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Role of the Neural Tissue in the Differentiation of Notochord from the Uninvaginated Part of the Dorsal Blastopore Lip of *Triturus*-Gastrula

By

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In the earliest gastrula of *Triturus*, the upper part of the blastopore lip consists of the presumptive materials of fore-gut and prechordal plate. These non-notochordal materials have the potency to differentiate into notochord (TAKAYA, '53). Our previous experiment (KATO & OKADA, '56) in relation to this phenomenon has demonstrated that such a potency can be realized only when these materials are explanted in the vesicle of the presumptive ectoderm from the early gastrula, while they differentiate according to their prospective fates when the flank epidermis of the neurula is used as the envelope. In the former case, moreover, the differentiation of notochord is always accompanied with the simultaneous production of the neural tissue from the ectoderm. This fact seems to suggest some relationship between the differentiation of the notochord from such non-notochordal materials and that of the neural tissue from the ectoderm. With the aim of examining this point closely, the present work was undertaken to see first whether the tissue which has differentiated into the neural one is also responsible for a transformative differentiation of the notochord from the non-notochordal materials.

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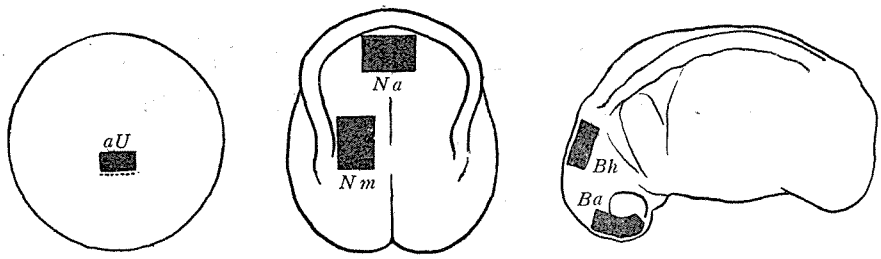
Meterials and Methods

The materials used were the embryos of *Triturus pyrrhogaster* in varied developmental stages. The explants consisted of the pieces from the upper part of the blastopore lip of the earliest gastrula (the piece will be designated as *aU* in the following description like in our previous paper, '56) and from the various parts of the neural tissue, that is, from the following part: (1) the anterior-most part of the medullary plate of neurula at stage 17 in OKADA & ICHIKAWA's table, (2) the middle part of it, (3) the dorsal half of the fore-brain of the embryo at stage 26 or (4) the dorsal half of the hind-brain of the embryo at the same



St. 11 St. 17 St. 26

Fig. 1. Scheme of embryos showing the areas from which wrapped piece were removed. Piece isolated from the black area of embryo at st. 11 was combined with each piece taken from the black areas of embryo at st. 17 or st. 26, and explanted within the vesicle of flank epidermis of the early neurula. *aU*: the upper part of the blastopore lip, *Na*: the anterior-most part of the medullary plate, *Nm*: the middle part of the medullary plate, *Ba*: the dorsal half of the fore-brain, *Bh*: the dorsal half of the hind-brain.

stage. Locations and sizes of these pieces are shown in Fig. 1.

The explants were covered with the wrapper of the flank epidermis taken out of the early neurulae, and they were cultured in HOLTFRETER's solution for 10–14 days.

Results

Series I. *Explantation of a piece* aU *with the anterior-most part of the medullary plate at stage 17*

Ten out of 27 available cases showed the differentiation of the notochord (37%); but other mesodermal tissues such as muscle and mesenchyme were also encountered with lower frequencies (muscle 4%, mesenchyme 15%). A large amorphous mass of havily yolk-laden cells of endoderm remained in all explants.

The neural structures derived from the added neural material were found in all cases. As to their regional character, the archencephalic structure equipped with an eye and a nose was predominant and appeared in 15 cases, the deuterencephalic sometimes with an ear vesicle occurred in 3 cases, and both of the two structures in 3 cases. Remaining 6 specimens had the neural mass alone, regional character of which was uncertain.

The notochord occurred always in the presence of either of the archencephalic or the deuterencephalic or both of them. It was found always in direct contact with or close to the neural tissue (cf. Fig. 2), but it can not be pointed out that any special correlation exists between the regionality of neural structures and the production of the notochord.

Series II. *Explantation of a piece* aU *with the middle part of the medullary plate at stage 17*

In this series, notochord was obtained in 10 out of 28 available cases (36%),

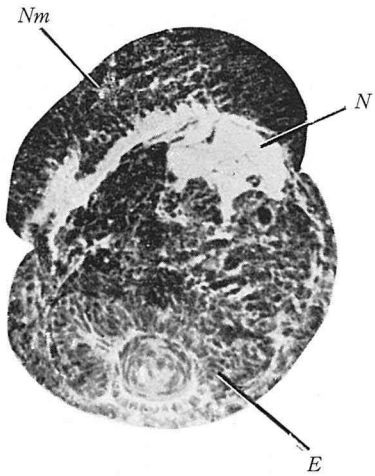


Fig. 2

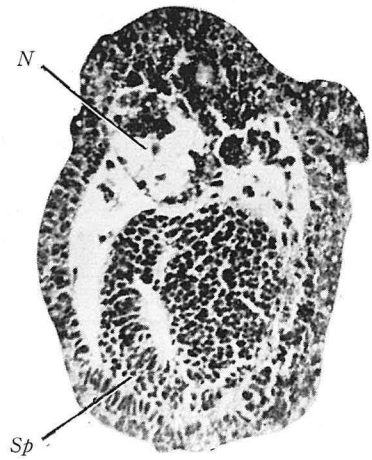


Fig. 3

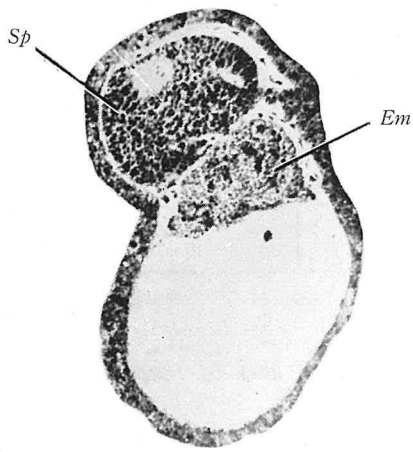


Fig. 4

Fig. 2. Differentiation of the notochord in the explant of a piece *aU* combined with a small piece from the anterior-most part of the medullary plate of the neurula. *N*: notochord, *Nm*: neural mass, *E*: eye.

Fig. 3. Differentiation of the notochord in the explant of a piece *aU* combined with a small piece from the middle part of the medullary plate of the neurula. *N*: notochord, *Sp*: spinal cord.

Fig. 4. Differentiation of a piece *aU* combined with a small piece taken from the middle part of the medullary plate of neurula. *Em*: an amorphous mass of endoderm from piece *aU*, *Sp*: spinal cord.

muscle in 3 (11%), and mesenchyme in 6 (21%). The undifferentiated endodermal mass was contained in all explants. The results as to the chordal, mesodermal and endodermal differentiations were almost similar to those obtained in the previous series.

Every specimen had the neural tissue, the regional character of which was generally deuterencephalic or spinocaudal; i.e., the former was found in 12, the latter in 8, and both of them in 3 explants. Remaining 5 cases contained only undefinable neural mass.

Series III. *Explantation of a piece* aU *with the dorsal half of the fore-brain at stage 26*

All of 16 specimens had neither notochord nor muscle, but mesenchyme in 6 cases (38%). Undifferentiated endodermal mass was met with in all specimens. As to the neural differentiation, the archencephalic structure was found in 5 specimens, while the remaining 11 specimens had only an undefinable neural mass respectively.

Table 1. The tissues differentiated in the explants.

Experimental series	I	II	III	IV
No. of available explants	27	28	16	15
No. of explants containing mesodermal tissues				
Notochordal	10 (37)	10 (36)		1 (7)
Muscular	1 (4)	3 (11)		1 (7)
Mesenchymal	4 (15)	6 (21)	6 (38)	4 (27)
No. of explants containing endodermal tissues	27 (100)	28 (100)	16 (100)	15 (100)
No. of neural structure differentiated in explants				
Archencephalic	18 (67)		5 (31)	
Deuterencephalic	6 (22)	15 (54)		4 (27)
Spino-caudal		11 (39)		
Undefinable	6 (22)	5 (18)	11 (69)	11 (73)

The number in the parenthesis indicates the percentage of the formation of respective structure.

Series IV. *Explantation of a piece* aU *with the dorsal half of the hind-brain at stage 26*

Notochord and muscle were found respectively only in one specimen out of 15 available explants (7%), and the mesenchymal cells were included in 4 explants (27%). The undifferentiated endoderm was the common occurrence throughout all cases. Neural differentiation took place in all explants, of which 4 had the deuterencephalic structure with or without ear vesicle, and 11 contained merely an undefinable mass each.

Discussion

As was reported in our previous paper (KATO & OKADA, '56), the uninvaginated part of the dorsal blastopore lip of the earliest gastrula (piece *aU*), consisting of the presumptive materials of the fore-gut and the prechordal plate, is able to differentiate into the notochord under such experimental conditions that the neural

differentiation simultaneously takes place from the wrapping ectoderm through the inductive action of these materials. The results of the present experiments verify that the differentiation of the piece *aU* towards the notochord is also possible in the presence of such tissue that had already been differentiated into the neural material. Namely, when the piece *aU* was explanted together with a small piece taken out of the anterior-most part or the middle part of the open medullary plate of the neurula, the occurrence of notochord was frequently encountered (37% and 36% in the respective series of the experiments, cf. Table 1 & Fig. 5). Further, the notochord appeared always in contact with or close to the neural tissue (cf. Figs. 2 & 3). The fact seems to imply that the intimate relation exists between the presence of neural tissue and the differentiation of notochord from the piece *aU*. The same fact was observed also by the author's unpublished explantation experiment; i.e., when a piece *aU* was combined with a small piece of the undetermined competent ectoderm from the earliest gastrula (st. 11) and wrapped with the determined ventral ectoderm isolated from the early neurula, the differentiation of notochord occurred only in the case where the neural tissue was produced.

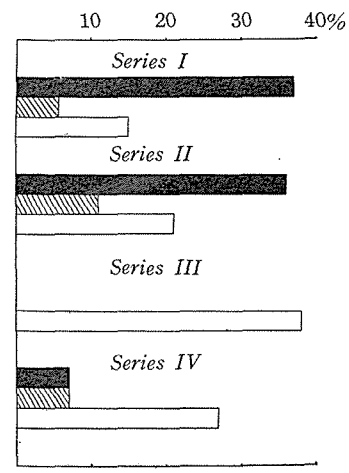


Fig. 5. Diagrammatic representation of the mesodermal frequencies differentiated from a piece *aU* in different experimental series. The frequency is obtained by percentage of the number of explants containing each tissue against the total number of explants in each series. black: notochord, oblique line: muscle, white: mesenchyme.

However, we must pay our attention to the fact that the notochord was hardly formed from the piece *aU* when the added neural material was taken from the embryos at the tail bud stage (Series III & IV, cf. Table 1 & Fig. 5). Taking the difference of age into consideration of the combined neural tissue, we may say that the advanced neural tissue is not so effective as the tissue just differentiated in the transformative differentiation of notochord from the non-notochordal materials. In our previous explantation (KATO & OKADA, '56), the notochordal differentiation occurred as high as 79% (34 out of 43 available cases), when the piece *aU* was wrapped with the undetermined ectodermal envelope. In this sort of explant, the transformative differentiation of notochord from the piece *aU* will proceed synchronously with the induction of the neural tissue from the ectodermal envelope.

Judging from these experimental results, it may be assumed that the tissue in the process of determination and differentiation towards neural character exerts the strong influence upon the piece *aU* to give rise to the notochord, but that the influence gradually decreases according as its neural differentiation advances.

Summary

Using the embryos of *Triturus pyrrhogaster*, a piece from the uninvaginated part of the dorsal blastopore lip of the earliest gastrula was explanted within the wrapper of the flank epidermis of the early neurula together with the following neural material: (1) the anterior-most part of the medullary plate of the neurula, (2) the middle part of it, (3) the dorsal half of the fore-brain of the embryo at the tail bud stage or (4) the dorsal half of its hind-brain. The piece of dorsal blastopore lip sometimes showed the differentiation into notochord when combined with (1) or (2), whereas the combination of this piece with (3) or (4) hardly resulted in the production of notochord. The discussion was done with respect to the correlation between the differentiation of notochord from the non-notochordal materials and that of neural tissue from the competent ectoderm.

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