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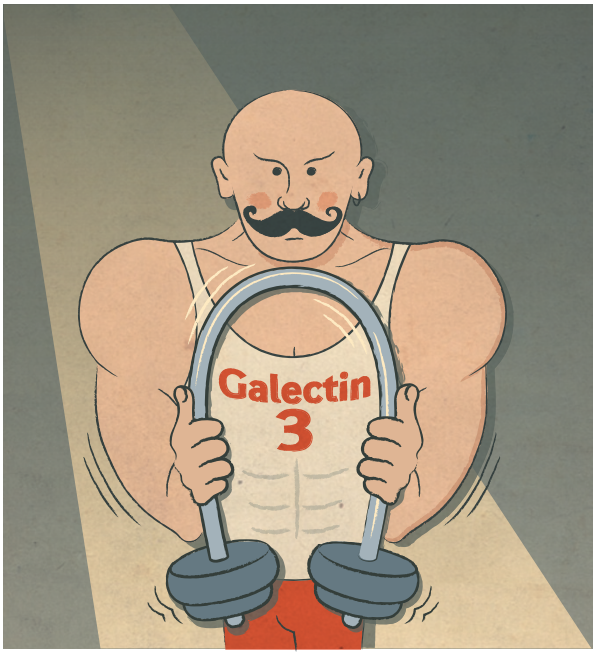
ENDOCYTOSIS

# Bend it like galectin 3

> GAL3 functions as an endocytic adaptor that co-clusters glycosylated cargos and GSLs

In addition to clathrin-dependent endocytosis, several protein cargos are internalized in the absence of a clathrin coat — a process that is known as clathrin-independent endocytosis. Clathrin-independent carriers (CLICs), which are morphologically distinct from conventional clathrin-coated vesicles, have been identified as the main carrier vesicles involved in this pathway; however, how cargo proteins are sorted and CLICs are generated at the plasma membrane remained unclear. Lakshminarayan *et al.* now show that galectin 3 (GAL3) binds to both glycosylated cargos and membrane glycosphingolipids (GSLs; complex sphingolipids with a carbohydrate head group), which leads to membrane bending and CLIC biogenesis.

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The authors set out to investigate the contribution of GSLs to endocytosis in mammalian cells and showed that depletion of these membrane lipids resulted in less CLICs, whereas the formation of other endocytic vesicles was unaffected. GSL depletion also inhibited uptake of the CLIC cargo molecule CD44, but not uptake of cargo through clathrin-dependent pathways, which suggests a role for GSLs in clathrin-independent endocytosis. Electron tomography revealed that the carbohydrate-binding lectin GAL3 localized to membrane invaginations that exhibited the characteristic morphologies of CLICs. Moreover, GAL3 recruitment to these structures was dependent on the presence of GSLs, which suggests a functional link between these molecules.

Depletion of GAL3 using siRNA inhibited CD44 uptake; this effect was rescued by the addition of exogenous GAL3 but not by a GAL3 mutant lacking the amino-terminal domain involved in oligomerization, which suggests that GAL3 oligomerization is important for cargo internalization. In addition, GAL3 depletion reduced the formation of CLICs. These results suggest that both GAL3 and GSLs are required for CD44 endocytosis and CLIC biogenesis.

The addition of an inhibitor that prevented *N*-glycosylation led to reduced GAL3 binding to the cell surface. Moreover, uptake of a mutant CD44 protein that lacked all *N*-glycosylation sites was abolished. These findings led the authors to propose that GAL3 was recruited to the plasma membrane by binding to glycosylated cargo molecules and that this association was required for the endocytic function of GAL3.

Finally, the authors established that GAL3 initiated the GSL-dependent formation of invaginations directly at the plasma membrane, and they went on to assess whether GAL3 had a direct role in membrane bending using a liposome reconstitution system. GAL3 was targeted to lipid membranes, and the authors found that GAL3 clusters and tubular invaginations only formed in the presence of GSLs. Furthermore, they showed that inward membrane bending was dependent on an interaction between GAL3 and the GSL carbohydrate group.

Thus, the authors propose a model for GAL3-driven CLIC biogenesis whereby GAL3 functions as an endocytic adaptor that co-clusters glycosylated cargos and GSLs at the plasma membrane, which leads to both membrane bending and clathrin-independent formation of the endocytic pit.

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**ORIGINAL RESEARCH PAPER** Lakshminarayan, R. *et al.* Galectin-3 drives glycosphingolipid-dependent biogenesis of clathrin-independent carriers. *Nature Cell Biol.* http://dx.doi.org/10.1038/ncb2970 (2014)