

# Day-1 Chick Development

Guojun Sheng\*

The first day of chick development takes place inside the mother hen (in utero), during which the embryo progresses from fertilization to late blastula/early gastrula formation. The salient features of developmental anatomy in this period are conserved among the sauropsids (birds and reptiles). Many of these features are also shared in prototherian (monotreme) embryos, whereas metatherian (marsupial) and eutherian (placental) embryos display significant variations. Important for understanding the evolution of early development in amniotes, the knowledge of cellular and molecular mechanisms regulating in utero chick development may also offer valuable insight into early lineage specification in prototherians and conserved features in mammalian early development. This commentary provides a snapshot of what is currently known about intrauterine chick development and identifies key issues that await further clarification, including the process of cellularization, allocation of maternal determinants, zygotic gene activation, mid-blastula transition, cell layer increase and reduction, radial symmetry breaking, early lineage segregation, and role of yolk syncytium in early patterning. *Developmental Dynamics* 243:357–367, 2014. © 2013 Wiley Periodicals, Inc.

**Key words:** chicken; intrauterine development; amniotes; monotremes; marsupials; eutherians; birds; reptiles; cleavage; blastula; gastrulation

Submitted 9 September 2013; First Decision 22 October 2013; Accepted 22 October 2013

## A CASE FOR STUDYING THE FIRST DAY OF CHICK DEVELOPMENT

The amniotes consist of two major groups of vertebrate animals, the synapsids (prototherian, metatherian, and eutherian mammals) and sauropsids (reptiles and birds) (Fig. 1A). Two species, the mouse representing the mammals and the chick representing the birds/reptiles, are currently used as the benchmark developmental models for the amniotes. The list of chick model's contributions to developmental biology is long (Stern, 2005). Missing from this list is any study on developmental processes taking place before oviposition. This period of development has been variably termed preovipositional, oviductal, or intrauterine (the last of which

will be used here), and encompasses stages of rapid cell cleavages and pre-gastrulation development. In this regard, intrauterine chick development is broadly equivalent to the pre-implantation development of the mouse embryo. During this period, a mouse embryo will complete the process of early lineage specification and generate three cell populations (trophoblast, epiblast, and hypoblast) that make up the blastocyst (Stephenson et al., 2012; Schrode et al., 2013).

Understanding how this period of development is regulated is obviously important. However, intrauterine stage chick embryos are generally inaccessible unless the materials can be readily resourced from poultry facilities affiliated with agriculture or biotechnology institutions. Why is it desirable to use the chick model to

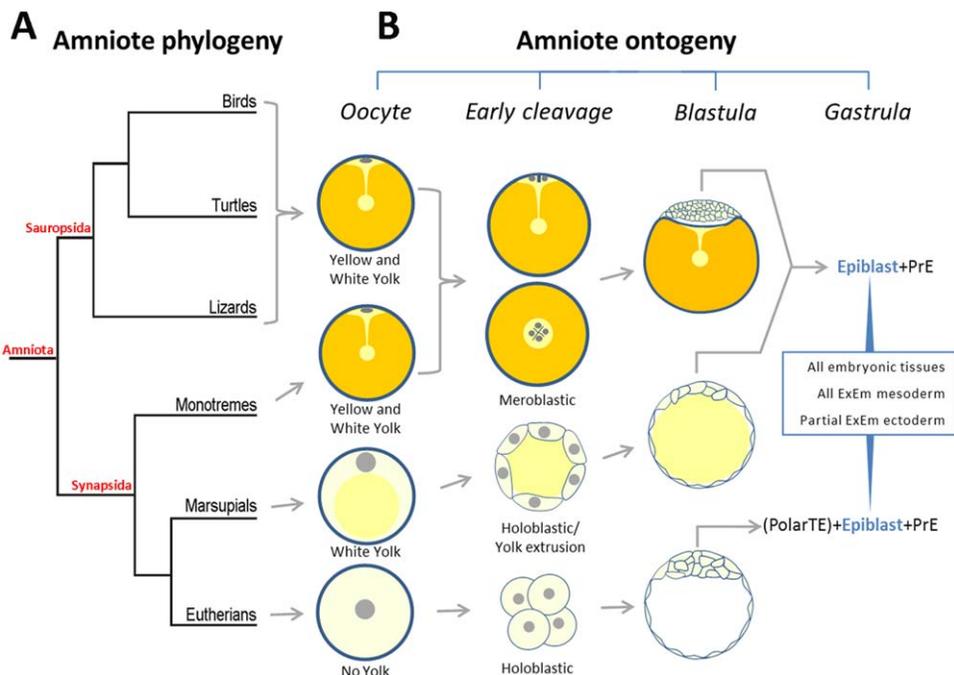
study the intrauterine/preimplantation period of amniote development? Answer to this question lies in understanding phylogenetic diversifications in early amniote development. It is well-known that the yolk-less feature of a eutherian egg is a derived attribute of evolution. The marsupials have moderately yolky eggs (Selwood, 1992; Menkhorst et al., 2009) and the monotremes have an egg organization very similar to that of the birds and reptiles (Flynn and Hill, 1938) (Fig. 1B). Ancestral mammalian eggs likely resembled a chicken egg more than the mouse one. Early cleavage pattern of a mammalian embryo also varies, with the monotreme pattern again being more similar to the avian/reptilian one than to either the eutherian or marsupial one (Flynn and Hill, 1938, 1947; Eakin and

Laboratory for Early Embryogenesis, RIKEN Center for Developmental Biology, Kobe, Hyogo, Japan

\*Correspondence to: Guojun Sheng, Laboratory for Early Embryogenesis, RIKEN Center for Developmental Biology, Kobe, Hyogo 650-0047, Japan. E-mail: sheng@cdb.riken.jp

DOI: 10.1002/dvdy.24087

Published online 29 October 2013 in Wiley Online Library (wileyonlinelibrary.com).



**Fig. 1.** Phylogenetic and ontogenetic relationships between the mammals and birds. **A:** Amniote phylogeny. The Synapsida (mammalian) group contains the monotremes, marsupials, and eutherians. The sauropsida (reptilian and avian) group contains the birds, crocodiles (omitted), turtles, lizards, snakes (omitted), and tuatara (omitted). **B:** Amniote ontogeny, showing four early stages: mature oocyte before fertilization, early cleavage, blastula, and gastrula. The monotremes, the reptiles, and the birds have similar oocyte organization, with yellow and white yolk. Marsupial eggs have white yolk only, and the composition of marsupial white yolk differs from that in sauropsid/monotreme eggs. Eutherian eggs do not have yolk in the traditional sense. Early cleavages are meroblastic (side and top views) and are similar in monotreme and reptilian (including bird) embryos. Marsupial embryos are holoblastic, but in many marsupial species early blastomeres extrude acellular yolk into a central cavity. Eutherian embryos are holoblastic. Monotreme, reptilian, and avian blastulae have a multilayered blastoderm positioned on top of a big yolk cell. Blastula-stage marsupial embryos (blastocysts) are unilaminar, and by late blastula-stage pluriblast cells become morphologically distinguishable and will give rise to epiblast and hypoblast cells. Eutherian blastulae have mural trophectoderm cells being morphologically distinct from polar trophectoderm and inner cell mass cells. The polar trophectoderm cell lineage is a eutherian invention, but is not present in all eutherian mammals. A two-layered structure (an upper epiblast and a lower hypoblast) is conserved in reptilian/avian, monotreme, and marsupial early gastrulae. TE, trophectoderm; PrE, primitive endoderm/hypoblast; ExEm, extraembryonic.

Behringer, 2004) (Fig. 1B). Moreover, at late blastula/early gastrula stages, monotreme and marsupial embryos do not have their pluripotent cell population (epiblast/pluriblast) covered by a polar trophectoderm (Raubert's) layer present in most eutherian mammals and defined as part of the trophectoderm layer which surrounds and is in close association with the inner cell mass/epiblast (Flynn and Hill, 1947; Selwood, 1992; Selwood and Johnson, 2006; Frankenberg et al., 2013) (Fig. 1B). Instead, monotremes and marsupials have a single-cell thick epiblast layer in direct contact with the *zona pellucida* and a thin layer of hypoblast underneath it, similar to the organization known in a postovipositional chick embryo. Of interest, the polar trophectoderm is not a conserved structure in the eutherian clade. It is absent in the tenrec and elephant shrew (Wimsatt,

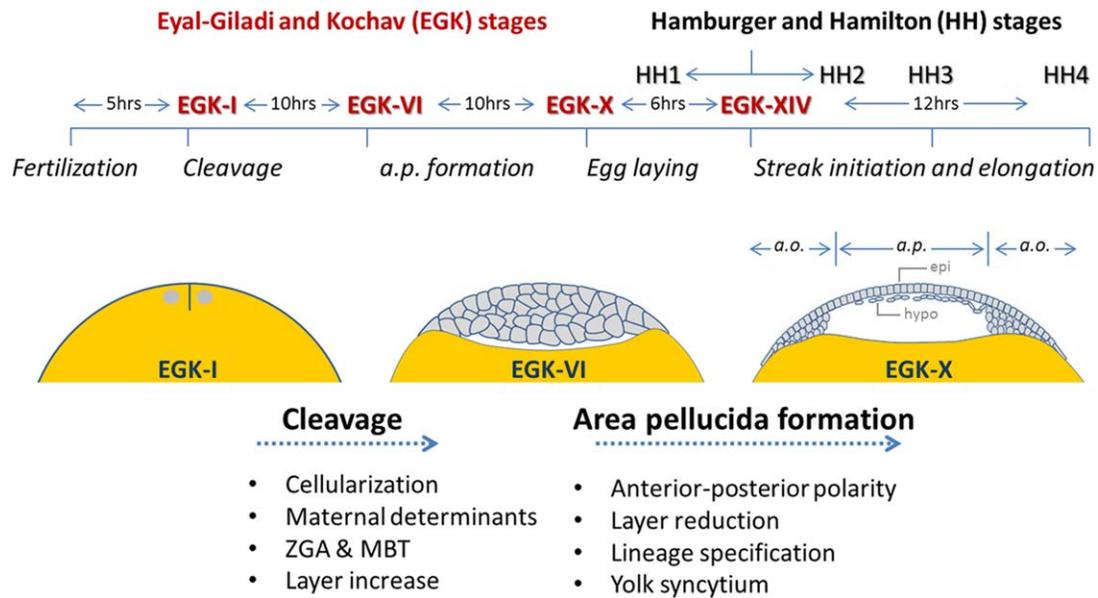
1975; Eakin and Behringer, 2004), and formed but quickly lost in the rabbit, pig, cattle, dog, cat, and many other species (Williams and Biggers, 1990; Flechon et al., 2004; Vejlsted et al., 2005; Blomberg et al., 2008; Hassoun et al., 2009; Wolf et al., 2011).

Two conclusions can be drawn from above-discussed phenotypic diversity seen in mammalian early development. First, the "preimplantation" development from cleavage to late blastula stage in basal extant mammalian lineage (monotremes) is morphogenetically similar to the intrauterine development in the chick. Second, the "preimplantation" development in many therian mammals differs from that in the mouse, the conventional mammalian model. Given that the monotremes are not likely to become technically amenable models for embryological studies, knowledge of day-1 chick develop-

ment may provide useful insight into the variable and conserved features of mammalian early development.

## OVERVIEW OF INTRAUTERINE CHICK DEVELOPMENT

Avian oviduct has five functional regions: the infundibulum, magnum, isthmus, uterus and vagina. After ovulation, a mature chicken oocyte is fertilized at the infundibulum. The fertilized oocyte passes through the magnum which secretes egg white, isthmus which makes soft shell and uterus which adds hard shell before oviposition induced by vaginal muscle contraction. The entire process takes approximately 25 hr. The first cleavage starts approximately 5 hr after fertilization (Olsen, 1942; Gipson, 1974), and the rest of preovipositional



**Fig. 2.** Intrauterine chick development. Two staging systems, the EGK and HH, are currently used to delineate the developmental stage of early chick embryos. The EGK stages are used for intrauterine and prestreak stages. The intrauterine period, covering approximately 25 hr in total, is divided into 10 stages: EGK-I to EGK-X. From fertilization to first cell division: 0–5 hr. EGK-I to -VI: cleavage stages (5–15 hr). EGK-VII to EGK-X: area pellucida formation period (15–25 hr). *a.p.*, area pellucida; *a.o.*, area opaca; epi, epiblast; hypo, hypoblast. See the text for detail of the developmental processes.

development (20 hr) takes place in the uterus. Chick development is traditionally described according to two staging systems, the Hamburger and Hamilton (HH) and the Eyal-Giladi and Kochav (EGK) (Eyal-Giladi and Kochav, 1976; Kochav et al., 1980; Hamburger and Hamilton, 1992; Bellairs and Osmond, 2005; Wong et al., 2013), the latter of which is used for intrauterine embryos (Fig. 2).

Eyal-Giladi and Kochav divided the prestreak development (HH stage 1) into 14 stages (denoted by roman numerals as EGK-I to -XIV) (Eyal-Giladi and Kochav, 1976; Kochav et al., 1980). The intrauterine period covers EGK-I to -X. EGK-I to -VI are collectively called “cleavage” stages and take approximately 10 hr. EGK-VII to -X are collectively called “formation of the *area pellucida*” (*a.p.* formation) stages and take another 10 hr (Fig. 2). Early divisions are meroblastic. Cellularization and subgerminal cavity formation start from between EGK-II to EGK-III. The blastoderm becomes multilayered in the center from EGK-III and reaches its thickest (5–6 cell layer thick) at EGK-VI, after which the number of cell layers in the *area pellucida* decreases gradually to 1–2 at EGK-X, with an upper continuous, single cell-layered

epiblast and a lower discontinuous layer of hypoblast cells. The peripheral cells, precursors to the *area opaca*, remain multilayered and maintain close contact with the underlying yolk cell.

Aside from these two publications from Eyal-Giladi and colleagues (Eyal-Giladi and Kochav, 1976; Kochav et al., 1980), earlier work on intrauterine development from other authors was summarized in their first staging paper (Eyal-Giladi and Kochav, 1976). Work published afterward will be discussed in appropriate sections below.

### KEY ISSUES OF INTRAUTERINE CHICK DEVELOPMENT IN NEED OF FURTHER CLARIFICATION

Compared with other vertebrate models (zebrafish, *Xenopus*, and mouse), very little is known about the cell and molecular biology of early development in the chick. Such knowledge would enable us to gain a glimpse of the ancestry of and diversity in mammalian and amniote pregastulation development. In the rest of this commentary, I will list and discuss several key issues in need of further clarification. Concerning the **cleav-**

**age period** of intrauterine development: (1) How do open blastomeres cellularize and are there maternal determinants allocated asymmetrically during the cellularization process? (2) When does zygotic gene activation start and what is the equivalent stage of mid-blastula transition? (3) What are the cellular and molecular mechanisms regulating initial separation of inner and outer layers and later increase in cell layer number? Concerning the ***a.p.* formation period** of intrauterine development: (4) When is the A-P polarity first established in the blastoderm? (5) What is the main mechanism driving cell-layer reduction and what is the earliest step in epiblast/hypoblast cell lineage segregation? (6) Is the yolk cell a syncytium and, if so, does it play a role in early patterning and lineage segregation?

### Cellularization and Maternal Determinants

The first cell to complete the cellularization process, i.e., with “basal” separation from the yolk, does so at EGK-II to -III, when the embryo has more than 16 “apical-laterally” enclosed cells (Eyal-Giladi and Kochav, 1976; Bellairs et al., 1978; Kochav et al.,

1980; Lee et al., 2013). This process continues until the end of the cleavage phase (EGK-VI), when all cells, including the most peripheral ones, are cellularized. Gipson (Gipson, 1974) and Bellairs et al. (1978) observed three types of furrow morphology associated with early cleavages: V- or U-shaped shallow furrow, the pendulum-shaped deep furrow and slit-shaped smooth and tight furrow (Fig. 3A). Both the shallow and pendulum-shaped furrows have numerous furrow wall protrusions, likely reflecting the process of new membrane addition similar to what is known during the cellularization process in *Drosophila* (Lecuit and Wieschaus, 2000) and in meroblastic cleavages in zebrafish (Li et al., 2006) and squid (Arnold, 1969) embryos. The pendulum-shaped furrow has an elaborate structure at its base called furrow base body, which in addition to supplying new membrane may function as an equivalent of midbody in driving furrow deepening (Gipson, 1974) or in controlling the transition from vertical to horizontal furrow burrowing (Bellairs et al., 1978). The slit-shaped furrow represents cell-cell contacts being established while the cellularization process is still in progress, a phenomenon similarly observed during zebrafish meroblastic cleavage and *Drosophila* cellularization.

Cytokinesis lags behind nuclear division, but most cyokinetic events are associated with zygotic mitosis. The exceptions are the pseudocleavage furrows (not discussed here) induced by supernumerary sperm nuclei (Lee et al., 2013) and the edge cells. It is unclear whether the edge cells complete the cellularization process with no further nuclear division (thus leaving no nucleus to the remaining yolk cell) or with an additional round of nuclear division (leaving syncytial nuclei in the yolk cell). Cell biology of meroblastic cleavage in the chick (as outline above) and in other model systems (Mitchison et al., 2012) is still very poorly understood. Many important features of a young chick embryo, such as the presence of a subgerminal cavity, separation of the inside and outside environment, polarization of early blastomeres, and possible asymmetric distribution of maternal determinants (discussed

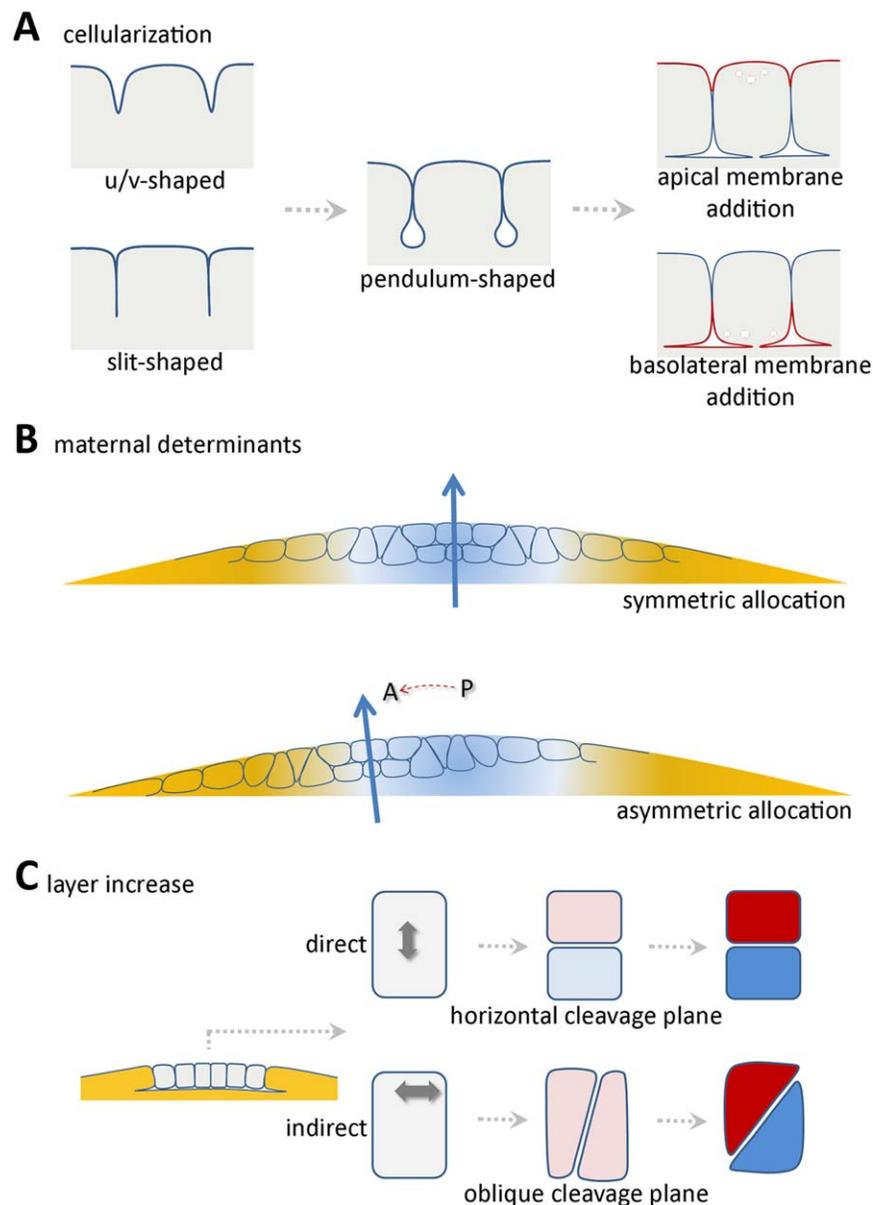
below), are fundamentally linked to the cellularization process.

Whether maternal determinants play a role in early patterning in the chick embryo is still debated. Specification of chicken primordial germ cells was shown to correlate with asymmetric distribution of maternally deposited Vasa-positive structures (Tsunekawa et al., 2000). Embryonic dorsoventral (D-V) patterning, corresponding to anteroposterior (A-P) polarization of the blastoderm, in quail and chick embryos was hypothesized to be regulated by asymmetric distribution of different ooplasm at early cleavage stages (Callebaut, 1987, 2005). Ooplasmic materials surrounding the germinal vesicle, the oocyte nucleus, are heterogenous. Callebaut and colleagues classified them into four types: the  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  ooplasm based on histochemical staining and radiolabeling data, and suggested that asymmetric inheritance of the  $\gamma$  and  $\delta$  ooplasm during cleavage stages provides positional cues for subsequent A-P polarization. The  $\gamma$  ooplasm is derived from the perinuclear mitochondria-rich “ticos” (thymidine-incorporating cytoplasmic organelles) and the  $\delta$  ooplasm is the cytoplasmic material on the surface of the nucleus of Pander, the white yolk substance located below the germinal vesicle. The  $\gamma$  and  $\delta$  ooplasm represent more “primitive” cytoplasmic materials than the yellow yolk which is deposited during late stages of oocyte maturation. Although it is plausible from an evo-devo perspective that maternal determinants in an avian egg are localized to these ooplasm, it is unclear whether or how asymmetric inheritance of these ooplasm determines the A-P polarity. During EGK-I to -IV, the center of cell proliferation, where the first two cleavage furrows meet and where the smallest blastomeres concentrate, is often located off the geometric center of the embryo. Dividing blastomeres may therefore inherit ooplasm materials asymmetrically as they cellularize (Fig. 3B). Like the amphibian Nieuwkoop center, this ooplasm asymmetry may lead to signaling asymmetry which later determines the future dorsal side at posterior end of the blastoderm. Significance of this

hypothesis is uncertain because artificial tilting of the blastoderm after EGK-VI can reorient embryonic D-V axis with high efficiency (Kochav and Eyal-Giladi, 1971) and multiple D-V axes can be experimentally induced in postovipositional embryos (Lutz, 1949; Spratt and Haas, 1960; Bertocchini et al., 2004; Bertocchini and Stern, 2012). An alternative explanation, reconciling both the ooplasm-mediated predetermination hypothesis and the regulative nature of D-V axis formation at *a.p.* formation and postovipositional stages, is that maternal asymmetry determinants may be localized not in dividing blastomeres during cellularization, but in yolk cell cortex after cellularization, as reported in pregastrula zebrafish embryos (Carvalho and Heisenberg, 2010). Molecular nature of the putative maternal determinants in either scenario (blastomere-inherited or yolk cortex-inherited) is unknown.

### Zygotic Gene Activation (ZGA) and Mid-blastula Transition (MBT)

ZGA occurs in two waves in vertebrate embryos (Tadros and Lipshitz, 2009): an early/minor wave as the earliest sign of ZGA and a late/major wave representing a robust and large-scale increase in ZGA. In the zebrafish, the minor ZGA starts at cleavage stages (64-cell stage) and the major one at early blastula stage (Mathavan et al., 2005). In the mouse, the minor ZGA starts at one-cell stage and the major one at two-cell stage (Wang and Dey, 2006); whereas in human, the minor ZGA starts at two-cell stage and the major ZGA at the four- to eight-cell stage (Vassena et al., 2011; Xue et al., 2013; Yan et al., 2013). Timing of ZGA onset is influenced by nucleocytoplasmic ratio, maternal clock, transcript abortion, and epigenetic modification (Tadros and Lipshitz, 2009), the first two of which are related to oocyte organization, early cleavage pattern and developmental speed. In this respect the chick embryo resembles more the fish and frog embryos than the mouse or human embryos. Radioisotope labeling (Wylie, 1972) and electron microscopy (Raveh et al., 1976) studies,



**Fig. 3.** Cellularization, maternal determinants, and formation of cell layers. **A:** Model of cytokinesis. Early furrows are u/v-shaped (without immediate formation of cell–cell junctions) or slit-shaped (with junctional interactions). The pendulum-shaped furrows are at an intermediate stage. Furrow base body, not drawn here, is located at the base of the pendulum and functions to drive vertical deepening or horizontal burrowing of the furrow. Completion of the cellularization process requires a supply of new plasma membrane, which can be provided either apically (before tight junction formation) or basolaterally (after tight junction formation). **B:** Ooplasm is deposited in the oocyte in a radially symmetric manner. In one scenario, dividing blastomeres inherit these ooplasm symmetrically (top). However, early cleavages are known to be radially asymmetric, possibly due to off-center pronuclear fusion, gravitational effects on yolk materials, or stochastic variation in peripheral extension of meroblastic cleavage furrows. This results in an asymmetric inheritance of maternal determinants (bottom). The side of the embryo inheriting more  $\gamma$  and  $\delta$  ooplasmic materials (surrounding and within in the nucleus of Pander, respectively) (Callebaut, 2008) will become or be biased to become posterior. Arrows: Hypothetical axis from yolk center to blastoderm center. **C:** Two possible causes for the increase in cell layer number from one to two. Direct cause: Horizontal (parallel to blastoderm surface) cleavage plane leads to the formation of outer and inner daughters. Indirect cause: Vertical (orthogonal to blastoderm surface) cleavage plane (not shown) or oblique cleavage plane (shown) generates two surface daughters, one of which with smaller surface area may be biased to become an inner cell.

using techniques which are quite insensitive by modern-day standards, suggested that there is no prominent ribosomal RNA synthesis before EGK-III. One may predict based on these lines of evidence that the minor ZGA

in the chick starts at mid-cleavage stages (EGK-II to -IV) and the major ZGA at late cleavage stages (EGK-IV to -VI). The juncture between the cleavage period and the *a.p.* formation period, EGK-VI, can thus be viewed as

the equivalent of mid-blastula transition (MBT) in *Xenopus*. Cell-counting analysis revealed that an EGK-V embryo contains approximately 2,400 cells and an EGK-VII embryo has 36,000 cells (Park et al., 2006).

Although divisions are known to be asynchronous after the 4th cleavage (Perry, 1987), these numbers translate into approximately the 11th cell cycle at EGK-V and the 15th cell cycle at EGK-VII, making it plausible that an MBT-like event occurs at EGK-VI. The increase in cell number proceeds at a much slower rate afterward, reaching approximately 54,000 at EGK-X (Park et al., 2006), probably due to the lengthening of cell cycle duration in the second half of intrauterine development (Emanuelsson, 1965).

### Increase in the Number of Cell Layers

Blastomeres located in the middle complete the cellularization process at EGK-II to -III, and further cell divisions result in the formation of a multilayered organization in central blastoderm, approximately two cells thick at EGK-III to -IV and reaching a maximum of five to six cells thick at EGK-VI. More peripheral regions undergo a similar, but delayed and less pronounced, process of layer increase. The most peripheral part of the blastoderm is always one-cell thick. When a centrally located blastomere separates itself from the yolk by forming a horizontal “basal” membrane, the yolk counterpart is anuclear. Thus the increase in layer number is the outcome of either rearrangement or proliferation of already cellularized blastomeres (Fig. 3C). Changing from a mono-layered to a bi-layered structure produces an inside cell population sealed off from the external milieu. A similar process in the mouse initiates the separation of cell types at the morula stage (Stephenson et al., 2012; Hirate et al., 2013). Asymmetric cleavage during the transition from 4-cell to 16-cell stage generates inside and outside daughters, whereas symmetric cleavage produces two outside or inside cells (Sasaki, 2010). The type of division can be predicted based on the orientation of mitotic plane: those parallel to the surface of the morula being asymmetric (by generating an inner, inner cell mass-biased daughter and an outer, trophectoderm-biased daughter) and those orthogonal to the surface being symmetric. Symmetric division of a surface blastomere, gen-

erating two outer daughters, can secondarily give rise to inner cells through postdivision modifications of cell movement and cell adhesion (Parfitt and Zernicka-Goetz, 2010). In *Xenopus*, inner cell population forms through a similar parallel-to-surface orientation of mitotic planes after 64-cell stage, while orthogonal-to-surface orientation of the mitotic plane generates two surface daughter cells (Chalmers et al., 2003). A third type observed in *Xenopus*, the oblique division, produces one daughter with smaller surface area which is strongly biased to undergo asymmetric division in the next cell cycle (Chalmers et al., 2003). In the chick, horizontal (parallel-to-surface) orientation of the cleavage plane was also hypothesized to be the cause for layer increase (Kochav et al., 1980), although there is no direct evidence either in favor of or against this model.

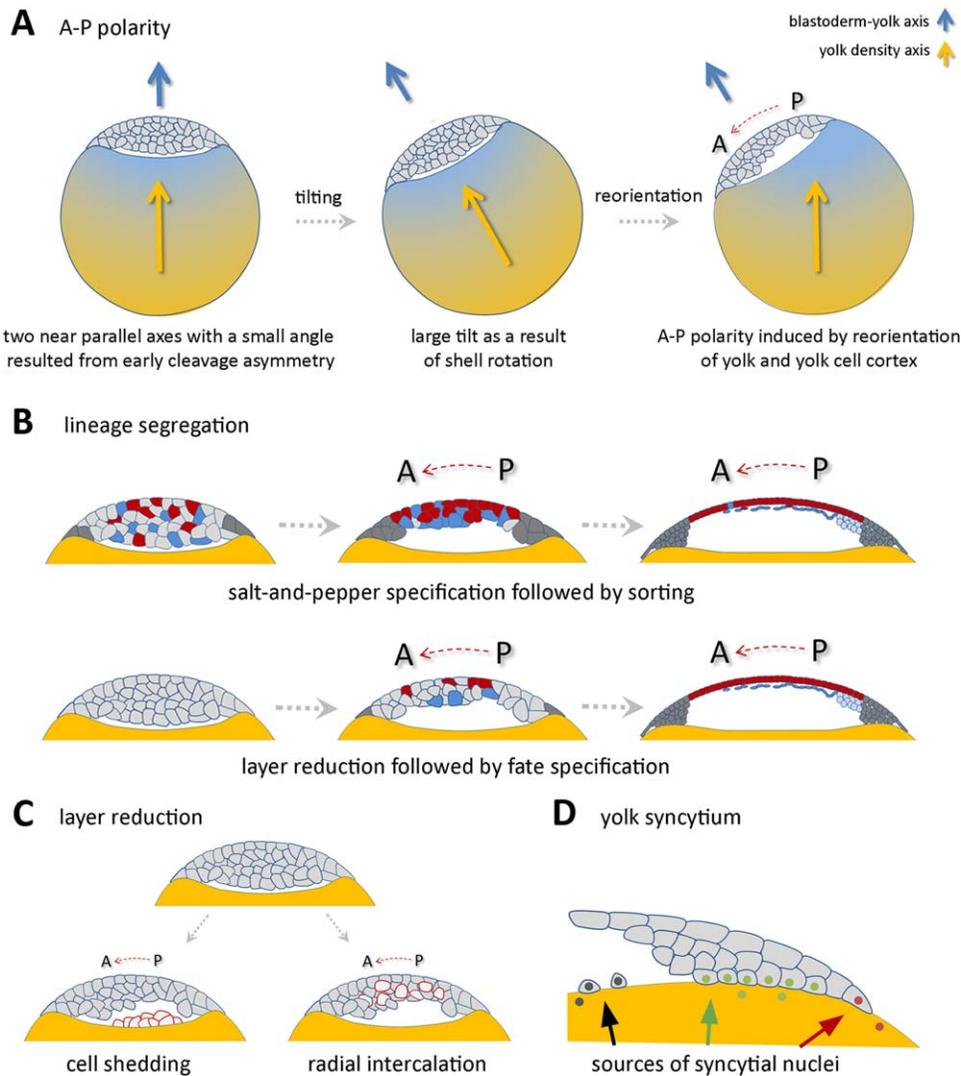
### A-P Polarity of the Blastoderm (Radial Symmetry Breaking)

Although often described as such, chick embryos obtained from freshly laid eggs are not radially symmetrical. At EGK-X, morphological asymmetry along the A-P axis of the blastoderm is apparent with the asymmetric distribution of hypoblast islands, and molecular asymmetry is also readily detectable in both the epiblast and hypoblast cells (Peter, 1938; Spratt and Haas, 1960; Eyal-Giladi and Kochav, 1976; Sheng and Stern, 1999; Bertocchini and Stern, 2012; Alev et al., 2013b). At the beginning of the *a.p.* formation period, EGK-VII, initiation of layer reduction has been proposed to start from the posterior side of future *area pellucida* (Clavert, 1960; Kochav et al., 1980; Watt et al., 1993). As in most telolecithal eggs, the abembryonic side of chicken yolk is heavier than the embryonic side, and a developing embryo positions itself on top of the yolk if left undisturbed. Vintemberger and Clavert, in a series of papers published in 1950s and 1960, proposed that bilateral symmetry (i.e., A-P polarity of the blastoderm and D-V polarity of the embryo) is established by egg rotation in utero (Fig. 4A). The forming egg

shell is rotated along its long axis in the uterus (10–15 times per hr counter clock-wise if viewed from the isthmus side) with no matching rotation of the egg yolk, resulting in the formation of coiled chalazae (two strands of dense egg white materials connecting the vitelline membrane around the yolk with the outer thick albumen underneath the soft shell) and a constant tilt of the blastoderm relative to its gravitationally dictated position (Fig. 4A). The highest point of the blastoderm will eventually become the posterior side (the so-called von Baer’s rule). Results from artificial tilting of EGK-VI to -X embryos supported this hypothesis (Kochav and Eyal-Giladi, 1971; Eyal-Giladi and Fabian, 1980; Eyal-Giladi, 1991), suggesting that molecular determinants of radial-symmetry breaking is sensitive to gravitational cues at late cleavage and early *a.p.* formation stages. These observations do not rule out the possibility of radial-symmetry breaking being determined or biased by pronuclear fusion point or maternal ooplasm segregation (Fig. 3B), but they do strongly argue in favor of a regulative mechanism underlying initial radial-symmetry breaking. The molecular nature of the earliest determinants of A-P axis formation, either maternally biased at early cleavage stages or gravitationally regulated at early *a.p.* formation stages, is unknown.

### Layer Reduction and Lineage Segregation

From EGK-VII, the number of cell layers in the blastoderm decreases from initial five to six at EGK-VI to one to two at EGK-X. This process starts from the central-posterior region of the blastoderm and spreads to all areas above the subgerminal cavity, but not to the *area opaca*. Cell shedding was proposed to be the cause for layer reduction (Kochav et al., 1980, Fabian and Eyal-Giladi, 1981) (Fig. 4C). Radial intercalation, as an alternative although not mutually exclusive mechanism, may also play a role in this process (Fig. 4C). During the process of *a.p.* formation, the central-most, thickest area takes longer time to appear translucent than the rest of the blastoderm located above the subgerminal cavity.



**Fig. 4.** A-P polarization, lineage segregation, layer reduction, and yolk syncytium formation. **A:** A-P polarity. Despite possible biases imposed at an earlier stage (see Fig. 3B), anterior-posterior polarity of a chick blastoderm first becomes visible at EGK-VII. This is a regulative process. Egg shell is rotated counter clock-wise in utero (if viewed from the isthmus side), resulting in a tilt of the blastoderm (middle) away from its preferred position (left). Gravitational forces reorient the yolk and yolk cortical materials, and side of the embryo in closer contact with the nucleus of Pander ooplasm (top side) will become posterior. **B:** Two possible mechanisms for the segregation of the epiblast, hypoblast and *area opaca* cell lineages. In one scenario, lineage specification starts when the blastoderm is multilayered. The epiblast and hypoblast lineages are mixed in a salt-and-pepper manner. Sorting takes place during layer reduction, eventually resulting in bilaminar organization with an upper epiblast and a lower hypoblast layer. In the second scenario, lineage specification follows layer reduction. **C:** Two possible mechanisms for layer reduction: cell shedding and radial intercalation. **D:** Three possible sources of syncytial nuclei. Black arrow: shed cells on the floor of subgerminal cavity. Green arrow: germ wall deep layer cells. Red arrow: edge cell.

This central area is called *area alba* in the turkey (Gupta and Bakst, 1993) and an equivalent structure, although not named, is also present in the chick (Kochav et al., 1980). Eventually, at EGK-X, most regions of the *area pellucida*, including the *area alba*, become 1–2 cell thick, whereas the *area opaca* remains multilayered (Kochav et al., 1980; Andries et al., 1983; Gupta and Bakst, 1993; Watt et al., 1993). A notable exception is the region of the *area pellucida* at the

posterior marginal zone, located between the Koller's sickle (a crescent-shaped lower layer cell population closely associated with the overlying epiblast) and the posterior germ wall. This part of the *area pellucida* is often more than two cells thick, and the dynamic nature of its morphogenesis and signaling is among the most prominent and debated issues of early postovipositional development (Eyal-Giladi, 1991; Eyal-Giladi et al., 1992; Calle-

baut et al., 1998; Stern and Downs, 2012). This aspect of cell-layer reduction is not discussed in detail here.

Two general cell-biological features of the *area pellucida* at EGK-X, however, deserve further discussion. First, the one-cell thick areas have only the upper, epiblast layer; while the two-cell thick areas contain an upper epiblast layer and a lower loose aggregate of primary hypoblast cells (hypoblast islands). Epiblast cells in one-cell thick areas are polarized with

signs of basement membrane deposition, whereas epiblast cells in two-cell thick areas do not have a basement membrane and lag behind in the epithelialization process (Bortier et al., 1989; Harrisson et al., 1991; Lawson and Schoenwolf, 2001; Nakaya et al., 2008; Nakaya et al., 2011, 2013). Second, primary hypoblast islands have an anterior-sparse/posterior-dense distribution (Kochav et al., 1980; Watt et al., 1993), opposite to the initiation of layer reduction, which starts from the posterior side. The initiation of cell shedding or intercalation and the speed of its continuation may, therefore, be regulated separately.

The epiblast was hypothesized to be reduced to one cell thick, and the primary hypoblast cells were derived by means of poly-ingression from the upper, epiblast layer cells (Peter, 1938; Fabian and Eyal-Giladi, 1981; Eyal-Giladi, 1991; Watt et al., 1993). Support for this model is indirect (bottle-shaped cells spanning both layers) and pregastrulation development in the chick does not pass through any stage with a one-cell thick epiblast without associated hypoblast cells. A plausible interpretation is that primary hypoblast cells are “left-overs” of the layer reduction process. An incompletely epithelialized epiblast, with no basement membrane and labile epithelial junctions, can still accommodate accretion of cells from the lower layer, resulting in bottle-shaped cell morphology seen in sections or by surface labeling. Instead, a well-epithelialized epiblast with forming basement membrane will resist further cellular addition, leaving non-epithelialized lower layer cells as primary hypoblast cells. The difference in hypoblast island density along the A–P axis could, therefore, be simply due to a difference in the rate of epithelialization of the uppermost layer during the process of layer reduction.

Viewed from an evolutionary perspective, however, the poly-ingression hypothesis still deserves to be tested carefully. In monotremes, the multilayered blastoderm thins out into a single-layered structure, from which the hypoblast cells are thought to come (Flynn and Hill, 1947), although direct evidence is also lacking in this case. In marsupials, the blastoderm is single-layered from the beginning of

blastocyst formation and the hypoblast cells are generated from the epiblast/pluriblast by ingression, presumably from both the middle and border regions of the epiblast/pluriblast (Selwood, 1992; Kress and Selwood, 2006; Frankenberg et al., 2013). In eutherian mammals, hypoblast and epiblast cells separate from each other from an initially mixed inner cell mass population and before the epiblast cells adopt an epithelial structure (Stephenson et al., 2012; Xenopoulos et al., 2012). It, therefore, remains possible that hypoblast precursor cells in the chick are initially located in a salt-and-pepper manner in the multilayered blastoderm (including in the upper-most layer) and secondarily aggregate or ingress during the layer reduction/lineage sorting process.

The epiblast and hypoblast layers will give rise to different embryonic and extraembryonic cell types later in development. Cellular changes leading to their formation (i.e., *a.p.* formation and layer reduction) take place concomitant with changes in gene expression profiles. By the time a chick embryo reaches EGK-X, at least three cell populations with distinct molecular signatures have been delineated: the epiblast, hypoblast, and the *area opaca* cells (Fig. 4B). Each population has robust expression of lineage-specific markers, many of which have been well-characterized in mammalian models, such as *Nanog*, *PouV*, *Dnmt3B*, and *Lin28* in epiblast (Lavial et al., 2007; Shin et al., 2011; Alev et al., 2013a), *Hex* in hypoblast (Yatskiyevych et al., 1999), and *Eomes* in *area opaca* cells (Pernaute et al., 2010). It is unclear how early these lineage specific genes initiate their expression and whether their expression precedes or follows morphological changes of these three cell populations (Fig. 4B). It is also worth noting that instead of defining cellular fates, specific expression of lineage markers at these early stages likely marks cellular states, the labile and dynamic bias toward these cellular fates.

### Yolk Syncytium and Blastoderm-Yolk Contact

It is yet to be determined whether the membrane enclosed structure sur-

rounding the remaining yolk, after the completion of cellularization at EGK-VI, is a syncytium or not. In the zebrafish, yolk syncytium forms by fusion of the yolk cell and deep layer marginal blastomeres, and plays critical roles in embryonic induction and morphogenesis (Carvalho and Heisenberg, 2010). In monotremes, the yolk cell at late blastula is also a syncytium (Flynn and Hill, 1947). In the chick, there are three possible sources of zygotic nuclei which may contribute to the formation of a yolk syncytium: the most peripheral part of the blastoderm, deep blastomeres in the germ wall, and shed blastomeres in the *area pellucida* (Fig. 4D).

At cleavage stages, peripheral open cells are in contact with the yolk both basally and peripheral-laterally. Nuclear division in these cells generates a proximally located daughter which cellularizes like other centrally located blastomeres and a peripherally located one which remains as an open cell. Cycles of nuclear division and cytokinesis reduce the size of the open cell successively. If the last cellularization step takes place before nuclear division, no daughter nucleus is left in the yolk cell; and if it happens after nuclear division, each peripheral-most cell will leave a daughter nucleus in the yolk cell. The second source is the blastomeres located close to the yolk cell membrane. From EGK-III, a subgerminal cavity forms in central blastoderm between the yolk cell membrane and cellularized central blastomeres. This cavity expands as cellularization progresses peripherally. The expansion, however, lags behind the cellularization process, and the yolk cell stays in close contact with peripheral deep layer blastomeres even after complete cellularization. This area of contact is maintained during early development and is called the germ wall. It is unclear whether some of the deep layer cells in the germ wall can fuse with the yolk cell as seen in the zebrafish. The inner margin of the germ wall is a dynamic, shifting boundary between the *area pellucida* and *area opaca*. It is also an important center for early patterning. It is unclear what roles the yolk cell plays in the maintenance of this boundary or in its associated molecular signaling.

Deep cells in the *area pellucida* do not contact the yolk cell membrane. However, *area pellucida* cells that are “shed” during the process of layer reduction drop to the floor of the subgerminal cavity and associate with the yolk cell tightly (Andries et al., 1983). These shed cells are also distributed asymmetrically along the A–P axis. Whether some of them fuse with the yolk cell and whether they play any role in the function of yolk syncytium are unknown. Electron microscopy studies of EGK-X to -XIV chick and quail embryos (Andries et al., 1983) indicate that the cortical regions of the yolk cell on the floor of the subgerminal cavity are rich in mitochondria, microfilaments, and membrane invaginations and protrusions, suggesting that yolk cell cortex and its associated cellular and molecular activities are an integral part of avian early development.

#### TECHNICAL LIMITATIONS AND POSSIBLE SOLUTIONS

The chick is a mature developmental biology model. Tools for RNA/protein localization, cell labeling/tracing, imaging, overexpression/knockdown, and transcriptomics/proteomics analyses are all available. Recent progresses in avian transgenesis (McGrew et al., 2004; van de Lavoie et al., 2006; Park and Han, 2012; Nishijima and Iijima, 2013) make it possible to generate chicken lines with ubiquitous or promoter-specific green fluorescent protein expression, allowing sophisticated imaging and molecular analyses in the future. The only major limitation in studying intrauterine development is the access to these embryos. Large poultry facilities are common in most countries, but basic developmental biologists often do not have access to these facilities. Some universities still maintain poultry farms affiliated with their agriculture or biotechnology departments. Scientists working in these departments are best suited for bridging this gap.

Three methods have been used to retrieve intrauterine eggs for early stage embryos. They are the physically induced retrieval method (Olsen, 1942; Eyal-Giladi and Kochav, 1976; Park et al., 2006; Lee et al., 2013),

hormonally induced retrieval method (Olsen, 1942; Watt et al., 1993) and direct sacrifice of mother hens (Emanuelsson, 1965; Gipson, 1974; Bellairs et al., 1978). Only eggs with soft or hard shell (from four-cell stage onward) can be obtained through the first two techniques; while the third one can be used to obtain eggs from fertilization to one- to two-cell division stages. Retrieved eggs can be cultured *in vitro*, with or without the shell, to postovipositional stages with good survival rates (Vintemberger and Clavert, 1960; Kochav and Eyal-Giladi, 1971; Perry, 1988; Naito and Perry, 1989; Naito et al., 1990, 1995). Embryos collected from intrauterine eggs can be used directly for transcriptomics/proteomics investigations, fixed for gene expression and protein localization studies, or cultured *in vitro* for labeling, lineage tracing, and functional analyses. Finally, an alternative approach to circumvent the limited access of intrauterine chick embryos may be to use embryos from other avian species. The turkey (Gupta and Bakst, 1993) and zebra finch (unpublished data) lay their eggs at much earlier stages than the chick. Both species have their genome sequenced (Dalloul et al., 2010; Warren et al., 2010), and the early development, from cleavage to hypoblast formation, of the turkey embryo has also been described (Gupta and Bakst, 1993).

#### CONCLUSIONS

Intrauterine chick development is divided into 10 EGK stages (EGK-I to -X). EGK-I to -VI are collectively called cleavage stages, during which the cell number increases from 1 to approximately 10,000 and the embryo attains the equivalent of mid-blastula stage. The *area pellucida* forms between EGK-VII to -X, during which the embryo establishes bilateral symmetry and specifies three basic cell lineages, the epiblast, hypoblast, and *area opaca*. Intrauterine development in the chick is similar to the “preimplantation” (cleavage to late blastula) development in the monotremes, and the knowledge gleaned from studies of day-1 chick embryos will enable a better mechanistic

understanding of early development in diverse mammalian species.

#### ACKNOWLEDGMENTS

I thank Dr. Gary Schoenwolf for the invitation to write this commentary, and Drs. Claudio Stern, Patrick Tam, Jae Yong Han, members of Laboratory for Early Embryogenesis, and the reviewers for critical comments.

#### REFERENCES

- Alev C, Nakano M, Wu Y, Horiuchi H, Sheng G. 2013a. Manipulating the avian epiblast and epiblast-derived stem cells. *Methods Mol Biol* 1074:151–173.
- Alev C, Wu Y, Nakaya Y, Sheng G. 2013b. Decoupling of amniote gastrulation and streak formation reveals a morphogenetic unity in vertebrate mesoderm induction. *Development* 140:2691–2696.
- Andries L, Vakaet L, Vanroelen C. 1983. The subgerminal yolk surface and its relationship with the inner germ wall edge of the stages X to XIV chick and quail embryo. A SEM study. *Anat Embryol (Berl)* 166:453–462.
- Arnold JM. 1969. Cleavage furrow formation in a telolecithal egg (*Loligo pealii*). I. Filaments in early furrow formation. *J Cell Biol* 41:894–904.
- Bellairs R, Lorenz FW, Dunlap T. 1978. Cleavage in the chick embryo. *J Embryol Exp Morphol* 43:55–69.
- Bellairs R, Osmond M. 2005. The atlas of chick development. Amsterdam: Elsevier.
- Bertocchini F, Skromne I, Wolpert L, Stern CD. 2004. Determination of embryonic polarity in a regulative system: evidence for endogenous inhibitors acting sequentially during primitive streak formation in the chick embryo. *Development* 131:3381–3390.
- Bertocchini F, Stern CD. 2012. *Gata2* provides an early anterior bias and uncovers a global positioning system for polarity in the amniote embryo. *Development* 139:4232–4238.
- Blomberg L, Hashizume K, Viebahn C. 2008. Blastocyst elongation, trophoblastic differentiation, and embryonic pattern formation. *Reproduction* 135:181–195.
- Bortier H, De Bruyne G, Espeel M, Vakaet L. 1989. Immunohistochemistry of laminin in early chicken and quail blastoderms. *Anat Embryol (Berl)* 180:65–69.
- Callebaut M. 1987. Ooplasmic localization and segregation in quail germs: fate of the four ooplasm. *Arch Biol* 98:441–473.
- Callebaut M. 2005. Origin, fate, and function of the components of the avian germ disc region and early blastoderm: role of ooplasmic determinants. *Dev Dyn* 233:1194–1216.
- Callebaut M. 2008. Historical evolution of preformistic versus neoformistic (epigenetic) thinking in embryology. *Belg J Zool* 138.
- Callebaut M, Van Nueten E, Van Nassauw L, Bortier H, Harrisson F. 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 blastoderms. *Reprod Nutr Dev* 38:449–463.
- Carvalho L, Heisenberg CP. 2010. The yolk syncytial layer in early zebrafish development. *Trends Cell Biol* 20:586–592.
- Chalmers AD, Strauss B, Papalopulu N. 2003. Oriented cell divisions asymmetrically segregate aPKC and generate cell fate diversity in the early *Xenopus* embryo. *Development* 130:2657–2668.
- Clavert J. 1960. Determinisme de la symetrie bilaterale chez les oiseaux. IV Existence d'une phase critique pour la symétrization de l'oeuf. *Son stade*. *Arch Anat Microsc Morphol Exp* 49:345–361.
- Dalloul RA, Long JA, Zimin AV, Aslam L, Beal K, Blomberg Le A, Bouffard P, Burt DW, Crasta O, Crooijmans RP, Cooper K, Coulombe RA, De S, Delany ME, Dodgson JB, Dong JJ, Evans C, Frederickson KM, Flicek P, Florea L, Folkerts O, Groenen MA, Harkins TT, Herrero J, Hoffmann S, Megens HJ, Jiang A, De Jong P, Kaiser P, Kim H, Kim KW, Kim S, Langenberger D, Lee MK, Lee T, Mane S, Marcias G, Marz M, Mcelroy AP, Modise T, Nefedov M, Notredame C, Paton IR, Payne WS, Perteira G, Prickett D, Puiu D, Qiao D, Raineri E, Ruffier M, Salzberg SL, Schatz MC, Scheuring C, Schmidt CJ, Schroeder S, Searle SM, Smith EJ, Smith J, Sonstegard TS, Stadler PF, Tafer H, Tu ZJ, Van Tassell CP, Vilella AJ, Williams KP, Yorke JA, Zhang L, Zhang HB, Zhang X, Zhang Y, Reed KM. 2010. Multi-platform next-generation sequencing of the domestic turkey (*Meleagris gallopavo*): genome assembly and analysis. *PLoS Biol* 8:pii: e1000475.
- Eakin GS, Behringer RR. 2004. Diversity of germ layer and axis formation among mammals. *Semin Cell Dev Biol* 15: 619–629.
- Emanuelsson H. 1965. Cell multiplication in the chick blastoderm up to the time of laying. *Exp Cell Res* 39:386–399.
- Eyal-Giladi H. 1991. The early embryonic development of the chick, as an epigenetic process. *Crit Rev Poultry Biol* 3: 143–166.
- Eyal-Giladi H, Debby A, Harel N. 1992. The posterior section of the chick's area pellucida and its involvement in hypoblast and primitive streak formation. *Development* 116:819–830.
- Eyal-Giladi H, Fabian B. 1980. Axis determination in uterine chick blastodiscs under changing spatial positions during the sensitive period for polarity. *Dev Biol* 77:228–232.
- Eyal-Giladi H, Kochav S. 1976. From cleavage to primitive streak formation: a complementary normal table and a new look at the first stages of the development of the chick. I. General morphology. *Dev Biol* 49:321–337.
- Fabian B, Eyal-Giladi H. 1981. A SEM study of cell shedding during the formation of the area pellucida in the chick embryo. *J Embryol Exp Morphol* 64:11–22.
- Flechon JE, Degrouard J, Flechon B. 2004. Gastrulation events in the pre-streak pig embryo: ultrastructure and cell markers. *Genesis* 38:13–25.
- Flynn TT, Hill JP. 1938. The development of the monotremata. IV. Growth of the ovarian ovum, maturation, fertilisation, and early cleavage. *Trans Zool Soc Lond* 24:445–623.
- Flynn TT, Hill JP. 1947. The Development of the Monotremata. VI. The later stages of cleavage and the formation of the primary germ-layers. *Trans Zool Soc Lond* 26:1–151.
- Frankenberg S, Shaw G, Freyer C, Pask AJ, Renfree MB. 2013. Early cell lineage specification in a marsupial: a case for diverse mechanisms among mammals. *Development* 140:965–975.
- Gipson I. 1974. Electron microscopy of early cleavage furrows in the chick blastodisc. *J Ultrastruct Res* 49:331–347.
- Gupta SK, Bakst MR. 1993. Turkey embryo staging from cleavage through hypoblast formation. *J Morphol* 217: 313–325.
- Hamburger V, Hamilton HL. 1992. A series of normal stages in the development of the chick embryo. 1951. *Dev Dyn* 195:231–272.
- Harrisson F, Callebaut M, Vakaet L. 1991. Features of polyingression and primitive streak ingression through the basal lamina in the chicken blastoderm. *Anat Rec* 229:369–383.
- Hassoun R, Schwartz P, Feistel K, Blum M, Viebahn C. 2009. Axial differentiation and early gastrulation stages of the pig embryo. *Differentiation* 78:301–311.
- Hirate Y, Hirahara S, Inoue K, Suzuki A, Alarcon VB, Akimoto K, Hirai T, Hara T, Adachi M, Chida K, Ohno S, Marikawa Y, Nakao K, Shimono A, Sasaki H. 2013. Polarity-dependent distribution of angiominin localizes hippo signaling in preimplantation embryos. *Curr Biol* 23:1181–1194.
- Kochav S, Eyal-Giladi H. 1971. Bilateral symmetry in chick embryo determination by gravity. *Science* 171:1027–1029.
- Kochav S, Ginsburg M, Eyal-Giladi H. 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* 79:296–308.
- Kress A, Selwood L. 2006. Marsupial hypoblast: formation and differentiation of the bilaminar blastocyst in *Sminthopsis macroura*. *Cells Tissues Organs* 182:155–170.
- Lavial F, Aclouque H, Bertocchini F, Macleod DJ, Boast S, Bachelard E, Montillet G, Thenot S, Sang HM, Stern CD, Samarut J, Pain B. 2007. The Oct4 homologue PouV and Nanog regulate pluripotency in chicken embryonic stem cells. *Development* 134:3549–3563.
- Lawson A, Schoenwolf GC. 2001. New insights into critical events of avian gastrulation. *Anat Rec* 262:238–252.
- Lecuit T, Wieschaus E. 2000. Polarized insertion of new membrane from a cytoplasmic reservoir during cleavage of the *Drosophila* embryo. *J Cell Biol* 150: 849–860.
- Lee HC, Choi HJ, Park TS, Lee SI, Kim YM, Rengaraj D, Nagai H, Sheng G, Lim JM, Han JY. 2013. Cleavage events and sperm dynamics in chick intrauterine embryos. *PLoS ONE*. Doi: 10.1371/journal.pone.0080631.
- Li WM, Webb SE, Lee KW, Miller AL. 2006. Recruitment and SNARE-mediated fusion of vesicles in furrow membrane remodeling during cytokinesis in zebrafish embryos. *Exp Cell Res* 312:3260–3275.
- Lutz H. 1949. Sur la production expérimentale de la polyembryonie et des monstruosités doubles chez les Oiseaux. *Arch Anat Microsc Morphol Exp* 38:88–144.
- Mathavan S, Lee SG, Mak A, Miller LD, Murthy KR, Govindarajan KR, Tong Y, Wu YL, Lam SH, Yang H, Ruan Y, Korzh V, Gong Z, Liu ET, Lufkin T. 2005. Transcriptome analysis of zebrafish embryogenesis using microarrays. *PLoS Genet* 1:260–276.
- McGrew MJ, Sherman A, Ellard FM, Lillico SG, Gilhooley HJ, Kingsman AJ, Mitrophanous KA, Sang H. 2004. Efficient production of germline transgenic chickens using lentiviral vectors. *EMBO Rep* 5:728–733.
- Menkhorst E, Nation A, Cui S, Selwood L. 2009. Evolution of the shell coat and yolk in amniotes: a marsupial perspective. *J Exp Zool B Mol Dev Evol* 312: 625–638.
- Mitchison T, Wuhr M, Nguyen P, Ishihara K, Groen A, Field CM. 2012. Growth, interaction, and positioning of microtubule asters in extremely large vertebrate embryo cells. *Cytoskeleton (Hoboken)* 69:738–750.
- Naito M, Nirasawa K, Oishi T. 1990. Development in culture of the chick embryo from fertilized ovum to hatching. *J Exp Zool* 254:322–326.
- Naito M, Nirasawa K, Oishi T. 1995. An in vitro culture method for chick embryos obtained from the anterior portion of the magnum of oviduct. *Br Poult Sci* 36:161–164.
- Naito M, Perry MM. 1989. Development in culture of the chick embryo from cleavage to hatch. *Br Poult Sci* 30:251–256.
- Nakaya Y, Sukowati EW, Alev C, Nakazawa F, Sheng G. 2011. Involvement of dystroglycan in epithelial-mesenchymal transition during chick gastrulation. *Cells Tissues Organs* 193:64–73.
- Nakaya Y, Sukowati EW, Sheng G. 2013. Epiblast integrity requires CLASP and Dystroglycan-mediated microtubule anchoring to the basal cortex. *J Cell Biol* 202:637–651.
- Nakaya Y, Sukowati EW, Wu Y, Sheng G. 2008. RhoA and microtubule dynamics control cell-basement membrane interaction in EMT during gastrulation. *Nat Cell Biol* 10:765–775.
- Nishijima K, Iijima S. 2013. Transgenic chickens. *Dev Growth Differ* 55:207–216.

- Olsen MW. 1942. Maturation, fertilization, and early cleavage in the hen's egg. *J Morphol* 70:513–533.
- Parfitt DE, Zernicka-Goetz M. 2010. Epigenetic modification affecting expression of cell polarity and cell fate genes to regulate lineage specification in the early mouse embryo. *Mol Biol Cell* 21:2649–2660.
- Park HJ, Park TS, Kim TM, Kim JN, Shin SS, Lim JM, Han JY. 2006. Establishment of an in vitro culture system for chicken preblastodermal cells. *Mol Reprod Dev* 73:452–461.
- Park TS, Han JY. 2012. piggyBac transposition into primordial germ cells is an efficient tool for transgenesis in chickens. *Proc Natl Acad Sci U S A* 109:9337–9341.
- Pernaute B, Canon S, Crespo M, Fernandez-Tresguerres B, Rayon T, Manzanares M. 2010. Comparison of extraembryonic expression of *Eomes* and *Cdx2* in pregastrulation chick and mouse embryo unveils regulatory changes along evolution. *Dev Dyn* 239:620–629.
- Perry MM. 1987. Nuclear events from fertilisation to the early cleavage stages in the domestic fowl (*Gallus domesticus*). *J Anat* 150:99–109.
- Perry MM. 1988. A complete culture system for the chick embryo. *Nature* 331:70–72.
- Peter K. 1938. Untersuchungen über die Entwicklung des Dotterentoderms. 1. Die Entwicklung des Entoderms beim Huhnchen. *Z Mikr Anat Forsch* 43:362–415.
- Raveh D, Friedlander M, Eyal-Giladi H. 1976. Nucleolar ontogenesis in the uterine chick germ correlated with morphogenetic events. *Exp Cell Res* 100:195–203.
- Sasaki H. 2010. Mechanisms of trophectoderm fate specification in preimplantation mouse development. *Dev Growth Differ* 52:263–273.
- Schrode N, Xenopoulos P, Piliszek A, Frankenberg S, Plusa B, Hadjantonakis AK. 2013. Anatomy of a blastocyst: cell behaviors driving cell fate choice and morphogenesis in the early mouse embryo. *Genesis* 51:219–233.
- Selwood L. 1992. Mechanisms underlying the development of pattern in marsupial embryos. *Curr Top Dev Biol* 27:175–233.
- Selwood L, Johnson MH. 2006. Trophoblast and hypoblast in the monotreme, marsupial and eutherian mammal: evolution and origins. *Bioessays* 28:128–145.
- Sheng G, Stern CD. 1999. *Gata2* and *Gata3*: novel markers for early embryonic polarity and for non-neural ectoderm in the chick embryo. *Mech Dev* 87:213–216.
- Shin M, Alev C, Wu Y, Nagai H, Sheng G. 2011. Activin/TGF-beta signaling regulates *Nanog* expression in the epiblast during gastrulation. *Mech Dev* 128:268–278.
- Spratt N, Haas H. 1960. Integrative mechanisms in development of the early chick blastoderm. I. Regulative potentiality of separated parts. *J Exp Zool* 145:97–137.
- Stephenson RO, Rossant J, Tam PP. 2012. Intercellular interactions, position, and polarity in establishing blastocyst cell lineages and embryonic axes. *Cold Spring Harb Perspect Biol* 4:pii.
- Stern CD. 2005. The chick; a great model system becomes even greater. *Dev Cell* 8:9–17.
- Stern CD, Downs KM. 2012. The hypoblast (visceral endoderm): an evo-devo perspective. *Development* 139:1059–1069.
- Tadros W, Lipshitz HD. 2009. The maternal-to-zygotic transition: a play in two acts. *Development* 136:3033–3042.
- Tsunekawa N, Naito M, Sakai Y, Nishida T, Noce T. 2000. Isolation of chicken vasa homolog gene and tracing the origin of primordial germ cells. *Development* 127:2741–2750.
- Van De Lavoie MC, Diamond JH, Leighton PA, Mather-Love C, Heyer BS, Bradshaw R, Kerchner A, Hooi LT, Gessaro TM, Swanberg SE, Delany ME, Etches RJ. 2006. Germline transmission of genetically modified primordial germ cells. *Nature* 441:766–769.
- Vassena R, Boue S, Gonzalez-Roca E, Aran B, Auer H, Veiga A, Izpisua Belmonte JC. 2011. Waves of early transcriptional activation and pluripotency program initiation during human preimplantation development. *Development* 138:3699–3709.
- Vejlsted M, Avery B, Schmidt M, Greve T, Alexopoulos N, Maddox-Hyttel P. 2005. Ultrastructural and immunohistochemical characterization of the bovine epiblast. *Biol Reprod* 72:678–686.
- Vintemberger P, Clavert J. 1960. Sur le déterminisme de la symétrie bilatérale chez les oiseaux XIII Les facteurs de l'orientation de l'embryon par rapport à l'axe de l'oeuf et la règle de Von Baer, à la lumière de nos expériences d'orientation dirigées sur l'oeuf de poule extrait de l'utérus. *C R Soc Biol* 154:1072–1076.
- Wang H, Dey SK. 2006. Roadmap to embryo implantation: clues from mouse models. *Nat Rev Genet* 7:185–199.
- Warren WC, Clayton DF, Ellegren H, Arnold AP, Hillier LW, Kunstner A, Searle S, White S, Vilella AJ, Fairley S, Heger A, Kong L, Ponting CP, Jarvis ED, Mello CV, Minx P, Lovell P, Velho TA, Ferris M, Balakrishnan CN, Sinha S, Blatti C, London SE, Li Y, Lin YC, George J, Sweedler J, Southey B, Gunaratne P, Watson M, Nam K, Backstrom N, Smeds L, Nabholz B, Itoh Y, Whitney O, Pfenning AR, Howard J, Volker M, Skinner BM, Griffin DK, Ye L, McLaren WM, Flicke P, Quesada V, Velasco G, Lopez-Otin C, Puente XS, Olender T, Lancet D, Smit AF, Hubley R, Konkel MK, Walker JA, Batzer MA, Gu W, Pollock DD, Chen L, Cheng Z, Eichler EE, Stapley J, Slate J, Ekblom R, Birkhead T, Burke T, Burt D, Scharff C, Adam I, Richard H, Sultan M, Soldatov A, Lehrach H, Edwards SV, Yang SP, Li X, Graves T, Fulton L, Nelson J, Chinwalla A, Hou S, Mardis ER, Wilson RK. 2010. The genome of a songbird. *Nature* 464:757–762.
- Watt JM, Pettitte JN, Etches RJ. 1993. Early development of the chick embryo. *J Morphol* 215:165–182.
- Williams BS, Biggers JD. 1990. Polar trophoblast (Raubers' layer) of the rabbit blastocyst. *Anat Rec* 227:211–222.
- Wimsatt WA. 1975. Some comparative aspects of implantation. *Biol Reprod* 12:1–40.
- Wolf XA, Serup P, Hyttel P. 2011. Three-dimensional localisation of NANOG, OCT4, and E-CADHERIN in porcine pre- and peri-implantation embryos. *Dev Dyn* 240:204–210.
- Wong F, Welten MC, Anderson C, Bain AA, Liu J, Wicks MN, Pavlovskaya G, Davey MG, Murphy P, Davidson D, Tickle CA, Stern CD, Baldock RA, Burt DW. 2013. eChickAtlas: an introduction to the database. *Genesis* 51:365–371.
- Wylie CC. 1972. The appearance and quantitation of cytoplasmic ribonucleic acid in the early chick embryo. *J Embryol Exp Morphol* 28:367–384.
- Xenopoulos P, Kang M, Hadjantonakis AK. 2012. Cell lineage allocation within the inner cell mass of the mouse blastocyst. *Results Probl Cell Differ* 55:185–202.
- Xue Z, Huang K, Cai C, Cai L, Jiang CY, Feng Y, Liu Z, Zeng Q, Cheng L, Sun YE, Liu JY, Horvath S, Fan G. 2013. Genetic programs in human and mouse early embryos revealed by single-cell RNA sequencing. *Nature* 500:593–597.
- Yan L, Yang M, Guo H, Yang L, Wu J, Li R, Liu P, Lian Y, Zheng X, Yan J, Huang J, Li M, Wu X, Wen L, Lao K, Li R, Qiao J, Tang F. 2013. Single-cell RNA-Seq profiling of human preimplantation embryos and embryonic stem cells. *Nat Struct Mol Biol* 20:1131–1139.
- Yatskiyevych TA, Pascoe S, Antin PB. 1999. Expression of the homeobox gene *Hex* during early stages of chick embryo development. *Mech Dev* 80:107–109.