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Reggie-1 and reggie-2 localize in non-caveolar rafts in epithelial cells: Cellular localization is not dependent on the expression of caveolin proteins

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Abstract

Reggie-1 and reggie-2 are highly conserved and widely expressed proteins associated with membrane rafts. The molecular function of reggies remains to be clarified, but recent data indicate that they are involved in various cellular processes such as insulin signaling, phagocytosis and actin remodeling. However, there is discrepancy in the literature if reggies are associated with caveolae or non-caveolar rafts. Reggies are expressed and raft associated also in many cells which do not contain caveolae, such as neurons and lymphocytes. However, it is not clear if the function or localization of reggies are dependent on the presence of caveolae and expression of caveolin-1 protein. In this study, we directly addressed this question in epithelial cells. We could show that ectopic expression of caveolin-1 does not result in any change in the cellular localization of reggie-1, which is present at the plasma membrane also in the absence of caveolin-1. On the other hand, caveolin-2, which localizes in caveolae, is dependent on caveolin-1 expression in order to be localized at the plasma membrane. Although reggie-1 and reggie-2 strongly interact with each other, we did not detect a direct interaction between caveolin-1 and reggies by means of a yeast two-hybrid assay, nor could reggies be co-immunoprecipitated with caveolin-1. Furthermore, endogenous reggie-1 and -2 were found not to colocalize with caveolin-1 in epithelial cells. Thus, our data indicate that reggies are localized in microdomains different from caveolae, and the function of reggies is different from and independent of caveolin-1.

Keywords: Rafts; Caveolae; Microdomain; Flotillin; Reggie; Caveolin

Introduction

Membrane rafts are small microdomains of the cell membrane, enriched in cholesterol and glycosphingolipids (Simons and Ikonen, 1997). Rafts are insoluble in cold detergents such as Triton X-100, a feature which has been used for their isolation. The suggested functions of rafts include endocytosis and transcytosis of small molecules such as folate, the receptor of which is concentrated in rafts. In addition, rafts have been shown to play a role in many signaling processes of the cell, such as signaling through growth factor receptors and GPI-anchored proteins. The cytoplasmic side of rafts is heavily enriched with various signaling proteins, and rafts have therefore been suggested to function as

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signaling centers of the cell (for review, see Simons and Toomre, 2000).

Rafts are heterogeneous in nature, and different types of rafts have been shown to exist, the composition and morphology of which varies substantially. Caveolae are a specific subtype of rafts. They are small, invaginated structures that are abundant in many cell types, including endothelial and some epithelial cells. Other cells such as neurons, lymphocytes and thyrocytes do not contain any morphologically recognizable caveolae but rather other, non-invaginated raft types. Caveolae are characterized by the family of caveolin proteins, which consists of three members termed caveolin-1, -2 and -3. Caveolin-1 and -2 are relatively ubiquitous, whereas caveolin-3 has been shown to be expressed only in muscle cells and in astrocytes. Expression of caveolin-1 or -3 alone is sufficient to result in the formation of caveolar invaginations also in cells that normally do not exhibit any caveolae (Fra et al., 1995; Lipardi et al., 1998), whereas caveolin-2 alone cannot induce caveolae. Fischer rat thyroid (FRT) cells, an epithelial cell line of the thyroid, do not express any endogenous caveolin-1, whereas some caveolin-2 can be detected in these cells (Lipardi et al., 1998; Zurzolo et al., 1994). In the absence of caveolin-1, caveolin-2 localizes in the perinuclear TGN region of the cells, being unable to translocate to the plasma membrane (Lipardi et al., 1998; Mora et al., 1999). However, when these cells are transfected with caveolin-1, caveolin-2 is partially recruited to the plasma membrane together with caveolin-1, with some caveolin-2 remaining in intracellular structures (Mora et al., 1999). In addition, caveolin-1 expression also results in the formation of morphologically recognizable caveolae in FRT cells.

The non-invaginated rafts are substantially less well characterized than caveolae. However, in many cell types lacking caveolae, non-invaginated rafts have also been suggested to contain a typical set of proteins that can be used for their identification. One such example is the reggie/flotillin family which consists of two proteins that are related to each other and show a very profound evolutionary conservation (Lang et al., 1998; Schulte et al., 1997). Reggies are also referred to as flotillins in the literature, with reggie-1 being identical with flotillin-2 and reggie-2 with flotillin-1 (Bickel et al., 1997; Volonte et al., 1999). In addition, a truncated form of reggie-1 has been described in epidermal keratinocytes as epidermal surface antigen (ESA), a protein putatively functioning in cell adhesion (Schroeder et al., 1994).

Reggies were originally described as axonal proteins upregulated upon regeneration of goldfish retinal ganglion cells (Schulte et al., 1997) and were later shown to be associated with membrane rafts and to be ubiquitously expressed (Lang et al., 1998; Neumann-Giesen et al., 2004; Stuermer et al., 2001). We have shown that reggie-1 is associated with membrane rafts by means of myristoylation and palmitoylation and that lipid modifications and oligomerization are necessary for raft association (Neumann-Giesen et al., 2004). Our recent findings also show that reggie-1 is phosphorylated by Src kinases upon stimulation of cells with EGF. In addition, reggie-1 promotes cell-matrix adhesion (Neumann-Giesen et al., 2007).

Although the raft association of reggies has been verified by many studies, there is some discrepancy in the literature as to whether reggies localize in caveolae or in a non-caveolar raft. Reggies/flotillins have been described to be localized in non-caveolar rafts (Morrow et al., 2002; Neumann-Giesen et al., 2004; Rajendran et al., 2003; Stuermer et al., 2001), but some studies also suggest a localization in caveolae and physical association with the caveolin molecules (Bickel et al., 1997; Volonte et al., 1999). A recent study has shown that reggie-2/flotillin-1 is associated with endocytosis which takes place by means of a clathrin- and caveolin-independent mechanism (Glebov et al., 2006). Reggies are widely expressed and raft associated also in cells that do not contain caveolae, for example, in neurons and lymphocytes. However, the question of the identity of the reggie rafts and the dependency of their function on caveolae remains to be answered. In this study, we especially wanted to directly address the question if the cellular localization and function of reggies is dependent on the expression of caveolin-1 and thus also on the formation of caveolae. For this purpose, we have chosen the FRT cells as a model system in order to study if the ectopic expression of caveolin-1 in cells that do not contain endogenous caveolin-1 would result in a change in the cellular localization of reggies.

We here show that endogenous reggies and caveolin-1 are localized in different microdomains in epithelial cells. Importantly, ectopic expression of caveolin-1 does not affect the cellular localization or function of reggie-1. Since reggies cannot be co-immunoprecipitated with caveolin-1 and do not interact with caveolin-1 in a yeast two-hybrid assay, existence of molecular complexes containing both proteins in the epithelial cells used in this study seems unlikely. Thus, our results provide further evidence for the hypothesis that reggies and caveolins reside in different rafts and are functionally independent.

Materials and methods

Cell lines and antibodies

FRT cells and FRT cells stably transfected with caveolin-1 (FRT-Cav, (Mora et al., 1999)) were cultured in F12 Coon's medium with 10% fetal calf serum (FCS).