MANY FACES OF MYOSIN : PERSPECTIVE FROM CELL MIGRATION

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Cell migration is interesting, intricate and necessary cellular behaviour. Without it there will be no development, wound healing, no immune systems so on and so forth (1). On a negative side cell migration also contribute to morbidity and mortality through birth defects, metastasis, immune deficiencies, inflammatory diseases etc. (2-4). Our knowledge on cell migration gathered mostly from in vitro studies where single cell moves on petriplates without obstruction. (5-7). Cell migration happens in a few distinct steps; cells polarize, protrude towards the direction of migration, anchor/adhere at the front, contract the cell body and then finally retract the back. Needless to say that each of these processes involve extremely complex molecular machineries and each of them is connected spatially and temporally. We do know a great deal about cell migration in vitro and the molecular processes but understanding of the same process in a complex environment is still wanting to great extent. Replicating in vivo environment in vitro is still a challenge (8). To complicate the matter even further, many cells actually migrate in diverse kinds of interconnected groups not individually. Some maintain a loose connection between them, like trunk neural crest cells and mouse embryonic germ cells. (9, 10). Some cells maintain a more stable cell-cell connection like cranial neural crest cells. (11, 12). Other cells like Keratinocytes stay very tightly connected as a sheet as they fill the wound. A cell can also guide/lead a group to their destination like, blood vessels and Drosophila melanogaster trachea and salivary glands. Instances like these raises some even further fundamental questions like how do these cells coordinate their behaviour within themselves and with surrounding cells; what are the molecules that mediate these communication. The powerful combination of genetics and live imaging has made the border cells of the D. melanogaster ovary an important model for the cohort type of collective cell migration. Drosophila border cells are a genetically tractable model to address how actomyosin contraction regulates the cell shape, organization and movement of collectives in vivo. Border cells undergo a developmentally regulated migration during oogenesis (1). A central pair of polar cells (specialized follicle cells) recruits 4-8 epithelial-derived cells to form the border cell cluster. Border cells subsequently detach as a group from the follicular epithelium and migrate between the germline-derived nurse cells to reach the large oocyte [Figure 1A] (13).

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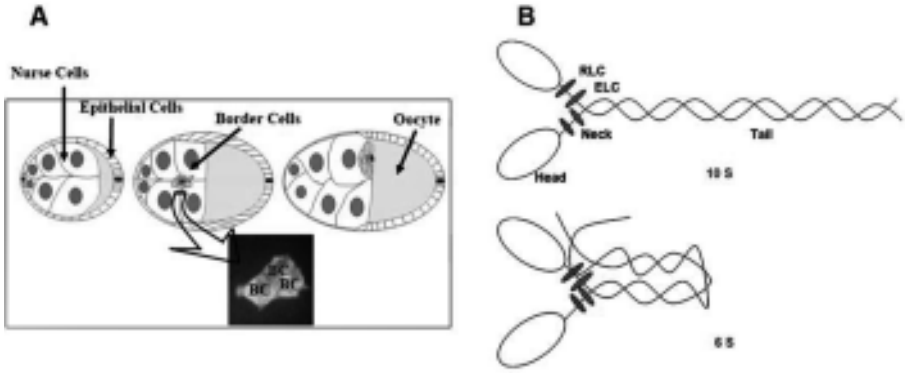

Figure 1: (A) A schematic representation of border cell migration during *Drosophila* ovary development. (B) A schematic representation of Mycosin II structures.

Another aspect of cell migration that receives comparatively less attention is mechanical factors affecting/regulating migration (14). To illustrate the point, mechanical factors associated with actomyosin tension, cellular behaviour, matrix properties, shear and cyclic stress, all of these factors affect/regulate cell migration directly or with conjuncture of biochemical pathways. Among them, intracellular tension is one of the most prominent (15, 16). This is created within actomyosin structures at both leading and trailing edges of the cells, stress fibres of non-muscle cells and sarcomere like structures in smooth muscle cells by constant active interaction between myosin II and actin cytoskeleton initiated by chemical and or mechanical stimuli. Actomyosin tension is essential in maintaining the cell body, disassembling of focal adhesions at the trailing edge of the cell during cell migration, protrusions and retractions (14).

The word "myosin" (myo + ose + in) means within muscle and was used to denote proteins with ATPase activity found originally in striated and smooth muscle cells (17). The term "myo" was originated from "mys" to denote muscle in Greek. More than 140 myosins are reported in eukaryotes except in red algae and diplomonadprotists (18). Most of the myosins have distinct head, neck and tail domains. They are divided into 35 classes based on phylogenic analysis of their conserved heads, domain architectures, specific amino acid polymorphisms and organismal distributions (19). Each class of myosins received a roman numeral. If more than one myosin of the same class is expressed in an organism, they are named in an alphabetical order according to their discovery. For the duration of this paper we will focus on myosin II.

Myosin II are mostly located at the cortical region of the cytoplasm of quiescent cells except in the nuclei of proliferating myoblasts (20). Both functional and mechanical roles of non-muscle myosin II are widely studied in migrating cells for the past two decades. Many laboratories reported that myosin IIA and myosin IIB have specific roles in regulating cell shape changes and interaction with matrix during migration. During migration cells prefer to make periodic lamellipodia protrusions and retractions. What is interesting is that Myosin IIB seems to promote lamellipodia and growth cone extension while Myosin IIA

retracts cell membrane. Distribution of Myosin IIA and IIB also differs correspondingly (21, 22). The specific roles of myosin IIC motor protein in driving cell migration are not clearly understood. Human skin wound healing requires keratinocytes migrating towards the wound and Myosin II is necessary for that migration (22). Myosin II mediated mechanical forces have been implicated in operating the activity of contractile vacuoles to expel additional water and toxic materials from the soil-living amoeba in hypo-osmotic conditions. Myosin II motor proteins have also been implicated in the mediation of viral infection, microparticle secretion and cell death (19), however, their specific roles and underlying mechanisms remain unclear.

Each Myosin II motor protein is a heterohexamer consisting of six non-covalently associated polypeptides. The polypeptides are encoded by a single myosin II and two different non-myosin genes. Each heterohexamer (525 kDa) is made of a myosin II heavy chain (MHC, Zipper-Zip in Drosophila) homodimer, two essential light chains (ELC), and two regulatory light chains (RLC or Spaghetti squash-Sqh in Drosophila). While MHC with 226 kDa molecular weight is encoded by a myosin II gene, both ELC with 16 kDa and RLC with 22 kDa molecular weights are considered as non- myosin proteins of myosin II complex. Both ELC and RLC are commonly found in all myosin II complexes. The MHC can be subdivided into distinct head, neck, and tail functional domains. Catalytic/motor head is at the N-terminal; it has binding sites for actin and ATP. The head domain undergoes an ATP-dependent conformational change for interaction with actin filaments, converting cellular free-energy into protein motion or mechanical work. Both ELC and RLC bind to the flexible neck region of MHC. Neck region is followed by a coiled-coil tail, the C-terminal part of MHC. Thus, myosin II heterohexamer has two globular heads or motor domains with a single coiled-coil tail structure. Myosin II complex attains a compact folded conformation due to a "proline-kink" at the junction of head and rod domains, and attachment of its C-terminal tail domain to RLC [Figure 1B] (19). Thus, the myosin II complex with compact folded structure sediments at 10 S (Svedberg) and therefore called 10S form. The myosin II complex in 10S form shows high binding affinity for ADP and inorganic phosphate (Pi), and virtually no enzyme activity (23, 24). However, the activated myosin II complex exists in an elongated conformation due to its C-terminal tail detachment from RLC. The activated myosin II complex in an elongated form sediments at 6 S and therefore called 6S form (25). Myosin II motor proteins with elongated conformation tend to assemble into highly ordered parallel and anti-parallel thick filaments due to intermolecular interactions between coiled-coil tail domains. Thus, RLC-controlled tail-domain filamentation and motor domain interaction with actin filaments are the most important aspects of cell strategy for converting ATP released free-energy into force and mechanical work using myosin II motor proteins.

Role of RLC phosphorylation in regulating myosin II activity in many cell and tissue types is extensively investigated since its discovery in rabbit skeletal muscle myosins more than three decades ago (26). RLC perhaps does not exist alone but when remains associated with the neck region of MHC undergoes reversible phosphorylation on its S1, S2, T9, T18 (T20 in Drosophila), and S19 (S21 in Drosophila) amino acids in order to turn-on and turn-off myosin II motor complexes in cells. RLC phosphorylation on S19 alone or on both T18 and S19 amino acids turns-on myosin II motor complex by increasing its ATPase activity and extended 6S conformation that allows simultaneous assembly into thick filaments (21;

27-29). However, RLC phosphorylation does not affect myosin II motor domain affinity for actin filaments (30). RLC phosphorylation on S1, S2, and S9 or dephosphorylation on T18 and S19 amino acids turns-off myosin II complex by allowing acquisition of monomeric 10S compact conformation and no filamentation. RLC reversible phosphorylation is tightly regulated by both myosin specific phosphatase and a wide variety of kinases including myosin light chain kinase (MLCK/MYLK), Rho-associated coiled-coil-containing kinase (ROCK), leucine zipper interacting protein kinase (ZIPK) or death associated protein kinase 3 (DAPK3), citron kinase or citron rho-interactive kinase (CRIK) or Serine/threonine-protein kinase 21 (STK21), myotonic dystrophy kinase-related CDC42-binding kinase (MRCK/CDC42BP). These kinases are known to phosphorylate RLC on T18 and S19 amino acids to activate myosin II complexes in different cell types (19). Interestingly, all these kinases display specific intracellular localizations and respond to a wide variety of signal transduction pathways in order to phosphorylate RLC and activate myosin II motor proteins in many cell types. Both intracellular site-specific RLC reversible phosphorylation and myosin II activation are tightly controlled by protein phosphatase 1 (PP1), a ubiquitously expressed myosin specific phosphatase (31-33). ROCK also directly phosphorylates LIM kinase and MYPT1, a regulatory subunit of PP1 in many types of cells and tissues (19). MYPT1 phosphorylation inactivates PP1 and this leads to a marked increase in RLC phosphorylation and myosin II activation. MYPT1 phosphorylation is also regulated by ZIPK, MRCK, and PKC in many cell and tissue types. Recently Par-1, a polarity protein, has also been shown to regulate myosin phosphatase (34). Our personal observations also lead us to believe that kinases phosphorylates RLC at different sites and therefore affect different aspects of cell migration and shape.

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