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SWAP-70 associates transiently with macropinosomes

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Abstract

Cells accomplish the non-selective uptake of extracellular fluids, antigens and pathogens by the endocytic process of macropinocytosis. The protein SWAP-70 is a widely expressed, pleckstrin-homology (PH) domain-containing protein that marks a transitional subset of actin filaments in motile cells. Here we report that the protein SWAP-70 associates transiently with macropinosomes in dendritic cells and NIH/3T3 fibroblasts. The association of SWAP-70 with macropinosomes is preceded by the accumulation of Rac-GTP and followed by that of Rab5. Three regions of SWAP-70, the N-terminal region, the PH domain and the C-terminal region, contribute in a combinatorial manner to the transient association with newly formed macropinosomes in the cell periphery and occasionally with aged macropinosomes on their passage to the cell center. These data identify SWAP-70 as a transient component of early macropinosomes.

Keywords: SWAP-70; Macropinocytosis; Dendritic cells; Rac; Rab5

Introduction

Macropinocytosis is a specialized form of endocytosis that mediates the non-selective uptake of solute macromolecules, nutrients and antigens (Swanson and Watts, 1995). It also regulates the composition of plasma membrane constituents. Some pathogenic bacteria such as *Salmonella typhimurium* and *Shigella flexneri* exploit macropinocytosis to invade their host cells (Alpuche-Aranda et al., 1994; Sansonetti, 2001). Macropinocytosis is initiated by membrane ruffling that is dependent on actin polymerisation. Circular ruffles of the plasma membrane close by membrane fusion to form large endocytic vesicles of up to 5 μm in diameter, called macropinosomes. Macropinosomes move from the cell periphery to the perinuclear region. They fuse with endosomes and mature to lysosomes or regurgitate their content back to the extracellular medium (Racoosin and Swanson, 1993; Hewlett et al., 1994; Sallusto et al., 1995; Hamasaki et al., 2004). However, relatively little is known about the detailed life cycle(s) of macropinosomes and the molecular constituents that regulate it. Actin is concentrated in the membrane ruffles and dissociates within a relatively short time after vesicle

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formation (Lee and Knecht, 2002). For the internalization step, actin dynamics and actin-binding proteins such as profilin, Scar, Arp2/3, coronin, Aip, myosins and α-actinin-4 play important roles (Araki et al., 2000; Cardelli, 2001). The monomeric G-protein Rab5 was found to be indispensable for circular ruffling and macropinocytosis (Lanzetti et al., 2004). The activity of two additional proteins, phosphatidylinositol-3-OH kinase and Rac, are required simultaneously with Rab5. Another member of the Rab family, Rab 34, has also been observed to participate in macropinosome formation and to localize in ruffles and nascent macropinosomes (Sun et al., 2003). In addition to Rac, the Rac and Cdc42 effector p21-activated kinase 1 (PAK1) as well as RhoG and its exchange factor SGEF were reported to promote ruffling and macropinocytosis (Dharmawardhane et al., 2000; Ellerbroek et al., 2004). Internalized macropinosomes recruit Rab5 and its effector rabankyrin-5 (Schnatwinkel et al., 2004).

In most cell lines macropinocytosis can be induced by various growth factors such as epidermal growth factor (Haigler et al., 1979), platelet derived growth factor (PDGF) (Davies and Ross, 1978) and macrophage colony-stimulating factor (Racoosin and Swanson, 1989, 1992). Mitogenic agents like the phorbol ester phorbol 12-myristate 13-acetate (PMA) (Swanson, 1989; Keller, 1990) or diacylglycerol (Keller, 1990) and oncogenic H-ras or v-src also induce extensive membrane ruffling and concomitant macropinocytosis (Bar-Sagi and Feramisco, 1986; Veithen et al., 1996; Amyere et al., 2000). Dendritic cells (DCs) and macrophages are specialized cells of the immune system that constitutively perform macropinocytosis. Immature bone marrow-derived dendritic cells take up antigens by phagocytosis (Inaba et al., 1993; Svensson et al., 1997), clathrin-mediated endocytosis and macropinocytosis (Sallusto et al., 1995). Macropinocytosis serves as a highly efficient route for non-selective antigen uptake. After the detection of microbial products or pro-inflammatory cytokines the immature DCs differentiate into mature DCs that are highly efficient antigen-presenting cells. This maturation step is accompanied by the downregulation of constitutive macropinocytosis (Mellman and Steinman, 2001).

The protein SWAP-70 has been demonstrated to regulate cellular actin dynamics and organization (Shinohara et al., 2002a; Hilpelä et al., 2003; Sivalenka and Jessberger, 2004; Ihara et al., 2006). It encompasses a pleckstrin-homology (PH) domain that binds to the PI3-kinase product phosphatidylinositol-3,4-bisphosphate. A functional PH domain is necessary, but not sufficient, for the localization of SWAP-70 to a specific subset of fine, loose actin filament arrays in motile cells (Hilpelä et al., 2003). The protein SWAP-70 is not essential as SWAP-70-deficient mice are vital and do not show any overt phenotype (Borggrefe et al., 2001). However, mast cells from SWAP-70-deficient mice demonstrate a reduced FcεRI-triggered degranulation and alterations in c-kit-induced activation, migration and cell–cell adhesion (Gross et al., 2002; Sivalenka and Jessberger, 2004). In SWAP-70-deficient primary fibroblasts membrane ruffling was impaired in response to epidermal growth factor (Shinohara et al., 2002a; Ihara et al., 2006).

Here we demonstrate that SWAP-70 associates transiently with macropinosomes. We have determined the regions of SWAP-70 that contribute to its association with macropinosomes and we have established a sequential association of Rac-GTP, SWAP-70 and Rab5 with macropinosomes.

Materials and methods

Cultivation of dendritic cells

Mouse bone marrow was collected from tibias and femurs of C57BL/6 mice, dissolved in phosphate-buffered saline (PBS) and centrifuged. After hypotonic lysis of erythrocytes for 3 min at room temperature, the cells were passed through a 70-μm nylon mesh. Cells were resuspended in RPMI 1640 medium containing 5% fetal calf serum (FCS) (Biochrom, Berlin, Germany), 2 mM L-glutamine, 50 μg/ml gentamicin (PAA, Cölbe, Germany), 0.1 mM nonessential amino acids, 1 mM sodium pyruvate (Biochrom), and 50 μM β-mercaptoethanol (Sigma-Aldrich, Steinheim, Germany). Cells (0.6×10^6/ml) were cultured in 75-cm^2 bottles in the presence of 150 U/ml granulocyte macrophage colony-stimulating factor (GM-CSF) and 75 U interleukin-4 at 37 °C under 5% CO_2 in a humidified atmosphere. Two-thirds of the media were changed on days 3 and 6 of culture.

Plasmids

Generation of XFP-SWAP-70 mutant constructs has been described elsewhere (Hilpelä et al., 2003). To construct mCherry-SWAP-70, the SWAP-70 cDNA was ligated into the BamHI and XhoI sites of the mCherry vector (Shaner et al., 2004). The PakCrib domain was amplified by PCR from GST-Pak-CD (Sander et al., 1998) to construct CFP-PakCrib. The primers used were MB655 (GCGAATTCTTCCATTTTACCTGGAGAT) and MB656 (GCGGATCCCTATTTCTGGCTGTTGGATGTCTT). The PCR fragment was cloned into EcoRI and BamHI restriction sites of the pECFP-C1 vector (BD Biosciences Clontech, Heidelberg, Germany). The plasmid mRFP-actin was a gift of Dr. K. Rottner (GBF, Braunschweig, Germany).