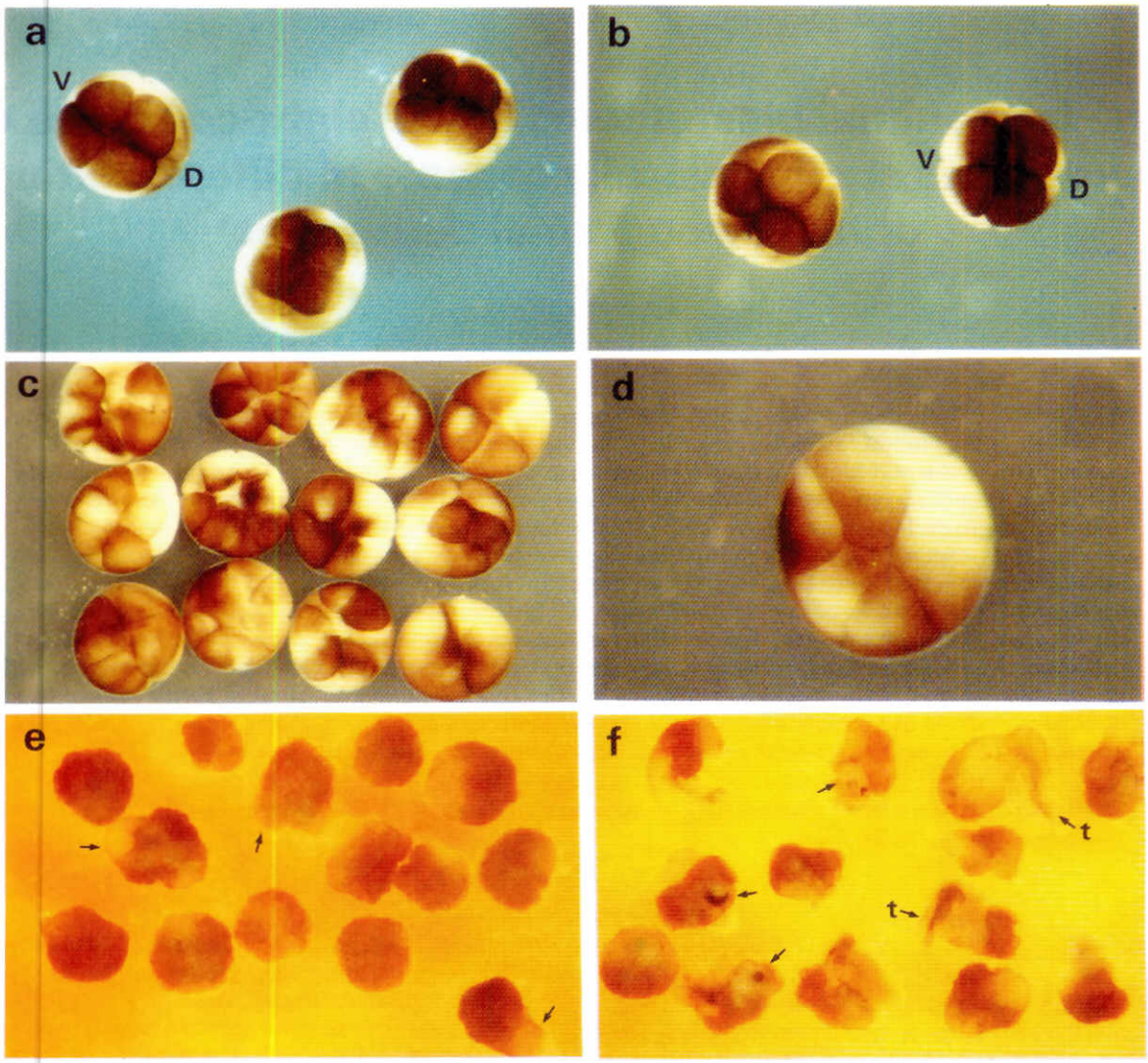

Fig. 1. Macroscopic views of early cleavage stages and derivatives of isolated 4 animal blastomeres of the eight-cell stage. (a) *8-cell stage embryos with stereotypic cleavage pattern. d, presumptive dorsal side, v, presumptive ventral side.* **(b)** *Two 8-cell stage embryos, one with "regular" cleavage pattern, the other with one blastomere reaching into the equatorial part of the embryo.* **(c)** *Embryos with irregular cleavage pattern. They do not form the typical 8-cell stage shown in many textbooks. Nevertheless they will continue to divide and develop into normal larvae.* **(d)** *One of the embryos with irregular cleavage pattern at higher magnification.* **(e)** *4 animal blastomeres isolated after a culture period of 3 days at 20° C. Most of them have developed into atypical epidermis (compare with Fig. 2a). Few contain vesicles in addition to the compact parts (arrows).* **(f)** *4 animal blastomeres isolated after a culture period of 3 days at 20°C. Macroscopic view of cases, which have differentiated to mesodermal and neural tissues. t, tail-like structures, eye fragments (arrows, compare with Fig. 2c,d).*

remained however an open question as to at which stage and in which embryonic regions gradients of inducing factors are established.

In an earlier paper we showed that the 4-animal blastomeres of an eight-cell stage of *Triturus alpestris* differentiate into atypical epidermis (Grunz, 1977). However, in 30% of the cases we also found notochord and somites. This result was unexpected and could depend on an irregular cleavage pattern. To see whether the differentiation of notochord and somites from the animal blastomeres can also be observed in other species we have now repeated these experiments on 8-cell stage embryos of *Xenopus laevis*. Also, in this species the eggs show strong variations in the cleavage pattern (see Fig. 1). However, we carefully selected only those 8-cell stages which showed a stereotypic radial cleavage for our studies. We rejected those 8-cell stages whose third cleavage furrow extended far toward the geometric equator. Also eggs with other cleavage irregularities were excluded.

Results

Differentiation of the four animal blastomeres

For the isolation of the 4 animal blastomeres only embryos with stereotypic radial cleavage were selected (Fig. 1a). It is important to mention that this type described in many normal tables, is not the

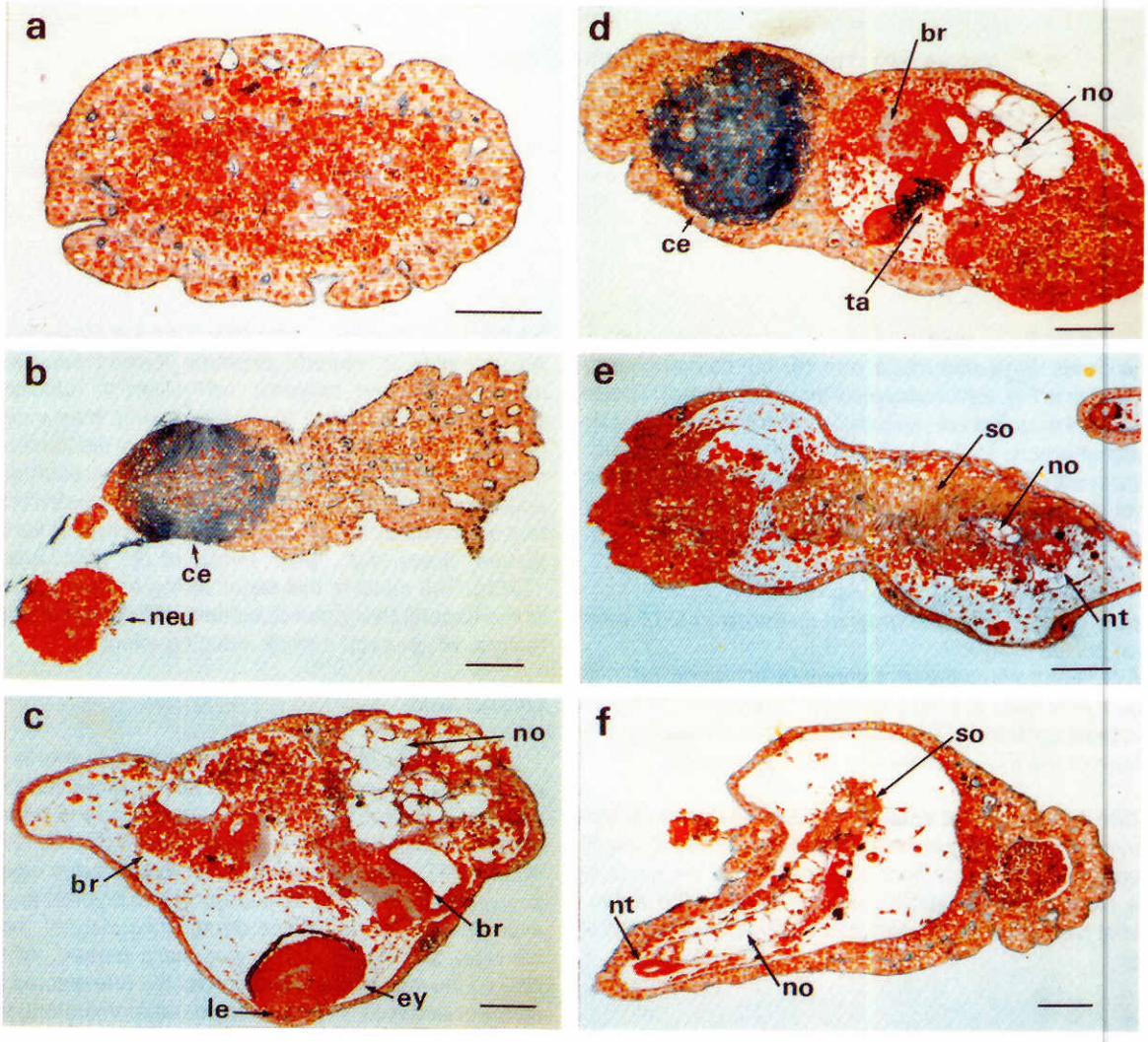

Fig. 2. Histological sections of 4 animal blastomeres isolated after a culture of 3 days at 20° C. (a) *4 animal blastomeres which have formed atypical epidermis.* **(b)** *4 animal blastomeres which have formed atypical epidermis with a cement gland (ce) and neural tissue (neu).* **(c)** *4 animal blastomeres which have differentiated into notochord (no), brain structures (br) and eye (ey) with lens(le).* **(d)** *4 animal blastomeres which have differentiated into notochord (no), brain structures (br), eye fragment with tapetum(ta) and large cement gland (ce).* **(e)** *4 animal blastomeres which have differentiated into large amounts of somites (so), small notochord (no) and neural tube (nt).* **(f)** *4 animal blastomeres which have differentiated into notochord (no), somites (so) and neural tube (nt). Bars, 0.1 mm.*

main and usual cleavage type. Strong variations in the cleavage pattern exist (Fig. 1b,c,d). However, even these embryos without regular radial cleavage develop to normal larvae. In the present experiments with *Xenopus* embryos only the "regular" type was carefully selected. In these radial symmetrically dividing embryos the border of the embryonic pigment reaches the geometric equator on the ventral side of the eight-cell stage. As in our earlier paper using *Triturus alpestris* embryos, the isolated animal blastomeres showed various degrees of differentiation.

In 49% of the cases, the 4 animal blastomeres differentiated exclusively into atypical epidermis (Figs. 1e, 2a). The so-called atypical epidermis is ciliated like typical epidermis(Grunz *et al.*, 1975). However, the cells do not show the linear arrangements typical linear arrangement for normal epidermis, which depends on underlying mesenchyme (Holtfreter, 1934, 1939). Notochord was found in 41% of the cases, somites in 38% and brain structures in 51%. All cases but one with notochord contained somites and all cases with notochord also contained mesenchyme, together with brain eye fragments, which were found in 9 cases (24% of total cases, see Table 1). Three types of differentiation patterns could be observed. The first one contained neural structures in addition to atypical epidermis only (Fig. 2b). The second differentiated into relatively large parts of notochord with small amounts of somites (Fig. 2c,d). The third type formed large amounts of somites accompanied by small quantities of notochord (Fig. 2e).

To exclude the possibility that the culture medium, especially the fetal calf serum, (FCS) may contain inducing activity, we isolated and cultivated the 4 animal blastomeres in 67% L-15 omitting the FCS. The results were comparable to the series performed with L-15 plus 10% FCS (cases with atypical epidermis

TABLE 1

DIFFERENTIATIONS* OF THE FOUR ANIMAL BLASTOMERES OF EIGHT-CELL STAGE

Experimental conditions	series	number of cases (n)	atypical epidermis	epidermis	mesenchyme	cement	neural structures gland	neural structures brain	eye neuroid	eye (tapetum)	ear lens	notochord vesicles	somites
L-15+10% FCS	1	37	100	41	41	95‡	51	—	24	8	8	41 (22‡,19#)	38 (19‡, 19#)
L-15 without FCS	2	13	100	54	54	92‡	38	8	38	15	8	31 (31‡)	54 (23‡, 31#)

*% of total cases; ‡ large; # small.

only 46%; notochord 31%, somites 54%, neural structures 46%). In 23% of the cases large amounts of somites but no notochords were found (similar to Fig. 2e,f, however without notochord). These cases represented a caudal tail-like pattern in contrast to 31% with a more anterior character (notochord, somites and forehead structures) (compare with Fig. 2c,d). Also in this series we found three main types of explants represented by Fig. 2b-f. Ear vesicles (a deuterencephalic structure) were found in only 8% of the cases in both series.

Differentiation of competent ectoderm cultured in L-15 plus FCS (control series 1)

In a control series we cultured competent ectoderm (animal caps of stage 8 embryos) in L-15 plus FCS. All explants differentiated into atypical epidermis (Table 2). This result shows that the culture medium or the FCS contains no inducing activity.

Differentiation of competent ectoderm placed into the debris of the removed 4 vegetal blastomeres (control series 2 and 3)

To exclude the possibility that the debris of the vegetal blastomeres exert mesodermalizing activity towards the neighbouring animal blastomeres we performed the following control experiments.

Series 2

Animal caps of stage 8 embryos were placed with the former blastocoelic side towards the yolk-rich leakage material of the 4 vegetal blastomeres, which was cut off from the 4 animal blastomeres. At the same time, when the animal blastomeres had rejected the last parts of the destroyed vegetal blastomeres, the animal caps were transferred into another culture vessel filled with Holtfreter solution. All explants developed into ciliated epidermis. In 22% of the cases the explants have formed small vesicles in addition to the atypical epidermis, which contained mesenchyme and coelomic epithelium (Table 2). However, dorsal mesodermal tissues (notochord and well differentiated somites) or neural structures were never observed.

Series 3

To exclude the possibility that the fetal calf serum may contain inducing activity we performed similar experiments as in series 2 omitting the fetal calf serum. The results were similar to those for series 2. However, vesicles with coelomic epithelium in addition to atypical epidermis were formed only in one case (4%, Table 2).

Differentiation of competent ectoderm placed into the debris of 4 damaged animal blastomeres (series 4)

Animal caps of stage 8 embryos were placed with the former blastocoelic side into the debris of the 4 destroyed animal blastomeres after their separation from the 4 vegetal blastomeres. As in series 2, several explants formed vesicles containing mesenchyme and coelomic epithelium in addition to atypical epidermis (29%, Table 2). These results may indicate that the debris of the vegetal as well as the animal blastomeres may exert a weak mesodermalizing effect on competent ectoderm. Coelomic epithelium (mesothel) and mesenchyme can be induced in competent ectoderm by low concentrations of activin or fibroblast growth factors (Slack *et al.*, 1987; Grunz *et al.*, 1988; Asashima *et al.*, 1990b). The result of this series indicates that not only the debris of the vegetal blastomeres contain diffusible mesoderm inducing factors, which exert a weak inducing effect.

Discussion

Here it is shown that the four animal blastomeres isolated from eight-cell-embryos develop into dorsal mesodermal and neural structures in over 50%. These data coincide with those of earlier experiments (Grunz, 1977; Kageura and Yamana, 1983; Gallagher *et al.*, 1991). The results received with *Triturus alpestris* in 1977 showed that 70% differentiated only into atypical epidermis. However, in 30% of the cases dorsal mesodermal (notochord and somites) and neural derivatives were formed. At that time we offered the following explanation for the unexpected results: since different embryos showed considerable variations in their cleavage mode, we speculated that minor amounts of the presumptive mesodermal area (marginal zone) were included in those animal blastomeres, partially reaching into the zone of the egg equator. Also, since in these experiments 8-cell stages with stereotypic radial cleavage with the 3rd-furrow well over the equator were selected I now think that the interpretation was too cautious and missed the main points. The experiments strongly suggest that a gradient of mesodermal determinants, which already exists in very early cleavage stages, reaches into the animal half. This coincides with the fate map of a 32 cell *Xenopus* embryo (Nakamura and Kishiyama, 1971). From this fate map it can be inferred that the dorsal animal blastomeres of the eight cell stage provide progeny to part of the notochord and a small part of the somites. This view is supported by the results of other authors (Dale and Slack, 1987; Moody, 1987; Gallagher *et al.*, 1991; Hainski and Moody, 1992). That only about 50% of the 4 animal blastomeres differentiate to notochord and somites could be due to the fact that the position of the radial superequatorial furrow which separates the 4 animal from the 4 vegetal blastomeres varies (even if embryos of stereotypic radial cleavage are selected), and that more or less determinants at the animal end of the gradient would be included into the animal blastomeres. Regulatory events which implicate factors which counteract differentiation of dorsal mesoderm (see below) could perhaps suppress differentiation, if the amount of mesoder-

mal determinants is too low. In some cases during the first day of culture small parts of the animal blastomeres were rejected during further cleavage. The loss of mesodermal determinants (quantitative and qualitative) may cause the differentiation of atypical epidermis. Ventral mesodermal tissues were not found as differentiation products of the 4 animal blastomeres. But this was not expected. According to the fate map of Nakamura and Kishiyama (1971) the 4 animal blastomeres of eggs with a regular cleavage pattern do not include the future ventral mesoderm (i.e. the ventral marginal zone).

Meanwhile, similar isolation experiments performed with *Xenopus laevis* by Kageura and Yamana (1983) showed that 53 % of their animal explants elongated. Although they did not perform histological analysis, these explants probably contained mesodermal tissues. The latter experiments and the results in this paper confirm our earlier data and indicate that the formation of mesodermal and neural derivatives is not the result of an erroneous preparation or selection of eggs with irregular cleavage symmetry. In the prevailing opinion an active center (so-called Nieuwkoop center) in the endoderm of the vegetal half will induce the ectodermal half to form mesoderm after Harrison stage 7 (morula) (Nieuwkoop, 1969a). The dorsal mesoderm (the Spemann organizer) then induces the overlaying neuroectoderm to form the central nervous system during gastrulation. There are indications that the final dorsalization of Spemann's organizer takes place during the early steps of gastrulation (Grunz, 1993a). From the experiments presented in this paper it can be concluded that the animal hemisphere is not only the permissive part, which receives all its instructions from the presumptive endoderm of the vegetal hemisphere, but also that in the animal blastomeres mesodermal determinants are present in the 8-cell stage.

However, another possibility has to be considered. The factors which determine mesoderm (see below) very likely act after binding to receptors on plasma membranes. A factor secreted from the vegetal blastomeres could be bound to receptors on the adjacent plasma membranes of the animal blastomeres. Since after egg rotation no general mixing of the cytoplasm occurs due to the stable gradient of yolk platelets, this would mean that mesoderm determinants are located in the marginal zone adjacent to the animal blastomeres and not only in an inducing center in the presumptive endoderm. But recent experiments of Fujisue *et al.* (1993) further support the view that mesoderm determinants are actually located in the animal cytoplasm even at very early stages. They have shown that cytoplasm taken from the vegetal pole of the egg before cortical rotation (which specifies the future dorsal axis, Ancel and Vintemberger, 1948; Vincent and Gerhart, 1987), induces a second axis after injection into ventral vegetal blastomeres of another embryo. After cortical rotation this activity diminishes and mesoderm inducing activity appears in the dorsal equatorial and to a lesser extent in the supraequatorial dorsal cytoplasm. After UV-irradiation, which blocks cortical rotation (Vincent and Gerhart, 1987) inducing activity does not appear in the equatorial and supraequatorial cytoplasm. The authors suggest that the inducing activity is transferred towards the marginal zone by the cortical rotation. The change of the inducing activity of the different cytoplasmic regions after cortical rotation could, however, also depend on a different distribution of molecules which form non-biologically active complexes with mesodermal determinants (proteins or glycoconjugates, Tiedemann *et al.*, 1961; Born *et al.*, 1987) or of factors which counteract the activity of dorsal mesodermalizing factors (BMP-4, Köster *et al.*, 1991 and Jones *et al.*, 1992; Xwnt-8, Christian and Moon, 1993). The results of Fujisue *et al.* (1993) correspond with the results reported in this communication and show that mesodermal determinants are located very early in animal cytoplasm. The notion that vegetal-animal gradients of mesodermal determinants are established early is also supported by cell transplantation and explantation experiments in the 16-64 cell stage (Gimlich and Gerhart, 1984; Gimlich, 1986; Gallagher *et al.*, 1991).

The best candidates for mesodermal determinants belong to the TGF-ß superfamily of growth factors (Knöchel *et al.*, 1987; Rosa *et al.*, 1988; Dawid *et al.*, 1990). Depending on their concentration

TABLE 2

DIFFERENTIATION* OF ANIMAL CAPS (STAGE 8)

Experimental conditions	series	number of cases (n)	atypical epidermis	epidermis	mesenchyme	cement gland	neural structures brain	neural structures neuroid	eye (tapetum)	lens	ear vesicles	notochord	coelomic epithelium
L-15+10% FCS	1	24	100	—	—	25 #	—	—	—	—	—	—	—
L-15+ 10% FCS, explants in contact with damaged vegetal blastomeres of the 8-cell embryo	2	18	100	28	28	33 #	—	—	—	—	—	—	22
L-15 (67 %), explants in contact with damaged vegetal blastomeres of the 8-cell embryo	3	26	100	4	4	4#	—	—	—	—	—	—	4
L-15 (67 %), explants in contact with damaged animal blastomeres of the 8-cell embryo	4	22	100	33	33	—	—	—	—	—	—	—	29

* % of total cases; #small

(Grunz, 1983) activin A (Asashima *et al.*, 1990a, 1991; Chertov *et al.*, 1990; Jones *et al.*, 1993), activin B (Nakamura *et al.*, 1992), as well the XTC-factor (Smith *et al.*, 1988; Grunz *et al.*, 1989) and the vegetalizing factor (Geithe *et al.*, 1981), which are activin homologues (Asashima *et al.*, 1990b; Smith *et al.*, 1990; Tiedemann *et al.*, 1992), induce endoderm and different mesodermal organs. Activins (or closely related proteins or their precursors) have been found in *Xenopus* oocytes and early embryos (Asashima *et al.*, 1991; Dohrmann *et al.*, 1993; Rebagliati and Dawid, 1993). Vg1, a protein preferentially found in the vegetal half (Melton, 1987; Weeks and Melton, 1987; Tannahill and Melton, 1989) has as a full length protein with no mesoderm inducing activity. The carboxyl end of this protein (110 terminal amino acids) has a high sequence homology to activin. However, it is not or only slightly processed to a mesoderm inducing dimer (Dale *et al.*, 1993; Thomsen and Melton, 1993). Other factors such as FGF (Knöchel *et al.*, 1987; Slack *et al.*, 1987; Grunz *et al.*, 1988; Isaacs *et al.*, 1992); noggin (Smith *et al.*, 1993) and Wnt proteins (McMahon and Moon, 1989; Christian and Moon, 1993) are needed for the formation of different types (dorsal or ventral) of mesodermal tissues. Receptors for b-FGF have been found mainly in the animal hemisphere and the dorsal marginal zone (Ding *et al.*, 1992; Amaya *et al.*, 1993). Recently it has been discovered that in addition to factors prelocated in the marginal zone, factor(s) from the presumptive ectoderm are needed for the differentiation of mesodermal organs (Tiedemann, 1993). This further supports the view that the mesodermal marginal zone is different from the presumptive ectoderm from the beginning of development.

At least some of these factors in the active form and/or their receptors are likely to be distributed in gradients (or, more correctly, form a graded distribution, because of the cell borders). How the gradients are formed is an unanswered question. As suggested by Crick (1970), diffusion is the most plausible mechanism for the establishment of gradients. Transfilter experiments have shown that secreted mesoderm determining factors are diffusible (Grunz and Tacke, 1986). Furthermore there is substantial evidence that cell interactions and cell communication are essential for mesoderm differentiation (Minuth and Grunz, 1980; Sargent *et al.*, 1986). However, additional mechanisms for the distribution of the factors have to be considered. Intracellular factors can be (loosely) associated with membranes of the endoplasmic reticulum (Tannahill and Melton, 1989) and perhaps also cytoskeletal proteins, so that they may be redistributed during cell cleavage.

The results presented above demonstrate that by the 8-cell stage, the factors are distributed in such a way that 4 animal blastomeres are intrinsically capable carrying out a developmental program, which in normogenesis is typical for the dorsal marginal zone and the presumptive ectoderm.

Materials and Methods

Embryos were obtained by artificial insemination. To prevent any distortion during the first two cleavage stages 8-cell-embryos with regular and symmetric cleavage pattern were dejellied immediately after the selection with 3% cysteinium chloride. They were carefully rinsed in Holtfreter solution and transferred into Leibovitz-medium with or without 10% fetal calf serum on petri dishes that had been coated with 1% agar. The vitelline membrane was removed with sharpened watch-makers forceps. The further preparation was performed as described elsewhere (Grunz, 1977). The 8-cell stage was turned upside down and the vegetal blastomeres were disintegrated with fine glass needles with ball-like melted tips starting from the point of intersection of the 2 vertical cleavages at the vegetal pole. Large parts of the vegetal blastomeres were cut off with sharp tipped glass needles. The animal blastomeres rejected the remaining fragments of the vegetal blastomeres within 20-30 minutes. The yolk-rich debris was sucked off and the animal blastomeres were carefully transported with a Spemann pipette to a debris-free area of the agar coated vessel. Next, the culture medium was replaced by fresh L-15 (50%+10% fetal calf serum) to exclude inducing effects originating from the debris. To exclude the possibility that fetal calf serum in the medium contains inducing activity, FCS was omitted in one series. In this case the concentration of L-15 to 67% was raised to compensate the reduction of osmolarity (50% L-15+10% FCS: 170 mOsm; 67 % L-15 without FCS: 190 mOsm). The L-15 was replaced stepwise by Holtfreter solution after 4 hours, when the animal blastomeres had divided several times and had formed round spheres. In several cases we observed that up to the next day small parts of the animal blastomeres were rejected. In another series we cultivated competent ectoderm (animal caps) of stage 8 (Nieuwkoop and Faber, 1967) in 50% L-15 with 10% FCS for 24 hours to prove that FCS has no inducing activity. In a further control series we placed competent ectoderm in the leakage material of the destroyed vegetal blastomeres of the eight-cell stage embryo for 30 minutes. Explants and fragments of all series were cultured for 3 days at 20°C prior to the fixation in Bouin's solution. After gross staining in borax-carmine, the tissue was dehydrated and embedded in paraffin. Sections (6 µm) were stained with aniline-blue-orange G.

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References

AMAYA, E., STEIN, P.A., MUSCI, T.J. and KIRSCHNER, M.W. (1993). FGF signalling in the early specification of mesoderm in *Xenopus*. *Development 118:* 477-487.

ANCEL, P. and VINTEMBERGER, P. (1948). Recherches sur le déterminisme de la symétrie bilaterale dans l' oeuf des amphibians. *Bull. Biol. Fr. Belg. 31 (Suppl.):* 1-182.

ASASHIMA, M. (1975). Inducing effects of the presumptive endoderm of successive stages in *Triturus alpestris*. *Roux Arch. Dev. Biol. 177:* 301-308.

ASASHIMA, M., NAKANO, H., SHIMADA, K., KINOSHITA, K., ISCHII, K., SHIBAI, H. and UENO, N. (1990a). Mesodermal induction in early amphibian embryos by activin A (erythroid differentiation factor). *Roux Arch. Dev. Biol. 198:* 330-335.

ASASHIMA, M., NAKANO, H., UCHIYAMA, H., DAVIDS, M., PLESSOW, S., LOPPNOW-BLINDE, B., HOPPE, P., DAU, H. and TIEDEMANN, H. (1990b). The vegetalizing factor belongs to a family of mesoderm inducing proteins related to erythroid differentiation factor. *Naturwissenschaften 77:* 389-391.

ASASHIMA, M., NAKANO, H., UCHIYAMA, H., SUGINO, H., NAKAMURA, T., ETOS, Y., EJIMA, D., NSIHIMATSU, S.I., UENO, N. and KINOSHITA, K. (1991). Presence of activin (erythroid differentiation factor) in unfertilized eggs and blastulae of *Xenopus laevis*. *Proc. Natl. Acad. Sci. USA 88:* 6511-6514.

BORN, J., DAVID, M. and TIEDEMANN, H. (1987). Affinity chromatography of embryonic inducing factors on heparin-sepharose. *Cell Differ. 21:* 131-136.

CHERTOV, O.Y., KRASNOSEL'SKII, A.L., BOGDANOV, M.E. and HOPERSKAYA, O.A. (1990). Mesoderm-inducing factor from bovine amniotic fluid: purification and N-terminal amino acid sequence determination. *Biomed. Sci. 1:* 499-506.

CHRISTIAN, J.L. and MOON, R.T. (1993). Interaction between Xwnt-8 and Spemann organizer signalling pathways generate dorsoventral pattern in the embryonic mesoderm of *Xenopus*. *Genes Dev. 7:* 13-28.

CRICK, F. (1970). Diffusion in embryogenesis. *Nature 225:* 420-422.

DALE, L. and SLACK, J.M.W. (1987). Fate map for the 32-cell stage of *Xenopus laevis*. *Development 99:* 527-551.

DALE, L., MATTHEWS, G. and COLMAN, A. (1993). Secretion and mesoderm inducing activity of the TGF-ß related domain of *Xenopus* Vg1. *EMBO J. 12:* 4471-4480.

DAWID, I.B. (1992). Mesoderm induction and axis formation in *Xenopus laevis*. *BioEssays 10:* 687- 691.

DAWID, I.B., SARGENT, T.D. and ROSA, F. (1990). The role of growth factors in embryonic induction in amphibians. *Curr. Top. Dev. Biol. 24:* 261-288.

DING, X-Y., MCKEEHAN, W.L., XU, J. and GRUNZ, H. (1992). Spatial and temporal localization of FGF receptors in *Xenopus laevis*. *Roux Arch. Dev. Biol. 201:* 334-339.

DOHRMANN, C.D., HEMMATI-BRIVANLOU, A., THOMSEN, G.H., FIELDS, A., WOOLF, T.M. and MELTON, D. (1993). Expression of activin mRNA during early development of *Xenopus laevis*. *Dev.Biol. 157:* 474-483.

FUJISUE, M., KOBAYAKAWA, Y. and YAMANA, K. (1993). Occurrence of dorsal axis-inducing activity around the vegetal pole of an uncleaved *Xenopus* egg and displacement to the equatorial region by cortical rotation. *Development 118:* 163-170.

GALLAGHER, B.C., HAINSKI, A.M. and MOODY, S.A. (1991). Autonomous differentiation of dorsal axial structures from an animal cap cleavage stage blastomere in *Xenopus*. *Development 112:* 1103-1114.

GEITHE, H-P., ASASHIMA, M., ASAHI, K-H., BORN, J., TIEDEMANN, H. and TIEDEMANN, H. (1981). A vegetalizing inducing factor. Isolation and chemical properties. *Biochim. Biophys. Acta 676:* 350-366.

GIMLICH, R.L. (1986). Acquisition of developmental autonomy in the equatorial region of the *Xenopus* embryo. *Dev. Biol. 115:* 340-352.

GIMLICH, R.L. and GERHART, J.C. (1984). Early cellular interactions promote embryonic axis formation in *Xenopus laevis*. *Dev. Biol. 104:* 117-130.

GRUNZ, H. (1977). The differentiation of the four animal and the four vegetal blastomeres of the eight-cell stage of *Triturus alpestris*. *Roux Arch. Dev. Biol. 181:* 267-277.

GRUNZ, H. (1983). Change in the differentiation pattern of *Xenopus laevis* ectoderm by variation of the incubation time and concentration of vegetalizing factor. *Roux Arch. Dev. Biol. 192:* 130-137.

GRUNZ, H. (1993a). The dorsalization of Spemann's organizer takes place during gastrulation in *Xenopus laevis* embryos. *Dev. Growth Differ. 35:* 21-28.

GRUNZ, H. (1993b). Factors controlling the determination of the body plan in the amphibian embryo. *Acta Biol. Exp. Sinica 26:* 317-341.

GRUNZ, H. and TACKE, L. (1986). The inducing capacity of the presumptive endoderm of *Xenopus laevis* studied by transfilter experiments. *Roux Arch. Dev. Biol. 195:* 467-473.

GRUNZ, H., BORN, J., DAVIDS, M., HOPPE, P., LOPPNOW-BLINDE, B., TACKE, L., TIEDEMANN, H. and TIEDEMANN, H. (1989). A mesoderm-inducing factor from a *Xenopus laevis* cell line: chemical properties and relation to the vegetalizing factor from chicken embryos. *Roux Arch. Dev. Biol. 198:* 8-13.

GRUNZ, H., McKEEHAN, W.L., KNÖCHEL, W., BORN, J., TIEDEMANN, H. and TIEDEMANN, H. (1988). Induction of mesodermal tissue by acidic and basic heparin binding growth factors. *Cell Differ. 22:* 183-190.

GRUNZ, H., MULTIER, A.M., HERBST, R. and ARKENBERG, G. (1975). The differentiation of isolated amphibian ectoderm with or without treatment of an inductor. A scanning electron microscope study. *Roux Arch. Dev. Biol. 178:* 277-284.

GURDON, J.B., MOHUN, T.J., FAIRMAN, S. and BRENNAN, S. (1985). All components required for the eventual activation of muscle-specific actin genes are localized in the subequatorial region of an uncleaved amphibian egg. *Proc. Natl. Acad. Sci. USA 82:* 139-143.

HAINSKI, A.M. and MOODY, S.A. (1992). *Xenopus* maternal RNAs from a dorsal animal blastomere induce a secondary axis in host embryos. *Development 116:* 347.

HOLTFRETER, J. (1934). Formative Reize in der Embryonalentwicklung der Amphibien, dargestellt an Explantationsversuchen. *Arch. Exp. Zellforsch. 15:* 281-301.

HOLTFRETER, J. (1939). Studien zur Ermittlung der Gestaltungsfaktoren in der Organentwicklung der Amphibien. *Roux Arch. Dev. Biol. 139:* 227-273.

ISAACS, H.V., TANNAHILL, D. and SLACK, J.M.W. (1992). Expression of a novel FGF in the *Xenopus* embryo — a new candidate inducing factor for mesoderm formation and anteroposterior specification. *Development 114:* 711-720.

JONES, E.A., ABEL, M.H. and WOODLAND, H.R. (1993). The possible role of mesodermal growth factors in the formation of endoderm in *Xenopus laevis*. *Roux Arch. Dev. Biol. 202:* 233-239.

JONES, M.C., LYONS, K.M., LAPAN, P.M., WRIGHT, C.V.E and HOGAN, A.L.M. (1992). DVR-4 (Bone morphogenetic protein 4) as a posterior-ventralizing factor in *Xenopus* mesoderm induction. *Development 115:* 639-647.

KAGEURA, H. and YAMANA, K. (1983). Pattern regulation in isolated halves and blastomeres of early *Xenopus laevis*. *J. Embryol. Exp. Morphol. 74:* 221-234.

KNÖCHEL. W, BORN, J., HOPPE, P., LOPPNOW-BLINDE, B., TIEDEMANN, H., TIEDEMANN, H., MCKEEHAN, W. and GRUNZ, H. (1987). Mesoderm inducing factors: their possible relationship to heparin binding growth factors and transforming growth factor-ß. *Naturwissenschaften 74:* 604-606.

KÖSTER, M., PLESSOW, S., CLEMENT J.H., LORENZ, A., TIEDEMANN, H. and KNÖCHEL, W. (1991). Bone morphogenetic protein 4 (BMP-4), a member of the TGF-ß family, in early embryos of *Xenopus laevis:* analysis of mesoderm inducing activity. *Mech. Dev. 44:* 191-200.

McMAHON, A.P. and MOON, R.T. (1989). Expression of the protooncogene int-1 in *Xenopus* embryos leads to duplication of the embryonic axis. *Cell 58:* 1075-1084.

MELTON, D.A. (1987). Translocation of a localized maternal mRNA to the vegetal pole of *Xenopus* oocyte. *Nature 328:* 80-82.

MINUTH, M. and GRUNZ, H. (1980). The formation of mesodermal derivates after induction with vegetalizing factor depends on secondary cell interactions. *Cell Differ. 3:* 229-238.

MOODY, S.A. (1987). Fates of the blastomeres of the 32-cell stage *Xenopus* embryo. *Dev. Biol. 122:* 300-319.

NAKAMURA, O. (1962). The period of acquisition of self-differentiation of the presumptive mesoderm region. *Jpn. J. Exp. Morph. 15:* 61-62.

NAKAMURA, O. (1978). Epigenetic formation of the organizer. In *Organizer — A Milestone of a Half-Century from Spemann* (Eds. O. Nakamura and S. Toivonen). Elsevier/North-Holland, Amsterdam, pp. 179-218.

NAKAMURA, O. and KISHIYAMA, K. (1971). Prospective fates of blastomeres at the 32 cell stage of *Xenopus laevis* embryos. *Proc. Jnp. Acad. 47:* 407-412.

NAKAMURA, O., TAKASAKI, H. and ISHIHARA, M. (1971). I. Formation of the organizer from combinations of presumptive ectoderm and endoderm. *Proc. Jpn. Acad. 47:* 313-318.

NAKAMURA, T., ASASHIMA, M., ETO, Y., TAKIO, K., UCHIYAMA, H., MORIYA, N., ARIIZUMI, T., YASHIRO, T., SUGINO, K., TITANI, K. and SUGINO, H. (1992). Isolation and characterization of native activin-B. *J. Biol. Chem. 267:* 16385-16389.

NIEUWKOOP, P.D. (1969a). The formation of the mesoderm in urodelean amphibians. I. Induction by the endoderm. *Roux Arch. Dev. Biol. 162:* 341-373.

NIEUWKOOP, P.D. (1969b). The formation of the mesoderm in urodelean amphibians. II. The origin of the dorso-ventral polarity of the mesoderm. *Roux Arch. Dev. Biol. 163:* 298-315.

NIEUWKOOP, P.D. (1973). The "organization center" of the amphibian embryo: its origin, spatial organization, and morphogenetic action. *Adv. Morphogen. 10:* 1-39.

NIEUWKOOP, P.D. and FABER, J. (1967). *Normal Table of Xenopus laevis (Daudin).* North Holland, Amsterdam.

ÔGI, K. (1967). Determination in the development of the amphibian embryo. *Sci. Rep. Res. Inst. Tohoku Univ. (Biol.) 33:* 239-247.

ÔGI, K. (1969). Regulation capacity in the early amphibian embryo. *Res. Bull. Gen. Educ. Nagoya Univ. 13:* 31-40.

REBAGLIATI, M.R. and DAWID, I.B. (1993). Expression of activin transcripts in follicle cells and oocytes of *Xenopus-laevis*. *Dev. Biol. 159:* 574-580.

ROSA, F., ROBERTS, A.B., DANIELPOUR, L.L., SPORN, M.B. and DAWID, I. (1988). Mesoderm induction in amphibians: the role of TGF-ß2 like factors. *Science 239:* 783-785.

SARGENT, T.D., JAMRICH, M. and DAWID, I.B. (1986). Cell interactions and the control of gene activity during early development of *Xenopus laevis*. *Dev. Biol. 114:* 238-246.

SLACK, J.M.W., DARLINGTON, B.G., HEATH, J.K. and GODSAVE, S.F. (1987). Mesoderm induction in early *Xenopus* embryos by heparin-binding growth factors. *Nature 362:* 197-200.

SMITH, J.C., PRICE, B.M.J., VAN HIMMEN , K. and HUYLEBROECK, D. (1990). Identification of a potent *Xenopus* mesoderm inducing factor as a homologue of activin A. *Nature 345:* 729-731.

SMITH, J.C., YAQOOB, M. and SYMES, K. (1988). Purification, partial characterization and biological effects of the XTC mesoderm-inducing factor. *Development 103:* 591-600.

SMITH, W.C., KNECHT, A.K., WU, M. and HARLAND, R.M. (1993). Secreted noggin mimics the Spemann organizer in dorsalizing *Xenopus* mesoderm. *Nature 361:* 547-549.

SPEMANN, H. (1936). *Experimentelle Beiträge zu einem Thema der Entwicklung.* Springer, Berlin.

SPEMANN, H. and MANGOLD, H. (1924). Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren. *Roux Arch. EntwMech. Org. 100:* 599-638.

TANNAHILL, D. and MELTON, D.A. (1989). Localized synthesis of the Vg1 protein during early *Xenopus* development. *Development 106:* 775-785.

THOMSEN, G.H. and MELTON, D.A. (1993). Processed Vg1 protein is an axial mesoderm inducer in *Xenopus*. *Cell 74:* 433-441.

TIEDEMANN, H. (1975). Substances with morphogenetic activity in differentiation of vertebrates. In *The Biochemistry of Animal Development Vol. III* (Ed. R. Weber). Academic Press, New York, pp 258-287.

TIEDEMANN, H. (1978). Chemical approach to the inducing agents. In *Organizer — A Milestone of a Half-Century from Spemann* (Eds. O. Nakamura and S. Toivonen). Elsevier/North-Holland, pp. 91-116.

TIEDEMANN, H. (1990). Cellular and molecular aspects of embryonic reduction. *Zool. Sci. 7:* 171-186.

TIEDEMANN, H., BECKER, U. and TIEDEMANN, H. (1961). Über die primären Schritte bei der embryonalen Induktion. *Embryologia 6:* 204-218.

TIEDEMANN, H., GRUNZ, H., KNÖCHEL, W. and TIEDEMANN, H. (1993). Inducing factors and the molecular mechanism of differentiation in early amphibian development. *Ontogenez 24:* 5-25.

TIEDEMANN, H., LOTTSPEICH, F., DAVIDS, M., KNÖCHEL, S., HOPPE, P. and TIEDEMANN, H. (1992). The vegetalizing factor. A member of the evolutionary highly conserved activin family. *FEBS Lett. 300:* 123-126.

TIEDEMANN, Hildegard (1993). Mesoderm differentiation in early amphibian embryos depends on the animal cap. *Roux Arch. Dev. Biol. 203,* 28-33.

VINCENT, J.P. and GERHART, J.C. (1987). Subcortical rotation in *Xenopus* eggs: an early step in embryonic axis specification. *Dev. Biol. 123*: 526-539.

WEEKS, D.L. and MELTON, D.A. (1987). A maternal mRNA localized to the vegetal hemisphere in *Xenopus* eggs codes for a growth factor related to TGF-ß. *Cell 51:* 861-867.

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