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Short Contribution

The final determination of *Xenopus* ectoderm depends on intrinsic and external positional information

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ABSTRACT Traditionally the whole animal cap (ventral plus dorsal ectoderm) of amphibian blastula and gastrula stages was considered as a homogeneous cell mass, because both the isolated dorsal and ventral ectoderm without induction differentiated into ciliated (atypical) epidermis. Recent results suggest a predisposition of the dorsal and ventral ectoderm. We used a special experimental approach, i.e. injection of activin as inducer into the blastocoel of intact *Xenopus* blastulae before the isolation of animal caps and fluorescein-dextran-amine (FDA) as a lineage tracer. In recombinants of FDA-labeled and unlabeled ectoderm we showed that the cells of the dorsal ectoderm mainly differentiate into neural tissue and notochord when they remain at their original dorsal position. In contrast, when small pieces of dorsal ectoderm are transplanted to the ventral part of animal caps, most of the descendants form epidermis. However, when small pieces of the ventral ectoderm are transplanted to the dorsal side, they significantly contribute to neural tissue and notochord. These results suggest that the prepattern in *Xenopus* animal caps of the late blastula and early gastrula stages is labile and reversible. Still more important is the fact that the fate of individual cells depends on the site of their localization within the animal cap. This means that cells in the dorsal most or ventral most part of the animal cap, respectively, will not randomly differentiate into all cell types, but predominantly into dorsal or ventral derivatives, respectively.

KEY WORDS: *predisposition, dorsal and ventral ectoderm, activin, pattern formation, cell lineage*

The establishment of the body axis (the dorso-ventral and anteroposterior polarity) is a central question in developmental biology (Grunz, 1996). It is generally accepted that the dorsal and ventral sides of the embryo are determined by the action of (maternal) factors or/and genes predominantly expressed either on the ventral or dorsal side of the embryo. In the traditional view the animal hemisphere (prospective brain and epidermis) has been considered as a naive target tissue which receives its (instruction) information for neural determination from the involuting chordamesoderm (Spemann and Mangold, 1924; Holtfreter, 1933; Grunz *et al.*, 1975). Moreover, the whole animal cap was considered as an uniform entity. However, newer studies indicate that the dorsal halves of animal caps are able to form dorsal mesoderm and neural tissue in response to activin, while ventral halves differentiate into ventral mesoderm derived structures (Sokol and Melton, 1991). The authors concluded from these data that the difference in the response to activin must be due to a predisposition of the dorsal and ventral ectoderm. Several transcription factors and secreted proteins are good candidates as dorsalizing and ventralizing molecules (Grunz and Tacke 1989, 1990; Smith and Harland, 1992; Sasai *et al.*, 1994, 1996; Gawantka *et al.*, 1995; Wilson and Hemmati-Brivanlou, 1995; Bouwmeester *et al.*, 1996; De Robertis and Sasai, 1996; Schmidt *et al.*, 1996).

Apparently factors responsible for the determination of the dorsal side of the embryo are already distributed in animal/vegetal and dorsal/ventral gradients during early cleavage (Grunz, 1977, 1994; Kageura and Yamana, 1986; Cardellini, 1988; Gallagher *et al.*, 1991; Li *et al.*, 1996; Miller and Moon, 1996; Yost *et al.*, 1996; Larabell *et al.*, 1997). However, so far it has not been possible to show which cells within an activin-treated animal cap of the late blastula or early gastrula will form the different cell types within the terminal differentiated explant. Since at certain concentrations and incubation times of the inducer animal caps form a wide range of different cell types (Grunz, 1979, 1983; Fukui and Asashima, 1994; Green *et al.*, 1992, 1994; Wilson and Melton, 1994), it is not clear which cells of a single animal cap will contribute to epidermis, notochord, somites, mesenchyme, etc. So it is of interest whether dorsal or ventral ectodermal cells will participate in a random way in the formation of the terminal differentiated structures within an animal cap. We addressed this question in detail using a special experimental approach with recombinants of FDA-labeled and unlabeled animal caps isolated from early gastrula, injected at the late blastula stage with activin (Fig. 1).

Abbreviations used in this paper: FDA, fluorescein-dextran-amine; BSA, bovine serum albumin.

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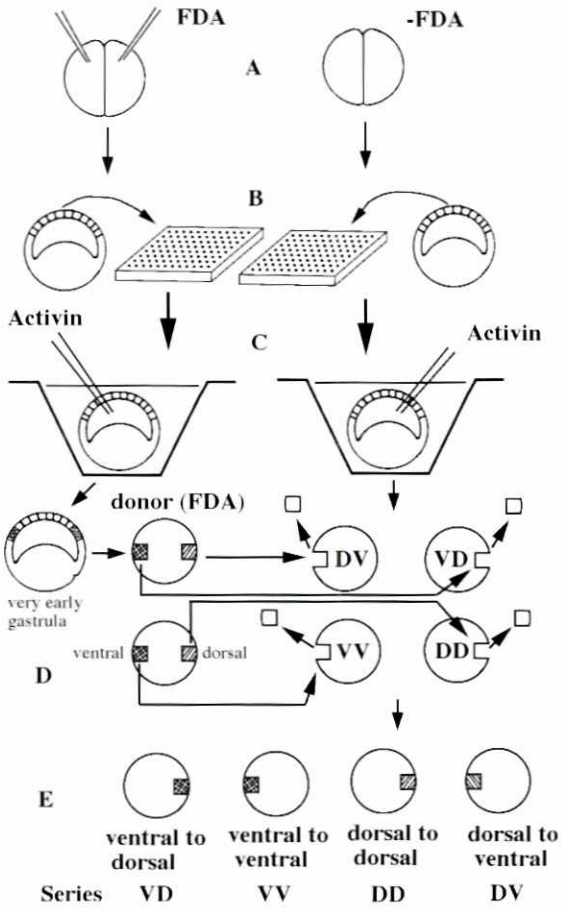

Fig. 1. Preparation of recombinants from FDA-labeled donor ectoderm and unlabeled recipient animal caps. (A) *Injection of FDA into both blastomeres of 2-cell embryos.* **(B)** *Transfer of the labeled and unlabeled embryos into Terasaki-microtest plates.* **(C)** *Injection of activin into the blastocoel of both FDA-injected and unlabeled embryos (late blastula; stage 9, Nieuwkoop and Faber, 1956).* **(D)** *Isolation of animal caps and immediate (orthotopic or heterotopic) transplantation of tiny pieces of FDA-labeled into ventral or dorsal most areas, respectively, of unlabeled ectoderm at stage 10.* **(E)** *Resulting recombinant animal caps. The explants (animal caps) of the 4 resulting series were cultured for up to 3 days at 20°C.*

When small pieces of FDA-labeled dorsal most ectoderm were transferred to the ventral part of the animal cap (D→V series), interestingly, most of them contributed to epidermis (67%) just like those of V→V series (compare Fig. 2A,B with E,F), but rarely to neural tissue (6%), notochord (1%) and other tissues (Table 1). However, when labeled ventral most ectoderm was grafted to the dorsal side of an unlabeled animal cap (V→D series) a considerable amount of them contributed to neural tissue (58%) and notochord (16%) (Fig. 2C,D), but rarely to epidermis (6%) and other tissues.

In the control series (orthotopic transplantation), a small piece of labeled ventral most ectoderm was transferred to the same ventral position of an unlabeled ectoderm (V→V), 66% of the lineage tracer was observed in the epidermis (Fig. 2E,F). However, labeled cells were rarely found in neural tissue (3%), notochord (1%) and other tissues.

However, when a small piece of FDA labeled dorsal most ectoderm was transferred to the same area of an unlabeled ectoderm (D→D), the labeled cells mainly differentiated into neural tissue (50%) and notochord (40%), but very rarely into epidermis (1%) and other tissues (Fig. 2G,H; Table 1).

In this paper we tried to answer the question which descendants within the induced animal cap will form, for example, the peripheral epidermis or notochord and neural tissue in the center of the explant. It could be argued that only the cells at the periphery of the animal cap (cortical cells) will form epidermis, while the cells in the central part (core of the animal cap) would differentiate into notochord and neural structures. Alternatively, all cells of the animal cap could randomly participate in the formation of the different tissues. Our data described above show that apparently neither of these assumptions can be confirmed. A random distribution of the FDA-labeled ectodermal cells can be excluded since we found the descendants of ventral most ectoderm (orthotopic transplantation, V→V series) preferentially in epidermis.

The suggestion that only the peripheral ectodermal cells of an isolated animal cap will form epidermis could also be ruled out. The descendants of those ectodermal pieces, isolated from dorsal most peripheral ectoderm (orthotopic transplantation, D→D series) rarely differentiated into epidermis, but mainly into notochord and neural structures (Table 1).

Summarizing, our data suggest that the final determination of individual cells depends on their position within the animal hemisphere (Wolpert, 1969; Gurdon, 1988; Meinhardt, 1996), which

TABLE 1

RECOMBINANTS OF SMALL PIECES OF FDA-LABELED DONOR ECTODERM AND UNSTAINED RECIPIENT ANIMAL CAPS AFTER TREATMENT WITH ACTIVIN

Series	number of cases (n)	epidermis %		mesenchyme %		neural %		notochord %		muscle %		yolk-rich tissue %	
D→V	34	67	(100)	4	(100)	6	(100)	1	(97)	11	(100)	11	(100)
V→D	19	6	(100)	3	(100)	58	(100)	16	(95)	12	(100)	7	(100)
V→V	25	66	(100)	5	(100)	3	(100)	1	(84)	10	(100)	15	(100)
D→D	25	1	(100)	2	(100)	50	(100)	40	(80)	4	(100)	4	(100)

Data represent the percentages of FDA-labeled cells in the different differentiations of the individual explants (for detailed description see Experimental Procedures and Fig. 1). As expected, all explants of all series show similar percentages (figures in parentheses) of the various differentiations, since the *in vivo*- activin-treatment of the ectoderm (injection into the blastocoel of intact blastulae) was identical in all cases and the isolation and recombination of FDA-labeled donor ectoderm and unlabeled recipient ectoderm took place after the activin-treatment. D→V, dorsal most part (donor) transplanted into ventral most part of host animal cap; V→D, ventral most part (donor) transplanted into dorsal most part of host animal cap; V→V, ventral most part (donor) transplanted into ventral most part of host animal cap; D→D, dorsal most part (donor) transplanted into dorsal most part of host animal cap.

makes them differentially susceptible to cell autonomous factors and gradients of secreted proteins (Sasai *et al.*, 1995; Piccolo *et al.*, 1996). In further experiments the fate of single cells in the animal cap and the whole embryo may be studied by special techniques.

Experimental Procedures

Eggs and embryos

Eggs were obtained by injection of female *Xenopus laevis* with 1000 IU human chorion gonadotropin (Schering AG, Berlin, Germany) 6 to 8 h prior to artificial insemination. Forty minutes after insemination the fertilized eggs were dejellied with 3.5% cysteine hydrochloride (pH 7.4), thoroughly washed with Holtfreter solution to which penicillin/streptomycin had been added, and transferred into Steinberg solution in larger culture dishes coated with 1% agar. All embryos were staged according to Nieuwkoop and Faber (1956).

Labeling with fluorescein-dextran-amine

Regular middle 2-cell stage embryos were selected and both blastomeres of each embryo were injected at the animal side with 15 nl each of the lineage label FDA (50 mg/ml fluorescein-dextran-amine in water; Gimlich and Braun, 1985). During and one hour after injection they were kept in Holtfreter solution containing 3% w/v Ficoll (type 400; Pharmacia) which prevents leakage of cytoplasm and simplifies injection by shrinkage of the perivitelline space. One hour later the embryos were transferred to Holtfreter solution omitting the Ficoll. Microinjection was performed using a microinjection system (PV 830 Pneumatic PicoPump; World Precision Instruments, Inc., Sarasota, FL, USA) and a Leica binocular microscope.

Treatment of embryos with activin A

Human recombinant activin was dissolved in 65% L-15 solution (pH 7.4) containing 0.1% BSA at final concentrations of 20 ng/ml and 200 ng/ml, respectively. At late blastula stage 9, the vitelline membranes of both the normal and labeled embryos were removed with fine watchmaker's forceps under a stereomicroscope (Leica). The whole embryos were then transferred to the wells of Terasaki-plates (PS-microtest plate 60K, Greiner) filled with 10 µl L-15 with added activin (20 ng/ml). 120 nl of the activin solution with higher concentration (200 ng/ml) was injected into the blastocoel of the embryos using the PV 830 Pneumatic PicoPump microinjection system. Under these special *in vivo* conditions (injection of activin into the blastocoel of intact embryos) we could prevent the curling up of ectoderm during activin treatment, which quickly takes place after microdissection. This point is very important for the exact performance of the grafting experiments (see below).

After one hour, the Terasaki-plate was filled up carefully with a relatively large amount of Holtfreter solution and embryos were transferred to agar-coated Petri dishes.

Grafting

The very beginning of gastrulation of activin-treated embryos was detected by a faint trace of pigment concentration at the blastopore lip area. At this stage a clear distinction between the dorsal and ventral ectoderm is possible. The ectoderm of both labeled and unlabeled embryos was dissected out with fine glass needles. A piece of defined size (0.1 mm x 0.1 mm) of labeled donor was removed and immediately transferred to the same (orthotopic) location or opposite (heterotopic) location of an unlabeled host ectoderm, where a piece of the same size was replaced by the transplant (Fig. 1). The donor tissue quickly integrated in the plane of the host ectoderm prior to curling up. The recipient animal cap was then cultured in Holtfreter solution at 20°C until intact embryos had reached stage 42.

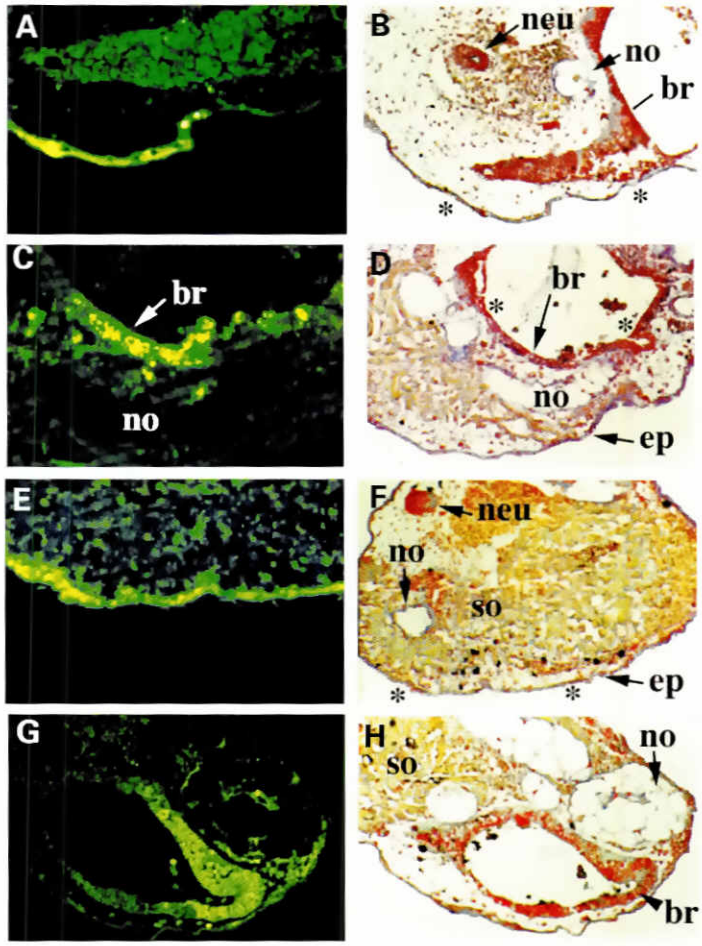

Fig. 2. Fluorescence localization of the derivatives of FDA-labeled ectodermal cells after activin treatment. *Typical representatives of all 4 series are included in this panel. Exactly the same sections are shown as fluorescence image (left) and after staining with standard histological techniques (right).* **(A)** *D→V series (heterotopic transplantation: dorsal FDA-labeled donor ectoderm transplanted to the unlabeled ventral area of the recipient animal cap). FDA-labeled progenitor cells have differentiated into epidermal cells. There are no further labeled cells in the not visible part of the section.* **(B)** *D→V series (the same section shown in* **(A)** *at lower magnification after a second staining for light microscopy). The fluorescent part, shown in* **(A)** *is marked by asterisks. no, notochord; br, neural structures; neu, neural tube-like structure.* **(C)** *V→D series (heterotopic transplantation: FDA-labeled ventral donor ectoderm transplanted to the unlabeled dorsal area of the recipient animal cap). FDA-labeled progenitor cells have differentiated into neural and notochord cells. There are no further labeled cells in this section. br, neural structures no, notochord.* **(D)** *V→D series (the same section shown in* **(C)** *at lower magnification after a second staining for light microscopy). The fluorescent part, shown in* **(C)** *is indicated by asterisks. no, notochord; br, neural structures; ep epidermis.* **(E)** *V→V series (orthotopic transplantation: ventral FDA-labeled donor ectoderm transplanted to the unlabeled ventral area of the recipient animal cap). FDA-labeled progenitor cells have differentiated into epidermal cells. There are no further labeled cells in the not visible part of the section.* **(F)** *V→V series (the same section shown in* **(E)** *at lower magnification after a second staining for light microscopy). The fluorescent part, shown in* **(E)** *is indicated by asterisks. no, notochord; br, neural structures; ep, epidermis.* **(G)** *D→D series (orthotopic transplantation: dorsal FDA-labeled donor ectoderm transplanted to the unlabeled dorsal area of the recipient animal cap). FDA-labeled progenitor cells have differentiated into neural cells and notochord. The neural structure in this section consists of both FDA-labeled donor and unlabeled host cells.* **(H)** *D→D series (the same section shown in* **(G)** *at slightly lower magnification after a second staining for light microscopy). The fluorescent cells, shown in* **(G)**, *can easily be identified as neural structures (br) and notochord (no). This section contains no labeled somites (so).*

Histological preparation and scoring

The explants were fixed for 12 h at 4°C in 4% freshly prepared paraformaldehyde buffered at pH 7.4 with sodium cacodylate (Gimlich and Braun, 1985). Samples were dehydrated through a graded ethanol series and embedded in paraffin. Sections (6 μm) were mounted in 80% glycerol with 4% n-propylgallate and scored with a Leitz photomicroscope equipped with epi-fluorescence. Only explants which showed a similar differentiation pattern were selected (Table 1, figures in parentheses). The percentage of each labeled tissue was scored in comparison to all other labeled tissues within every individual explant. On the basis of every individual case available in each series the average percentages of labeled tissues of all cases were calculated (Table 1). For example, in D→D series 50% of FDA-labeled cells were observed in neural structures and further 40% in the notochord, but 1% in epidermis only. After fluorescence analysis the specimens were stained with aniline blue-orange G for normal histological examination described elsewhere (Grunz, 1970, 1983). Exactly the same sections were checked in the fluorescence and the light microscope.

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