Studies on the Differentiating Potencies of the Dorsal Part of the Blastoporal Lip in Triturus-Gastrula (I) : Differentiation of Mesodermal Tissues in Relation to the Neural Tissue

AUTHOR(S):

Kato, Kenichi

CITATION:

Kato, Kenichi. Studies on the Differentiating Potencies of the Dorsal Part of the Blastoporal Lip in Triturus-Gastrula (I) : Differentiation of Mesodermal Tissues in Relation to the Neural Tissue. Memoirs of the College of Science, University of Kyoto. Series B 1958, 25(1): 1-10

ISSUE DATE:

1958-10-15

URL:

http://hdl.handle.net/2433/258517

MEMOIRS OF THE COLLEGE OF SCIENCE, UNIVERSITY OF KYOTO, SERIES B,
Vol. XXV, No. 1, Article 1 (Biology), 1958

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

I. Differentiation of Mesodermal Tissues in Relation to the Neural Tissue

By

Ken-ichi KATO

Zoological Institute, College of Science, University of Kyoto[1)]

(Received July 16, 1958)

A piece taken from the part anterior to the blastoporal lip in an early Amphibian gastrula can differentiate into the notochord and muscle in HOLTFRETER's solution (HOLTFRETER, '36, '38a, b). This appears to indicate that the part is already established certain predispositions for differentiating to notochordal and muscular tissues. But, HOLTFRETER ('38a) has shown that the piece consisting of the presumptive caudal somite and notochord, so far it is taken from the early gastrula, frequently produces a mere epidermis associated with or without connective tissue. Furthermore, the dorsal part of the early gastrula has been demonstrated to be still capable of differentiating various tissues such as neural and epidermal ones other than mesodermal derivatives (BRUNS, '31; LOPASCHOV, '35; TÖNDURY, '36; HOLTFRETER, '36). These findings show the high regulative power of the dorsal part of the blastoporal lip. In other words, the presumptive mesoderm dorsal to the blastoporal lip will require adequate conditions in order to differentiate into any definite tissues.

The present experiment was undertaken to know by means of explantation the precise distribution of these differentiating potencies in the dorsal part of the blastoporal lip.

Before going further, the author wishes to acknowledge his gratitude to Prof. Dr. M. ICHIKAWA, under whose direction and encouragement the present study was performed. He should like to express here his cordial thanks to Mr. N. IKUSHIMA and Mr. T. S. OKADA for their kind advice and criticisms, and also to Prof. Dr. T. SHIN-IKÉ of the Osaka Dental College for his kindness in the publication of this paper.

Material and Methods

As the material, the embryos of *Triturus pyrrhogaster* were used at various stages of gastrulation. Developmental stages of embryos were referred to OKADA and

1) Present address: Biological Institute, Osaka Dental College, Hirakata, Osaka Prefecture, Japan.

ICHIKAWA's standard table of the normal stages in the development of this newt.

The uninvaginated dorsal area of the blastoporal lip of the early gastrula at st.11 was divided into 3 parts as is shown in Fig. 1. The part just above to the blastoporal lip was designated as *aU*, the next as *bU*, and the most distal part as *cU*. The successive positions of these parts during the gastrulation and their presumptive fates were traced by applying the vital staining with Nile blue sulfate and neutral red[2]. The staining revealed that the proximal part *aU* occupies mainly the region of presumptive foregut and prechordal plate, and the middle one *bU* includes the region of notochord and sometimes even of a part of the prechordal plate. The distal part *cU* mainly spreads over the notochord and caudal somite. General sequence of shifting of these parts is shown diagrammatically in Fig. 1.

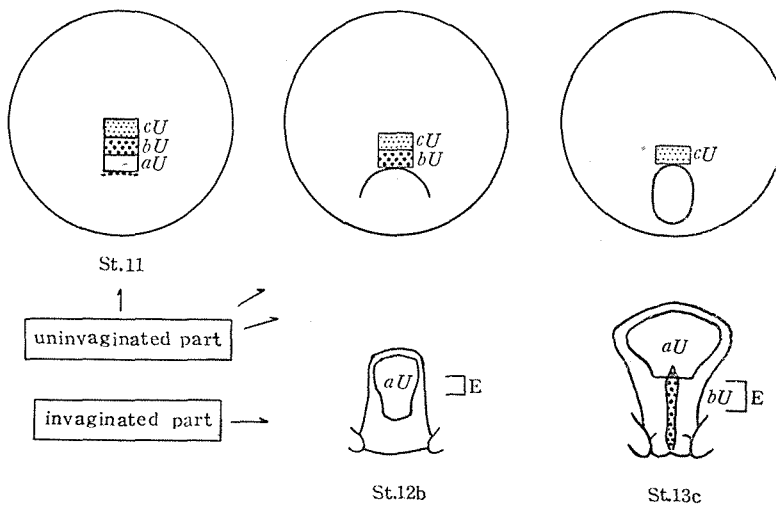

Fig. 1. Diagrammatic representation of the successive positions of the dorsal part of the blastopore during the course of gastrulation. Each part was cut out and wrapped with a piece of presumptive ectoderm from early gastrula. E in the lower figures of sts. 12b and 13c indicates the length of a piece to be isolated from the invaginated part.

Precisely speaking, however, the dimension of actually occupied region of the three parts during the course of development is wider than those given in this figure and differs a little from individual to individual. In general, when the gastrula advances to st. 12b, the part *aU* becomes invaginated, the parts *bU* and *cU* remain uninvaginated. When the embryo grows to st. 13c, a main part of *bU* has invaginated and a small part still remains outside as a lip-like thickening, while the part *cU* locates just dorsal to the blastoporal lip.

2) OKADA and HAMA ('45) investigated likewise on the fates of the dorsal parts of the blastoporal lip of the gastrula at various stages.

On isolating the piece to be explanted, every piece was made in approximately same size regardless of stages. Therefore, the part *bU* at st. *12b* or *13c*, for instance, could not cover completely the part corresponding to *bU* at previous stage. The piece isolated from the embryo at a given stage was wrapped with a piece of the presumptive ectoderm taken from the early gastrula (st.12a or st.12b). The explants were cultured for 10 to 15 days in sterilized HOLTFRETER's solution.

Experimental Results

Group I. *Explantation of Part aU*

Series aU at st. 11. A part *aU* at this stage is located in an uninvaginated area just anterior to the pigmented groove suggesting the place of the blastopore. As is given in the upper row of Table 1, the notochord was obtained in as many as 36 out of 47 available explants (77%), and it was frequently found either with the muscular tissue (27 cases), or with a mass of yolk-laden cells (14 cases). In the explants where the notochord appeared was found always the neural tissue, mostly deuterencephalic or spinocaudal. Notochord appeared usually in contact with such neural structures or with other undefinable neural fragment or mass, but it was not found near the archencephalic structure, especially in the vicinity of the eye and nose.

On the other hand, there were 11 cases in which the notochord failed to develop (23%). These explants except one lacked muscle. The test piece remained a mass or fragment of yolk-laden cells, or differentiated into mesenchymal cells containing many yolk-granules. In 9 cases neural formation occurred, but its regional character was all archencephalic, and in one case of them an undefinable fragment of neural tissue was induced in addition.

Table 1. Tissues differentiated in the explants of *aU*.

Notochordal	Muscular		Mesenchymal	Endodermal	Neural				
					archen-cephalic	deuteren-cephalic	spino-caudal	undefin-able	absent
Results of the explantation of *aU at st. 11*									
+36(77%)	metameric	18	10	4	1	10	14	2	
	non-metameric	9	7	5	5	2	2	2	
	absent	9	3	5	5	3	1	3	
−11(23%)	non-metameric	1	1	1				1	
	absent	10	4	10	8			1	2
Results of the explantation of *aU at st. 12b*									
+ 1(4%)	metameric	1	1					1	
−30(96%)	absent	30	10	28	13			7	10

Series aU at st. 12b. The part *aU* at this stage occupies the invaginated area. Occurrence of the notochord and muscle markedly decreased in comparison with the former series (only one out of 31 available explants, 3%). Mesenchyme was found in 8 cases (26%). In most cases in which neither notochord nor muscle appeared, the test material remained an amorphous mass of yolk-laden cells or it differentiated into some mesenchymal cells.

The regional character of the neural tissue induced in this series was archencephalic in 13 cases and undefinable in 7 cases. It must be noted here that an explant, in which the notochord was encountered, produced a small neural fragment in direct contact with the notochord.

The results of this Group I prove the following facts: (1) When the part *aU* is locating in the uninvaginated area of the blastoporal lip, it is able to produce the notochord and muscle, the former being more frequently than the latter, (2) it is a rule that the notochord appears together with the simultaneous production of the neural tissue in direct contact with the spinocaudal or deuterencephalic structure, but it never occurs near the archencephalic structure such as eye, nose or typical forebrain, and (3) when invaginated, the part *aU* becomes incapable of producing the mesodermal tissue such as notochord and muscle, but it aquires the specific inducing power of the archencephalic structure.

Group II. *Explantation of Part bU*

Series bU at st. 11. The part *bU* at this stage is situated in the area more distal to the blastoporal lip than the part *aU*. The results are presented in the upper row of Table 2. Notochordal differentiation took place in 22 out of 62 available specimens (35%), of which it was accompanied with muscle in 20 cases, with mesenchyme in 16 cases, and with a mass of yolk-laden cells in 12 cases. Neural induction occurred without exception in these cases: the spinocaudal structure appeared in 18 explants, the deuterencephalic one in 8 cases, and the undefinable neural mass or fragment in 6 specimens. The notochord, as was found in the explants of the first group, generally developed in the immediate vicinity of such neural structure. But, in a few cases a small piece of notochord appeared apart from the neural tissue. These small pieces had no notochordal sheath. The muscular differentiation was not always recognized in contact with the neural tissue.

The explants in which the notochord failed to develop were as many as 40 cases (65%), and almost all of them failed also to produce muscle (39 cases). The mesenchyme appeared sometimes, but a small mass of yolk-laden cells was present in all cases, except one that showed the so-called atypical epidermal structure. Neural tissue was sometimes found (24 cases) mainly as an undefinable fragment (21 cases).

In this series, one interesting point with respect to the mesenchymal differentiation was noted in relation to the presence or absence of the neural tissue; i. e., the mesenchymal cells appeared more often when the neural tissue was present than when absent, as is shown below.

Table 2. Tissues differentiated in the explants of *bU*.

Notochordal	Muscular		Mesenchymal	Endodermal	Neural				
					archen-cephalic	deuteren-cephalic	spino-caudal	undefin-able	absent
Results of the explantation of *bU at st. 11*									
+22 (35%)	metameric	15	13	8		8	13	4	
	non-metameric	5	3	2		2	3	1	
	absent	2	1	2			2	1	
−40 (65%)	non-metameric	1	1	1				1	
	absent	39	12	38		3		21	16
Results of the explantation of *bU at st. 12b*									
+25 (83%)	metameric	20	19	3	1	9	14	6	
	non-metameric	3	3	1		1	2	1	
	absent	2	2			2	1	1	
−15 (17%)	metameric	1	1	1		1			
	absent	4	3	3	2	1		1	1
Results of the explantation of *bU at st. 13c*									
+5 (100%)	metameric	3	1	2		2	3		
	non-metameric	2		1		2	1		

(a) Number of explants in which the mesenchymal cells and the neural tissue appeared simultaneously ..... 25 out of 46 cases (54%).

(b) Number of explants in which the mesenchymal cells appeared without being accompanied with the neural differentiation.....4 out of 16 cases (25%).

Series bU at st. 12b. The part *bU* arrives at the uninvaginated area above the blastoporal lip. In this series of explantation, the notochord developed also in contact with the neural tissue. The neural tissue was spinocaudal, deuterencephalic or undefinable according to case. Frequency of the notochordal formation was found as high as 83% (25 out of 30 available cases, cf. the middle row of Table 2). In 25 cases in which the notochord appeared, the muscle and mesenchyme appeared also very frequently (the former in 23, the latter in 24). A mass of yolk-laden cells, however, was encountered with only in one case, and a well-developed pharyngeal tissue in 3 cases.

In five specimens containing no notochord, the muscular tissue developed in one, but the mesenchyme in 4 cases. Neural tissue was met with in 4 explants, 2 of which showed the archencephalic structure, remaining one was the deuterencephalic, and still other remaining one the small neural fragment (cf. Table 2).

Series bU at st. 13c. This part occupies the most caudal area of the archenteric roof at this stage. The number of available cases was a few, but all of 5 explants showed the production of notochord (cf. the lower row of Table 2). Muscle was also

found in all cases, but the mesenchyme occurred only in one case. Undefinable small fragment of endodermal cells appeared in 3 cases. All specimens contained either the deuterencephalic or the spinocaudal structure which occurred in contact with the notochord.

Group III. *Explantation of Part cU*

Series cU at st. 11. This part is located next to the presumptive neural plate, and most distal to the blastoporal lip. The results of this series are shown in the upper row of Table 3. Only one out of 35 available cases possessed a notochord (3%), and at a side of it a small neural tissue was induced and at an opposite side a small mass of yolk-laden cells was present.

Remaining 34 explants had neither notochord nor muscle, many of them differentiated into an atypical epidermis (25 cases). In 3 cases, the mesenchymal cells and a small piece of neural tissue were found. The regionality of the latter was the spinocaudal, deuterencephalic or undefinable structure. A mass of yolk-laden cells was found in 9 cases.

Table 3. Tissues differentiated in the explants of *cU*.

Notochordal	Muscular		Mesenchymal	Endodermal	Neural			
					deuteren-cephalic	spino-caudal	undefin-able	absent
Results of the explantation of *cU at st. 11*								
+ 1 (3%)	absent	1		1				
−34 (97%)	absent	34	3	9	1	2	1	31
Results of the explantation of *cU at st. 12b*								
+13 (59%)	metameric	5	4	2		4	1	
	non-metameric	5	2	3		3	3	
	absent	3		2			2	1
− 9 (41%)	absent	9	2	8	2	1	2	4
Results of the explantation of *cU at st. 13c*								
+ 9 (100%)	metameric	9	6		3	9		

Series cU at st. 12b. The part *cU* is situated in the uninvaginated area and somewhat away from the blastoporal lip. Thirteen out of 22 available explants included the notochord (59%) together with or without the muscular tissue. Mesenchyme appeared in 6 and a mass of yolk-laden cells in 7 isolates. The 13 cases also showed mostly the neural production such as spinocaudal structure and/or undefinable fragment (12 cases).

In 9 cases, the notochord and muscle did not occur, but a mass of yolk-laden

cells appeared in 8 of them. Neural induction took place in 3 out of the 8 cases (cf. the middle row of Table 3).

In this series, a tendency was also recognized that the mesenchyme appeared more frequently in the explants containing neural tissue than in those without neural tissue. That is, the mesenchyme was found in 7 out of 17 specimens in which the neural tissue was produced (41%), while it was met with only in one case among 4 explants without neural tissue (20%).

Series cU at st. 13c. This part becomes located just above the blastoporal lip. As is given in the lower row of Table 3, all of 9 specimens showed the occurrence of the notochord and segmented muscle, followed by the differentiation of the neural tissue of spinocaudal structure. Mesenchyme was found in 6 cases (67%). A mass of yolk-laden cells was not encountered in any cases.

Main results obtained in the experiments of Groups II and III are as follows: (1) the notochord and muscle occurred more frequently in the proximal explants than in the distal ones, (2) the same tendency was also found in the frequency of the neural induction, (3) the muscular differentiation took place as a rule in the presence of notochord and (4) the mesenchymal cells could differentiate more often in the presence of neural tissue than in the absence of it.

Discussion

Since SPEMANN's excellent experiments, it has been taken for granted that the dorsal part of the blastoporal lip of Amphibian gastrula is in a self-differentiating system in the sense that it can form by itself the mesodermal tissues such as notochord and muscle. The results of the present experiments confirm the above situation. But, it is found that the explants are removed the more proximal to the blastopore, the more often the production of notochord and muscle occurs, regardless of the developmental stages. In other words, the frequency of notochordal and muscular occurrence increases gradually as the origin of the explants shifts to the blastopore. This leads us to the conclusion that the potency to form notochord and muscle becomes more and more definitive during the course of gastrulation. However, it does not imply that the potency of a given part is restricted to its presumptive fate, because the part *aU at st.11* shows higher percentage of the production of notochord and muscle, although its presumptive fate is to form a prechordal plate. What kind of factor or factors does operate in strengthening of this potency? Although the present results are not enough to account for this question, some findings obtained seem to allow in some measure an assumption concerning the conditions under which the development of notochord, muscle and mesenchyme occurs.

1. *Notochordal differentiation*: In our previous experiments (KATO & OKADA, '56; KATO, '57), it was a rule that the piece taken from the dorsal part, corresponding to the part *aU at st.11* in the present experiment, was able to differentiate into notochord only in the presence of differentiating neural tissue. In the present

experiment, not only a part *aU* but also the various parts of the dorsal lip of the blastopore were examined (cf. Fig. 1). Nevertheless, the results of all series of the experiments are in good agreement with those of the previous experiments; that is, the notochord developed, regardless of the source of explants, in all explants in which the neural tissue was produced, with only one exception. But, in some explants the neural tissue was induced, but neither notochord nor muscle appeared. It should be mentioned, however, that this failure of the mesodermal occurrence was limited to the case in which the implanted piece was situated apart from the neural tissue induced. It was a rule that, when the notochord was found, it occurred in contact with the neural tissue. These results suggest two possibilities; one is that the differentiating neural tissue would exert some influence to make the piece differentiate towards the notochord and the other is that it would reinforce the notochord forming potency of the piece. The latter alternative seems likely.

Furthermore, the present results showed that in the specimens in which the archencephalic structure was induced the notochord developed less frequently, whereas in the case in which the deuterencephalic or spinocaudal one was produced, the notochord occurred more often. This phenomenon was especially marked in Group I. The part *aU* in this group changes, in the course of invagination, its neural structure inducing power from the dueterencephalic or spinocaudal to the archencephalic, and loses at the same time its notochord forming potency. A doubt may arise whether the archencephalic structure prevents the piece from developing towards notochord. In relation to this point, the previous experiment (KATO, '57) provides the answer. It revealed that when the part *aU at st.11*, referring to the terminology in this paper, was explanted together with a small piece of the neural tissue taken from either the anterior or the middle part of the plate of neurula, no marked difference was encountered in the percentage of the notochordal occurrence according to the regional difference of the added neural tissue. But, when the notochord developed in the explant of the former combination in which the archencephalic structure was expected, it appeared always only in contact with the undefinable neural mass, and away from the archencephalic structures such as eye and nose if they were produced. It is likely that even the anterior part of the neural plate would exert the same influence to the piece so as to differentiate into the notochord, so long as the part could not develop to the archencephalic structure and remained an undefinable mass.

2. *Muscular differentiation*: In general, well-developed muscular tissue was obtained in the explants in which both of the notochord and neural tissue differentiated. In contrast to this, in the absence of these tissues, the muscle, if any, was very small and very poor in its differentiation. That the muscular differentiation is dependent upon the notochordal differentiation has already been demonstrated by MURATORI ('39), YAMADA ('40), MUCHMORE ('51) and TAKAYA ('56). The effect of the neural tissue upon the muscular differentiation has also been reported by YAMADA ('39), although MUCHMORE ('51) denied such effect of the neural plate

upon the differentiation of the muscle. It seems likely that the production of notochord plus neural tissue is more effective for the muscular differentiation than the production of each alone. But it is difficult to estimate which of the notochord and neural tissue is more responsible for the muscular differentiation from the dorsal part, for the cases were very scarce in which the muscular differentiation took place together with either of them. This point will be a future problem to be examined.

3. *Mesenchymal differentiation*: As was found in the series *bU at st.11* of Group II and *cU at st.12b* of Group III, the mesenchyme differentiated more frequently in the presence of the neural tissue than in the absence of it, having nothing to do with the presence or absence of the mesodermal tissues such as notochord and muscle. According to NIEUWKOOP and NIGTEVECHT ('54) and HORI and NIEUWKOOP ('54), the neural crest derivatives including mesenchyme appear in the case in which rhombencephalic structure is induced. In the present experiment, however, the mesenchyme was found without marked difference in its frequency, regardless of whether the rhombencephalic structure is present or not. The explanted piece would have an ability to form the mesenchymal cells by itself, and the neural structure would cause the realization of this ability.

Judging from the above inspections we can safely assume that the production of neural tissue may act as the realizing factor for the differentiation of mesodermal tissue. In order to ascertain this point more closely, the culturing of the dorsal part may be necessary under the conditions in which the neural induction is suppressed. The forthcoming paper will be concerned with this problem.

Summary

(1) The dorsal part of the blastoporal lip of *Triturus*-gastrula at various stages was divided into three parts. They were wrapped with an ectodermal piece of young gastrula, and cultured in HOLTFRETER's solution.

(2) When the explants were taken from the part located in the uninvaginated area, the mesodermal tissues such as notochord and muscle produced, but the frequency of their production was much higher in the explants from the proximal part than in those from the distal one, regardless of the stages. Thus it is concluded that the potency to form the mesodermal tissues increases as the origin of the test piece shifts towards the blastoporal lip.

(3) These mesodermal tissues were often encountered in the explants in which the neural tissue occurred, whereas they were hardly recognized in the explants in which the neural tissue was not induced. Consequently, it is assumed that the production of the neural tissue may offer a favourable factor for the realization of the potency to form the mesodermal tissues from the dorsal part of blastoporal lip.

References

BRUNS, E., 1931. Roux' Arch., **123**: 682–718.
HOLTFRETER, J., 1936. Ibid., **134**: 466–550.
——— 1938a. Ibid., **138**: 522–656.
——— 1938b. Ibid., **138**: 657–738.
HORI, R. & P. D. NIEUWKOOP, 1954. Proc. Kon. Ned. Akad. v. Wet. A'dem, (C), **58**: 265–272.
KATO, K., 1957. Mem. Coll. Sci. Univ. Kyoto (B), **24**: 165–170.
——— & T. S. OKADA, 1956. Ibid., **23**: 1–9.
LOPASCHOV, G. V., 1935. Zool. Jb. Abt. Allg. Zool. u. Physiol., **54**: 299–312.
MAYER, B., 1935. Roux' Arch., **133**: 518–581.
MUCHMORE, W. B., 1951. J. Exp. Zool., **118**: 137–186.
MURATORI, G., 1939. Verh. Anat. Ges. Ergh. Anat. Anz., **87**: 430–435.
NIEUWKOOP, P. D. & G. V. NIGTEVECHT, 1954. J. Embryol. Exp. Morphol., **2**: 175–193.
OKADA, Y. K. & T. HAMA, 1945. Proc. Jap. Acad., **21**: 341–348.
——— & M. ICHIKAWA, 1947. Jap. J. Exp. Morphol., **3**: 1–6.
TAKAYA, H., 1956. Annot. Zool. Japon., **29**: 133–137.
TÖNDURY, G., 1936. Roux' Arch., **134**: 1–111.
YAMADA, T., 1939. Fol. Anat. Jap., **18**: 569–572.
——— 1940. Ibid., **19**: 131–197.