The Effect of Aging on the Neural Competence of the Presumptive Ectoderm and the Effect of Aged Ectoderm on the Differentiation of the Trunk Organizer in \textit{Cynops pyrrhogaster}

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Summary. The effect of aging on the neural competence of the presumptive ectoderm of the early gastrula, and the effect of aged ectoderm on the differentiation of the still uninvaginated dorsal blastoporal lip at the small yolk-plug stage – representing the trunk organizer – were examined by the sandwich method in \textit{Cynops pyrrhogaster}.

The presumptive ectoderm to be used as reaction system was taken from 0 to 36 h exogastrulae obtained by operation at the early gastrula stage and combined with trunk organizer. In the 0 to 12 h explants typical trunk-tail structures were formed. With further aging of the presumptive ectoderm a decrease in frequency of spinal cord, notochord, and muscle and a simultaneous increase in frequency of mesenchyme and mesothelium were observed. In the 30 and 36 h explants neural competence had largely disappeared, the frequency of notochord and muscle become very low and their differentiation very poor, whereas the frequency of mesenchyme and mesothelium reached very high levels.

We infer a reciprocal relationship between the induced spinal cord and the differentiation of notochord and muscle, as well as a transformation of notochordal material into mesenchyme and mesothelium under the influence of the aged ectoderm. The mode of action of the trunk organizer in normal development is discussed.

Key words: Aging – Neural competence – Trunk organizer – \textit{Cynops pyrrhogaster}.

Introduction

The trunk organizer has been defined as that region of the dorsal marginal zone which differentiates into notochord and somites and induces spinal cord

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when (1) transplanted into the presumptive ectodermal region of the early gastrula (Spemann, 1931; Okada and Takaya, 1942a, b), (2) inserted into the blastocoel (Spemann, 1931), or (3) wrapped in presumptive early gastrula ectoderm (Okada and Hama, 1943). It should however be realized that in normal development the trunk organizer exerts its influence upon the ectoderm in the middle to late gastrula and in even later stages.

Holtfreter (1938) reported that presumptive ectoderm explanted for various lengths of time in Holtfreter solution gradually loses its neural competence as tested by transplantation to the dorsal region of the early neurula.

Kato and Okada (1956) found that the uninvaginated dorsal lip of early Cynops gastrulae differentiated into notochord when wrapped in early gastrula ectoderm, but did not do so when wrapped in neurula flank epidermis. The uninvaginated dorsal lip of the small yolk-plug stage differentiated into notochord and somites irrespective of the age of the investing ectoderm. These experiments suggest that in normal development the trunk organizer is established only at the small yolk-plug stage.

The question remains open of how the trunk organizer functions in normal development. This paper deals with the change in neural competence of the ectoderm aged in exogastrulae and with the effect of this ectoderm on the differentiation of the trunk organizer.

Material and Methods

Gastrulae of Cynops pyrrhogaster (egg diameter: 2.3–2.4 mm) were used throughout. The culture medium was Holtfreter solution (pH 7.6) with 40 I. U./ml potassium penicillin G and 0.03 mg/ml streptomycin sulphate. Development took place at 20° C.

Care was taken that aging of the presumptive ectoderm took place in the embryo as a whole and that the aging ectoderm showed a smooth surface during the entire aging period. This was achieved by the following exogastrulation method. Just before gastrulation (0 h embryo) a transverse cut was made 0.4–0.5 mm away from the blastoporal rim and the inner yolk cell mass was pulled out through the slit (Fig. 1). In this way complete exogastrulae were obtained; the presumptive ectoderm maintained a smooth surface for 36 h after the operation and neither mesodermal nor endodermal cells were found in the blastocoel. Exogastrulation in hyperionic solution as described by Holtfreter (1933) was unsatisfactory, since complete exogastrulation occurred only in 40% of the cases.

Fig. 1. Method to produce exogastrulae. A transverse cut is made 0.4–0.5 mm above the blastoporal rim of the incipient gastrula and the inner yolk cell mass is pulled out from the slit. Dashed line indicates the future blastopore