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Mammalian Balbiani Body As a Sign of Ancestral Oocyte Asymmetry: Review

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Eutherian eggs underwent a secondary loss of the ooplasmic gradient of yolk and maternal morphogens. However, the oocytes of mammalian primordial follicles display an asymmetric organelle cluster recognized as Balbiani body. Distribution of components of this aggregate might be a clue for the function of Balbiani body in eutherians and some alterations of its dynamics could be associated with ovarian pathology, particularly in human.

Key words: oocyte polarity, mitochondria, Golgi complex, Xenopus, mouse.

Mammalian evolution is characterized by secondary loss of the ancestral amniotic egg with its cytoplasmic gradients. As a result, blastomeres of eutherians (placental mammals) lack morphogenetic signals of maternal origin, and their fates are determined solely by embryonic induction. In this respect, eutherians are a rare exception. In all other metazoan groups, embryonic cell fates are determined by two mechanisms, ooplasmic segregation of maternal morphogens being at least as important as embryonic induction [8]. Ooplasmic segregation, i.e. the specification of preformed mRNAs, regulator proteins and other important oocyte components (e.g. yolk) into gradients, is important because these components can perform their morphogenetic function only if they are unevenly distributed into the early blastomeres during cleavage. For that reason, the establishment of oocyte asymmetry is a key prerequisite for successful development throughout the metazoan kingdom, with eutherians as a secondary exception.

The oocyte structure thought to be responsible for determination of cytoplasmic polarity is the so called Balbiani body, first decribed more than a hundred years ago. It is found in oocytes of different invertebrate and vertebrate species – insects, echinoderms, fishes, amphibians and birds [9, 13, 20, 23]. The Balbiani body is a transient collection of organelles, inclusions, proteins and mRNAs located adjacent to the nucleus. In frogs and fishes, it assembles in oogonia or early primary oocytes and disperses prior to stage II of oogenesis [17, 18], while in eutherians, it is observed first in oogonia and then in primary oocytes until dictyate stage [15].

The main constituent organelles of the Balbiani body are endoplasmic reticulum, Golgi cisternae and mitochondria. In the model amphibian *Xenopus*, where it is best studied, it is an aggregation composed mostly of mitochondria, endoplasmic reticulum,

clustered Golgi cisternae and germinal granules. Other animals may have somewhat different structure and composition of this complex. For example, the Balbiani body of mouse oocytes contains a much smaller quantity of mitochondria and no germinal granules [19]. Another difference is that mRNAs are an important component of the Balbiani body in oocytes of Xenopus but have not yet been found in its mouse counterpart [18].

A number of studies have addressed the relation of the Balbiani body to cytoskeletal structures. It is invariably associated with the centrosome. In frog oogonia, Balbiani body formation starts with aggregation of mitochondria and germinal granules around the centrosome. During mitosis, its components associate with the centrosome in the vicinity of the cytoplasmic bridges interconnecting the daughter cells. At a later stage, in the oocyte, the position of Balbiani complex marks the vegetal pole [14]. Ultrastructural and immunocytochemical studies of mouse oocytes also suggest involvement of microtubules in the dynamics of Balbiani body formation and disassembly. In ovaries of newborn mice, where meiosis is still ongoing, elements of Balbiani body are associated with centrosomes in primordial follicles [15].

Among other cytoskeletal elements, intermediate filaments have been reported to colocalize with the Balbiani body, particularly in amniote oocytes. A recent study on bird eggs (Japanese quail, *Coturnix japonica*) shows the presence of vimentin and cytokeratin 5, in the mitochondrial aggregation of Balbiani [20]. In the human, presence of cytokeratin 19 has been shown as a single perinuclear aggregation in oocytes from developing and adult ovaries [21] which is likely to correspond to the Balbiani body. However, the presence and dynamics of intermediate filaments in mammalian oocytes is still insufficiently studied and subject to controversy [16].

The evolutionary conservation of the Balbiani body across so many animal phyla suggests important functions of this structure in oogenesis. Based on the regular presence of mitochondrial aggregates in this complex, some authors have suggested that it plays a role in selection of good-quality mitochondria for the zygote [6, 10]. However, other data indicate importance of the Balbiani body for the transport of RNAs towards the vegetal pole of fish and frog eggs and its involvement in segregation and/or storage of regulatory proteins and mRNAs encoding them [12, 14, 22]. Notably, some maternal mRNAs required at later stages to establish the germline of the embryo (*vasa, nanos, gasz, dazl*) localize to the Balbiani body of fish and frog oocytes [18]. This strongly suggests a morphogenetic function of this transient organelle at least in mesolecithal and telolecithal eggs.

Unlike *Xenopus*, eutherians have secondarily isolecithal eggs. Despite the well-known asymmetrical position of the spindle, they have no specific yolk accumulation and no known gradients of morphogens; the ancestral cytoplasmic gradients have been lost. Nevertheless, a transient perinuclear complex of cytoplasmic organelles, apparently corresponding to the Balbiani body of other vertebrates though not always explicitly identified with it, has been observed in oocytes from primordial follicles of eutherians [11, 15]. This complex includes Golgi cisternae, endoplasmic reticulum, mitochondria and proteins; however, no RNAs have been detected so far [3, 5, 7, 14, 15, 19]. In mouse primordial follicles, Balbiani body is formed by aggregation of Golgi cisternae surrounded by mitochondria and endoplasmic reticulum (EPR). Perinuclear mitochondrial accumulation occurs just before ovarian tissue differentiates into primordial follicles, and is present for a short period in early primordial follicles. In later follicle stages (growing follicles), mitochondria and EPR disperse and Balbiani body is not present [19]. Hence, as a dynamic structure, the Balbiani body found in immature eutherian oocytes corresponds to those of eggs with cytoplasmic gradient as well as to a hypothetic ancestral model of polar egg [15].

What about the function of the eutherian Balbiani body? Is it just an evolutionary relic of the polarity-establishing structure that was important in the ancestral amniotic egg? The failure to detect mRNAs in the mouse Balbiani body, as well as the dispersal of key regulatory proteins such as Dazl throughout the cytoplasm of primary oocytes, seems to point to that direction [2]. However, in accordance with the general rule that conservation of structure indicates preservation of function, new data cast doubt on the old concepts; particularly the Vasa regulator protein, initially thought to lack specific localization in the oocyte, has recently been reported to associate with the Balbiani body in prepubertal and pubertal human ovaries [1].

Clues for the function of Balbiani body may come also from alteration of its dynamics associated with pathology. Our study of ovarian tissue from patients with ovarian polycystosis has demonstrated atypical structure corresponding to the Balbiani body – a semicircular or circular granular aggregate at the nuclear periphery in immature oocytes [24]. The Golgi complex, endoplasmic reticulum, mitochondria and associated proteins as important cytoplasmic components presumably affect the development of mammalian follicles, and their intracellular distribution may be important for some aspects of ovarian pathology during prenatal and postnatal mammalian development.

Perspectives of future investigation on the Balbiani body in mammals are related to localization and dynamic changes of its structures in oocytes from different species and at different developmental stages. Ultrastructural and immunocytochemical studies must be done on fetal and neonatal as well as adult ovaries. In this respect, the mouse is the most convenient object, particularly with its temporal shift in oocyte meiotic maturation allowing observation of active prophase I oocytes in the first days after birth [4]. Studies on humans are more difficult because of practical and ethical considerations, but at the same time they provide the opportunity to investigate the Balbiani body not only in norm but also in pathology.

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