ABSTRACT

The establishment of cellular polarity is first critical step of directional cell migration. The process of cellular polarization requires many signaling pathways that are differently regulated at the cell front and at the rear side and enables creation of typical asymmetrical profile of migrating cell. During the polarization cell forms the leading edge and trailing rear and relocalizes the intracellular organelles to such a position that is optimal for directional movement. In many migrating cells cell nucleus is usually located at the cell rear and microtubule organizing center localizes between the nucleus and the leading edge of the cell. This cellular arrangement is prerequisite for directional cell migration. We have shown that during cell polarization cell also reorients the nucleus to the direction of migration. The nuclear reorientation is temporally restricted rotation of the cell nucleus that aligns the longer nuclear axis with the axis of migration. Nuclear reorientation promotes the establishment of cellular polarity and facilitates the movement of the cell.

The nuclear reorientation requires the physical linkage of the nucleus to cell cytoskeleton mediated by LINC (Linker of Nucleoskeleton and Cytoskeleton) complex. We have shown that LINC complex anchors the nucleus to actin stress fibers exposed above the nucleus and enables the nuclear reorientation to the direction of migration.

In migrating cells, actin forms several types of stress fibers: ventral fibers, dorsal fibers, transverse arcs and perinuclear actin fibers (perinuclear actin fibers are also referred as "perinuclear actin cap"). Ventral stress fibers are restricted to the basal side while dorsal stress fibers, transverse actin arcs and perinuclear actin cap filaments rise from the leading edge to the dorsal side of the cell, with perinuclear actin cap fibers being connected through LINC complex to the nuclear envelope. We have shown that during cell polarization, dorsal fibers, transverse arcs and perinuclear filaments form interconnected network crosslinked by actin binding protein α-actinin1. This network of actin fibers is anchored in adhesions at the cell front on one side and to the nuclear envelope on the other side thus mechanically links the nucleus with adhesions at the leading edge. Dorsal fibers and transverse arcs play central role in the actin cap assembly as they recruit preexisting peripheral stress fibers and move them to the apical side of the nucleus. Actin cap formation induces also nuclear reorientation to the direction of migration and, remarkably, actin cap promotes the actin arcs and dorsal fibers localization to the cell front. Our results thus suggest that the network of dorsal fibers, actin

cap and transverse arcs functions bi-directionally to regulate both, nuclear positioning and cell front organization.

The nuclear reorientation is controlled by coordinated regulation of two signaling axis: by LPA-mediated activation of small GTPase RhoA and by activation of integrin and FAK/Src and p190A-RhoGAP signaling. LPA stimulates receptors coupled with trimeric G-proteins that activate RhoA in the whole cell. Integrin and FAK signaling is activated predominantly at the cell front and represents primary polarity signal leading to the establishment of cellular polarity. Activation of FAK/Src complex subsequently stimulates RhoA inhibitor p190A-RhoGAP and induces its recruitment to the cell front. Cooperation of these two signaling axes dynamically regulates the activity of RhoA at the leading edge that allows the cell to massively reorganize the actin cytoskeleton. RhoA stimulates proteins from the formin family and induces the formation of interconnected actin network. Subsequent RhoA mediated contractility drives the perinuclear actin cap formation and nuclear reorientation.

In addition, we have found that presence of perinuclear fibers and nuclear reorientation correlate with the shape of motile cells and with mode of migration. Fibroblasts that reorient their nucleus to the direction of migration have elongated conical shape and perinuclear fibers are aligned with the longer nuclear axis and with the axis of migration. Such cells migrate using “inchworm” manner with front protrusions followed by rear retraction. In contrast, cells that do not possess perinuclear fibers, like U2OS, display fan-like shape, reorient the nucleus perpendicular to the direction of migration and their migration is characterized by persistent protrusion and constant cell body movement without tail retraction step. These data suggest that perinuclear actin fibers and nuclear reorientation determine the mode of migration.