Sweet control of cell migration, cytokinesis and organogenesis

Benjamin Podbilewicz

Why are proteins glycosylated? On the basis of new studies, I propose two models to clarify the specific functions of glycosylation in worms. The first explains how intra- and inter-cellular trafficking of an *N*-glycosylated disintegrin-metalloprotease guides somatic gonadal cells through their migratory route, determining the shape of an organ. The second explains how rigid coats of secreted chondroitin proteoglycans bend membranes to drive cytokinesis and epithelial invagination.

Although glycosylation of proteins is a frequent post-translational modification discovered many years ago, it is unclear why specific proteins go through this complex pathway of adding, and sometimes trimming, numerous sugars. Glycoproteins have been implicated in diverse intracellular functions from bacteria to mice[1,2], including organelle structure and function, sorting and trafficking, as well as in intercellular functions such as cell adhesion, migration, proliferation, extracellular matrix organization, differentiation and morphogenesis. Several congenital diseases resulting from defects in glycosylation also attest to the importance of this modification in humans. But what function of this modification underlies these diverse phenotypes? To date, glycosylation seems to be important for correct protein folding and for ligand–receptor interactions. A common theme in glycosylation disorders is defective maturation and processing of specific glycoproteins, resulting in organ- or system-restricted defects[1].

Two main types of protein glycosylation exist: *N*- and O-glycosylation. *N*-glycosylation results from a complex biochemical pathway requiring assembly and processing in sequential subcellular locations; these include the cytosol, endoplasmic reticulum and Golgi cisternae. In the endoplasmic reticulum, core sugars are added to asparagine residues on newly synthesized proteins destined for secretion. Resident enzymes in the endoplasmic reticulum and Golgi then remove and add different sugar residues to determine the final oligosaccharide structure. By contrast, *O*-glycosylation occurs in the Golgi only and does not include an 'assembly line' of enzymes in different organelles. This pathway generates a larger number of core structures and, as a result, there is greater diversity in *O*-glycans than in *N*-glycans.

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So why do mutations that result in specific glycosylation defects result in distinct phenotypes? Three recent papers studying *C. elegans* development give partial but exciting answers to these questions. On page 31 of this issue, Kiyoji Nishiwaki and colleagues[3] show that *N*-glycosylation of an ADAM (a secreted disintegrin and metalloprotease) family member is required for normal migration of two cells that determine the shape and size of the gonad in *C. elegans*. Earlier this year, two papers published in *Nature* also showed that *O*-glycosylation of chondroitin proteoglycans is essential for cell division and normal invagination of the vulva[4,5]. Together, these three papers open up a new chapter in glycobiology that will certainly help us to understand why sugar modifications on specific proteins and lipids are so important for fundamental biological processes.

Nishiwaki and colleagues[3] find that mutations in *mig-23* disrupt migration of mesodermal distal tip cells (DTC). Furthermore, they show that *mig-23* encodes a nucleoside diphosphatase (NDPase) that is localized to the Golgi membranes of muscle cells and is required for *N*-glycosylation of MIG-17/ADAM disintegrin-metalloprotease. Previously, it was shown that MIG-17 is required for DTC migration and that the phenotypes of *mig-23* and *mig-17* mutants were similar (Fig. 1)[6,7]. Thus, the authors set out to test whether MIG-23/NDPase affects DTC migration and organ morphogenesis through *N*-glycosylation of MIG-17/ADAM. Using elegant genetic, molecular and biochemical approaches, they found that *mig-23* and *mig-17* control DTC migration at the same step in the pathway[3], and that MIG-17 is underglycosylated and inactive in *mig-23* mutants. In further support of this model, MIG-23 has NDPase activity *in vitro* and can even rescue yeast double mutants that lack endogenous NDPase activity.

One possibility is that *N*-glycosylation of MIG-17/ADAM may be required for its correct folding and transport out of the *trans*-Golgi network, or for other steps in the secretory pathway of muscle cells (Fig. 2a). Although it appears that a recombinant MIG-17–green fluorescent protein (GFP) is secreted from muscle cells, even in a *mig-23* mutant background[3], greater resolution of the micrographs or electron microscopy is needed to confirm this. Once MIG-17 is in the extracellular space, it probably diffuses to the gonad where it binds to the basal lamina. If this metalloprotease is active, it may degrade an unidentified substrate in the basal lamina of the muscle, gonad and epidermis, which could be important for DTC migration[6]. The authors show that, in *mig-23*

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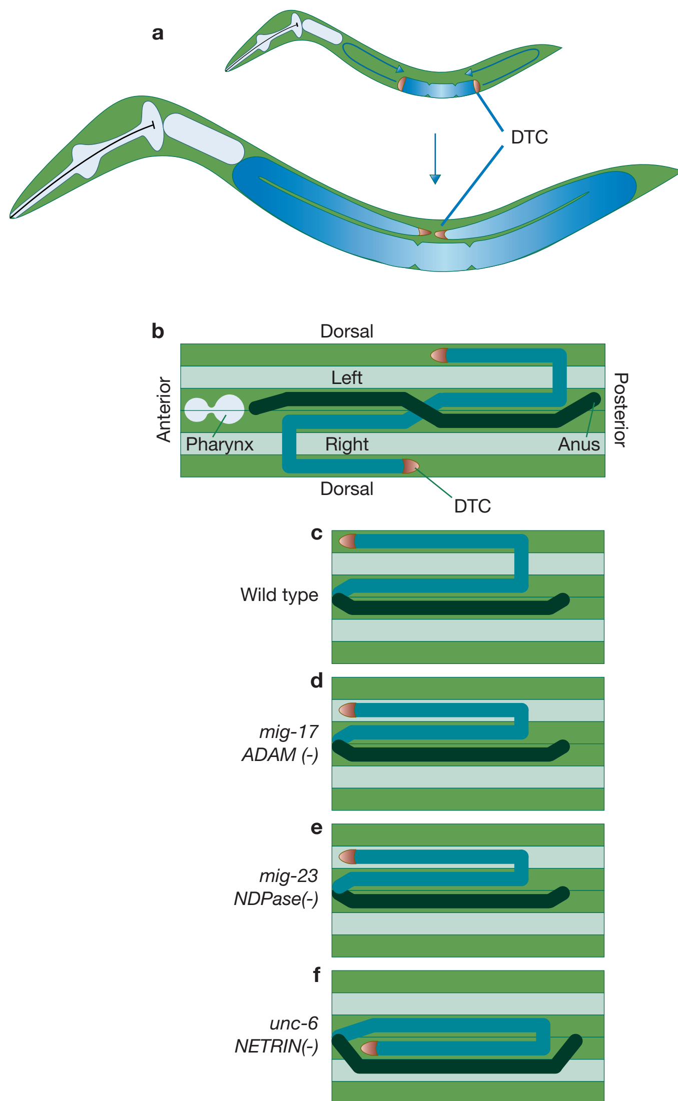

Figure 1 Migration of distal tip cells. (**a**) Migration of DTCs (red) starts in the mid-L2 stage (blue arrows) to generate U-shaped gonadal arms (blue) in the adult. **b**) Epidermis (light green) and muscles (green) are shown as a cylindrical projection of the worm opened along the dorsal midline in the adult. (**c**–**f**) As in **b**, but showing only the posterior DTC migration in wild-type (**c**), *mig-17(–)* (**d**), *mig-23(–)* (**e**) and *unc-6(–)* (**f**) animals. Dark green tube shows the position of the intestine.

mutants, MIG-17–GFP fluorescence on the surface of the gonad is reduced, and so they conclude that glycosylation is essential for its efficient localization here[3]. Moreover, punctate staining around the gonad is lost. This suggests several possibilities: first, wild-type MIG-17 may be bound by a surface receptor on the DTC and/or on the gonadal cells, perhaps triggering clustering and endocytosis of the metalloprotease, which removes it from the surface. Internalized MIG-17 could then be inactivated and degraded in lysosomes. Alternatively, it might be recycled through polarized secretion to a specific domain of the membrane that would allow directed migration of the DTCs towards the dorsal muscles (Fig. 2a). Clearly these events would need to be temporally and spatially regulated for correct migration. So although Nishiwaki and colleagues have shown that MIG-17 requires *N*-glycosylation in muscles for efficient secretion and binding to the somatic gonad, the mechanisms are unknown. Their analysis raises some interesting questions. How does glycosylated MIG-17/ADAM drive DTCs to migrate along such a complex pathway? Is active MIG-17 degrading basal lamina components to allow DTCs to move and migrate? What are the targets of MIG-17, and how is MIG-17 activated and inactivated in specific locations and times during cell migration?

Another question for further study is whether MIG-23/NDPase affects the glycosylation not only of MIG-17/ADAM, but also of other unidentified secreted and membrane-anchored proteins that control DTC migration. Candidates that could be put to the test include the predicted glycoproteins UNC-6/netrin, GON-1/ADAMTS, PAT-3/β-integrin, and UNC-71/ADM-1/ADAM[8–13], all of which are essential for cell migration.

Progress is also being made in understanding the function of *O*-glycosylation. Two *Nature* papers from the Horvitz[4] and Nomura[5] labs have identified *O*-glycosylation of chondroitin proteoglycans in worms as a different post-translational modification that controls morphogenesis and cytokinesis. In a previous genetic screen by Herman and Horvitz for vulva morphogenesis mutants, eight genes (*sqv-1* to *sqv-8*) were identified that result in a squashed vulva phenotype in *C. elegans*[14]. Horvitz and colleagues then found that embryos with strong *sqv* mutations die during embryogenesis with a defect in cytokinesis[4]. The molecular identities of seven of the *sqv* genes[4,15] indicate defects in biosynthesis of the glycosaminoglycans heparan sulphate and chondroitin, whereas the eighth gene (*sqv-5*) encodes a chondroitin synthase[4]. The mechanism by which chondroitin affects invagination of the vulva and cytokinesis is speculative (reviewed in ref. 15). Horvitz and colleagues proposed that glycosaminoglycans attached to the extracellular matrices drive the formation of fluid-filled spaces during both cytokinesis and vulval morphogenesis in *C. elegans*[4], analogous to the swelling of the fertilization membrane that is driven by secretion of glycosaminoglycans after sperm–egg fusion in the sea urchin.

Here I suggest that chondroitin might instead provide a rigid coat that drives membrane remodelling during both cell division and epithelial invagination. In the first case, the actin contractile ring mediates the initial stages of membrane invagination during cytokinesis. Then during the last stage of cytokinesis, which is actin-independent and involves membrane fission, the external chondroitin coat might provide the force that deforms the membranes, bending them to accomplish scission (Fig. 2b). This process would be similar to the budding of vesicles during endocytosis, in which protein coats containing clathrin and adaptor molecules may provide the force required for budding. This model implies that the site of fission during cytokinesis is a small isolated membrane domain deformed by the adjacent relatively

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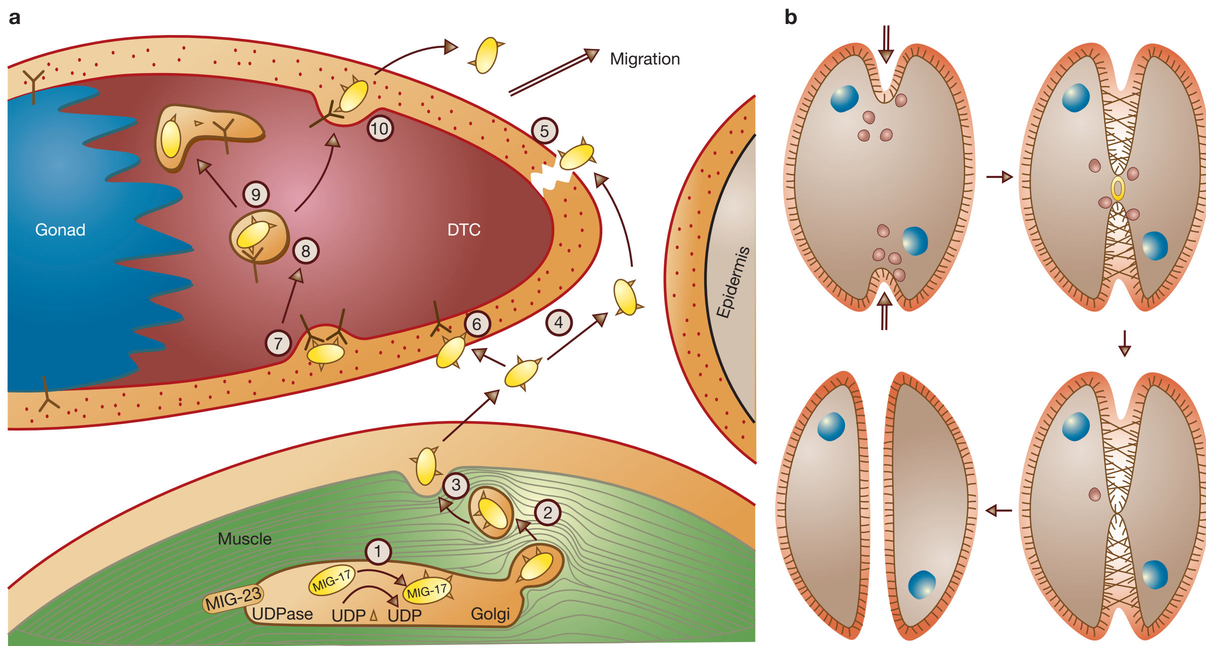

Figure 2 Models for glycosylated protein action in cell migration and cell division. (**a**) *N*-glycosylation of MIG-17/ADAM is necessary for cell migration. Normal MIG-23/NDPase activity in the Golgi apparatus of muscle cells (green) is required for normal *N*-glycosylation of MIG-17/ADAM (1). Correct sugars on MIG-17 may be necessary for sorting and intracellular trafficking (2). Secretion of MIG-17 (3) is followed by diffusion (4) to the basal laminae (orange) of gonadal cells (blue) and DTCs (red). MIG-17 may degrade components of the basal lamina (5) and/or binds to an unidentified receptor on the surface of gonadal cells (6). Receptor-mediated clustering of MIG-17 (7) results in endocytosis (8) and trafficking to lysosomes for degradation (9) or recycling to the leading edge of the DTC. Polarized exocytosis of MIG-17 (10) may cause cell migration at a particular time and in a defined direction. (**b**) Chondroitin *O*-glycosylation is necessary for cytokinesis. The dividing cell secretes chondroitin-glucosaminoglycans, resulting in the formation of an extracellular coat (orange). The actin contractile ring (yellow) mediates furrowing of the dividing cell. At the same time, secretion at the furrow contributes membranes and additional chondroitin. The chondroitin glues together the furrowing membranes, stabilizing the membrane invagination and bending the membrane. Membrane fission occurs because the chondroitin peptidoglycans crosslink the membranes, facilitating membrane remodelling and resulting in two daughter cells.

rigid structure of the chondroitin proteoglycan coat. Consistently, Nomura and colleagues observe that when chondroitin is not present in chondroitin synthase mutants or when its function is perturbed[5], the cells attempt to invaginate using their contractile rings but then the cleavage furrows regress. From this, I would suggest that a chondroitin coat outside the intracellular sites of membrane contact might drive membrane bending during cytokinesis. A similar function for protein coats has been proposed for viral and vesicular membrane fusion[16]. In addition, it is conceivable that newly secreted chondroitin proteoglycans could also help 'glue' the invaginating furrow and thereby maintain its rigidity. This adhesion energy might drive expansion of the membranes at the contact zone and provide a driving force for membrane fission. Polarized secretion at the site of invagination would also provide additional membranes[17], allowing membrane furrowing to continue until the actin contractile ring disappears and the membranes undergo fission (Fig. 2b). Does this mechanism also apply to the vulval morphogenesis phenotype observed by Horvitz and colleagues[14]? The vulval defects do not seem to result from a failure in cytokinesis or cell–cell fusion events during epithelial invagination[4]. Instead, it might be that a stiff extracellular coat of chondroitin proteoglycans secreted by invaginating epithelial cells could sculpt the vulval tube.

In humans, *O*-glycosylation defects are associated with at least four distinctive congenital disorders and *N*-glycosylation with twelve diseases that affect diverse organs[1]. Thus, it is not surprising that in worms, flies, fish, mice and humans, the specific *N*- and *O*-glycosylation of different targets controls many key developmental processes[1,2]. Hopefully, by combining classic genetic screens for genes that interact with *mig-23*/NDPase or *sqv-5*/chondroitin synthase with whole *N*- and *O*-glycome approaches, we may be able to identify additional targets for the glycosylation pathways that regulate cell migration, cytokinesis and organogenesis. □

1. Jaeken, J. & Matthijs, G. *Annu. Rev. Genomics Hum. Genet.* **2,** 129–151 (2001).
2. Selleck, S. B. & Schachter, H. *Biochim. Biophys. Acta* **1573,** 199–428 (2002).
3. Nishiwaki, K. *et al. Nature Cell Biol.* **6,** 31–37 (2004).
4. Hwang, H. Y., Olson, S. K., Esko, J. D. & Horvitz, H. R. *Nature* **423,** 439–443 (2003).
5. Mizuguchi, S. *et al. Nature* **423,** 443–448 (2003).
6. Nishiwaki, K., Hisamoto, N. & Matsumoto, K. *Science* **288,** 2205–2208 (2000).
7. Nishiwaki, K. *Genetics* **152,** 985–997 (1999).
8. Hedgecock, E. M., Culotti, J. G. & Hall, D. H. *Neuron* **2,** 61–85 (1990).
9. Su, M.-W. *et al. Development* **127,** 585–594 (2000).
10. Blelloch, R. & Kimble, J. *Nature* **399,** 586–590 (1999).
11. Lee, M., Cram, E. J., Shen, B. & Schwarzbauer, J. E. *J. Biol. Chem.* **276,** 36404–36410 (2001).
12. Podbilewicz, B. *Mol. Biol. Cell* **7,** 1877–1893 (1996).
13. Huang, X., Huang, P., Robinson, M. K., Stern, M. J. & Jin, Y. *Development* **130,** 3147–3161 (2003).
14. Herman, T. & Horvitz, H. R. *Cold Spring Harb. Symp. Quant. Biol.* **62,** 353–359 (1997).
15. Bulik, D. A. & Robbins, P. W. *Biochim. Biophys. Acta* **1573,** 247–257 (2002).
16. Chernomordik, L. V. & Kozlov, M. M. *Annu. Rev. Biochem.* **72,** 175–207 (2003).
17. Finger, F. P. & White, J. G. *Cell* **108,** 727–730 (2002).

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