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MESODERMAL COMPETENCE OF THE PRESUMPTIVE ECTO-NEURODERM AT VARIOUS DEVELOPMENTAL STAGES, IN *XENOPUS LAEVIS* (DAUDIN)

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R I N G K A S A N

Suatu studi perbandingan mengenai kapasitas berreaksi dalam membentuk mesoderm dari presumptif ekto-neuroderm dengan bertambahnya umur, telah dilakukan pada Xenopus laevis.

Telah dibuktikan, bahwa sebelum gastrulasi induksi mesoderm terutama menghasilkan struktur - struktur mesoderm dorsal. Selama proses gastrulasi berlangsung, hasil differensiasi cenderung untuk menggeser dari struktur-struktur mesoderm dorsal ke struktur-struktur mesoderm ventral.

Presumptif ekto-neuroderm telah mempunyai kompetensi untuk membentuk mesoderm pada stadium blastula muda dan kompetensi tersebut mulai menghilang pada stadium blastoporus berbentuk sepatu kuda.

A B S T R A C T

A comparative study on the reactive capacity of the presumptive ecto-neuroderm on mesoderm formation with increase of age has been done in Xenopus laevis.

It was proved that before gastrulation, mesoderm induction mainly produces dorsal mesodermal structures. During the gastrulation

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process the product of differentiation tends to shift from dorsal to ventral mesodermal structures.

The presumptive ecto-neuroderm has already a competence for mesoderm formation at the early blastula stage and this competence starts to fade out at the horse-shoe-shaped blastopore stage.

INTRODUCTION

In his isolation experiments Holtfreter (1938) has systematically investigated the developmental capacities of the three presumptive germ layers of the early urodelean and anuran gastrula, demonstrating the existence of pronounced differences among them. He found that the presumptive endoderm exhibits strong developmental capacities for various endodermal structures; the presumptive mesoderm shows a broad variety of developmental capacities for mesodermal, ecto-neurodermal as well as endodermal differentiation, while the presumptive ecto-neurodermal region shows hardly any developmental capacity. The latter forms only an atypical mass of ectodermal cells.

Focussing our attention on the development of the mesoderm, Yamada (1937, 1940) demonstrated that the medio-lateral organization of the mesoderm of *Triturus pyrrhogaster* depends upon an inductive action emanating from the medio-dorsal notochordal anlage and which spreads with decrement laterally and ventrally.

Toivonen (1953) showed that mesodermal structures of *Triturus vulgaris* can be induced in the gastrula ectoderm by using guineapig bone marrow as heterogenous inductor. Yamada (1958) found that bone marrow extract induced not only mesodermal but also endodermal structures in the gastrula ectoderm. This was later confirmed by several authors, e.g. Takata and Yamada (1960), Masui (1961), Ogi (1961), Engländer (1962) and Tseng (1963).

Studying the loss of mesodermal as well as neural competence of the presumptive ecto-neuroderm of *Triturus vulgaris* during gastrulation by using heterogenous inductors, Leikola (1963) has come to the conclusion that there are two kinds of competence: neural and mesodermal, the latter being lost earlier than the former.

Gebhardt and Nieuwkoop (1964), studying the mesodermizing action of lithium ions in axolotl, demonstrated that the blastula ectoderm has a much higher mesodermization competence than the gastrula ectoderm. The latter loses its competence at about the horse-shoe-shaped blastopore stage (stage 10½,

Harrison). The action of lithium ions was shown to be accompanied by extensive cytolysis of the ectodermal cells.

Also using lithium ions as inductor, Grunz (1968) investigated the endo- and mesodermal competence of the presumptive ectoderm at successive stages of development in two urodeles. He demonstrated that competence begins to appear at the morula stage, reaches a maximum at the middle to late blastula and terminates at the early gastrula stage. Whereas the ectoderm of *Ambystoma* forms endo- and mesodermal structures of tail character, that of *Triturus* chiefly produces those characteristic of the posterior and middle trunk region.

In his recombination experiments with micromeres and macromeres encircling the animal and vegetative poles of the morula to blastula stages of *Triturus pyrrhogaster*, Ogi (1967) observed that although the two parts did not contain any presumptive mesodermal cells, mesoderm formation occurred in the recombinates. He inferred the presence of a double gradient system in amphibian development analogous to that demonstrated in the sea urchin (Hörstadius, 1962; Runnström, 1966).

The present study was carried out to gain information on mesodermal competence of the presumptive ecto-neuroderm with further development, in *Xenopus laevis*. For this purpose experiments were designed to observe the reactive capacity of the presumptive ecto-neuroderm at various developmental stages.

MATERIALS AND METHODS

Throughout the investigation, eggs of *Xenopus laevis* (South African clawed frog) reared at the Hubrecht Laboratory, Utrecht-Holland, were used. The eggs were obtained by injecting the males and females with Physex (Leo Pharmaceutical Products, Ballerup-Denmark), a gonadotrophin. The injection was made into the dorsal lymph sac, piercing the skin of the thigh and the septum between the lymph sac of the thigh and the back. Two days before the eggs were required, the males were injected with 60 I.U. in 0.5 cc of aquadest. On the next afternoon they were once again injected with the same dose, and the females with 200 I.U. in 1 cc of aquadest. The animals were put in a covered container, sheltered from light, and provided with plastic bars for oviposition. Spawning occurred in the early morning, after the temperature had been raised artificially during the night and had passed the critical temperature which is 21°C.

To test the reactive capacity of the presumptive ecto-neuroderm before and during gastrulation to the inductive influence emanating from the endoderm, different ages of the

presumptive ecto-neuroderm ranging from early blastula to horse-shoe-shaped blastopore stage (stage 6½/7 to stage 11) were combined with endoderm of late blastula (stage 9, Nieuwkoop and Faber 1967). Both parts of the recombinates were reared as isolates for controls. The recombinates as well as the isolates were cultured for three days at 20°C in normal modified Barth's solution, to which 20.000 I.U./L penicillin and 0.1 g/L streptomycin were added as antibacterial agents.

All specimens were fixed in Smith's solution for six hours, then washed directly in 70% alcohol. They were then block-stained with borax-carmine and subsequently embedded in paraffin-wax through n-butyl alcohol and sectioned at 10/U. The sections were counterstained with aniline blue-orange G.

RESULTS

The isolates of the presumptive ecto-neuroderm (zone I-II) formed irregular masses of atypical ectoderm, whereas the isolates of presumptive endoderm (zone IV) each consisted of a solid mass of undifferentiated yolk-laden cells.

Recombinates were obtained by placing zone IV into the cup-shaped zone I-II (Fig. 1). The two parts soon adhered to each other firmly and the recombinates were then turned to their normal position. Development of all recombinates was mainly characterized by exogastrulation. The older the gastrula, the smaller became the area of free presumptive ectoderm. The combining of later stages of presumptive ectoderm with the endoderm of late blastula did not show any rejection phenomena.

In recombinates, ectodermal derivatives formed were epidermis, placodes, melanophores and neural tissues, the latter being present in the form of either a distinct neural tube or an irregular neural mass. The mesodermal structures consisted of muscle tissue, notochord, nephric tubules, blood cells and mesenchyme. Most of the endoderm remained undifferentiated and was found as solid mass of yolk-laden cells.

The experimental results are summarized in table 1. In all experimental series the percentage of cases with neural differentiation lies between that with notochordal and muscle differentiation. Using presumptive ectoderm as reactive tissue in pre-gastrulation stages, muscle was found in 100%, while notochord in 40 - 45% of the cases. The frequency of occurrence of muscle and notochord differentiation dropped very rapidly in gastrulation stage. In recombinates with presumptive ectoderm of the horse-shoe-shaped blastopore stage (stage 11), muscle differentiation was found in only 25% and notochord in 5% of the cases. Blood cells started to differentiate in recombinates with presumptive ecto-neuroderm of the

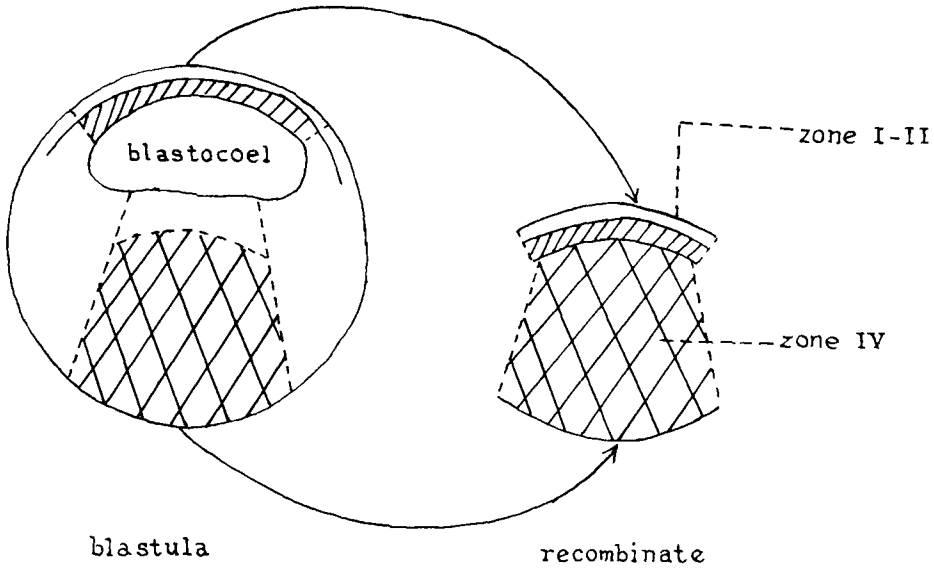

Fig. 1. Diagrammatic representation of the operation, showing the areas taken from the blastula to form the recombinate of the entire presumptive ecto-neuroderm (zone I - II) with the presumptive endoderm (zone IV).

early blastula stage (stage 7⁻) and was formed in 5% of the cases. In recombinates with older presumptive ecto-neuroderm the frequency of occurrence increased rapidly, especially that of the crescent-shaped blastopore stage.

At a later stage, the horse-shoe-shaped blastopore or stage 11, there was a pronounced drop. Yet, the frequency of blood cell occurrence in the latter case was still higher (35% of the cases) than that of other main mesodermal structures, such as notochord, muscle cells and nephric tubules. In recombinates of presumptive ectoderm of the crescent-shaped blastopore stage (stage 10½) poor differentiation of mesodermal structures was found; only mesenchyme and blood cells were formed (in 7 out of 20 cases). Moreover, in some recombinates only mesenchymal cells were formed (in 2 out of 20 cases). In some recombinates of the horse-shoe-shaped blastopore stage (stage 11) there was no mesodermal differentiation at all; recombinates consisted only of atypical ectoderm and an undifferentiated endodermal mass (5 out of 20 cases).

Table 1. The reactive capacity of the presumptive ectoderm of different stages combined with presumptive endoderm of late blastula. Stages according to Nieuwkoop and Faber (1967).

Number of recombinates containing:	Recombinates with presumptive ectoderm of:				
	Early blastula (st.6½/7)	Late blastula (st.9⁻)	Initial gastrula (st.10)	Crescent shaped blastopore (st.10½)	Horseshoe-shaped blastopore (st.11)
	20*	25*	20*	20*	20*
Epidermis	20 (100%)	25 (100%)	20 (100%)	20 (100%)	20 (100%)
Neural tissue	13 (65%)	19 (76%)	12 (60%)	7 (35%)	5 (25%)
Notochord	9 (45%)	10 (40%)	6 (30%)	3 (15%)	1 (5%)
Muscle	20 (100%)	25 (100%)	17 (85%)	10 (50%)	5 (25%)
Nephric tubules	9 (45%)	4 (16%)	2 (10%)	3 (15%)	2 (10%)
Blood cells	1 (5%)	8 (32%)	12 (60%)	14 (70%)	7 (35%)
Mesenchyme	19 (95%)	25 (100%)	19 (95%)	19 (95%)	10 (50%)
Mesothelium	8 (40%)	19 (76%)	17 (85%)	14 (70%)	5 (25%)
Melanophores	6 (30%)	17 (68%)	13 (65%)	12 (60%)	2 (10%)
Undifferentiated endoderm	20 (100%)	25 (100%)	20 (100%)	20 (100%)	20 (100%)

* Number of available recombinates

DISCUSSION AND CONCLUSIONS

In the analysis of mesoderm formation in a urodele, axolotl, Nieuwkoop (1969 a) came to the conclusion that the mesoderm is derived from the ectodermal part of the egg, under the inductive influence of the endoderm. The conclusions drawn from the quantitative findings of the experiments on *Xenopus laevis* blastulae correspond with those based on the study of axolotl (Sri Sudarwati and P.D. Nieuwkoop, 1971).

Isolates of the presumptive ecto-neuroderm formed irregular masses of atypical ectoderm in *Xenopus laevis*, which corroborates Holtfreter's findings (1938). As was already found by Ogi (1967) and confirmed by Nieuwkoop (1969), isolation of the central endodermal mass does not lead to any cellular differentiation in urodeles. This also holds for *Xenopus laevis*. When the central yolk mass is recombined with ectodermal cap, the mesoderm is newly induced (Sri Sudarwati and P.D. Nieuwkoop, 1971).

Taking into consideration the differentiation of the main mesodermal structures such as notochord, muscle, nephric tubules and blood cells, the present study reveals that before gastrulation the reactive capacity of the presumptive ectoderm is mainly the production of dorsal mesodermal structures. During the gastrulation process there is a tendency to shift the differentiation of dorsal to ventral mesodermal structures. This latter finding is in agreement with the result of the investigation done by Tseng (1963) on heterogenous induction, using urodelean ectoderm as reactive tissue and guinea-pig bone marrow as mesodermal inductor.

Yamada (1937, 1940) suggests that there is a mesodermal factor which controls the differentiation of the various mesodermal structures. The present investigation on the reactive capacity of the presumptive ecto-neuroderm with increase in age gives supplementary information to the matter. It is probable that the ectodermal cells before gastrulation possess the reactive capacity to form all the mesodermal differentiations. It may develop into dorsal as well as lateral and ventral structures under the action of a high concentration of that factor. However, the presence of large amounts of notochord and muscle suppresses the differentiation of the lateral and ventral mesoderm. Accompanying the increase in age, the ectodermal cells may undergo certain changes of permeability or biosynthetic activities, so that they can no longer be acted upon by the mesodermal factor at a high concentration. When the condition to differentiate into notochord and muscle is not fulfilled, the ectodermal cells mainly form lateral and ventral mesodermal structures.

The present study also reveals that the presumptive ecto-neuroderm in *Xenopus laevis* has already a competence for mesoderm formation as early as the early blastula stage (stage

7⁻, Nieuwkoop and Faber, 1967). The blastula ectoderm (stage 9⁻) has the highest mesodermization competence. During gastrulation there is a continuous decrease in the capacity of the ectoderm to form mesodermal structures. Both the size and frequency of the structures formed generally decrease with the age of the ectoderm. There was not only a general decrease in reactivity, but also a qualitative change towards epidermal formation. Mesodermization competence starts to fade out in recombinates with ectoderm of the horse-shoe-shaped blastopore stage (stage 11).

SUMMARY

1. Mesoderm formation of *Xenopus laevis* (Daudin) has been studied in order to gain a better insight to the process in amphibians.
2. The reactivity of the presumptive ecto-neuroderm in mesoderm formation at various embryonal stages, starting with the early blastula (stage 6½/7, Nieuwkoop and Faber, 1967) to late gastrula (stage 11) has been studied.
3. With the increase in age of the presumptive ecto-neuroderm used in the experiments, there is a shift in the differentiation of the dorsal to ventral mesodermal structures.
4. The size and frequency of the occurrence of mesodermal structures generally decrease with the increase in age of the presumptive ecto-neuroderm.
5. There was not only a general decrease in reactivity, but also in qualitative change towards epidermal formation.
6. The presumptive ecto-neuroderm has already a competence for mesoderm formation as early as the early blastula stage and this competence starts to fade out at the horse-shoe-shaped blastopore stage.

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