Belg. J. Zool. — Volume 123 (1993) — issue 2 — pages 107-112 — Brussels 1993

Manuscript received on 1 February 1993

UNEQUAL CAUDOCEPHALIC OOPLASMIC UPTAKE AND ECCENTRIC FORMATION OF THE SUBGERMINAL SPACE BELOW UNINCUBATED QUAIL BLASTODERMS PRESENTING A KOLLER'S SICKLE

by

MARC CALLEBAUT

RUCA, Laboratory of Human Anatomy and Embryology,
Groenenborgerlaan 171, B-2020 Antwerp, Belgium

SUMMARY

By radioactive ooplasmic yolk layer labeling during late oogenesis, we demonstrated that below unincubated quail blastoderms the subgerminal space extends much more peripherally in the cranial then in the caudal direction, where it ends abruptly against Koller's sickle. Our autoradiographic and histochemical investigations indicate that Koller's sickle develops by a progressive local colonisation of the centro-caudal subgerminal ooplasm, which does not take place in the cranial diametrically opposite anti-sickle region. The deep paracentral uptake of voluminous ooplasmic areas by large encircling extensions of its cells is a characteristic feature of the sickle region.

Key words : avian ooplasm, quail blastoderm, Koller sickle, caudo-cephalic axis.

INTRODUCTION

The caudal sickle named after KOLLER (1882), already described before by RAUBER (1876), can clearly be observed alive under the stereomicroscope, from the exterior in about 30 % of unincubated laid quail blastoderms (FARGEIX, 1964 ; LUTZ, 1964). It develops in the highest (future caudal) half of the blastodisc of temporally obliquely or vertically placed extracted uterine quail egg yolks (CALLEBAUT, 1991, 1993). From the middle part of the sickle region the primitive streak will develop after early incubation (KOLLER, 1882). We have shown by maternal injections of tritium labelled protein precursors that the semi-solid ooplasmic layers playing a role in the formation of the avian germ disc were disposed in/and around the nucleus of Pander as the peels in an onion-bulb (CALLEBAUT, 1974, 1983). By the use of that labeling method it was possible in the present study to demonstrate an unequal caudocephalically directed uptake of ooplasm in the blastoderm of laid unincubated eggs presenting a Koller's sickle. After appropriate fixation (Calcium

formalin) and staining (Unna), we were able to visualize clearly the massive capture of underlying ooplasm by the encircling movement of the blastomeres in the region of Koller's sickle.

MATERIAL AND METHODS

Fifteen fertilized Japanese quail females, selected for regularly laying eggs with blastoderms presenting a Koller's sickle (Stage 1 ; VAKAET, 1962, 1970), were injected 3 times (every 2 days) with 0,5 mCi L- (4, 5-3H) leucine (164 Ci/mM, Amersham, England).

The eggs laid during the days following the injection were opened. After removal of the surrounding albumen and rinsing in Ringer solution most of their yolks with blastoderms presenting a Koller's sickle were fixed *in toto* in calcium formalin (according to SILVERTON and ANDERSON, 1961). For comparison some yolks were also fixed in Susa after Heidenhain (ROMEIS, 1948) without sublimate, in acetic acid-alcohol (1 : 3 v) or in an acetic acid-formaldehyde 35 %-alcohol 95 % (1 : 4 : 15 v) mixture (AFA).

The localization of Koller's sickle was indicated with a linear charcoal mark on the vitelline membrane just behind the blastoderm rim. After 1 night of fixation at room temperature the egg yolks were placed in tap water (the acetic-acid and AFA fixed blastoderms were placed in 70 % alcohol) and during the following days dehydrated. In 95 % alcohol, the germs still adherent to their vitelline membrane were excised with some surrounding yolk and after clearing in xylene, embedded in paraffin.

Eight µm thick sectioning was performed parallel with the caudocephalic axis of the germ(perpendicular to the linear charcoal mark behind Koller's sickle). The blastoderms in the paraffin were placed vertically, perpendicular to the microtome knife edge during sectioning, to avoid a dorsoventral compression. Comparable sections of similar blastoderms of not injected females were used as controls both before and after the autoradiographic procedure. The radioactively labeled and some control sections were dipped in nuclear emulsion L4 (Ilford, England). After 1 month of exposure in the dark the autoradiographs were developed according to CARO and VAN TUBERGEN (1962). Thereafter the autoradiographs were coloured with Unna.

RESULTS

On the autoradiographs of mediosagittal sections through unincubated blastoderms (fixed in calcium formalin) of eggs laid 6 days after the first and 4 days after the second injection the respectively corresponding labeled ooplasmic layers (layers 1 and 2 on Fig. 1) were clearly seen. In the neighbourhood of the germ their form remained no longer symmetrical as was originally the case in the oocyte. At the future cranial side of the germ (Figs. 1, 2), the second labeled layer was seen

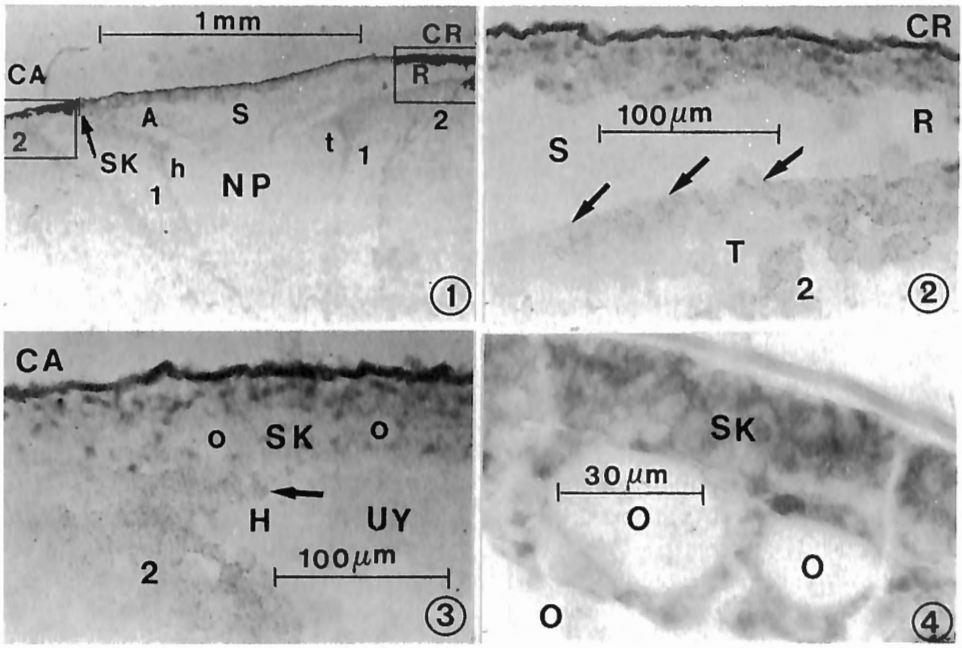

Fig. 1-4. — Microphotographs taken from Unna stained sections of calcium formalin fixed quail blastoderms.

1. — Autoradiograph of a mediosagittal section through an unincubated quail germ on its egg yolk presenting two labeled ooplasmic layers (numbered 1 and 2 formed respectively 6 and 4 days before laying) CA : caudal and CR : cranial side of the germ ; layer 1 is seen to surround the bootshaped nucleus of Pander (NP) ; t : cranial toe-like and h : caudal heel-like part of NP ; S : subgerminal space extending more peripherally as a recess (R) at the cranial side ends abruptly in A (axilla form) since it is closed at the caudal side by the sickle of Koller (SK) ; layer 2 is caudally seen to end against the Koller sickle material (see also Fig. 3) whereas cranially it ends below the cranial shallow recess of the subgerminal space (see also Fig. 2).

2. — Enlargement of the cranial part of Fig. 1 (cranial rectangle). Note the long hook-like recurved part (indicated by 3 arrows) of the labeled ooplasmic layer 2 surrounding a toe-like area (T) of unlabeled ooplasm (UY) below the widely open subgerminal space (S) and its cranial recess (R).

3. — Enlargement of the caudal part of Fig. 1 (caudal rectangle). Note the much shorter hook-like part (arrow on its point) of labeled layer 2 surrounding a heel-like part (H) of unlabeled ooplasm (UY) below the sickle of Koller (SK) ; O : islands of ooplasm without nuclei in Koller's sickle.

4. — Mediosagittal section through sickle of Koller (SK) of unincubated quail blastoderm to show the uptake of voluminous parts of ooplasm (upper o's) by an encircling movement or extension of the sickle cells. The contact zone between the sickle and the underlying ooplasm (lower left O) is seen to be rectilinear.

to form a long hook-like structure below the widely open subgerminal space which extends more peripherally as a narrow recess. By contrast at the future caudal side the hook form was much less developed or absent (Figs.1, 3). Here the subgerminal space was obliterated by the yolk rich cells of Koller's sickle. The first (deepest) layer which surrounds the nucleus of PANDER (1817) (Fig. 1) was seen to present an analogous but less obvious form (parallel with the second labelled layer) corresponding to the bootshape we have previously described (CALLEBAUT 1983, 1987). After autoradiographic processing of similar sections through non radioactive control blastoderms no background labeling was observed.

On the sections through non radioactive calcium formalin fixed control blastoderms which were not submitted to the autoradiographic procedure and were only stained with Unna, the contrast between the cellular borders of the blastoderm and the subgerminal ooplasm was very sharp. So it was clearly seen that voluminous ooplasmic areas were locally taken up into the sickle by an extensive encirclement of its deeper cells (Fig. 4). These areas corresponded to the nuclei free zones seen after autoradiographic coating with nuclear emulsion (Fig. 3). The interface contact zone between the sickle and the underlying ooplasm remained nearly plane (Fig. 4). After fixation in AFA or acetic alcohol and Unna or iron hematoxylin and eosin staining the blastoderms and subgerminal cavity were seen to be somewhat compressed and deformed and the distinction between the blastodermal cell borders and the colonized underlying ooplasm was not well demarcated.

DISCUSSION

The present study indicates that the deep paracentral colonization of voluminous yolky ooplasmic areas by large encircling movements of the blastomeres is a characteristic feature of Koller's sickle region in quail blastoderms. So the Koller's sickle may develop as the result of the deep penetration of blastomeres in the ooplasm of the upper germ wall after the eccentric formation of the area pellucida (CALLEBAUT, 1993) or perhaps also by a more central proliferation of cells obliterating locally the subgerminal space. Unincubated quail eggs treated with 3H-thymidine at room temperature presented an unequal incorporation pattern with a more intensive incorporation at the caudal side of the germ, mainly in the nuclei of Koller's sickle (CALLEBAUT, 1989) indicating their higher DNA synthesis rate.

Although we showed that a cytoplasmic continuity no longer existed between the cells of the unincubated quail blastoderm and the underlying ooplasm, our observations indicated that the deep sickle cells can hold both structures together by means of their prominent protrusions. It is clear that the penetration of the sickle cells is different from that of the more peripheral yolk endoderm which originates later from the outer part of the germ wall (area opaca) and in which according to VAKAET (1962) no cellular boundaries existed.

The present study indicates that at the level of Koller (or Rauber) sickle a larger quantity of centrally localized ooplasm (closer to the original animal pole of the oocyte) is taken up into the germ. So an unequal asymmetric distribution of the original β and/or γ ooplasms (CALLEBAUT, 1987) takes place which can be determining for the further development of the germ. At the end of the intrauterine period some centrifugal expansion occurs in the upper layer forming the main part of the area opaca (CALLEBAUT and MEEUSSEN, 1988). The localization of the sickle of Koller however, remains unchanged. The early or primary eccentricity is somewhat « paradoxical » and is diametrically opposite to the eccentricity usually seen after laying or during the first hours of incubation (CALLEBAUT, 1993). CLAVERT (1960, 1962) and KOCHAV *et al.* (1980) have not seen the parallelism existing between the structure of the avian germ, the subgerminal cavity and the underlying yolk. The latter authors dissected out the germ together with underlying yolk and removed the vitelline membrane before fixation and so disturbed some early spatial relationships. Moreover at that moment, knowledge about the formation of the avian yolk layers in the germ disk was still incomplete. From their study KOCHAV *et al.* (1980) concluded that at the moment of area pellucida formation there occurs a massive cell shedding process. They presumed that all the deeper cells of the germ fall into the subgerminal cavity and finally assemble in its lowest (future cranial) part under influence of gravity. I have never seen any proof of this hypothesis, since after trypan blue induced fluorescent labeling of the yolk in quail eggs the labeled cells or yolk masses, both in the caudal and cranial part of the subgerminal space, always remained localized in the prolongation of the labelled subgerminal yolk layers (CALLEBAUT, 1987). This was also particularly the case for the Koller sickle region in which locally voluminous densely trypan blue labeled yolk masses remained visible, confirming our present observations.

ACKNOWLEDGEMENTS

The author is very grateful to Mrs. E. Van Nueten, Mrs. S. Van Rompaey and Mr. F. De Bruyn for excellent technical assistance and to Mrs. L. Debie for typing the manuscript.

REFERENCES

CALLEBAUT, M. (1974) — La formation de l'oocyte d'oiseau. *Archiv. Biol. (Brux.)*, **85** : 201-233.

CALLEBAUT, M. (1983) — The constituent oocytal layers of the avian germ and the origin of the primordial germ cell yolk. *Arch. Anat. Microsc.*, **72** : 199-214.

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

CALLEBAUT, M. (1989) — Incorporation of 3H-thymidine in unincubated quail blastoderms presenting a sickle of Koller. *Med. Sci. Res.*, **17** : 873-874.

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. (1993) — Early eccentricity in gravitationally oriented quail germs. *Eur. J. Morphol.*, **31** : 5-8.

CALLEBAUT, M. and C. MEEUSSEN (1989) — The area opaca of the avian blastoderm is mainly formed by centrifugal expansion. *Med. Sci. Res.*, **16** : 617-618.

CARO, L. and R. VAN TUBERGEN (1962) — High-resolution autoradiography. *J. Cell Biol.*, **15** : 173-188.

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

CLAVERT, I. (1962) — Symmetrization of the egg in vertebrates. In : *Advances in morphogenesis 2*. AP, NY London : 27-60.

FARGEIX, N. (1964) — La caille domestique (*Coturnix coturnix japonica*). Ponte et étude de l'œuf au stade non incubé. *Arch. Anat. Histol. Embryol. norm. et exp.*, **1** (4) : 275-281.

KOCHAV S. M. GINSBURG and H. EYAL-GILADI (1980) — From cleavage to primitive streak formation : a complementary normal table and a new look at the first stages of the development of the chick II. Microscopic anatomy and cell population dynamics. *Dev. Biol.*, **98** : 441-473.

KOLLER, C. (1882) — Untersuchungen über die Blätterbindung im Hühnerkeim. *Arch. Mikrosk. Anat., 20* : 171-211.

LUTZ, H. (1964) — Le stade non incubé du blastoderme d'oiseau. *Arch. Anat. Histol. Embryol.*, **47** : 531-537.

PANDER, C. (1817) — *Historiam metamorphoseos quam ovum incubatum prioribus quinque diebus subit* : F.E. Nitribitt, Wirceburgi (69 pp).

RAUBER, A. (1876) — *Über die stellung des Hühnchens im Entwicklungsplan* W. Engelsmann, Leipzig (28 pp).

ROMEIS, W. (1948) — *Mikroskopische Technik*. 15. Aufl. Liebnitz, München (695 pp).

SILVERTON, R. and M. ANDERSON (1961) — *Handbook of Medical Laboratory Formulae*. Butterworths, London (676 pp).

VAKAET, L. (1962) — *Pregastrulatie en gastrulatie der Vogelkiem. Morfologische en experimentele studie*. Arscia, Brussel (244 pp).

VAKAET, L. (1970) — Cinephotomicrographic investigations of gastrulation in the chick blastoderm. *Arch. Biol. (Liège)*, **81** : 387-426.