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# CYSTOCYTE AND LYMPHOCYTE DERIVED FUSOMES/SPECTROSOMES: ANALOGIES AND DIFFERENCES: A MINI-REVIEW

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**Abstract:** Structures analogous to Drosophila spectrosomes were found in mammalian lymphocytes. Repasky and colleagues discovered an intracellular spectrin-rich structure in lymphoid cells, which had far-reaching parallels with the fusome/spectrosome of *D. melanogaster* germ cells. This fact implies that spectrosomes may be characteristic not only of insect germ cells, but also that an analogous structure may play an important role in other cell types.
The term "spectrosome" was first used by Lin and Spradling in 1995 to describe a large sphere of fusomal material in *D. melanogaster* germline stem cells and their differentiated daughter cells – cytoblasts. In the *D. melanogaster* ovary, membrane skeletal proteins such as ankyrin, $\alpha/\beta$ spectrin as well as adducin-like Hts protein(s) were found in this specific organelle – spectrosome/fusome. These orgalelles are involved in the creation of mitotic spindles and *D. melanogaster* cyst formation and oocyte differentiation, but the role of analogous spectrin-based aggregates found in nucleated cells still remains unclear.

**Key Words:** Spectrin, Spectrosome, Fusome, Membrane Skeleton, Cystocytes

## INTRODUCTION

Spectrin is commonly classified as a structural protein which is mainly involved in the structural organisation of the erythrocyte membrane skeleton [1-3]. However, recent reports emphasise the fact that spectrin may also be involved in the regulation of many physiological processes [4-14]. Spectrin is a multidomain

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protein composed of an α and β heterodimer that self-associates head-to-head to form a 200 nm tetramer filament, which is its functional form in the cells. Spectrin controls membrane organisation, stability and shape, and links the membrane bilayer to all main filament systems and motors of intracellular transport [10, 11, 15]. The presence of spectrin at/in various cellular compartments suggests that the role of this protein may be not only structural but also physiological. Particularly interesting are the subcellular structures termed "spectrosomes" (fusomes), which are rich in spectrin and other membrane skeletal proteins as well as in other components of regulatory functions such as protein kinase C isoforms as well as proteins belonging to the HSP70 class.
The aim of this short review is to present potential structural and physiological analogies and differences between large intracellular spectrin-rich structures found both in mammalian haematopoietic cells and in insect germlines.

## SPECTRIN AND MEMBRANE SKELETON

The mammalian erythrocyte membrane skeleton is organised as a specific meshwork formed by five to seven extended spectrin molecules linked to actin protofilaments (~ 40 nm in length). The spectrin-actin network is linked to the membrane bilayer by association of spectrin with ankyrin, which, in turn, is bound to the cytoplasmic domain of anion exchanger. Anion exchanger is associated also on their cytoplasmic domain with band 4.2. The spectrin-actin network is also connected to the characteristic complex between proteins 4.1, p55 and glycophorin C/D. Adducin, tropomyosin and tropomodulin play an important role in stabilisation of the spectrin-actin network. A major function of the spectrin skeleton in the erythrocyte is to provide mechanical support for the membrane, which allows surviving of these cells in the circulation [10, 11, 15].
The human erythrocyte is best understood in terms of the membrane skeleton and the role of spectrin in this structure. Since the isoforms of spectrin were found in non-erythroid cells, a question whether the erythrocyte paradigm could have relevance for other cell types is still under dispute [14-18]. Although the detailed structure of many membrane skeletal components analogues of erythroid cells is known, the main structural features of the membrane skeleton as well as the molecular organisation and function of its particular components in non-erythroid cells may be different and remain to be explored.

## LYMPHOCYTE SPECTRIN (SPECTROSOMES)

The search for analogies and differences in the structure and function of the membrane skeleton in erythroid and non-erythroid cells resulted in very intriguing observations, which were made by Repasky and colleagues. Analysis of spectrin distribution in various lymphoid and myeloid cell lines revealed distinct patterns of spectrin localisation in these cell types in response to specific extracellular stimuli. In majority of analysed cell lines spectrin was

symmetrically distributed in the submembraneous region of the cell, while in some cell lines, a large aggregate of spectrin, termed "a spectrosome", was present. Moreover, specific redistribution of submembraneous spectrin was promoted by certain agents: phorbol myristate acetate, mezerin, ionophore A23187, concanavalin A, interferon α, as well as by physical treatment, e.g. high temperature exposition. It was also found that ankyrin, PKC βII, PKC θ and HSP70 translocate from the soluble cytosolic/submembraneous to a particulate fraction upon cell stimulation. In addition, this large multiprotein aggregate remains Triton X-100 insoluble [19-26].

Detailed descriptions and characterisation of the nature of spectrin-based lymphocyte aggregates still have to be made. Most of the studies on the "lymphocytary" spectrin date from the mid-1980s - mid-1990s, but the results obtained from the analysis of spectrin distribution in neoplastic cells (Acute Lymphoblastic Leukaemia and non-Hodgkin Lymphoma) undergoing chemotherapeutically induced apoptosis show that aggregation of spectrin followed up by aggregation of PKC θ occur in such cells [27]. Moreover, normal lymphocytes grown in the presence of cytostatics, showed the same pattern of spectrin and PKC θ distribution. These facts imply that both proteins are involved in the programmed cell death process, however, further analysis should be performed.

## INSECT CELL SPECTROSOME/FUSOME

Spectrin in non-mammalian cells is also involved in the formation of a large cytoplasmic structure [28]. This structure, termed "a spectrosome", which was first visualised by light microscopy [28, 29], is associated with cyst formation in *D. melanogaster* and other insects. This structure was first described in the ovary of a dividing beetle *Dytiscus marginalis* by A. Giardina in 1901, and later in ovarian stem cells and cytoblasts of *D. melanogaster* [30]. In Drosophila germ cells, the fusome exists as a characteristic intracellular region rich in membrane vesicles and fibrils excluding the mitochondrion and ribosomes [31-33].

Division of a germline cell of *D. melanogaster* and most insects yields in the creation of stem cells and daughter cytoblasts, which will form oocytes and "nurse cells" as well. Resulting cytoblasts undergo four rounds of mitosis with incomplete cytokinesis. It results in a syncytium where the cells (sixteen cystocytes) are incompletely separated and interconnected by a fusome. During the four cystocyte mitoses the fusome forms a large, extended, aborescent structure interconnecting all the cells and cysts [33-36] (Fig.1).

## STRUCTURE OF SPECTRIN-BASED AGGREGATES

α/β-spectrin, ankyrin, actin and the adducin-like *hu-li tai shao* (Hts) protein were found in fusomes [28-36]. Fusomes are filled with small membrane-bound vesicles the content of which is unknown, although they are deficient in

ribosomes and mitochondria. Most fusome components were also found in the stem cells precursor of the fusome – a spectrosome; however, the spectrosome is enriched in ankyrin and deficient in small vesicles. This may suggest that fusomal membrane skeletons undergo specific changes during cyst formation and maturation. In the *D. melanogaster* ovary, cyst formation starts with asymmetrical dividing of germline stem cells, and a new stem cell and a cytoblast are formed. The cytoblast immediately begins a program of cell division and divides four times, and forms a cluster of cystocytes. Cytokinesis is incomplete and characteristic bridges – ring canals, interconnect cystocytes. Although cystocytes share a common cytoplasm, one of them differentiates into an oocyte and the others become nurse cells. Various components, including mRNA, are transferred through the ring canals. Stem cells and cystoblasts contain a large complex of fusomal material – a spectrosome. During the cystoblast mitoses the spindles associate with the fusome and after the mitoses the fusomes form a large, branched structure, extending through the ring canals so that all cells of the cyst are interconnected [33-40] (Fig.1).

Although all the above information gives some insight into the basic structure of the fusome/spectrosome, detailed information regarding the molecular organisation of the components of the fusome remains unclear.

Detailed molecular organisation and structure of lymphocyte spectrin-rich aggregates has also to be evaluated. Regarding the structure of the lymphocyte spectrosome, it is only known that PKC βII, PKC θ, HSP70 and RACK1 (receptor for activated C-kinase) colocalise with spectrin upon lymphocyte activation and that the lymphocyte spectrosome also contains membrane vesicles as well as ankyrin [19-26, 41]. The fact that in spectrosomes found in activated lymphocytes aggregation of spectrin is associated with aggregation of certain important regulatory proteins may suggest that such aggregation is an important phenomenon for signal transduction in this cell type.

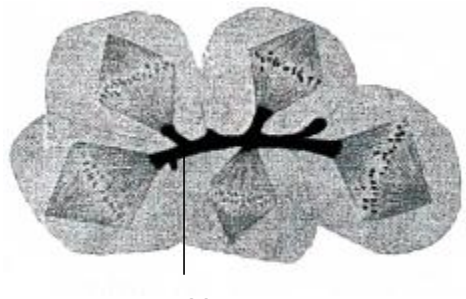


Fig. 1. Schematic representation of an insect (wasp) developing ovarian germline cysts in the metaphase (after Maziarski, 1913, and Lin, 1994; modified by the authors). The dark structure in the centre of the cyst is the fusome.

## POSSIBLE PHYSIOLOGICAL FUNCTIONS

Fusomes were proposed to play several important functions in *D. melanogaster* oogenesis: they may block cytokinesis by formation of channels and interconnected cysts of cells, they may be involved in the control of inter-cystocyte connections by determination of mitotic spindles orientations. Fusomes also take part in the synchronisation of the cystocyte division and in the creation (by interacting with microtubules and centrosomes during the late interphase in both mitotic and meiotic cysts) of specific polarity leading to distinguishing one cell as an oocyte [28-40].

The functional role of the lymphocyte cytoskeleton during specialised cellular responses and activities still remains unclear. It was shown that although cytoskeletal components of the erythrocyte membrane skeleton were identified in nucleated cells, there is significant lack of consensus regarding the organisation of spectrin and ankyrin in this cell type [10, 11, 15]. These proteins, always present at the cell periphery in erythrocytes, in the lymphocytes, after extracellular stimuli, spectrin and ankyrin can be aggregated in a discrete filamentous cytoplasmic structure – a spectrosome found in the vicinity of the *trans* Golgi region. The physiological function of a lymphocyte spectrosome is not yet known, however, the particular role of its components has been suggested. It was proposed that two distinct distribution patterns of spectrin within lymphocytes may be connected to variation of membrane stability and lipid asymmetry between the plasma membranes of the resting and/or the activated lymphocyte and may be associated with the presence of specific lipid domains in this cell type [42-44]. Activation of PKC βII results in translocation of this protein to a membrane-rich, insoluble, particulate fraction. Finding that PKC inhibitor - calphostin C inhibits the antigen–induced redistribution of spectrin and PKC βII suggested that accessibility of the regulatory domain of PKC to DAG (diacyloglicerol) might be required for the movement of these proteins to the cytoplasmic/submembraneous aggregate. It was suggested that spectrin might provide a framework allowing PKC βII to be distributed to the sites where its substrates are located. Focal concentration of spectrin and PKC βII is often associated with the *trans* Golgi apparatus or the nuclear envelope, and this may suggest that PKC βII is required for phosphorylation of newly synthesised substrates [22]. Double immunofluorescence studies demonstrated that ankyrin colocalises with spectrin and PKC βII, therefore also ankyrin may be directly involved in anchorage of proteins and therefore indirectly involved in cellular signal transduction pathways [23]. Also HSP70 was found in a large cytoplasmic aggregate in lymphocytes, but its role in this structure awaits discovery [24-26]. The formation of this HSP70/spectrin/PKCβII/ankyrin containing organelle was induced by conditions that activate PKC and lead to lymphocyte activation. PKC θ and RACK1 were also found to colocalise with spectrin in the spectrosome [26, 41].

The fact that the lymphocyte spectrosome appears during lymphocyte interaction with macrophages, mitogen or chemokine stimulation, cell-mediated cytotoxicity and increased concentration of adhesion and effector molecules [26, 45, 46], suggests that this structure could also be important for cell interactions.

## CONCLUSIONS

Both insect and lymphocyte spectrosomes are spectrin-based structures and their roles in intracellular pathways suggest that spectrin may be both a framework in these structures and perhaps, via its regulatory SH3 and PH domains, may be directly involved in signal transduction pathways. Despite molecular structure and component differences between germline cells and lymphocyte spectrosomes, the fact that such organelles exists and have important functions in the cell physiology, makes them interesting subjects for further structural and functional studies.

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