# Gametic synapses, nanotubes and sperm RNAs - Redefining the origin of maternal determinants

Malgorzata Kloc, Jacek Z. Kubiak, Szczepan M. Bilinski

**Gametic synapses, nanotubes and sperm RNAs – redefining the origin of maternal determinants.**

Malgorzata Kloc[1, 2, 3]*, Jacek Z Kubiak[4], Szczepan M Bilinski[5]

[1]The Houston Methodist Research Institute, Houston, TX, USA;
[2]Department of Surgery, The Houston Methodist Hospital, Houston, TX, USA;
[3]Department of Genetics, University of Texas M D Anderson Cancer Center, Houston, TX, USA;
[4]CNRS UMR 6290, Institute of Genetics and Development of Rennes, Cell Cycle Group, University of Rennes 1, IFR 140 GFAS, Faculty of Medicine, 35 043, Rennes, France;
[5]Institute of Zoology, Jagiellonian University, Krakow, Poland

**Email addresses:** mkloc@houstonmethodist.org
jacek.kubiak@univ-rennes1.fr
szczepan.bilinski@uj.edu.pl

**Corresponding author information**:
Malgorzata Kloc
The Houston Methodist Hospital, Department of Surgery,
6550 Fannin St., Houston, TX 77030
Tel. 713.441.6875
Fax 713.790.3755
e-mail: mkloc@houstonmethodist.org



## Abstract

The female germline cells, i.e., the oocytes/eggs, contain a subpopulation of unique organelles and molecules (RNA and proteins) collectively called "the maternal determinants" that are indispensable for the determination of cell fate in the developing embryo. Although it has been known for some time that somatic cells deliver low-molecular-weight molecules to the oocyte/egg, the paradigm has been that the larger molecules and organelles are synthesized by the female germline cells without input from the surrounding somatic cells. However, recent discoveries of novel types of intercellular connections such as gametic synapses and tunneling nanotubes, allowing the transfer of large, externally derived molecules to the oocyte/egg, may dismantle the paradigm of the transcriptional/translational self-containment of the female gamete and add novel and unexpected aspects to the

origin and identity of maternal determinants. In addition, the discovery that sperm delivers various RNAs to the egg suggests that sperm may not only epigenetically modify the egg genome but also influence or modify information contained in the maternal determinants.

**Key words:** maternal determinants, germ cells, oocyte, sperm, RNA, tunneling nanotubes

## Commentary

The female germline cells, i.e., the oocytes/eggs, contain, in addition to the generic organelles and molecules common to all cell types, a subpopulation of unique organelles (such as for example Balbiani body in Xenopus and mouse; Kloc et al. 1998; 2004, 2008; Lei and Spradling, 2016 ) and molecules (RNA and proteins) collectively called "the maternal determinants", which are inherited through the female germline and are indispensable for the determination of cell fates and for proper embryonic development. By definition, the maternal determinants are synthesized by the female germline cells without any molecular or organellar input from the surrounding somatic cells. In all animals, the maternal determinants accumulate in the oocyte cytoplasm during the long and elaborate process of oogenesis. There are two major types of oogenesis. In one type (for example in *Drosophila* and some other insect species), the cystoblast/pre-oogonium divides into the oocyte and accompanying nurse cells; in the other type (for example in *Xenopus,* majority of mammals, and some insect species) a single cystoblast/pre-oogonium changes into a single oocyte, or it divides but with all of its descendants becoming oocytes. In the first type of oogenesis, the oocyte and also the nurse cells contribute to the maternal determinants accumulated in the oocyte, and in the second type, all maternal determinants are synthesized by the oocyte itself. Because in both types of oogenesis the maternal determinants are produced exclusively by the descendants of cystoblast/pre-oogonium, the inescapable conclusion has always been that all maternal determinants are female germline-derived (Fig. 1).

Although it has been known for some time that somatic cells surrounding the oocyte/egg deliver, via gap junction, the low-molecular-weight components regulating oocyte growth and meiosis progression (Eppig, 1991; Eppig and Wigglesworth, 2000, Eppig et al. 2002, 2005), the common belief has been that large molecules such as high-molecular-weight proteins, RNAs and organelles are exclusively made by the oocyte/egg. However, this dogma may fall apart in view of recent discoveries showing delivery of large exogenous molecules to female germ cells. Studies of fully-grown bovine oocytes showed that in addition to heterologous gap junctions (responsible for the transfer of small molecules), the somatic cumulus cells are connected to the oocyte via synapse-like connections (gametic synapses, also called trans-zonal projections, TZPs). The gametic synapses transfer various mRNAs and long non-coding RNAs to the oocyte through the synaptic-like vesicles (Macaulay et al. 2014). The very large diameters (2 μm) of TZPs suggest that they are potentially also able to deliver various organelles such as mitochondria or viruses (which may introduce epigenetic genome modifications) to the oocyte. In addition, the authors showed that in *in vitro* reconstituted complexes of oocytes and cumulus cells, the cumulus cells are able to deliver transcripts of transfected plasmids to the oocytes (Macaulay et al. 2016). Although the gametic synapses superficially resemble the newly discovered intercellular connections called tunneling nanotubes (TNTs) between various somatic cells (Rustom et al., 2004; McCoy-Simandle et al., 2016), they are probably gamete-specific structures. Gametic synapses are much larger than TNTs (TNTs are ~ 50-500 nm in diameter) and, in contrast to TNTs, their membranes are not fused with the oocyte membrane and they are long-lasting (Macaulay et al. 2014; Rustom et al., 2004; McCoy-Simandle et al., 2016). Long-lasting TNT-like structures connecting somatic follicular cells to the oocyte have also been described in insects (Tworzydlo et al. 2010), which suggests the universality of these types of connections within animal kingdom. There are also studies showing that, at least in mammals, various miRNAs and proteins can be delivered from a somatic source to the egg

via extracellular microvesicles and exosomes extruded to the ovarian follicular fluid (Hung et al., 2015, da Silveira et al., 2012).

Another potential source of exogenous molecules delivered to the egg is the sperm. Again, the paradigm had been that sperm just activates the egg and delivers highly condensed and transcriptionally silent chromatin. However, recent studies have established that sperm contains vast quantities of various types of RNA: mRNAs, tRNA, and long and short noncoding RNAs (Goodrich et al. 2013). Some of these RNAs enter the egg cytoplasm during fertilization and are able to epigenetically modify the egg's chromatin and ultimately influence the embryo's phenotype and heredity (Rassoulzadegan and Cuzin, 2015; Leslie, 2016). Thus, the existence of such transgenerational signals in the sperm changes our conventional view of paternal transmission and heredity. In addition to epigenetic effects on egg chromosomal DNA, one can also easily imagine that the sperm-derived molecules, similar to the RNAs/proteins/organelles delivered by gametic synapse or nanotubes, may influence or participate in reshaping the information contained in the maternal determinants present in the egg cytoplasm. All these new discoveries lead to the following fascinating questions: Are the maternal determinants really exclusively maternally derived? Do they also contain somatic and/or sperm-derived molecules and/or organelles in addition to maternal components? Are the maternal determinants somehow modified by these exogenously derived components?

Outside the scope of this commentary but crucial for the idea of strict delineation between germline and soma, there is also an issue of a transfer of information between somatic cells and sperm. Studies have demonstrated the existence of cytoplasmic processes and tubulobulbar complexes, as well as an extensive crosstalk between Sertoli cells (equivalent of follicular and nurse cells of oocytes) and spermatogonia/spermatocytes (Segretain and Decrossas, 1991; Chalmel et al., 2014). This therefore introduces the question of whether somatic components modify only the maternal information in the egg or also the paternal information in the sperm.

We hope that this commentary will inspire studies seeking the answers to these questions.

## References


1. Chalmel F, Com E, Lavigne R, Hernio N, Teixeira-Gomes AP, Dacheux JL, Pineau C. (2014). An integrative omics strategy to assess the germ cell secretome and to decipher sertoli-germ cell crosstalk in the Mammalian testis. *PLoS One.* **9**, e104418.
2. de Asis AM, Pires M, Lyon K, Vogl AW (2013. A network of spectrin and plectin surrounds the actin cuffs of apical tubulobulbar complexes in the rat. *Spermatogenesis.* **3**, e25733.
3. da Silveira JC, Veeramachaneni DN, Winger QA, Carnevale EM, Bouma GJ (2012). Cell-secreted vesicles in equine ovarian follicular fluid contain miRNAs and proteins: a possible new form of cell communication within the ovarian follicle. *Biol Reprod.* **86**, 71.
4. Eppig JJ and Wigglesworth K (2000). Development of Mouse and Rat Oocytes in Chimeric Reaggregated Ovaries after Interspecific Exchange of Somatic and Germ Cell Components. *Biol Reprod*. **63**, 1014–1023
5. Eppig JJ (1991). Intercommunication between mammalian oocytes and companion somatic cells. *Bioessays* **13**, 569 –574.
6. Eppig JJ, Wigglesworth K, Pendola FL. (2002). The mammalian oocyte orchestrates the rate of ovarian follicular development. *Proc Natl Acad Sci USA.* **99**, 2890–2894.
7. Eppig JJ, Pendola FL, Wigglesworth K, Pendola JK. (2005). Mouse oocytes regulate metabolic cooperativity between granulosa cells and oocytes: amino acid transport. *Biol Reprod.* **73**, 351–357.
8. Goodrich RJ, Anton E, Krawetz SA. (2013). Isolating mRNA and small noncoding RNAs from human sperm. *Methods Mol Biol*. **927**, 385-396

9. Hung WT, Hong X, Christenson LK, McGinnis LK. (2015). Extracellular Vesicles from Bovine Follicular Fluid Support Cumulus Expansion. *Biol Reprod.* **93**,117.
10. Kloc M, Bilinski S, Dougherty MT, Brey EM, Etkin LD. (2004) Formation, architecture and polarity of female germline cyst in Xenopus. *Dev Biol.* 266; 43-61.
11. Kloc M, Jaglarz M, Dougherty M, Stewart MD, Nel-Themaat L, Bilinski S. (2008). Mouse early oocytes are transiently polar: three-dimensional and ultrastructural analysis. *Exp Cell Res.* 314; 3245-54
12. Kloc M, Larabell C, Chan AP, Etkin LD. (1998). Contribution of METRO pathway localized molecules to the organization of the germ cell lineage. *Mech Dev*. 75; 81-93.
13. Lei L, Spradling AC. (2016). Mouse oocytes differentiate through organelle enrichment from sister cyst germ cells. *Science*. 352; 95-9.
14. Leslie M. (2016). EPIGENETICS. Sperm RNA fragments modify offspring metabolism. *Science.* **351,**13
15. Macaulay AD, Gilbert I, Caballero J, Barreto R, Fournier E, Tossou P, Sirard MA, Clarke HJ, Khandjian ÉW, Richard FJ, Hyttel P, Robert C. (2014). The gametic synapse: RNA transfer to the bovine oocyte. *Biol Reprod.* **91**, 1-12
16. Macaulay AD, Gilbert I, Scantland S, Fournier E, Ashkar F, Bastien A, Saadi HA, Gagné D, Sirard MA, Khandjian ÉW, Richard FJ, Hyttel P, Robert C. (2016). Cumulus Cell Transcripts Transit to the Bovine Oocyte in Preparation for Maturation. *Biol Reprod*. **94**, 1-11.
17. McCoy-Simandle K, Hanna SJ, Cox D. (2016). Exosomes and nanotubes: Control of immune cell communication. *Int J Biochem Cell Biol*. **71**,44-54
18. Rassoulzadegan M, Cuzin F. (2015). Epigenetic heredity: RNA-mediated modes of phenotypic variation. *Ann N Y Acad Sci*. **1341**, 172-175
19. Rustom A, Saffrich R, Markovic I, Walther P, Gerdes HH. (2004). Nanotubular highways for intercellular organelle transport. *Science.* **303**, 1007-1010.
20. Segretain D, Decrossas B. (1991). The Sertoli-spermatid processes in the mouse: new cellular structure. Biol Cell. 1991;71(3):321-324.

21. Tworzydlo W. Kloc M. Bilinski SM. (2010). Female germline stem cell niches of earwigs are structurally simple and different from those of Drosophila melanogaster. *J Morphol* 271, 634-640

**Figure Legend**

**Figure 1.** Diagram of female germline origin (left panel) versus female germline-soma/sperm origin (right panel) of maternal determinants.
The left panel shows different types of oogenesis, in which the cystoblast (pre-oogonium) transforms directly into the oocyte or divides several times, creating a cyst of oocytes or a cyst of oocyte with nurse cells connected by intercellular bridges. In all these cases, the maternal determinants (RNAs, proteins and organelles) are produced exclusively by female germline cells (either the oocyte alone or the oocyte and nurse cells) without the participation of surrounding somatic cells. The right panel shows oocytes containing not only female germline-derived determinants but also molecules and organelles delivered via gametic synapses/nanotubes from the surrounding somatic cells. After ovulation and the loss of the surrounding somatic cells the egg becomes fertilized by incoming sperm. The sperm may introduce its RNAs and/or proteins to the egg cytoplasm. All these exogenous components (somatic cell-derived and/or sperm-derived) may influence the composition and/or function of maternal determinants.

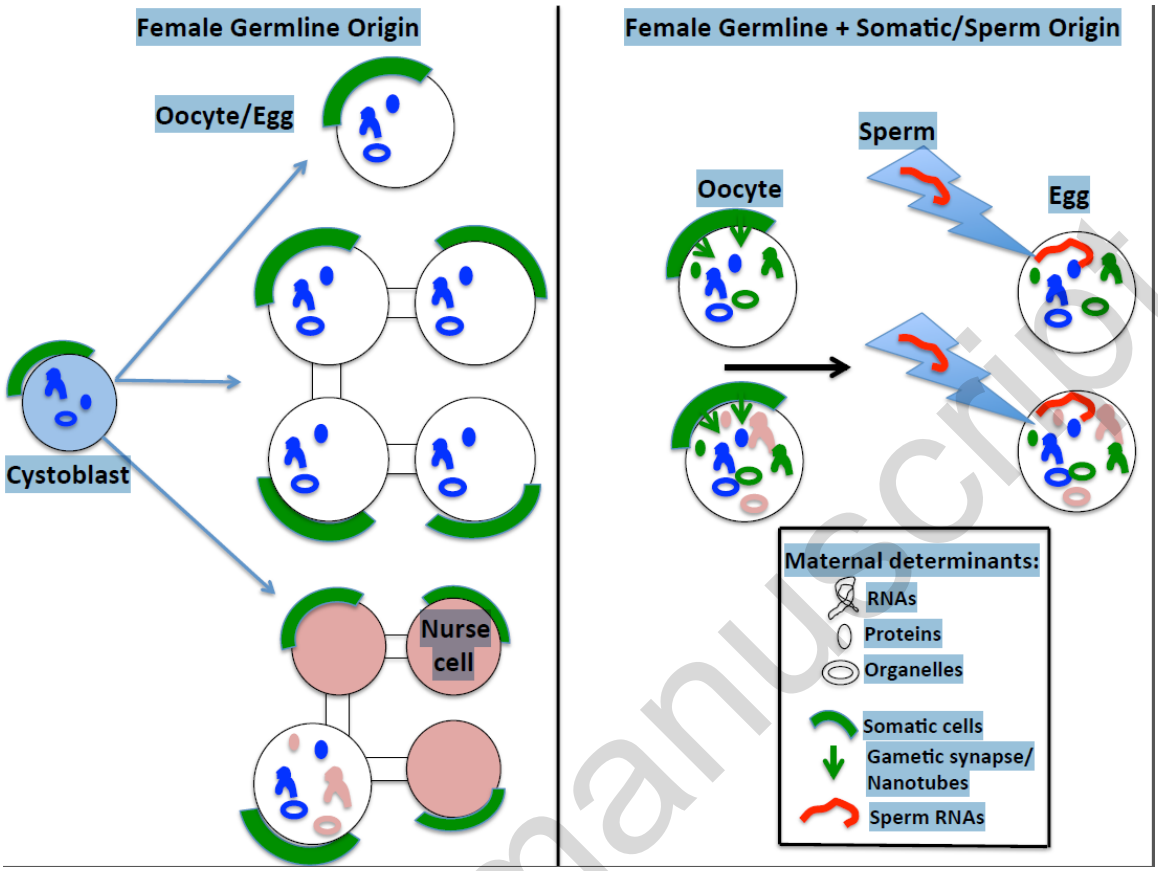


Fig. 1.

Diagram of female germline origin (left panel) versus female germline- soma/sperm origin (right panel) of maternal determinants.

The left panel shows different types of oogenesis, in which the cystoblast (pre-oogonium) transforms directly into the oocyte or divides several times, creating a cyst of oocytes or a cyst of oocyte with nurse cells connected by intercellular bridges. In all these cases, the maternal determinants (RNAs, proteins and organelles) are produced exclusively by female germline cells (either the oocyte alone or the oocyte and nurse cells) without the participation of surrounding somatic cells. The right panel shows oocytes containing not only female germline-derived determinants but also molecules and organelles delivered via gametic synapses/nanotubes from the surrounding somatic cells. After ovulation and the loss of the surrounding somatic cells the egg becomes fertilized by incoming sperm. The sperm may introduce its RNAs and/or proteins to the egg cytoplasm. All these exogenous components (somatic cell-derived and/or sperm-derived) may influence the composition and/or function of maternal determinants.