EXPERIMENTAL BIOLOGY

On the Common Mechanisms of Cambial Somatic and Sex Cells Proliferation and the Notion of the Cambial Cells

T. M. Yavisheva and S. D. Shcherbakov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 1, pp. 94-97, January, 2012
Original article submitted October 5, 2010

Study of the early embryogenesis process based on the previously suggested scheme of epithelial cambial cell work has shown that cambial (or stem) cells originate from the primary ectoderm and intensely express RhoA protein and slightly express Src kinase. This explains their stem properties and the absence of differentiation. Primary sex cells are separated stem cells getting into an environment which expresses (to a certain measure) Src kinase in them and causes their polarization and specialization. In order to induce differentiation of cambial cells in a certain direction, expression of Src kinase should be stimulated to the needed degree in each tissue.

Key Words: *Src kinase; RhoA; early embryogenesis*

In our previous study we suggested, on the base of a vast scope of experimental findings and published data, a scheme of epithelial cambial cell (CC) work in health and some diseases [4] and evaluated some functional parameters determining their stem characteristics. The basic characteristics of CC determined by embryogenesis remained unclear. Fertilization is an absolutely unique phenomenon, but it is realized through the same mechanisms of cell signal transmission which regulate intracellular processes in somatic cells [1]. Based on the previously suggested scheme of CC functioning we studied the early embryogenesis process and detected some mechanisms and ways of their emergence and differentiation.

We previously showed that polarization of a somatic cell is induced by stimulation of Src kinase (one of the main regulators of cell proliferation, differentiation, and migration [4]). Being fixed to the substrate, the cell turns to the membrane with the site where the Golgi complex (producing numerous membranes on which Src is located) is situated. Since Src kinase is involved in the assembly of microtubules and actin microfilaments, a new active edge of the cell is forming here, due to which the cell spreads further. Hence, Src kinase provides cell and nucleus stretching and looping of certain sites of chromosomes essential for transcription. However, the greater is cell compression, the lower is its stretching. It is known that a cell is compressed due to contraction of the cortical actin-myosin complex. The RhoA enzyme (one of minor G proteins) is the key protein in this process. Hence, cell differentiation is determined by two key proteins, Src kinase and RhoA [4]. If the expression of Src is normal (moderately reducing RhoA) — more formines are stimulated, which generate nonbranching filaments essential for the formation of actin cords and stress fibrils. The cell spreads parallel to the basal membrane, and chromosome sites are looped close to the telomeres, thus determining the epidermal differentiation of cells. If the expression of Src kinase is high, RhoA

N. N. Blokhin Russian Oncological Research Center, the Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** Javisheva@rambler.ru. T. M. Yavisheva

activity reduces, as Src phosphorylates p190RhoGAP protein inactivating RhoA. The formation of active formines and activity of Rho kinase is therefore reduced, which leads to relaxation of the cortex and reduction of stress fibrils. In this case, the cell cannot spread horizontally and it stretches sharply vertically, and the chromosome sites close to the centromeres are looped, determining the mesodermal differentiation of cells. If Src expression is low, formines act as classical capping proteins or nucleate the protein from a new fixed point, and the formation of stress fibrils is therefore reduced, but a cell spasm increases at the expense of RhoA and Rho kinase intensification, which stimulates myosin interactions with actin filaments. The cells in this case stretch somewhat vertically. Hence, the intensity of Src expression *vs.* the expression of RhoA determines the specific differentiation of cells.

Src is poorly expressed in CC, which leads to more intense expression of RhoA and absence of cell stretching and differentiation [4]. Pluripotent CC and primary sex cells (PSC) have much in common during their early development and originate from the proximal compartment of the epiblast, presumptive ectoderm [6], that is, before its transformation into the epidermis and neural tube. Hence, similarly as CC, PSC intensely express RhoA and poorly express Src kinase. Later PSC migrate into the yolk sac, germinative ovarian epithelium, and primordial gonadal mesenchyma, releasing growth factors similar to the fibroblast growth factor. We showed previously that in somatic cells these factors stimulate significantly the Src kinase in the active cell edge [4]. Hence, in oogonia Src kinase is stimulated not in the entire cell, but just in its active edge, that is, closer to the Golgi complex. This leads to accumulation of Src and inactivation of RhoA in these sites. Hence, the initial polarity of oogonias is forming at the stage when they are located in the gonadal epithelium and stroma, with their vegetative pole in the site with high expression of Src kinase and low of RhoA. The animal pole forms on the opposite side, where RhoA expression is high and that of Src is low. The mitotic division of oogonias continues as long as the total RhoA expression in the cell remains significantly higher than that of Src kinase, as high level of RhoA is essential for elimination of proliferation inhibitors and formation of the contractile ring [4]. Higher expression of Src kinase and reduction of RhoA in the ovarian stroma lead to reduction of formine formation and elimination of proliferation inhibitors, as a result of which the mitosis cease. Hence, in order to resume the gonocyte mitotic activity, the expression of RhoA in them should be elevated. Gonadotropic hormones regulate the follicular activity in adult age, the most significant of these hormones is the pituitary luteinizing hormone (LH) [3]. The cell membranes of the developing follicles have LH receptors. These cells produce testosterone under the effect of LH; the hormone enters the granular cells and transforms into estrogen there which, in turn, stimulates the formation of LH receptors in granular cells. The LH receptors belong to the G-protein related receptor superfamily. Binding of these receptors to LH results in transactivation of epidermal growth factor receptors [5]. We have previously shown that these receptors are characterized by low affinity for SRC than fibroblast growth factor receptors [4]. Hence, moderate expression of Src and p190RhoGAP, inactivating RhoA, allows significant stimulation of RhoA in granular cells. Hence, expression of RhoA in oocyte increases in the animal pole under the effect of granular cells during ovulation (the maximum elevation of LH). Therefore, the biological essence of LH effect consists in RhoA stimulation, that is, the oocyte and the somatic cell start proliferation under the same. During ovulation, when the level of LH stimulating RhoA increases sharply, the contractile activity of the cortical layer of the oocyte animal pole increases in comparison with the vegetative pole. The division spindle will therefore pull up unevenly, with one arm to the animal pole. Since the microtubule organization center (MTOC) material contains much Src kinase, while RhoA expression in the animal pole is high, a compact layer of actin filaments forms at the site of the spindle arm contact with the ovum cytoplasm cortical layer at the expense of formine stimulation. And as RhoA stimulates Rho kinase, phosphorylating the myosin light chain, the formine contractility increases sharply, this leading to the formation of the division sulcus and the first directive body. A portion of the cytoplasm with MTOC material is cleaved during this process, which reduces Src kinase level in this region, and the division spindle therefore unfolds parallel to the plasmatic membrane. Hence, the next elevation of Src kinase level in this oocyte site is needed for separation of the second directive body in order to place the division spindle at an angle to the plasmatic membrane and for formine activation. This is provided for by the spermium acrosomal reaction and the cortical granules contents. The spermium acrosomal vesicle is homologous to the ovum cortical granules, is formed by the Golgi complex, and contains membrane-like structures on which Src kinase is located [2]. That is why the Src kinase and formines are stimulated at the site of the spermium head fusion with the oocyte, this leading to the formation of a compact tubercle. In addition, Src kinase is involved in the microtubules polymerization, and hence, a greater number thereof forms at the site of spermium penetration than in other sites. Because of high contractility of this site and a well-developed system of microtubules in it, the divi-

sion spindle unfolds at an angle to the ovum plasmatic membrane. The subsequent processes are the same as during division of the first directive body.

Since MTOC pericentriolar material is partially detached with cleavage of the second directive body and the Src kinase level in this region reduces, the actin-myosin complex and microtubules are more pronounced in sites of the spermium penetration. Because of the pulling force of microtubules and formines' contractile force the nucleus is pulled towards the site of spermium penetration with simultaneous rotation of the cortical layers of the cytoplasm. As a result, the main axis of the nucleus is established, reflecting the anatelophase orientation and serving as the standpoint for subsequent blastomer division and orderly looping of this or that chromosome site. The vegetative cytoplasm, in which Src is stimulated, somewhat shifts to the animal pole. But the nucleus and cytoplasm rotation is paralleled by a similar rotation of the Golgi complex (bound to the nuclear membranes). Hence, conditions for maximum expression of Src emerge at the interface of the animal and vegetative regions on the side contralateral to the side of spermium penetration. This expression gradually reduces towards the animal and vegetative poles of the ovum. The reduction of Src kinase expression is more pronounced in the direction to the animal pole than the vegetative one, as its activity in the latter one is initially high.

The maximum activity of Src in this region causes a significant reduction of RhoA expression. This leads to thinning and softening of the cortex in this region of the ovum, creates the needed conditions for gastrulation and subsequent emergence of the blastopor dorsal lip. Importantly that at the blastula stage the proliferation is more active towards the animal than to the vegetative pole of the ovum because of high expression of RhoA at the animal pole. This causes the formation of inner cell mass here. The vegetative pole cells differ basically from the animal pole cells by high expression of Src kinase and low proliferation and presumably serve as the trophoblast formation source. Presumably, the hypoblast forms due to the inductive effect of the trophoblast on the lower compartments of the inner cell mass and expression of Src in these cells. The epiblast cells formed after this are also heterogeneous by Src kinase expression in them. The activity of this enzyme is the highest in the blastopor dorsal lip (in the epiblast proximal compartment). Hence, the level of active formines and stress fibrils and of focal contact reduces in cells of this region because of low expression of RhoA, which impedes the cell stretching parallel to the basal membrane and increases their mobility. The microtubules are very well developed under conditions of high expression of Src kinase; this promotes vertical stretching of the cells and their mesodermal differentiation. Due to their high mobility and elongation, these cells are the first to penetrate into the blastocel through the proximal part of the strip and form the cord. As the activity of Src kinase in the epiblast cells is different, the invaginating cells differ by the degree of cell and nucleus stretching, that is, by differentiation, and also by mobility and order of entry in blastocel. The cord then stimulates the ectoderm to develop into the neural tube. The expression of Src kinase in the cord material is very high, while the sublying ectoderm expresses RhoA. Therefore, the expression of Src predominates over RhoA in this ectoderm region, this leading to RhoA reduction, formine and stress fibril formation. The ectoderm cells stretch vertically, but less than in the cord, as a result of which the neural plate forms. The inductive effect of the cord on the peripheral parts of the neural plate is weaker than on the central part, that is, the expression of Src kinase is the highest in the center and lesser at the periphery, while the expression of RhoA increases from the center to the periphery. As a result, the expression of RhoA sharply increases in the peripheral sections of the neural plate (neural rolls), this stimulating the actin-myosin complex and these cells' contractility. This generates a wave of contractions in all cells tightly connected by their apical terminals, which causes bending of the neural plate and formation of the neural tube from it. The neural rolls during this process move towards each other and fuse, thus forming the primordial neural crest cells. Virtually all components of the peripheral nervous system eventually form from these cells, as well as the adrenal cells, pigment cells, and also the cartilage, bone, and other connective tissues in the head [1]. Hence, the neural crest cells are pluripotent stem cells which develop from the presumptive ectoderm at the interface of strong expressed RhoA and poorly expressed Src kinase. That is why the nucleus is virtually unstretched and these cells virtually do not differentiate, which maintains their stem characteristics. Similar expression of RhoA and Src kinase is found in the somatic epithelial CC, PSC during the early development. Hence, stem cells are the cells originating from the ectoderm with strong expression of RhoA protein and slight expression of Src kinase. In order to induce the differentiation of these cells, Src should be expressed in them by bioactive factors expressing Src similarly as in the tissues producing them. A significant predominance of Src over RhoA induces the restructuring of the cell cytoskeleton and its vertical stretching from the basal membrane, looping of certain chromosome sites in accordance with their anatelophase orientation. This causes the formation of mesodermal cells. A moderate increase of Src and predominance of RhoA induces differentiation by the epithelial way, as well-developed stress fibrils

and microtubules stretch the cell parallel to the basal membrane and cause looping of other sites. Sex cells are also cambial cells, in which Src kinase has been stimulated, which has led to their specialization.

REFERENCES

1. B. Alberts, D. Bray, J. Lewis, *et al.*, *Molecular Biology of Cell* [in Russian], Moscow (1994).
2. S. Gilbert, *Developmental Biology* [in Russian], Moscow (1993).
3. A. P. Dyban, *Early Development of Mammals* [in Russian], Leningrad (1988).
4. T. M. Yavisheva and S. D. Shcherbakov, *Uspekhi Gerontol.*, **22**, No. 4, 605-613 (2009).
5. M. Conti, M. Hsieh, J. Y. Park, and Y. Q. Su, *Mol. Endocrinol.*, **20**, No. 4, 715-723 (2006).
6. M. P. De Miguel, F. Arnalich Montiel, P. Lopez Iglesias, *et al.*, *Int. J. Dev. Biol.*, **53**, Nos. 8-10, 1529-1540 (2009).