

A Mutual Relationship between the Explanted Piece
of Dorsal Blastoporal Lip and the Enveloping
Ectoderm in *Triturus*-Gastrula

By

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It has been well known that the pieces of dorsal blastoporal lip of amphibian gastrulae show differentiation into notochord, when cultivated *in vitro* with or without ectodermal envelope (Holtfreter, '36, '38a, '38b; Okada & Hama, '43, '45a). More recently, explanting the dorsal blastoporal lip taken out of varied stages of gastrulae with the ectodermal envelope, Takaya ('53) obtained always notochord even when the piece was removed from such uninvaginated part of dorsal lip of the earliest gastrula, that has no prospective material of notochord. On the other hand, the opposite result has been reported by one of the present authors (Okada, '54), i.e., notochord is never found to develop from the explant of dorsal lip of the earliest gastrula, endodermal structures and mesenchyme being encountered always. Discussing closely why the different results are obtained, we found that the reasons for it exist in the following two points; first, Takaya enveloped the piece of dorsal lip with the prospective ectoderm of early gastrula, while Okada did with the flap of belly ectoderm of neurula. The second is that the dorsal lip explanted by Takaya was isolated from the region of the prospective prechordal plate, although inclusion of some endodermal cells might be unavoidable, whereas Okada's piece was mainly obtained from the endodermal region. May we attribute the different results between us merely to the regional difference of the explanted piece? Or, does the stage difference of the ectoderm used as envelope cause the differentiation of the wrapped piece? To solve these questions the present experiments were performed.

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Materials and Methods

The materials used were gastrulae and neurulae of *Triturus pyrrhogaster*. The blastoporal lips to be explanted were taken from gastrulae in three different

stages, i.e., first from the earliest gastrula in which the blastopore just appeared as a pigmented groove (Okada & Ichikawa's stage 11), second from the gastrula with a semicircular blastopore (stage 12b) and third from the gastrula with a small yolk-plug (stage 13c). The dorsal lips of the latter two gastrulae were divided into uninvaginated and invaginated parts and each part was covered with a flap of ectoderm isolated either from a gastrula in stage 12a or 12b (the flap will be indicated as *G_{Ec}* in the following description), or from an early neurula in stage 16 (abbreviation: *N_{Ec}*). Locations and sizes of the explants are shown in the Fig. 1.

Explants were cultured for 10~15 days in Holtfreter's solution at a laboratory temperature.

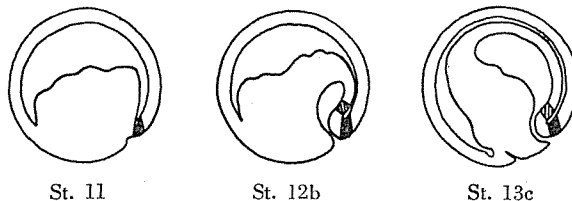


Fig. 1. Scheme of gastrulae showing the areas from which wrapped pieces were removed.

Experimental Results

Group I: *Explantation of Uninvaginated Part of the Dorsal Lip in St. 11* (abbreviation: *aU*)

Series aU wrapped with G_{Ec}: In most of 43 available cases, mesodermal structures such as notochord, muscle and mesenchyme were encountered (cf. Table 1). Among them, notochord was found in as many as 34 cases (79%, see Fig. 2). Muscular differentiation took place also in 26 cases (60%), but it is worthy to notice that the muscle occurred generally together with the notochord (Fig. 2). The metameric structure of muscle was found in 17 explants, while the other 9 cases contained a few muscle-bundles without segmentation. Mesenchyme was found in 23 cases (53%). Endoderm was met with in 21 specimens (49%) as an amorphous mass of heavily yolk-laden cells, and it showed hardly differentiation toward any definite structure. Presence or absence of the endoderm seemed to have nothing to do with the production of notochord and muscle.

All available explants contained the neural structures induced; i. e., the archencephalic with eye or nose was found in 16 specimens, the deuterocephalic with ear in 6, the spino-caudal in 8, both deuterocephalic and spino-caudal structures were contained in 7, and all of the three neural structures were shown in one explant. Besides, a small mass of neural tissue appeared in the remaining

5 specimens each, but it was difficult to say its regional character. Such unspecifiable neural mass is designated as "neural fragment" in the Table 1 and in the following description.

Of these neural structures induced, the spino-caudal differentiation occurred generally in the explants in which both notochord and segmented muscle developed. But, it was impossible from the present results to point out any particular tissue which would be in direct connection with the archencephalic or deuterencephalic induction.

Series aU wrapped with NEc: In this series, the frequencies in the occurrence of notochord and muscle showed a striking decrease against the previous series *aU wrapped with GEc*, i.e., notochord appeared only in 4 out of 41 available cases (10%) and muscle in 5 (12%). Muscle showed no segmentation. On the contrary, the enoderm was frequent occurrence (34 cases, 85%), although it persisted always as an undifferentiated mass (Fig. 3). The mesenchyme was found in 26 cases (63%).

As to the neural differentiation, a neural fragment was obtained only in 2

Table 1. Differentiation of the explants of the dorsal blastoporal lip in the envelope

Kind of wrapped piece		<i>aU</i>		<i>bI</i>		<i>bU</i>		<i>cI</i>		<i>cU</i>	
Kind of envelope		<i>GEc</i>	<i>NEc</i>	<i>GEc</i>	<i>NEc</i>	<i>GEc</i>	<i>NEc</i>	<i>GEc</i>	<i>NEc</i>	<i>GEc</i>	<i>NEc</i>
No. of available cases		43	41	31	26	25	27	5	5	9	5
Structures found within explants	Neural	Archencephalic	17 (40)	—	13 (42)	—	2 (8)	—	—	—	—
		Deuterencephalic	13 (30)	—	—	—	11 (44)	4 (80)	—	3 (33)	—
		Spino-caudal	15 (35)	—	—	—	16 (64)	4 (80)	—	9 (100)	—
		Fragmental	5 (12)	2 (5)	8 (26)	2 (8)	5 (20)	2 (8)	—	—	—
	Mesodermal	Notochordal	34 (79)	4 (10)	1 (3)	—	20 (80)	18 (67)	5 (100)	4 (80)	9 (100)
		Muscular	26 (60)	5 (12)	1 (3)	2 (8)	17 (68)	12 (44)	5 (100)	4 (80)	9 (100)
		Pronephric	—	—	—	—	2 (8)	1 (4)	—	1 (20)	1 (11)
		Mesenchymal	23 (53)	26 (63)	11 (35)	13 (50)	22 (88)	24 (89)	3 (60)	3 (60)	6 (67)
	Endodermal		21 (49)	34 (85)	28 (90)	25 (96)	6 (24)	19 (70)	2 (40)	2 (40)	—
										1 (20)	

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

cases (5%). The fact apparently indicates that the flank epidermis of neurula, which was employed as envelope in this series, has lost its neural competence almost completely.

The results of the two series of above experiment reveal that the differentiation of mesodermal structures from the explants taken out of the same source of blastoporal lip differs markedly according to the difference in stage of embryo from which the ectodermal envelope is taken out.

Group II. *Explantation of Invaginated Part of the Dorsal Lip in St. 12b (bI)*

Series bI wrapped with GEc: The origin of *bI* in this group nearly corresponds to the region that would be occupied by a piece *aU* in the previous series, if the latter was left in its normal site. Therefore, the prospective fate of *bI* is to coincide with that of *aU*. Nevertheless, the result of this series was largely different from that of series *aU wrapped with GEc*. Notochord and muscle were found developed only in one of 31 available explants (3%). Undifferentiated endodermal mass was met with in 28 cases (90%).

Neural induction occurred in 21 cases (68%), but its differentiation was archencephalic in 13 cases, and fragmental in 8 cases.

That the notochord-forming potency of the blastoporal lip of early gastrula is lost after its invagination, has been demonstrated by Hama ('49) and Takaya ('53) before us. Our results coincide with these authors'.

Series bI wrapped with NEc: Notochord and muscle were found only in 2 of 26 available explants (8%). Muscle did not show any sign of segmentation. Endoderm was contained in 25 specimens (96%), but its differentiation was undefinable at the time of fixation. Two explants had a neural fragment each, and one contained a free-lens.

In this group of *bI* explantation, the endodermal and mesodermal differentiations were nearly the same in both cases of *GEc* and *NEc*.

Group III. *Explantation of Uninvaginated Part of the Dorsal Lip in St. 12b (bU)*

Series bU wrapped with GEc: Frequent occurrence of notochord and muscle was met with in this series. Notochord occurred in 20 out of 25 explants (80%), muscle appeared in 17 cases (68%), generally accompanied with notochord and showing almost segmentation. Undefinable endodermal mass was found in 6 cases (24%).

Regarding the neural induction, the deuterencephalic and spino-caudal structures were the main products of this series. Four were the former structure, 9 the latter, and 7 both structures. In only 2 cases the archencephalic induction took place. Neural fragment alone was encountered in 5 cases.

Series bU wrapped with NEc: In this series notochord and muscle differentiated in many cases. Notochord was met with in 18 out of 27 available explants (67%). Of these explants containing notochord, 6 had a large quantity of segmented muscle and 6 a few muscle-bundles. The endodermal mass was present

in 19 cases (70%).

As to neural differentiation, we obtained the ear vesicle in 1 case, and neural fragment in 2 cases.

In this group of explantation of *bU*, the difference between the two series is small with respect to the mesodermal differentiation.

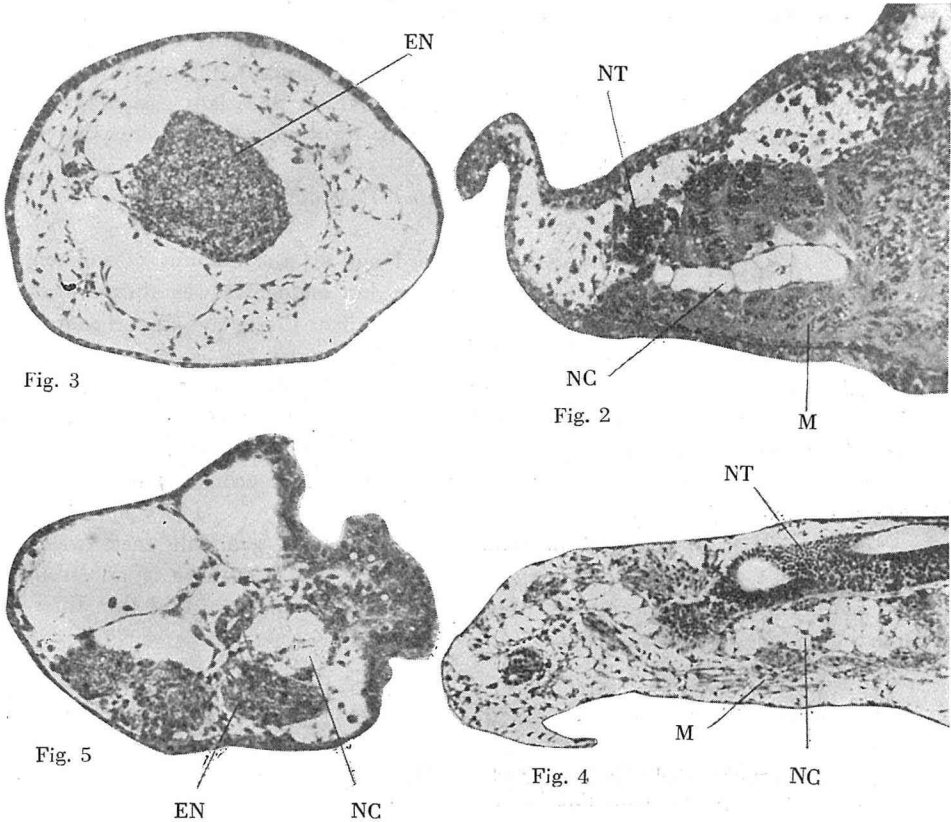


Fig. 2; Differentiation of notochord (NC), segmented muscle (M) and neural tube (NT) in the explant of *aU* with *GEc*.

Fig. 3; Presence of endoderm (EN) and mesenchyme in the explant of *aU* with *NEc*.

Fig. 4; Differentiation of notochord (NC), segmented muscles (M) and neural tube (NT) in the explant of *cI* with *GEc*.

Fig. 5; The explant of *cI* with *NEc*, showing notochord (NC) and endoderm (EN).

Group IV. *Explantation of Invaginated Part of the Dorsal Lip of St. 13c (cI)*

Series cI wrapped with GEc: The region from which piece *cI* was removed had been located just above the dorsal lip in the previous stage 12b, and re-

presented the piece *bU*. All 5 explants of this series had notochord and segmented muscle (Fig. 4). Endodermal mass was contained in 2 cases. Deuterencephalic or spino-caudal induction of neural tissues occurred in 4 specimens.

Series cI wrapped with NEc: Four out of 5 available specimens had notochord and muscle within (Fig. 5). The endodermal mass was encountered in 2 cases. All the explants of this series lacked the neural structure.

In the Explantation of *cI*, the difference of the enveloping ectoderm caused only a slight difference in the mesodermal and endodermal differentiations from the wrapped piece. The results indicate also that in the late gastrula the invaginated part of the dorsal lip has potency to form notochord, irrespective of the difference in the envelope.

Group V. *Explantation of Uninvaginated Part of the Dorsal Lip of St. 13c (cU)*

Series cU wrapped with GEc: Notochord and a large amount of segmented muscle were found in all 9 cases of this series, but endoderm was always absent. All explants showed spino-caudal induction, and in 3 of them, the deuterencephalic structure was also induced together with the spino-caudal.

Series cU wrapped with NEc: Four out of 5 available explants of this series included notochord and segmented muscle; the other one was so small that it contained only mesenchyme. A small mass of several undifferentiated endodermal cells was found in one specimen of the 4 just described. Neural induction did not occur in all cases.

In both series of the present group, the number of available cases was too small to discuss the results obtained. But, we may say that there is no essential difference between the experiments using *GEc* and *NEc* in regard to the differentiation of endoderm and mesoderm.

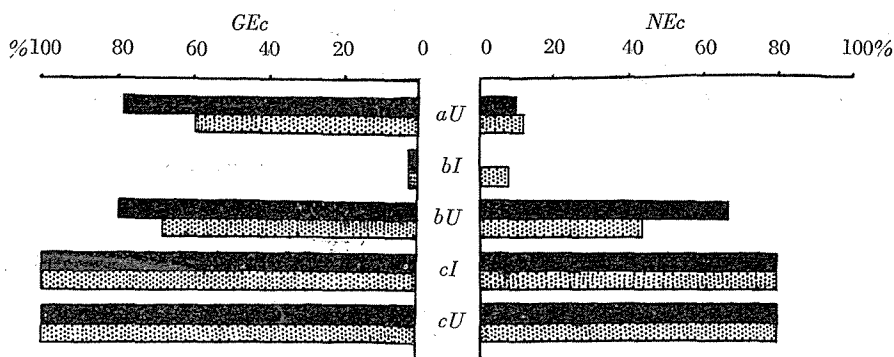


Fig. 6. Diagrammatic representation of the differentiation of notochord and muscle in the explants.
black: notochord, dotted: muscle

Discussion

That the blastoporal lip of every stage of gastrula before its invagination possesses notochord-forming potency has been reported by Takaya ('53). According to him, such a potency is not monopolized by prospective notochord region, but spreading over the uninvaginated prechordal area. The present results verified also that pieces taken out of the uninvaginated part of the dorsal lip in various gastrula stages, i. e., *aU*, *bU* or *cU*, can form notochord with very high frequencies (cf. Table 1 & Fig. 6).

But our stress must be laid on the fact that the frequencies of its production as well as of muscle differentiation were widely different according to the age of ectoderm used as the envelope of the explants, especially in the experimental series *aU* (cf. Table 1 & Fig. 6). When this piece was explanted in the envelope of gastrular ectoderm (*GEC*), notochord (79%) and muscle (60%) were formed frequently, whereas most of the explants enveloped with neurular ectoderm (*NEC*) lacked often these mesodermal structures. According to the precise observation by means of vital staining by Okada & Hama ('45b), our piece *aU* consists of prospective materials of foregut and prechordal plate. As, however, there exists no visible boundary between prospective prechordal and notochordal areas, a possibility of including some of notochordal material within the piece *aU* is not rejected. May we ascribe the notochordal formation in the explantation of *aU* solely to such material accidentally included within? If it were true, the frequencies in the notochordal production should be the same in both series *aU* wrapped with *GEC* and *NEC*. The fact lies far otherwise. Large difference in the notochordal frequencies between these two series makes us impossible to account for the source of notochord only from its own prospective material.

Concerning the source of notochords found in the experimental series *aU* wrapped with *GEC*, there remains still another question, i. e., whether they were derived from the envelope of *GEC* or from *aU* wrapped with it. That the prospective ectoderm of gastrula has potency to form notochord has been demonstrated by many workers. Especially, the notochords were often produced in the experiments in which some heterogenic inductors such as ventral skin of adult frog (Okada, '48), or kidney of guinea-pig (Toivonen, '40), were brought about into contact with gastrular ectoderm. In order to solve the above question, the piece *aU* stained previously with Nile-blue dye was explanted within the envelope of unstained *GEC* (unpublished). Microscopical observation of the serial sections of these explants revealed that the majority of cells of notochord and muscle kept blue dye, i. e., they were of *aU*-origin. The assumption of *aU*-origin of the notochord may be justified also by the fact that there were some cases in which notochord and muscle were present without accompanying endoderm and mesenchyme in the present series *aU* wrapped with *GEC*. The production of the latter two

tissues may be expected judging from the prospective fate of the piece *aU* if their developments are unable to switch over toward notochord and muscle.

To sum up, it may be safe to state that the endodermal and prechordal material locating at the dorsal margin of the blastopore just appeared has a potency to give rise to notochord and muscle, but the potency is simply realized, only when these materials are explanted within the envelopes of early gastrular ectoderm.

It is needless to say that neural induction always occurred, if *aU* was explanted within the competent ectodermal vesicle. Thus, it is open to doubt that "bedeutungsfremde" differentiation of notochord and muscle from piece *aU* is in some connection with the induction of neural structures from enveloping material. Closer analysis of the connection between these two processes was not carried out at present. But, in this connection the paper of Yamada ('39) may afford a clue for the explanation of our results, i. e., in his paper the analogous phenomenon to that described above is reported with respect to the differentiation of somite from its rudiment of *Triturus-neurula*. In his case the differentiation of somite from its own rudiment occurs only when the latter is explanted within the envelope of competent ectoderm and the neural induction takes place in the ectoderm.

Under these considerations, the present results may be explainable as follows: The development of the explant made of piece *aU* and competent ectoderm is affected each other by the reciprocal action of the two components. Namely, the envelope in which the neural differentiation is in progress through the inductive effect of wrapped *aU*, exerts in turn an influence upon *aU* so as to alter its fate toward the mesodermal differentiation.

Looking through the present data, we can note that in the explantation of the piece removed from the region of prospective notochordal material (*bU*, *cU*), the difference caused by the envelope of ectoderm in different stages is so slight that it is almost negligible. Differentiation of notochord was met with very frequently in these explants regardless of the age of envelope. The fact seems to indicate that the uninvaginated blastoporal lip of all stages of gastrulation has notochord-forming potency, but weak segregation of potencies begins already between prospective notochordal and non-notochordal areas.

This segregation may become definite when these areas constitute the archenteric roof. In the present experiments, the pieces of invaginated parts (*bI*, *cI*) showed generally differentiation along their prospective fates irrespective of the stage of envelopes used. The segregation of notochordal and non-notochordal materials after the invagination has been shown by the other authors (Hama, '49; Takaya, '53).

Summary

The small pieces taken out of the uninvaginated or invaginated part of the

dorsal lip of three stages of gastrulae were respectively explanted, after enveloped with either prospective ectoderm of early gastrula, or flank epidermis of neurula. Envelopes of different stages caused a difference in the differentiation from the wrapped piece of non-notochordal material of uninvaginated dorsal lip. When enveloped with the competent gastrular ectoderm, the piece gave rise to notochord and muscle, while it produced endoderm, when non-competent epidermis of neurula was used as the envelope. The same area, however, loses its notochord-forming potency after invagination.

The differentiation from prospective notochordal material was similar irrespective of the age of its envelope, and resulted in the production of notochord and muscle.

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