European Journal of Cell Biology 94 (2015) 309–315

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.com/locate/ejcb

# Recycling of galectin-3 in epithelial cells

Ellena Hönig, Katharina Schneider, Ralf Jacob*

*Department of Cell Biology and Cell Pathology, Philipps University of Marburg, Marburg, Germany*

ARTICLE INFO

*Keywords:*
Polarized sorting
Galectin
Rab
Endosome
Apical targeting
SNAP-tag

ABSTRACT

Galectins, a family of β-galactoside binding proteins, do not possess a signalling sequence to enter the endoplasmic reticulum as a starting point for the classical secretory pathway. They use a so-called unconventional secretion mechanism for translocation across the plasma membrane and/or into the lumen of transport vesicles. The β-galactoside binding protein galectin-3 is highly expressed in a variety of epithelial cell lines. Polarized MDCK cells secrete this lectin predominantly into the apical medium. The lectin re-enters the cell by non-clathrin mediated endocytosis and passages through endosomal organelles. This internalized galectin-3 plays an important role in apical protein trafficking by directing the subcellular targeting of apical glycoproteins via oligomerization into high molecular weight clusters, a process that can be fine-tuned by changes in the environmental pH. Following release at the apical plasma membrane, the lectin can reenter the cell for another round of recycling and apical protein sorting. This review will briefly address galectin-3-functions in epithelia and focus on distinct phases in apical recycling of the lectin.



## 1. Introduction

Posttranslational protein modification is an effective strategy of the cell to add protein information not encoded by the genome. Amongst numerous modifications, the addition of sugar moieties, called glycosylation, provides an enormous proteomic diversity. Not only the simple sequence of monosaccharides, but also the side of glycosidic linkage, the anomeric position and the ring size determine the coding potential in the first dimension. Considering the availability of more than one linkage point, branching of oligomers yields a second dimension to the sugar code (Gabius et al., 2002). Because of their hydrophilic nature glycans can also influence the solubility of modified proteins (Rudd and Dwek, 1997).

Glycan chains can be identified by sugar binding proteins termed lectins, which are classified according to their sequence and structural homology (Varki et al., 2009). The family of galectins recognizes β-galactosides by means of a highly conserved carbohydrate recognition domain (CRD) and consists of 15 family members, which are categorized by their domain composition (Fig. 1). This galectin-division into prototype, tandem repeat and chimeric type was introduced in Hirabayashi and Kasai (1993). Prototype galectins comprising galectins-1, -2, -5, -7, -11, -13, -14 and -15, contain a single CRD and act as monomers or dimers. Galectins of the tandem repeat type (galectins-4, -6, -8, -9 and -12) possess two covalently linked homologous but not identical CRDs. Galectin-3 is the only chimeric type variant in vertebrates and consists of a CRD and a proline- and glycine-rich N-terminal domain (Leffler et al., 2004).

The galectin CRD is composed of a 5-stranded (F1–F5) and a 6-stranded (S1–S6) β-sheet forming a β-sandwich structure (Seetharaman et al., 1998). The 6-stranded β-sheet forms the carbohydrate binding cleft, long enough to hold a linear tetrasaccharide and is therefore described to have the subsites A–D. There is as well a fifth site E, which is less defined by structure. Although the binding site for β-galactosides is the subsite C (Leffler et al., 2004), interaction between the CRD and the monosaccharide galactose is weak compared to an about 100 fold higher binding affinity for the disaccharide lactose (Leffler and Barondes, 1986). The extended binding region with the subsites A, B and D, E facilitates binding of complex glycans by interference with additional sugar residues. These regions are less conserved among the galectin family and are therefore responsible for different ligand specificities (Leffler et al., 2004).

In vertebrates galectin-3 is expressed in various tissues and cell types and is found in the cytoplasm, the nucleus, on the surface of cells and in the extracellular space where it accomplishes numerous cellular functions. Its expression can be modulated during cellular morphogenesis and various physiological conditions (Dumic et al., 2006). Recent findings revealed that galectin-3 is involved in intracellular trafficking pathways between the plasma membrane and

* Corresponding author. Tel.: +49 6421 286 6482; fax: +49 6421 286 6414.
*E-mail address:* jacob@staff.uni-marburg.de (R. Jacob).

http://dx.doi.org/10.1016/j.ejcb.2015.05.004
0171-9335/

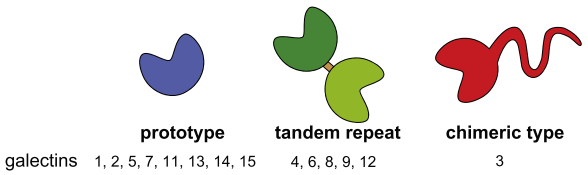


**Fig. 1.** Classification and structure of galectins. Members of the β-galactoside binding lectins are categorized according to their number and structure of the carbohydrate recognition domain (CRD). Prototype galectins contain a single CRD, galectins of the tandem repeat type contain two CRDs connected by a linker sequence and chimeric galectin-3 consists of a CRD and a flexible N-terminal domain.

endosomal organelles (Delacour et al., 2006; Lakshminarayan et al., 2014). This review now focuses on galectin-3 in apical trafficking of polarized epithelial cells.

## 2. Participation of galectin-3 in apical trafficking

Galectin-3 synthesized in the cytosol is secreted independently of the endoplasmatic recticulum (ER) into the extracellular milieu and secretion is not reduced by a blockade of the classical secretion pathway with brefeldin A (Lindstedt et al., 1993). In polarized Madin Darby canine kidney (MDCK) epithelial cells the lectin is secreted predominantly into the apical medium with a low efficiency of about 12% (Lindstedt et al., 1993; Sato et al., 1993). However, this release is blocked at an incubation temperature of 20 °C or by microtubule disruption with nocodazole, two striking characteristics of *post* Golgi trafficking in MDCK cells. A close connection between exocytic galectin-3 trafficking pathways and intracellular vesicular *post* Golgi transport is the accumulation of galectin-3 in vesicular structures emerging from the *trans*-Golgi network (TGN) destined for the apical membrane (Delacour et al., 2006). Even though secretion of galectin-3 itself is not impaired when the classical secretory pathway is blocked, the lectin is required for correct apical targeting of certain glycoproteins. For example, galectin-3 depletion missorts apical lactase-phlorizin hydrolase (LPH), the neurotrophin receptor p75 ($p75^{NTR}$) and gp114 to the basolateral domain of epithelial MDCK cells (Delacour et al., 2006) and inhibits apical secretion of Wnt11 (Yamamoto et al., 2013). Furthermore, LPH and dipeptidylpeptidase IV (DPPIV) are mislocalized in enterocytes of galectin-3 knockout mice (Delacour et al., 2008). However, galectin-3 is not a general receptor for apical sorting in epithelial cells. Apical sorting of the soluble glycosylated growth hormone (gGH), the sialomucin endolyn, MUC1 or *lipid-raft*-associated sucrase isomaltase (SI) is not influenced by depletion of galectin-3 (Delacour et al., 2006; Kinlough et al., 2011; Mattila et al., 2012; Mo et al., 2012). Neither does apical transcytosis of the transferrin receptor (TfR) depend on galectin-3 (Perez Bay et al., 2014). Knockdown of galectin-4 inhibits TfR transcytosis to the apical membrane (Perez Bay et al., 2014) and galectin-4 is also involved in the delivery of glycoproteins to the brush border membrane of enterocyte-like cells (Stechly et al., 2009). Galectin-9, another tandem repeat type galectin, is required for apical trafficking of sialylated endolyn (Mo et al., 2012). This suggests, that multiple mechanisms for glycan-mediated apical sorting exist in epithelial cells with diverging galectin-3 participation.

N-glycosyl- as well as O-linked sugar chains had been described as apical sorting signals before (Alfalah et al., 1999; Scheiffele et al., 1995; Yeaman et al., 1997). Among the galectin-3 binding partners LPH and $p75^{NTR}$ contain both N- and O-glycans (Naim and Lentze, 1992; Yeaman et al., 1997), whereas gp114 is only N-glycosylated (Le Bivic et al., 1993). Both glycan-variants could be involved in galectin-3 mediated apical sorting since galectin-3 binds to β-galactosides of N- (Carlsson et al., 2013) and in principle also of O-glycans. Nevertheless, galectin-1 and -3 bind nearly exclusively to complex N-glycans on the surface of CHO cells (Patnaik et al., 2006). The affinities of binding to complex N-glycans are proportional to the *N*-acetyllactosamine content of the glycan (Hirabayashi et al., 2002). Given the fact that glycan chains are added in the lumen of the ER or the *cis*-Golgi to the extracellular moiety of transmembrane or secreted proteins, it remains an open question how cytosolically synthesized galectin-3 traverses the membrane of secretory organelles or the plasma membrane for sugar interaction. Results from Lukyanov et al. demonstrate that galectin-3 is able to translocate across the membrane bilayer *in vitro*. They showed that galectin-3 directly interacts with membrane lipids and penetrates the lipid bilayer of liposomes (Lukyanov et al., 2005). It may be that simple, however, alternative pathways are provided by the cell that may also play a role in membrane translocation of galectin-3.

## 3. Unconventional galectin-3 secretion

Up to now, four distinct non-conventional transport pathways have been described (Nickel and Rabouille, 2009). The first one is direct translocation as best studied for the fibroblast growth factor 2 (Zehe et al., 2006). The second pathway for unconventional protein secretion involves the sequestration of a soluble cytoplasmic factor by secretory lysosomes as shown for interleukin 1β (Andrei et al., 1999). Vesicle-mediated secretion by microvesicles or exosomes comprise the third and fourth unconventional protein secretion pathways. Findings from Mehul and Hughes point to the third pathway and describe that an acylated galectin-3/chloramphenicol acyltransferase (CAT) chimera is released by microvesicle shedding in COS-7 cells (Mehul and Hughes, 1997). On the other hand, several proteomic approaches led to the identification of galectin-3 in exosomes derived from different cell types (Ogawa et al., 2008; Pisitkun et al., 2004; Thery et al., 2001; Welton et al., 2010). It thus seems that galectin-3 uses distinct pathways for unconventional secretion, which also depends on the individual cell type. However, the exact secretion mechanism of galectin-3 has not been identified for any of these pathways yet. Nevertheless, the Hughes lab was able to demonstrate that a short octapeptid in the N-terminus of galectin-3 was essential but not sufficient for unconventional secretion of an acylated fusion protein of the chloramphenicol acyltransferase (CAT) with N-terminal residues 1–120 of hamster galectin-3 (Menon and Hughes, 1999). Here, the YP(90)SAP(93)GAY sequence was found to be one essential determinant for efficient secretion. Interestingly, unconventional secretion of galectin-3 as well as of other cytosolic proteins was shown to be dependent on caspase-1 activity (Keller et al., 2008). This provides a potential regulation mechanism for the pool of extracellular versus intracellular galectin-3 (Viguier et al., 2014).

## 4. Roles of galectin-3 in the extracellular matrix

Extracellular galectin-3 can modulate cell adhesion to different extracellular matrix (ECM) components by lattice formation and cross-linking of matrix molecules and cell surface glycoproteins. The involvement of extracellular galectin-3 in cell adhesion correlates with its functions in collective cell migration during wound healing and tissue repair in epithelia. Galectin-3 binds directly to collagen IV *in vitro*, promotes adhesion of corneal epithelial cells onto collagen IV and enhances wound healing in corneal explants in a carbohydrate-dependent manner (Yabuta et al., 2014). Enhanced cell adhesion to collagen is supposed to be mediated by α2β1-integrins (Friedrichs et al., 2008, 2007). Another study showed that extracellular galectin-3 promotes cell migration by cross-linking complex N-glycans on α3β1-integrins on the surface of human corneal epithelial