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# Developmental potencies of area opaca and marginal zone areas of early chick blastoderms

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SUMMARY

The marginal zone, in pregastrulating chick blastoderms, has been defined as the intermediate ring between the epiblast proper and the most external region, the area opaca (Spratt & Haas, 1960). Azar & Eyal-Giladi (1979) have shown that the marginal zone of a stage XIII blastoderm has the capacity of regenerating an inductive layer which when in contact with a competent stage XIII epiblast can cause the formation of axial structures.

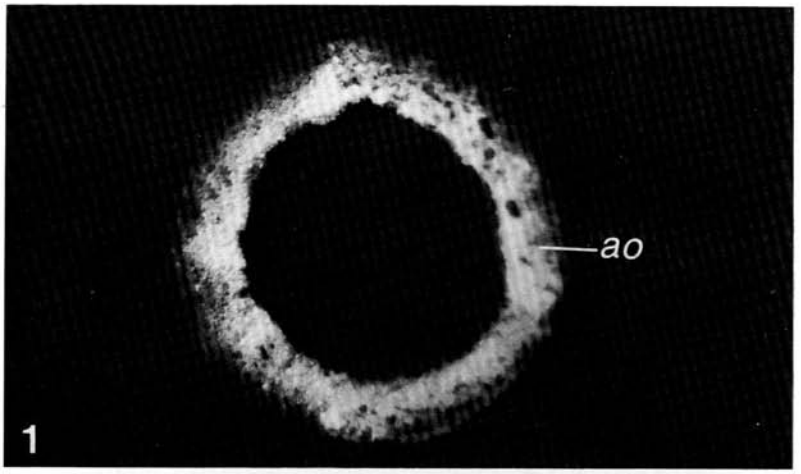
The present work demonstrates that at stage X (Eyal-Giladi & Kochav, 1976) the marginal zone can both induce and respond to its own inductive stimulus by forming an embryonic axis. By stage XIII the marginal zone seems to have lost its competence to respond to an inductive stimulus and cannot form an embryonic axis. It is further shown that at stages X–XIII the area opaca is neither competent to develop any sort of axial structures nor has the capacity to generate an inductive layer.

## INTRODUCTION

At stage X (Eyal-Giladi & Kochav, 1976) the chick blastoderm is composed essentially of one layer of cells, the area pellucida which is surrounded peripherally by a ring of denser looking cells the area opaca. For reasons that will become clearer at stage XIII, the area pellucida (ap) can arbitrarily be subdivided into a peripheral ring, the marginal zone, and a central disk of area pellucida. At stage XIII the blastoderm becomes a two layer system as an additional hypoblastic layer forms on the lower side of the central ap disk. The peripheral region of the area pellucida region which is not covered by the hypoblast is thus defined as the marginal zone. It is known that all embryonic structures develop from the epiblast (Vakaet, 1962; Rosenquist, 1966), and that the hypoblast induces and determines the orientation of the primitive streak (Waddington, 1932; Azar & Eyal-Giladi, 1981; Mitrani & Eyal-Giladi, 1981). Azar & Eyal-Giladi (1979) claimed that the inductivity of the hypoblast is probably dependent on a cellular contribution from the marginal zone.

In the present study we examine the developmental potencies of both the area opaca and the marginal zone areas of chick blastoderms of stages X–XIII. We show that the marginal zone at stage X is capable of generating both an inductive

*Key words*: induction, marginal zone, totipotency, hypoblast, chick embryo.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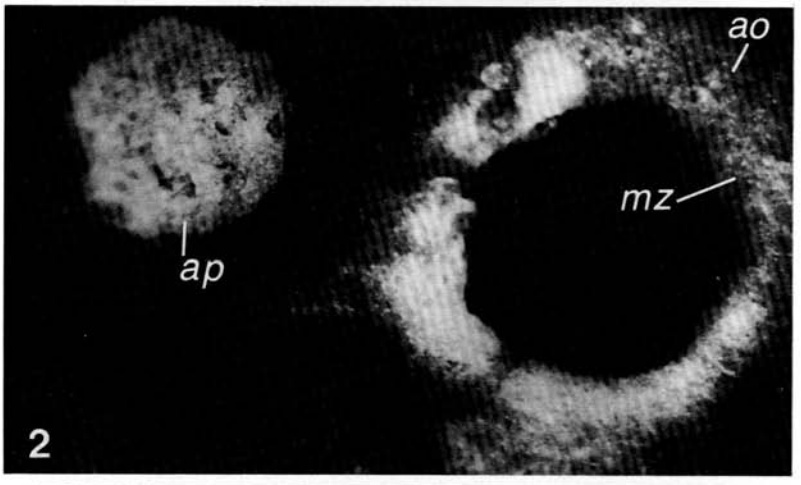
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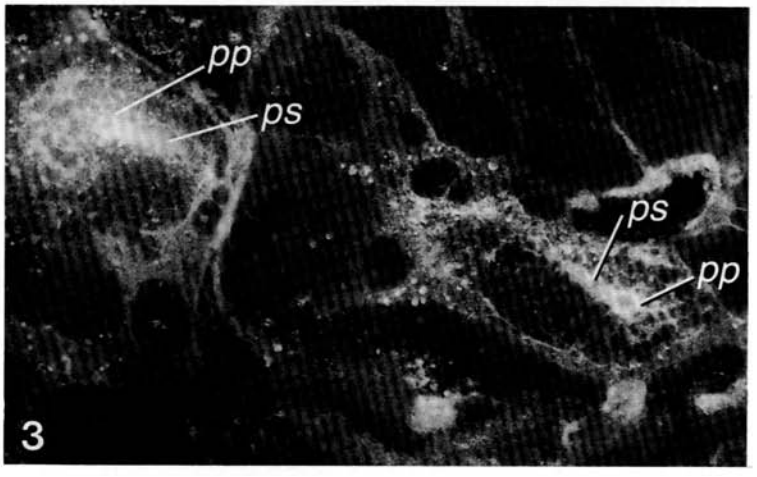
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element and a competent layer. This competent layer can respond to the inductive element by generating an embryonic axis. By stage XIII the marginal zone seems to have lost the competence to respond even to the inductive action exerted by a normal hypoblast. No developmental potential was detected in area opaca regions derived from either stage X or stage XIII blastoderms, with or without the influence of a normal stage XIII hypoblast.

## MATERIALS AND METHODS

Blastoderms were obtained from White Leghorn or hybrid New Hampshire × Leghorn eggs and were removed into Ringer's solution either at stage X or at stage XIII (Eyal-Giladi & Kochav, 1976). Only those blastoderms whose posterior side could be clearly marked, were used. The posterior side was marked with carbon. The various microdissections were performed using sharp tungsten needles. Blastoderms were grown on vitelline membranes using the New (1955) technique. All explants were grown on semisolid albumin at 37 °C for a period of 48 h.

### *Stage X*

#### *Area opaca explants*

The blastoderms were removed from the egg and the area pellucida was separated by a circular cut from the area opaca and removed. A peripheral narrow ring containing only area opaca cells, was either cultured whole or an incision was made at its anterior end so that the central hole could be made smaller by pushing both sides together and then removing the excess tissue derived from the two anteriolateral aspects of the area opaca. In some experiments, a hypoblast was removed with fine needles from a normal stage XIII blastoderm and layered onto the area opaca explant so that the posterior side of the hypoblast would coincide with the posterior side of the area opaca.

#### *Explants of the marginal zone plus the area opaca*

Explants were grown as indicated above. The blastoderms were operated so that only the central part of the area pellucida, corresponding to the epiblast proper, was removed leaving a wider ring containing the area opaca plus the marginal zone. As a control, in some experiments, the central epiblastic area was cultured as well next to the explant on the same vitelline membrane (see Figs 1, 2).

### *Stage XIII*

#### *Explants of the area opaca*

Area opaca of stage XIII blastoderms were prepared and cultured as for area opaca from stage X blastoderms. In some experiments a normal stage XIII hypoblast was also added to the explant as described above.

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Fig. 1. Stage X blastoderm whose central area pellucida has been surgically removed and the area opaca (*ao*) is ready to be incubated on a vitelline membrane (×25).

Fig. 2. Stage X blastoderm whose central area has been cut from the area pellucida and both explants: area opaca (*ao*) + marginal zone (*mz*) and central area pellucida (*ap*) are ready to be incubated on a vitelline membrane (×25).

Fig. 3. Stage X blastoderm whose central area has been removed from the area pellucida as shown in Fig. 2, and both explants have been cultured on the same membrane for 24 h. An axis can be seen developing from each of the explants (×25). *ps*, primitive streak; *pp*, primitive pit within Hensen's node.

*Explants of the marginal zone*

Marginal zone areas of stage XIII blastoderms were prepared and cultured as for marginal zone derived from stage X blastoderms, the only difference was that the removed central part was already a two-layer system containing epiblast and hypoblast. Due to the presence of the hypoblast, the boundaries of the marginal zone could be easily delineated. In some experiments a normal stage XIII hypoblast was overlaid onto the explant so that the posterior hypoblast aspect would overlay the posterior aspect of the marginal zone.

*Explants of the central region*

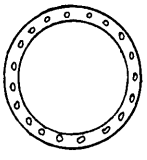
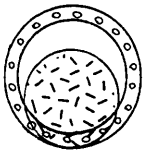
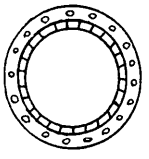
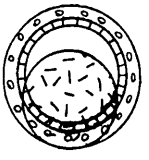
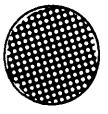
Explants of central regions from stage XIII blastoderms were prepared by removing the hypoblast so that only the central epiblastic region was cultured further.

## RESULTS

Results are summarized in Table 1. We found that the marginal zone at stage X is capable of generating both an inductive element and a competent layer which responds to the inductive element by generating an embryonic axis (see Fig. 3). By stage XIII the marginal zone seems to have lost the competence even to respond to the inductive action exerted by a normal hypoblast. We were unable to detect any developmental potential from area opaca regions derived either from stage X or stage XIII blastoderms.

In all cases in which embryonic axes were observed the degree of development was generally restricted to the formation of a primitive streak and in some cases a structure resembling a headfold stage was obtained.

Table 1. *Percentage of cases in which embryonic axes developed from each of the different experimental procedures*

| Stage of development | Area opaca | | Area opaca and marginal zone | | Central area |
|---|---|---|---|---|---|
| | | Hypoblast added* | | Hypoblast added* | |
| |  |  |  |  |  |
| X | 0 % (9) | 0 % (17) | 85 % (18)<br>39 %† (18) | –<br>– | 55 % (18)<br>39 %† (18) |
| XIII | 0 % (4) | 0 % (7) | 0 % (13) | 0 %(7) | 0 % (6) |

Number in parenthesis indicate number of experiments.
*A normal stage XIII hypoblast was layered onto the experimental explant.
† Cases in which axes were obtained both in the marginal zone explant and in the central area.

## DISCUSSION

Azar & Eyal-Giladi (1979) suggested that the cells of the primary hypoblast which are involved in the anteriorly directed growth from Koller's sickle (Vackaet, 1962, 1970; Spratt & Haas, 1965; Eyal-Giladi & Kochav, 1976) are the inductive component of the hypoblast and that they probably originate from the marginal zone. Recently Khaner & Eyal-Giladi (1984) showed that a small piece of stage X posterior marginal zone cells when transplanted to a different location in the marginal zone, can cause the development of an axis at their new position. On the other hand, Mitrani (1984) has shown that single cells derived from normal primary early chick embryonic cells can form colonies in soft agar in the absence of endogenous growth factors. At preprimitive stages the capacity to form colonies in soft agar is restricted essentially to the area opaca and the marginal zone areas (Einhorn & Mitrani, submitted). Colony-forming ability is considered the best *in vitro* correlate of cell tumorigenicity (Shin, Freedman, Risser & Pollack, 1975). Because of the 'undifferentiated' nature of tumour cells we were interested to see whether, in the embryo, the capacity of cells to form colonies in soft agar is in some way related to their developmental potential. We therefore decided to further examine the developmental potencies of both peripheral areas namely the marginal zone and area opaca separately at stages X–XIII as well as that of the central part of the epiblast.

At stage X a technical problem arises when determining the border between the marginal zone and the epiblast proper. Whilst the boundary between the area opaca and the marginal zone is clear, that between the marginal zone and the central area is somewhat arbitrary. We have attempted to circumvent this difficulty by cultivating the two explants together. The fact that both the explant of the central disk and the explant containing the marginal zone developed embryonic axes indicates that the results cannot be explained on the basis of having included too much of the central area when culturing the marginal zone or *vice versa*. Preliminary experiments (not shown) indicated that a smaller central area lacks the capacity of generating axial structures. The central area at stage X was known to have the capacity to form primitive-streak-inducing elements (Eyal-Giladi & Spratt, 1965). This capacity is then lost from the central area and remains only in the marginal zone at stage XIII (Azar & Eyal-Giladi, 1979). The present results show, conversely, that the marginal zone at stage X is also competent to generate an axis but it loses this capacity by stage XIII.

Spratt & Haas (1960) examined amongst other things the regulative potential of radially symmetrical parts consisting of area pellucida and area opaca cells. For one group of experiments (group A Table 9 of Spratt & Haas, 1960) the blastoderms were divided into a central disk containing 65% of the area pellucida and an external ring containing the area opaca and 35% of the area pellucida. From ten experiments only four developed embryos from the central region. In no case did axial structures develop from peripheral rings. It is at first difficult to explain why in such experiments no axial structures developed from the marginal-

zone-containing fragment. One can perhaps assume that those experiments are equivalent to the marginal zone experiments described above in terms of the size of the areas considered. There is however the problem of comparing the stages at which the experiments were performed. It is now clear that what Spratt & Haas then called 'unincubated' is now being acknowledged as stage XII–XIII (Eyal-Giladi & Kochav, 1976). It is therefore no surprise that they did not get any axial development from the peripheral 'marginal zone'-containing area. Their results are in agreement with our own data (not shown) which indicate that by stage XII the marginal zone has lost its potency to generate a competent epiblast. Lutz, Departout, Hubert & Pieau (1963) working with unincubated duck blastoderms found that even the most lateral segments of the area pellucida possessed striking capacities to develop axial structures. He suggested that the interior developmental potency found by Spratt's group on the unincubated chick blastoderm could be explained on the basis that, at the time of laying, the duck blastoderm is at an earlier developmental stage. By the time the chick blastoderm is laid, and certainly by stages XII–XIII, marginal zone areas of the chick blastoderm have restricted their capacities to generate axial structures.

The results obtained with the area opaca are difficult to explain. This area is not necessary for embryonic development and it has generally been associated with pulling and stretching the blastoderm as it grows radially (Bellairs, Boyde & Heaysman, 1969). The area opaca produces the highest frequency of clones, yet, it does not seem to have any developmental potencies. It is however interesting to note that the marginal zone at stage X, which has been shown to have the capacity to form colonies in soft agar, is now shown to have the capacity of generating also a competent epiblastic element from which axial structures can develop.

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