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Studies on the Differentiating Potencies of the Dorsal Part of the Blastoporal Lip in *Triturus*-Gastrula

II. On the Differentiation of Notochord

By

Ken-ichi KATO

Biological Laboratory, Osaka Dental College, Hirakata, Osaka-Prefecture

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In our previous paper (KATO and OKADA, '56) it was reported that the dorsal blastoporal lip of the earliest *Triturus*-gastrula, consisting mainly of the presumptive materials of the foregut and prechordal plate, is able to differentiate into notochord and muscle when explanted within the envelope of the competent presumptive ectoderm isolated from the early gastrula, but is unable when explanted within the envelope removed from the incompetent belly ectoderm of the neurula. The difference is attributed to the presence or absence of the neural tissue derived from the enveloping ectoderm. In other words, there is a close correlation between the differentiation of neural tissue and the mesodermal differentiation. The same situation occurred also in another experiment, in which the different parts in the dorsal blastoporal area of the early gastrula were tested for their developmental potency by explantation within the ectodermal vesicle taken out of the early gastrula (KATO, '58). Will the difference of the envelope be the cause for the different result in the differentiation of the presumptive notochordal area of the early gastrula?

This paper deals with the closer study of such neuro-mesodermal relationship in the differentiation of the mesodermal tissue from the dorsal blastoporal part in the course of gastrulation.

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Material and Method

The explantation of three different regions of the dorsal part of the blastopore was carried out, using the gastrulae of *Triturus pyrrhogaster*. The closer account

of the location and shift of the material to be tested in the course of gastrulation was already given in the author's previous paper ('58), and it can be summarized in the following Table 1.

Table 1. Sequence of shifting of the blastoporal areas during gastrulation.

Areas to be tested \ Stages of test areas	St. 11	St. 12b	St. 13c	Presumptive fate
aU	proximal to the blastopore	invaginated	invaginated	foregut and prechordal plate
bU	medial to the blastopore	proximal to the blastopore	invaginated	notochord
cU	distal to the blastopore	medial to the blastopore	proximal to the blastopore	notochord and a part of somite

The region to be tested, immediately after removal or after 4 hours preculturing in saline, was wrapped with the incompetent ectodermal piece taken from the presumptive belly region of the early neurula (stage 15 of OKADA and ICHIKAWA's standard table), and the explants thus made were kept in HOLTFRETER's solution for 12–15 days.

Results

I. *Differentiating potency of the explanted piece.*

(a) *Part aU.* In most cases the explanted material remained a mass or undifferentiated cells containing many yolk platelets, and only in a very few cases the development of notochord and muscle occurred. This was universal, regardless of whether the test piece was taken either before or after its invagination. Besides, a few specimens had a neural tissue.

(b) *Part bU.* Unlike the results of the previous series, the mesodermal tissues such as notochord and muscle were found in many cases. There existed, however, a considerable difference in the frequency of their appearance according to the stage at which the test piece was taken. Namely, the explants taken at stage 11, i.e., from the part medial to the blastopore, differentiated into notochord and/or muscle only in 24 per cent and 16 per cent of all cases respectively. On the other hand, when the *bU* material was removed at stage 12b, i.e., from the part located immediately above the blastopore, the frequency was increased to 77 per cent and 43 per cent respectively. Moreover, the developed structures of the mesodermal tissues were improved inversely to the decline of the undifferentiated mass or fragment.

There were specimens in which the neural tissue was established, and such an unexpected production of neural tissue occurred more often when the test piece was removed at stage 11 than when it was isolated at stage 12b. Here will arise a doubt as to the origin of the neural tissue. In order to ascertain this point, the

Table 2. Tissues developed in the explants.

Pieces to be tested / Differentiated tissues	*aU at st. 11*	*aU at st. 12b*	*bU at st. 11*	*bU at st. 12b*	*bU at st. 13c*	*cU at st. 12b*	*cU at st. 13c*
	Number of available cases						
	65	26	49	27	8	42	8
Notochord	5 (8)[1]	1 (4)	12 (24)	21 (77)	8 (100)	19 (45)[2]	8 (100)
Muscle	5 (8)	2 (8)	8 (16)	12 (43)	7 (88)	10 (24)	7 (88)
Mesenchyme	32 (49)	12 (46)	23 (47)	24 (88)	5 (63)	20 (48)	6 (75)
Neural tissue[3]	2 (3)	1 (4)	11 (22)	3 (11)	0	10 (24)	0
Atypical epidermis	0	0	2 (4)	0	0	7 (17)	0
Undifferentiated tissue	58 (89)	25 (96)	49 (100)	19 (70)	5 (63)	30 (71)	2 (25)

1) The number in parenthesis indicates the percentage against the total number of available cases.
2) Blood cells appeared in one specimen (*cf.* Fig. 2).
3) Archencephalic induction took place in one explant of the part *aU at st. 12b*; in other cases neural tissue appeared as a small mass of a neural tube or an amorphous fragment (*cf.* Figs. 4 and 5).

test piece was taken sometimes from the embryos previously stained with Nile blue sulphate and was enveloped with the unstained ectoderm. The results revealed that the neural tissue had been derived solely from the test piece. The xenoplastic combination using the ectoderm of *Rana*-neurula as enveloping material also confirmed this point. These evidences indicate that the part *bU at stage 11* has a potency to differentiate even into the neural tissue under favourable conditions.

(c) *Part cU.* As to the differentiation of the mesodermal tissue, the part *cU* behaved quite like the previous part *bU* in explantation, i.e., the notochord and muscle appeared very frequently in the explants coming from the part proximal to the blastopore at stage 13c (100% and 88% respectively), while the explants from the part medial to the blastopore at stage 12b gave rise to the mesodermal tissues in less than half of the total cases, i.e., 45% in the notochordal and 24% in the muscular differentiation. The neural differentiation took place only in the latter explants.

As mentioned above, the neural tissue was occasionally developed in spite of the belly ectoderm of neurula having been used as envelope. The production of this tissue occurred more frequently in the explants of the distal part than in those of

the proximal part. Consequently, the correlation of the neural tissue to the differentiation of the notochord was surmised in the former case, in which almost all of the explants with notochord had the neural tissue, too. Contrary to this, the explants from the part proximal to the blastopore produced often the notochord independent of the production of the neural tissue. These situations are summarized in Table 3. On the other hand, as is seen in the table, the muscular differentiation had nothing to do with the neural tissue, as was pointed out by MUCHMORE ('51).

Table 3. Differentiation of the notochord and muscle in relation to the presence or absence of the neural tissue in the explants from the presumptive notochordal areas (parts *bU* and *cU*).

Pieces to be tested / Differentiated tissues	Part apart from the blastopore			Part immediately above the blastopore		
	bU at st. 11	*cU at st. 12b*	Total	*bU at st. 12b*	*cU at st. 13c*	Total
Production of neural tissue	11	10	21	3	0	3
with notochord	9	9	18 (86)	3	0	3 (100)
without notochord	2	1	3 (14)	0	0	0
with muscle	1	3	4 (59)	3	0	3 (100)
without muscle	10	7	17 (81)	0	0	0
Non-production of neural tissue	38	32	70	24	8	32
with notochord	3	10	13 (19)	18	8	26 (81)
without notochord	35	22	57 (81)	6	0	6 (19)
with muscle	7	7	14 (20)	9	7	16 (50)
without muscle	31	25	56 (80)	15	1	16 (50)

The number in parenthesis indicates the percentage against the total number of explants with or without the neural tissue.

II. *Differentiating potency of the part apart from the blastopore after 4-hour culturing.*

The results of the experiments so far described indicate that the notochordal differentiation from the parts *bU* and *cU* occurred with high frequency, only when these parts come to occupy the area immediately above the blastopore. As to the cause which enhances this potency during the course of gastrulation, we may surmise two possibilities; one is the mere aging of the tissue and the other is the influence from the surrounding tissues. In order to examine the first possibility, the part *bU at st. 11* located medial to the blastopore had been previously cultured in HOLTFRETER's solution for about 4 hours. During this period this part would shift to the area immediately above the blastopore, if it would be left in the intact embryo. Then, the pre-cultured material was wrapped with an incompetent ectodermal

Table 4. Differentiation from the part *bU* removed at st. 11 and pre-cultured in HOLTFRETER's solution for 4 hours.

No. of available cases	Notochord	Muscle	Mesenchyme	Undifferentiated mass
17	3 (18%)	2 (12%)	9 (53%)	17 (100%)

piece. The results are shown in Table 4. A comparison of the results in Table 4 with those in Table 2 indicates that the pre-culturing does not enhance the mesodermal differentiation, but suppresses it slightly. Consequently, it seems unlikely that a mere aging of the part is an active cause for the enhancement of the notochordal differentiation. The second possibility of the surrounding influence would tentatively be considered as the cause in question, though the direct evidence for it is lacking.

Discussion

The results of the present experiments have demonstrated that the presumptive area of notochord can differentiate into a definitive notochord in explantation, when it occupies the area immediately above the blastopore, but that the same material fails very often to develop into the notochordal structure, if it is taken out when it lies apart from the blastopore. Thus clearly there exists a difference in the differentiating potency of the presumptive notochordal material between when it is distal to the blastopore and when proximal to it. As mentioned already, the cause for this difference can not be attributed to a mere lapse of time required for the shifting of the area from the distal location to the proximal site. The unknown influence exerted from the surroundings may be taken into consideration to explain the present results. Recently, from the biochemical, histochemical and electron-microscopical studies a wealth of data has been accumulated that the dorsal part of gastrula is the most active site in proteolysis and protein synthesis (BRACHET, '50; KARASAKI, '58). Although no work from such an angle of study has ever been reported about any difference between such small areas as studied in the present experiment, the data presented by ROUND and FLICKINGER ('58) seem to be suggestive in relation to the present results. They found that RNA content of the residual fraction of the dorsal blastoporal area of *Rana*-gastrula decreases during the course of gastrulation. From this, it seems permissible to assume that the activities in the protein breakdown and in the new protein synthesis differ between the distal and proximal parts of the dorsal blastoporal region. These differences revealed in the biochemical level might reflect the difference of the notochord forming potency obtained by the present experiments.

Summary

(1) The developmental potencies of the different parts of the blastoporal lip

isolated from the gastrulae at various stages were tested by explanting each within an ectodermal envelope removed from the early neurula.

(2) At the same stage of gastrula, there were differences in the developmental potency between the parts distal and proximal to the blastoporal lip. Occasional production of the neural tissue occurred only from the distal part, while the notochord and muscle were frequently developed from the proximal part.

(3) When the differentiation of the notochord from the distal part took place, it was mainly accompanied with the simultaneous production of the neural tissue.

(4) The pre-culturing of the distal part in HOLTFRETER's solution for 4 hours which is equivalent to the time of shifting of this part to the area just above the blastopore in the course of gastrulation, could not enhance the differentiation of the mesodermal tissues.

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Explanation of Figures

Abbreviations: Bl, blood cells; Ms, muscular bundle; N, notochord; Nt, neural tube or tissue; U, undifferentiated mass of cells.

Fig. 1. Differentiation of notochord and small muscular bundles in the explant of the part *bU* at stage 11. — Fig. 2. Differentiation of notochord and blood cells in the explant of the part *cU* at stage 13c. — Fig. 3. Differentiation of notochord and muscle in the explant of the part *bU* at stage 12b. — Fig. 4. Differentiation of notochord, small neural tube and undifferentiated mass of cells in the explant of the part *bU* at stage 11. — Fig. 5. Differentiation of notochord and small fragment of neural tissue in the explant of the part *cU* at stage 12b. — Fig. 6. Undifferentiated mass of cells in the explant of the part *bU* at stage 11.

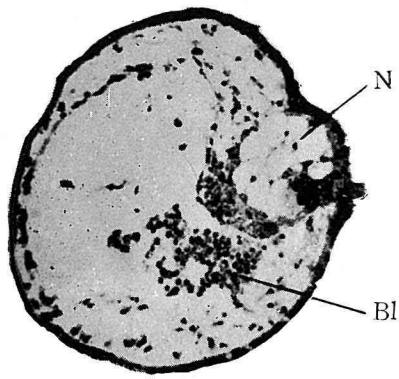

Fig.2

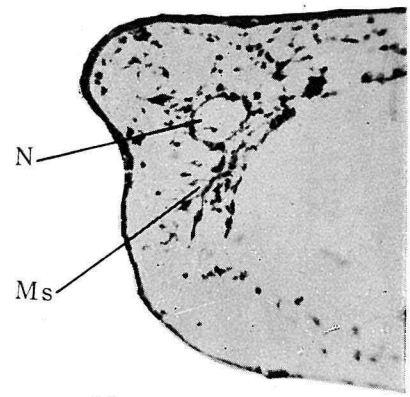

Fig.1

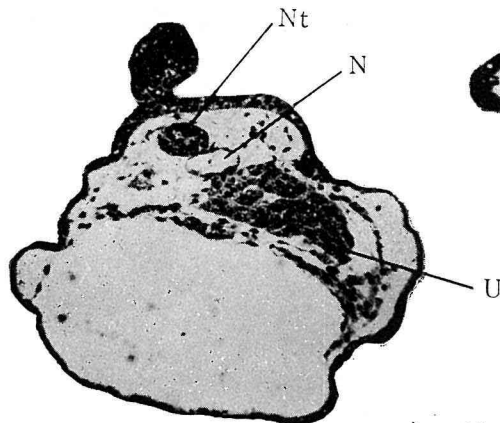

Fig4

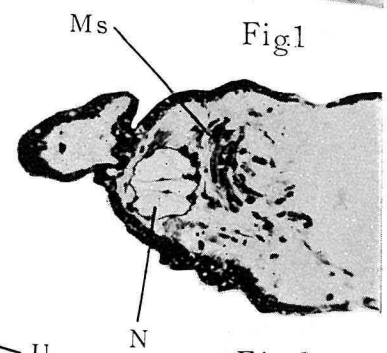

Fig 3

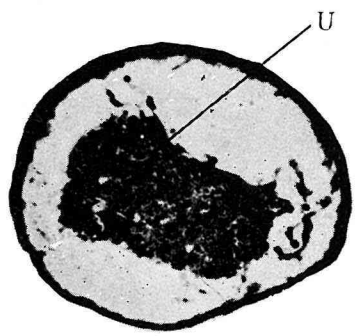

Fig.6

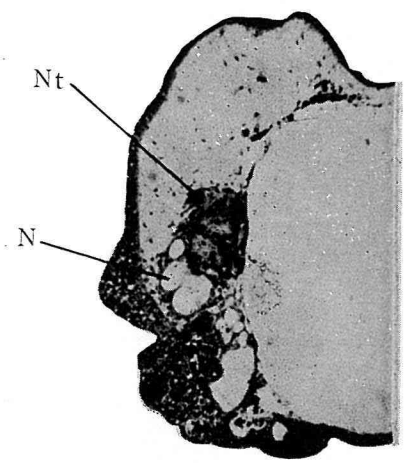

Fig.5