Guanine-nucleotide exchange factor that promotes loss of polygonal cell shape

Actin cytoskeleton dynamics determines cell shape and movement. Fibroblasts or epithelial cells, for instance, adhere and spread onto planar surfaces adopting an elongated polygonal shape. Underneath the cytoplasmic membrane, local activation of small GTPase enzymes of the Rho family regulates F-actin (filamentous) organization. Rho GTPases cycle between two configurations, GTP- or GDP-bound (Fig. 1). The GTP-bound forms are active, because they can associate with downstream effector proteins. The exchange activity of GDP for GTP is favored by proteins called guanosine-nucleotide exchange factors (GEFs). GTP hydrolysis, favored by GTPase-activating proteins (GAPs), closes the cycle by returning the GTPase to the GDP-bound form, unable to bind to their effectors (inactive). The prototypic members of the Rho GTPase family are RhoA, Rac1 and Cdc42. Among other functions, RhoA organizes F-actin in long bundles (stress fibers); Rac1 promotes flat projections containing a dense mesh of F-actin at the migrating leading edge (lamellipodia), and motile surfaces containing a meshwork of F-actin (membrane ruffles); and Cdc42 induces motile hair-like protrusions filled with bundled F-actin (filopodia).

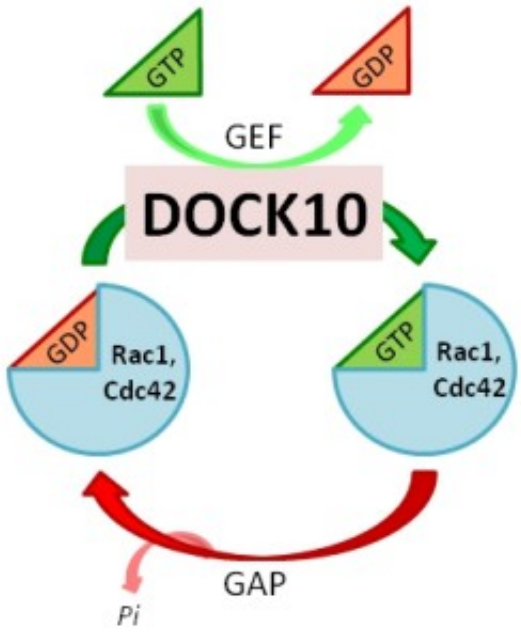

Fig. 1. The role of Dock10 as a GEF in the cycle of Rho GTPases Cdc42 and Rac1.

Rho GTPase regulation is complex: around 70 genes code for the Dbl-homology (DH) proteins, and 11 for the Dedicator of cytokinesis (Dock) proteins, the two groups of Rho GEFs. The Dock proteins are large in size, ranging between 1800 and 2200 amino acids. Dock1 to Dock5 activate

Rac1; Dock8, Dock9 and Dock11 activate Cdc42; and Dock6 and Dock7 activate both. In this paper, the ability of Dock10 to interact with and activate Rho GTPases was investigated by specific biochemical assays (GST pulldown). Among 10 GTPases tested, Dock10 interacted with and activated Rac1 and Cdc42.

To examine Dock10 effects in actin cytoskeleton and cell morphology, fluorescence and time-lapse microscopy were applied to human epithelial HeLa cell clones manipulated by transfection with specific plasmids in which we could switch off and on expression of Dock10 easily by adding or removing doxycycline, respectively. When Dock10 was off, HeLa cells had polygonal elongated morphology, with dynamic protrusive activity at the vertices. When Dock10 was on, HeLa cells transformed into a non-polygonal rounder shape, lost stress fibers, and gained filopodia and ruffling activity around the cell perimeter (Fig. 2). These observations are consistent with a previous study performed in a cell line which normally grows as round cells, in which a specific decrease in the levels of Dock10, mediated by RNA interference, induced cell elongation. Dock10 presence may be critical for formation of filopodia and ruffles, since it colocalized with F-actin in these structures.

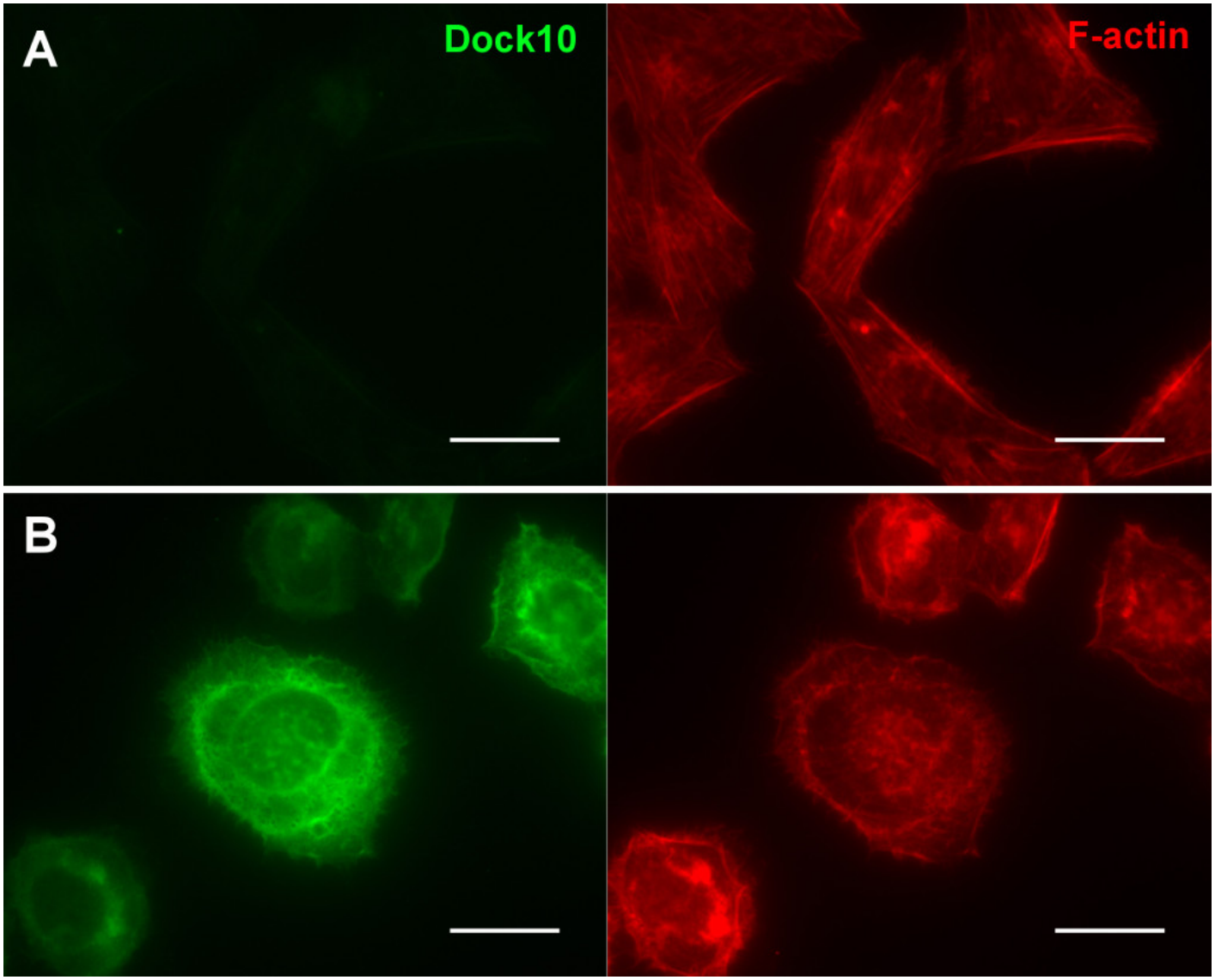

Fig. 2. HeLa cells double stained for fluorescence microscopy visualization of Dock10 protein and F-actin. HeLa cells with low levels of Dock10 displayed polygonal elongated shape and exhibit stress fibers, particularly thick at the cell edges (A). HeLa cells with high levels of Dock10 spread with more rounded contour, develop filopodia and membrane ruffles, and lose stress fibers (B). Scale bar, 25 µm.

To obtain further indications as to whether the phenotype induced by Dock10 is truly mediated through Cdc42 and Rac1, HeLa clones with inducible expression of constitutively active Cdc42 and Rac1, permanently loaded with GTP due to an amino acid substitution (Q61L) that inhibits GTP hydrolysis, were also created. The Cdc42 active mutant induced filopodia and contraction, and the Rac1 active mutant, ruffling and flattening. Similar to Dock10, both mutants induced loss of

elongation. Moreover, the co-expression of Dock10 with each of the mutants enhanced their respective phenotypes. In summary, this study confirms the suspected role of Dock10 as a Rho GEF, defining its specificity, and gains first insights into its cell shape regulatory function. Since a recent study indicates that Dock10 expression may be essential in cancer cells guiding tumor metastasis, future studies adressing the role of Dock10 in cell movement could find application in cancer research.

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Publication

Dock10, a Cdc42 and Rac1 GEF, induces loss of elongation, filopodia, and ruffles in cervical cancer epithelial HeLa cells.
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