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Early interaction between deep and superficial layers in avian blastodiscs : uptake of ooplasmic determinants

Marc Callebaut, Emmy Van Nueten, Fernand Harrisson and Hilde Bortier

University of Antwerp, Laboratory of Human Anatomy & Embryology, Groenenborgerlaan 171 B-2020 Antwerpen, Belgium
Corresponding author : Marc Callebaut : e-mail : marc.callebaut@ua.ac.be.

ABSTRACT. Parts from radially symmetric prelaid quail blastodiscs placed in culture in contact with isolated cranial quadrants from unincubated chicken blastoderms induce in the chicken upper layer the formation of a miniature embryo with primitive streak and neural plate. Both components (blastodisc parts and upper layer fragments) do not differentiate into an embryo when cultured apart. We concluded that the formation of the avian blastoderm is the result of an interaction between the deep part of the blastodisc and the uncommitted upper layer. Indeed during the culture period the deep part of the quail blastodisc differentiates into quail sickle endoblast and quail junctional endoblast, indicating the formation of Rauber's sickle material in the quail tissue which by induction gives rise to the development of an embryo in the neighbouring upper layer. The here observed induction phenomena result from placing early deep material on more advanced upper layer. This suggests an analogy with the phenomena observed after the eccentric, radially-symmetry-breaking displacement of the deep layer, with reference to the superficial layer which occurs during normal bilateral symmetrization under influence of gravity in undisturbed eggs *in utero*.

KEY WORDS : avian blastoderm, gastrulation, neurulation, Rauber's sickle, symmetrization, δ and γ ooplasms, ooplasmic determinants.

INTRODUCTION

From the experimental studies of LUTZ (1953), VINTEMBERGER & CLAVERT (1954) CLAVERT (1960), KOCHAV & EYAL-GILADI (1971) we know that the avian blastodisc before laying presents a radial symmetry. Studies (CALLEBAUT 1993a, b, c; CALLEBAUT & VAN NUETEN, 1994, 1995; CALLEBAUT et al., 1998) yielded new data about the structure and developmental events in avian intrauterine germ discs during bilateral symmetrization and in unincubated eggs. In the present article we also use the name blastodisc for an intrauterine germ disc. We have shown by the use of the quail chicken chimera technique that Rauber's sickle (RAUBER, 1876) is the early gastrulation organizer in avian blastoderms (CALLEBAUT et al., 1997a). During early incubation it only differentiates into : 1) junctional endoblast by local proliferation of its cells into the neighbouring subgerminal ooplasm; (CALLEBAUT et al., 2000c) and 2) sickle endoblast by centripetal and cranial growth, forming a one-cell-thick layer (CALLEBAUT & VAN NUETEN, 1994; CALLEBAUT et al., 1997b). Although Rauber's sickle and the Rauber's sickle-derived sickle endoblast and junctional endoblast have a very important and indispensable inductive function for the development of the embryonic tissues during gastrulation and neurulation (CALLEBAUT et al., 2003), they never give rise to cells of the embryo proper and therefore belong to the so-called extraembryonic part of the blastoderm (as is also the case for the Nieuwkoop center in amphibians : GUGER & GUMBINER, 1995). In conclusion, the avian Rauber's sickle fulfils the major postulate to be homologous with a functional Nieuwkoop center (NIEUWKOOP, 1969, 1973), namely, the potential for organizer induction without itself contributing to the new structure. Recently, this has been confirmed by molecular biology studies of goosecoid genes in avian blastoderms. Indeed, LEMAIRE et al. (1997) found strong expression of one of the goosecoid genes (GSX) in the upper layer above Rauber's sickle, suggesting induction by the latter. Thus, goosecoid expression was not found in Rauber's sickle, but above it. Later, these upper layers will ingress and form the primitive streak and Hensen's node, which are also GSX-expressing. The latter are close to the amphibian organizer in SPEMANN & MANGOLD'S definition (1924). Thus, Rauber's sickle and not Hensen's node (which develops much later as a complex secondary structure) is the early organizer of the avian embryo (CALLEBAUT & VAN NUETEN, 1994; CALLEBAUT et al., 1996, 1998). Moreover, during the peripheral migration of mesoblast, Rauber's sickle induces the formation of blood islands (CALLEBAUT et al., 2000a) and associated coelomic vesicles, followed by the development of vitelline blood vessels with primitive blood cells and the coelomic cavity including the heart and pericard (CALLEBAUT et al., 2004a). After culture of isolated chicken cranial quadrants (from unincubated avian blastoderms) always a preneural plate forms (CALLEBAUT et al., 2000b). Sections of the latter show a pronounced thickening of the upper layer above the deep layer composed of segregated endophyll. On sections the thickened UL is separated from the endophyll by a large space, and presents localized "banding" of the nuclei (ENGLAND, 1973) indicating primary neural induction (ENGLAND & LAWSON, 1993) in the absence of chordamesoderm (CALLEBAUT & VAN NUETEN, 1995). The latter developmental pattern with only the formation of a preneural plate was also seen after the culture of cranial quadrants from unincubated quail blastoderms (CALLEBAUT & VAN NUETEN, 1995). In a part of the isolated cra-

nial quadrants also a more or less developed primitive streak was seen. The reason for this different developmental behaviour was the occasional presence (proximity) or absence of Rauber's sickle horns in the cranial quadrants. Whole egg yolk balls from quail eggs extracted before bilateral symmetrization (in a white calcareous shell) develop normally during incubation in a humid atmosphere (CALLEBAUT, 1993a). Even when cultured *in toto* in egg white outside their egg shell, they will still develop (CALLEBAUT 1991; CALLEBAUT et al., 2000b). By contrast in the present study, we observed that blastodiscs (or part of them) extracted before bilateral symmetrization and separated from their subgerminal ooplasm and their egg yolk ball, do not develop when cultured *in vitro*. However parts of these unsymmetrized quail blastodiscs, placed in contact with the upper layer of isolated cranial quadrants from unincubated chicken blastoderms, induce a miniature embryo. In the present study we try to explain these phenomena by comparing them with the normal early interaction *in ovo* between upper and deeper part of the avian blastodisc.

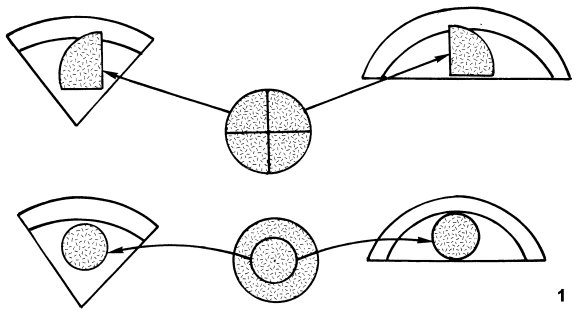


Fig. 1. – Schematic representation of the transplantation of fragments (quadrants or only the central part) of radially symmetric extracted quail blastodiscs (dotted seen in the middle region of the figure) on cranial chicken quadrants (left of the figure) or an anti-sickle regions (right of the figure).

MATERIAL AND METHODS

The used quail (*Coturnix coturnix japonica*) eggs were extracted from the uterus at the beginning of calcification i.e. approximately 10h after oviposition of the previous egg, according to STEPINSKA & OLSZANSKA (1983). From these extracted quail eggs the blastodiscs were removed and the whole blastodisc or parts of it were incubated *in vitro* according to the culture method of NEW (1955) or SPRATT (1947). Other quail blastodiscs were sectioned in quadrants or only, the central part of it was isolated (Fig. 1). These quadrants or central parts were then placed in culture with their deep side on the deep side of the isolated cranial quadrant or anti-sickle region of an unincubated chicken blastoderm. In a similar way they were cultured *in vitro*. Stereomicroscopic polaroid photographs were taken always in the same direction, at the beginning, during and at the end of the culture period (23-28h). Fixation was performed overnight in a modified Heidenhain's fixative (ROMEIS, 1948) containing 0,5g sodium chloride, 2g trichloric acid, 4ml acetic acid, 20ml formalin and 80ml water. After rinsing in tap water the blastoderm associations were placed *in toto* in Unna solution. After rapid dehydration in a graded alcohol series and embedding in paraffin wax they were sectioned perpendicular to the visible or presumed axis. The UNNA staining permitted eventual observation of the orientation of the embryo in the paraffin wax before sectioning. The deparaffinized 8 µm thick sections were Feulgen-stained after DEMALSY & CALLEBAUT (1967) in order to be able to identify the origin of the nuclei in the chimeric blastoderms. This allowed us to observe the typical central or subcentral chromatin granule in the nuclei of the grafted quail cells (CALLEBAUT, 1968; KOSHIDA & KOSIN, 1968; LEDOUARIN & BARQ, 1969) as well as to observe their relationship with the chicken tissues.

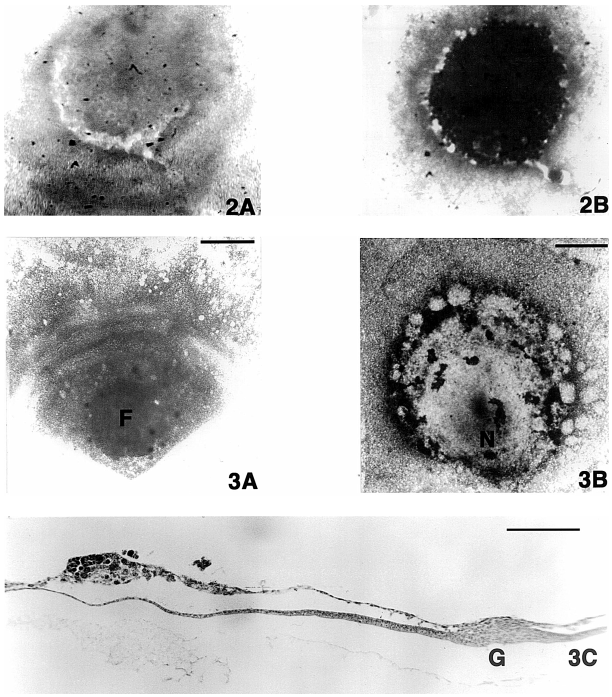


Fig. 2A. – Micrograph of a whole extracted prelaid radially symmetric quail blastodisc at the start of the culture (same magnification as Fig. 3A).
Fig. 2B. – The same blastodisc as seen in Fig. 2A. after 1 day of culture : no developmental phenomena have taken place; the disc has a denser aspect now (same magnification as Fig. 3A).
Fig. 3A. – On the cranial quadrant of an unincubated chicken blastoderm a central circular fragment (F) of a prelaid radially symmetric quail blastodisc has been placed; bar : 1mm.
Fig. 3B. – The chimera of Fig. 3A. after approximately 1 day of culture : note the development of a radially directed miniature embryo with visible neural plate (N) and primitive streak in its prolongation; bar : 1mm.
Fig. 3C. – Feulgen stained section through the embryo seen in Fig. 3B. shows the presence of a fully developed primitive streak with groove (G); bar : 200µm.

RESULTS

Culture of whole radially symmetric intrauterine quail germ discs (blastodiscs after extraction from the uterus) (n = 6)

Such a blastodisc at the start of the culture period is seen in Fig. 2A. After 23h of culture, no differentiation or

growth is observed (Fig. 2B). On sections nor a neural plate, nor a primitive streak is seen indicating the total absence of induction phenomena.

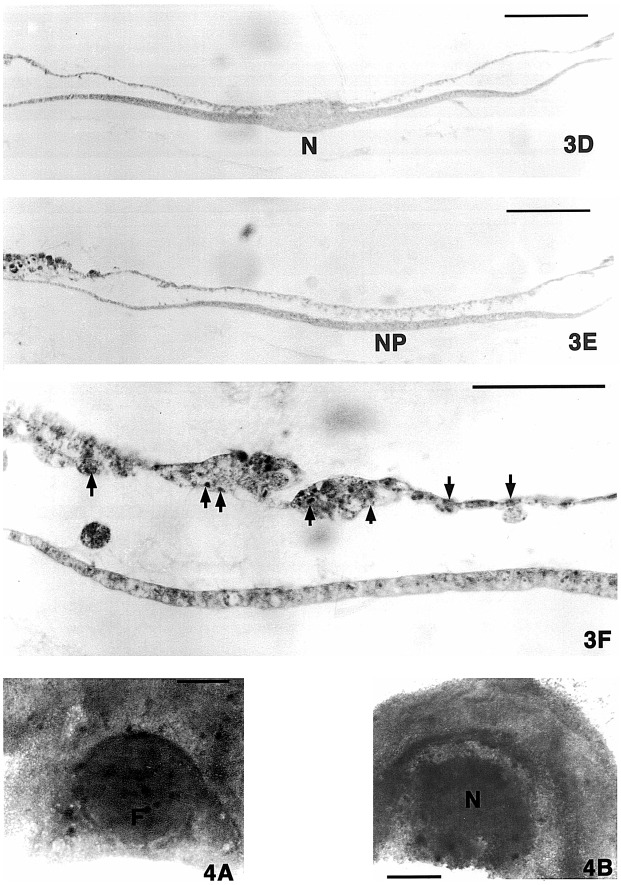


Fig. 3D. – Section through the nodus region (N) of a similar embryo as in fig. 3B.; bar : 200µm.
Fig. 3E. – Section through the neural plate (NP) region formed in the embryo of Fig. 3B.; bar : 200µm.
Fig. 3F. – Feulgen stained section through the primitive streak forming area of the embryo of Fig. 3B. Note laterally the voluminous junctional endoblast containing quail cells (indicated by upside directed arrows) and the flat one cell thick more medial sickle endoblast also containing quail cells (indicated by downside directed arrows); the upper chicken layer (epiblast) in which the primitive streak is induced is seen at the bottom of the figure; bar : 100µm.
Fig. 4A. – The central circular part (F) of a prelaid radially symmetric quail germ disc was placed on an isolated anti-sickle region of an unincubated chicken blastoderm at the start of the culture period; bar : 1mm.
Fig. 4B. – The quail-chicken chimera of Fig. 4A. after 20h of culture : a dense (pre)neural plate (N) has formed (confirmed by sectioning); bar : 1mm.

Placing the deep side of a fragment of a similar unsymmetrized quail blastodisc on the deep side of a chicken cranial quadrant in culture (Fig. 3A) (n = 14)

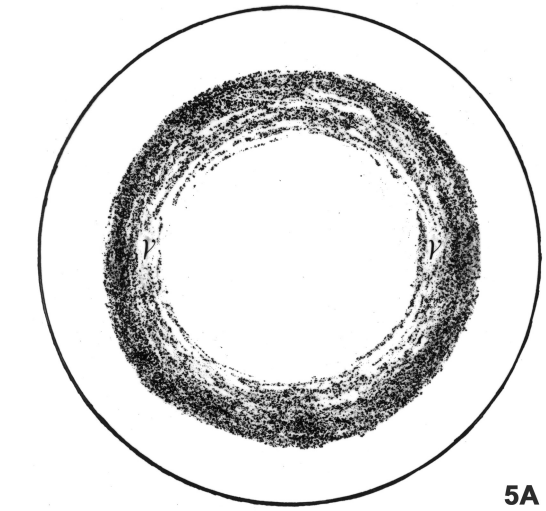
After culture a miniature embryo with a radially placed axis is observed (Fig. 3B). On sections this axis is seen to be partially formed by a primitive streak (Fig. 3C) with a more or less developed nodus (Fig. 3D). In other serial sections a neural plate localized centrally or peripherally (or both) form the primitive streak area, can be discerned (Fig. 3E). The primitive streaks are formed from chicken tissue and are seen to be in association with quail tissue. They are formed in the median part of the upper layer of the area limited by V-shaped quail junctional endoblast (derived from the locally developed Rauber's sickle (Fig. 3F). Quail sickle endoblast (Fig. 3F) is seen laterally from the primitive streak, as the result of the displacement by the concentrically expanding definitive chicken endoderm derived from the tip of the primitive streak. Central fragments of quail germ discs give in general a better embryonic development than quadrants.

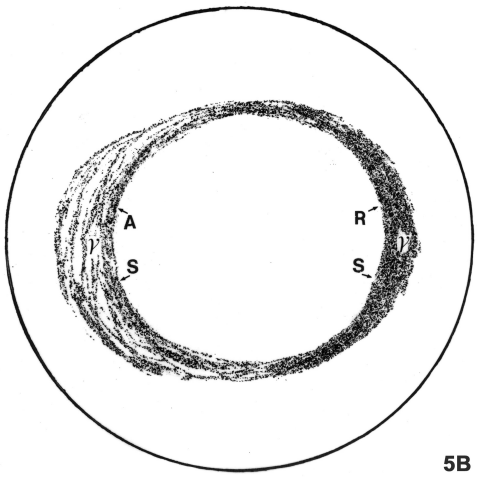
Placing a fragment of radially symmetric quail blastodisc on the deep side of a chicken anti-sickle region in culture (n = 8) (Fig. 4A)

In half of this associations a preneural plate was induced (Fig. 4B) but no primitive streak developed. In another half of the cases, no induction phenomena were observed. Here also a central part excised from a quail germ disc, induces more prominent phenomena in the chicken upper layer.

DISCUSSION

Our results show that whole radially symmetric extracted quail germ discs do not develop *in vitro* in conditions which permit normal development of bilaterally symmetrized unincubated blastoderms. By contrast a fragment of a similar radially symmetric quail blastodisc, placed with its deep side on the deep side of a chicken cranial quadrant, or anti-sickle region differentiates and induces gastrulation and neurulation phenomena. The miniature embryo which so develops is formed by a concerted action between both fragments of different origin and developmental stage. A circular centrally excised fragment of quail germ disc gives the best final development. That these central regions have a more powerful inducing effect on the chicken upper layer is probably the consequence of the presence of a greater part of nucleus of Pander material (containing δ-ooplasm surrounded by some γ-ooplasm : CALLEBAUT, 1987). The localization of the δ and surrounding γ ooplasm in the radially symmetric quail blastodisc and neighboring underlying ooplasm is represented schematically in Fig. 5A. After bilateral symmetrization, in the unincubated blastoderm, Rauber's sickle material is found in the γ ooplasm of the future caudolateral part of the germ disc (Fig. 5B). These ooplasmic layers extend vertically to the upper layer. In the diametrically oposed anti-sickle region the γ containing ooplasm is disrupted from the future cranial part of the blastoderm (which contains no γ ooplasm) (Fig. 5C). The ooplasmic layers in the future cranial region are horizontally disposed and are less condensed then in the caudal Rauber's sickle region as the result of the eccentric tilting of the ooplasm and egg yolk (CALLEBAUT, 1993a, b, c). In a recent study we have seen that the nucleus of Pander (before bilateral symmetrization) has a preneurulation and sometimes a concommited gastrulation effect (CALLEBAUT et al., 2004b). We know that the first cleavage furrows penetrate deeply in the superficial part of the





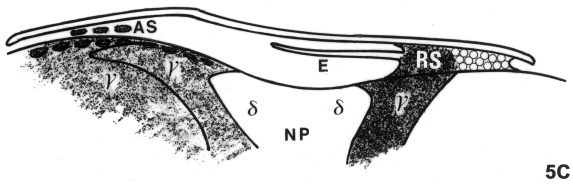


Fig. 5A. – Schematic representation of the circular localization (seen in vertical projection) of the γ ooplasm (γ) in the avian blastodisc before bilateral symmetrization. The γ ooplasm surrounds the more central δ ooplasm (white in the figure).
Fig. 5B. – Schematic representation of the ovoid localization of the γ ooplasm (seen in vertical projection) in the bilateral symmetrized blastoderm; the condensated Rauber's sickle (RS) and the expanded anti-sickle region (AS) have eccentrically formed in the γ ooplasm which surrounds the δ ooplasm.
Fig. 5C. – Schematic representation (simplified after Callebaut, 1993b) of the localization of the γ and δ ooplasm in a mediosagittal section of an unincubated avian blastoderm (bilaterally symmetrized) : caudally in the neighborhood of the Rauber's sickle (RS) the layers in the γ ooplasm are condensed vertically and adhere to the upper layer, whilst cranially in the anti-sickle region (AS) the layers are horizontally flattened and are not taken up by the blastoderm; E : endophyll and NP : nucleus of Pander both containing δ ooplasm (δ).

nucleus of Pander (composed of δ ooplasm) which gives rise to also δ ooplasm-containing endophyll and primordial germ cells (Callebaut, 1984). Preneurulation phenomena appear initially in the neighbourhood of δ ooplasm-containing structures i.e. nucleus of Pander and endophyll. The δ ooplasm seems thus to play an early role in (pre)neurulation phenomena, while the more peripheral and more superficial γ ooplasm influences early gastrulation. In the sickle-shaped region where the γ ooplasm, forming Rauber's sickle is taken up by the germ during and after bilateral symmetrization, gastrulation phenomena start with formation of a primitive streak close to the place where the spatially oblique uptake is maximal i.e. in the middle of Rauber's sickle (Callebaut, 1993c, 1994). A similar phenomenon takes place by the oblique formation of the subgerminal space (Callebaut, 1987, 1993b), through the nucleus of Pander (containing δ ooplasm). This makes that endophyll (also containing δ ooplasm) is only taken up in the caudal half of the germ. The latter is responsible for preneurulation phenomena. This indicates the fundamental influence of the uptake of ooplasmic determinants respectively for gastrulation (by γ ooplasm containing cells) and for preneurulation (by δ ooplasm) phenomena. From a comparative literature study, Eyal-Giladi (1997) concludes that the establishment of the axis in chordates (axialization with bilateral symmetrization) depends on the translocation of oocytal (maternal) determinants form the vegetal pole towards the future dorsocaudal side of the embryo. On arrival at their destination the activated determinants form in all chordates, an induction center homologous to the amphibian "Nieuwkoop center" (or avian Rauber's sickle) which later will respectively induce the formation of the "Spemann's organizer" or the avian Hensen's node. Our studies confirm this hypothesis. The placing of a fragment of quail germ disc on a cranial chicken blastoderm quadrant produces a more developed miniature embryo then after the placing on an isolated anti-sickle region. This can probably be explained by the quantity of involved chicken blastoderm. The presence of quail sickle endoblast and quail junctional endoblast in the present experiments demonstrates that both have a preponderant influence in the formation and orientation of the chicken primitive streak. The difference in developmental potencies *in vitro* between an unsymmetrized blastodisc which is removed from its underlying ooplasm and yolk and an unsymmetrized blastodisc which remains on its egg yolk ball seems due to the eccentric dislocation of the upper layer with reference to the underlying ooplasm during the oblique positioning of the egg *in toto* by which γ ooplasm is taken up forming Rauber's sickle (Figs 5B-C). Indeed a similar phenomenon occurs in our present experiments when early radially symmetric quail deep layer material and ooplasm comes in contact with the deep side of the chicken upper layer. So an artificial association of elements from originally differently localized regions takes place, resulting in the differentiation of deep material into Rauber's sickle derived junctional and sickle endoblast which finally organize and dominate the whole induced embryo (Callebaut et al., 2003), without forming part of the final embryonic tissues.

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REFERENCES

CALLEBAUT, M. (1968). Extracorporal development of quail oocytes. *Experientia*, 24 : 1242-1243.

CALLEBAUT, M. (1984). Avian primordial germ cells contain yolk from the nucleus of Pander. *IRCS Med. Sci.*, 12 : 730-731.

CALLEBAUT, M. (1987). Ooplasmic localization and segregation in quail germs : fate of the four ooplasms. *Arch Biol (Brux)*, 98 : 441-473.

CALLEBAUT, M. (1991). Methods for studying early development of avian germs on their egg yolk in a vertical or inverted position. *Eur. Arch. Biol.*, 102 : 197-199.

CALLEBAUT, M. (1993a). Early eccentricity in gravitationally oriented quail germs. *Eur. J. Morph.*, 31 : 5-8.

CALLEBAUT, M. (1993b). Unequal caudocephalic ooplasmic uptake and eccentric formation of the subgerminal space below unincubated quail blastoderms presenting a Koller's sickle. *Belg. J. Zool.*, 123 : 107-112.

CALLEBAUT, M. (1993c). Development of quail germs during and after gravitationally oriented bilateral symmetrization. *Eur. Arch. Biol.*, 104 : 135-140.

CALLEBAUT, M. (1994). Relationship between the avian blastoderm and the subgerminal ooplasm. *Eur. Arch. Biol.,* 105 : 111-123.

CALLEBAUT, M. & E. VAN NUETEN (1994). Rauber's (Koller's sickle) : The early gastrulation organizer of the avian blastoderm. *Eur. J. Morph.*, 32 : 35-48.

CALLEBAUT, M. & E. VAN NUETEN (1995). Gastrulation inducing potencies of endophyll and Rauber's sickle in isolated caudocranially oriented prestreak avian blastoderm quadrants (or fragments) in vitro. *Eur. J. Morph.*, 33 : 221-235.

CALLEBAUT, M., E. VAN NUETEN, H. BORTIER, F. HARRISSON & L. VAN NASSAUW (1996). Map of the Anlage fields in the avian unincubated blastoderm. *Eur. J. Morph.*, 34(5) : 347-361.

CALLEBAUT, M., E. VAN NUETEN, F. HARRISSON, L. VAN NASSAUW, A. SCHREVENS & H. BORTIER (1997a). Avian gastrulation and neurulation are not impaired by the removal of the marginal zone at the unincubated blastoderm stage. *Eur. J. Morph.*, 35 : 69-77.

CALLEBAUT, M., E. VAN NUETEN, H. BORTIER, F. HARRISSON, L. VAN NASSAUW & A. SCHREVENS (1997b). Spatial relationship between endophyll, primordial germ cells, sickle endoblast and upper layer in cultured avian blastoderms. *Reprod. Nutr. Dev.*, 35 : 293-304.

CALLEBAUT, M., E. VAN NUETEN, L. VAN NASSAUW, H. BORTIER & F. HARRISSON (1998). Only the endophyll-Rauber's sickle complex and not Cells derived from the caudal marginal zone induce a primitive streak in the upper layer of avian blastoderm. *Reprod. Nutr. Dev.*, 38 : 449-463.

CALLEBAUT, M., E. VAN NUETEN, F. HARRISSON & H. BORTIER (2000a). Development of the sickle canal, an unrecognized formation in the avian blastoderm, and its spatial relationship with the first appearing blood islands, induced by Rauber's sickle. *Belg. J. Zool.*, 130 : 143-156.

CALLEBAUT, M., E. VAN NUETEN, F. HARRISSON & H. BORTIER (2000b). Mechanisms of caudocephalic axis formation in the avian germ disc. *Belg. J. Zool.*, 130 : 67-79.

CALLEBAUT, M., E. VAN NUETEN, F. HARRISSON, L. VAN NASSAUW & H. BORTIER (2000c). Avian junctional endoblast has strong embryo –inducing and dominating potencies. *Eur. J. Morph*., 38 : 3-16.

CALLEBAUT, M., E. VAN NUETEN, H. BORTIER & F. HARRISSON (2003). Rauber's sickle generates only extraembryonic tissues (junctional- and sickle endoblast), and, by positional information, organizes and dominates the whole avian blastoderm (gastulation, neurulation and blood island formation). *Belg. J. Zool.*, 133 : 45-59.

CALLEBAUT, M., E. VAN NUETEN, H. BORTIER & F. HARRISSON (2004a). Induction of the avian coelom with associated vitelline blood circulation by Rauber's sickle derived junctional endoblast and its fundamental role in heart formation. *J. Morph.*, 259 : 21-32.

CALLEBAUT, M., E. VAN NUETEN, F. HARRISSON & H. BORTIER (2004b). Induction and improved embryonic development by the nucleus of Pander in associated avian blastoderm parts : the influence of δ or γ ooplasm. *J. Morph.*, 260 : 201-208.

CLAVERT, I. (1960). Déterminisme de la symétrie bilaterale chez les oiseaux. IV Existence d'une phase critique pour la symétrization de l'œuf. Son stade. *Arch. Anat. Microsc. Morph. Exp.,* 49 : 345-361.

DEMALSY, P. & M. CALLEBAUT (1967). Plain water as a rinsing agent preferable to sulfurous acid after the Feulgen nuclear reaction. *Stain technol.*, 42 : 133-136.

ENGLAND, M.A. (1973). The occurence of a band of nuclei in primary neural induction in the chick embryo. *Experientia*, 31 : 1449-1451.

ENGLAND, M.A. & A. LAWSON (1993). Natural wound formation : endodermal responses in experimental primary neural induction in the chick embryo. *Anat. Rec.*, 236 : 710-730.

EYAL-GILADI, H. (1997). Establishment of the axis in chordates : facts and speculations. *Development*, 124 : 2285-2296.

GUGER, K. & B. GUMBINER (1995). ß-Catenin has Wnt-like activity and mimics the Nieuwkoop signaling center in Xenopus dorsal-ventral patterning. *Dev. Biol*., 172 : 115-125.

KOCHAV, S. & H. EYAL-GILADI (1971). Bilateral symmetry in the chick embryo. Determination by gravity. *Science,* 171 : 1027-1029.

KOSHIDA Y. & I. KOSIN (1968). Intranuclear sex dimorphism in the feathers of six species of galliformes. *Cytologia (Tokyo)*, 33 : 230-240.

LE DOUARIN N. & G. BARQ (1969). Sur l'utilisation des cellules de la caille japonaise comme marqueurs biologiques en embryologie expérimentale. *CR Acad Sci Paris*, 269 : 1543-1546.

LEMAIRE, L., T. ROESER, J. IZPISÚA-BELMONTE & M. KESSEL (1997). Segregating expression domains of two goosecoid genes during the transition from gastrulation to neurulation in chick embryos. *Development*, 124 : 1443-1452.

LUTZ, H. (1953). L'orientation des axes embryonnaires dans la gémellité expérimentale chez les oiseaux et son déterminisme. *Bull. Biol. France et Belg.*, 86 : 34-67.

NEW, D.A.T. (1955). A new technique for the cultivation of the chick embryo. *J. Embryol. Exp. Morph.*, 3 : 326-331.

NIEUWKOOP, P. (1969). The formation of the mesoderm in urodelean amphibians. I Induction by the endoderm. *Roux Arch Dev. Biol.*, 162 : 341-373.

NIEUWKOOP, P. (1973). The "organizing center" of the amphibian embryo : its spatial organization and morphogenetic action. *Adv Morphogen*., 10 : 1-39.

RAUBER, A. (1876). Uber die Stellung des Hünchens im Entwicklungsplan. W ENGELMANN, Leipzig.

ROMEIS W. (1948). Mikroskopische technik 15 Aufl. Leibnitz, München.

SPEMANN, H. & H. MANGOLD (1924). Über die Induktion von Embryoanlagen durch Implantation artfremder Organisatoren. *Roux Arch. Entwicklungsmech.*, 100 : 599-638.

Spratt, N. (1947). A simple method for explanting and cultivating early chick embryos in vitro. *Science*, 106 : 452.

Stepinska, U. & B. Olszanska (1983). Cell multiplication and blastoderm development in relation to egg envelope formation during uterine development of quail (*Coturnix coturnix japonica*) embryo. *J. Exp. Zool.*, 228 : 505-510.

Vintemberger, P. & I. Clavert (1954). Sur le détermisme de la symétrie bilaterale chez les oiseaux. V Notion de l'existence, durant le séjour de l'œuf dans l'utérus d'une période critique déterminante dans la réalisation de la symétrie bilatérale. *C.R. Soc. Biol.*, 148 : 1489-1493.

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