Cooperation between the actin nucleators mDia1 and Arp2/3 complex controls membrane ruffling

T. Isogai [1], R. van der Kammen [1], D. Leyton-Puig [2], K. M. Kedziora [2], K. Jalink [2], and M. Innocenti [1] *

[1]Netherlands Cancer Institute , Division of Molecular Genetics, 1066 CX Amsterdam (NL)
[2]Netherlands Cancer Institute , Division of Cell Biology I, 1066 CX Amsterdam (NL)
m.innocenti@nki.nl
* Corresponding Author

Keywords: *actin cytoskeleton, mDia1, Arp2/3 complex, lamellipodia, ruffles.*

Abstract

Polymerization of branched actin filaments mediates the formation of lamellipodia and ruffles, protrusions of the plasma membrane involved in cell migration, phagocytosis, macropinocytosis and pathogen entry. Biochemical evidence indicates that Arp2/3 complex upon activation by WAVE complex and pre-existing mother actin filaments. Genetic studies demonstrate that the Arp2/3 and WAVE complexes are required for the formation of lamellipodia and ruffles. Although how the WAVE complex activates the Arp2/3 complex is well understood, nothing is known about the mother actin filaments.

Here we show that the actin nucleator mDia1 acts in concert with the Arp2/3 complex to promote the efficient formation of ruffles. Knockdown and rescue experiments revealed that EGF-induced membrane ruffling requires mDia1 and its actin-nucleation abilities. Although activated full-length mDia1 and Arp2/3 complex were poor actin nucleators, their combination triggered explosive polymerization of branched actin arrays *in vitro*. Thus, mDia1 polymerizes linear mother actin filaments igniting autocatalytic Arp2/3-complex-dependent nucleation of branched actin filaments and ruffling. Consistently, super-resolution microscopy localized WAVE and mDia1 within EGF-induced ruffles. Cooperation between mDia1 and the Arp2/3 complex in the making of ruffles is demonstrated by hyperactivation of the Arp2/3 complex and overexpression of mDia1 restoring EGF-induced ruffling in mDia1 knockdown and WAVE-complex knockdown cells, respectively. Given that the expression levels and the activity of mDia1 and the Arp2/3 complex dictate the contribution of either nucleator to membrane ruffling, it appears that cell-intrinsic and cell-extrinsic factors sculpt the lamellipodium/ruffle-making machinery.