

Sweet solution: sugars to the rescue

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Sugar pills are usually placebos, but Smith et al. (2002, this issue) use one to rescue designer mice unable to make GDP-Fucose. Dietary fucose enters a salvage pathway and spares the mice. Sound simple? Not so. Unknown genetic factors determine life or death.

Fucosylated sugar chains decorate cell surface glycoproteins including signaling receptors, where the sugars can have critical functions. The best-known example is that of selectin ligands, which mediate leukocyte rolling on endothelial cells (Ley, 2001). The discovery that Notch signaling involves fucose-based glycans (Moloney et al., 2000) heralds another exciting area in glycobiology, dubbed the “Cinderella science” in 2001 (Alper, 2001). Before that time, studies showed that stable fucosylated glycan expression in intestinal epithelial cells requires continuous dialogue with the gut’s commensal flora (Hooper and Gordon, 2001). In humans, congenital disorder of glycosylation II-c (CDG-IIc)* caused by mutations in the Golgi GDP-Fucose transporter results in global fucosylation defects, profound mental retardation, failure to thrive, recurrent infections, and leukocytosis (Becker and Lowe, 1999; Freeze, 2001; Lübke et al., 2001).

GDP-Fucose is mostly derived from the *de novo* pathway (Fig. 1). Mutant mammalian cell lines lacking the first enzyme (GMD) in the pathway lose fucosylation, but this does not affect their survival. Adding fucose to the medium supplements the salvage pathway and corrects the glycosylation defect (Fig. 1). Applying the same rationale, Smith et al. (2002) knocked out the *de novo* pathway using homologous recombination to ablate the FX gene in mice, and then attempted to restore normal fucosylation with exogenous fucose.

Heterozygous matings generated an expected ratio of null embryos at 3.5 d postcoitum (dpc). Significant losses occurred by 12.5 dpc, but normal looking 18.5-dpc nulls contained fucosylated glycans similar to wild-type embryos. Fibroblasts derived from the nulls could not synthesize fucosylated glycans unless given a modest 22- μ M exogenous fucose. Clearly, the null embryos in utero obtained fucose from a maternal or fraternal source via lysosomal catabolism of glycoconjugates or

direct import of the monosaccharide. Both routes are possible since plasma membrane and lysosomal membrane fucose transporters exist (Jonas et al., 1990; Wiese et al., 1994). Unfortunately, little is known about the availability, transport, or utilization of exogenous monosaccharides in any mammalian system, not to mention the embryonic environment.

Live pups died before, or soon after, weaning. Undaunted, the research team shot-gunned the null allele into a series of other strains hoping to generate viable progeny. Fortunately, one approach produced two males that survived into adult life when reared on fucose. These prized survivors were mated with heterozygous females and produced a substantial proportion of null pups (~30%). Surprisingly, fucose supplements during gestation did not increase litter size or survival. Based on these crosses, they concluded that one or more, yet to be identified, genes accounted for this success. Recent and more selective breeding produced an even greater percentage of null progeny.

FX null mice were small at weaning, but grew normally when given fucose-supplemented water and chow. Without fucose, null mice had excessive diarrhea, and the histopathology of the colon resembled inflammatory bowel syndrome with crypt destruction, abscesses, and inflammatory cell infiltrates. Providing fucose halted diarrhea within 9 d, and withdrawing it provoked diarrhea again ~16 d later. The reasons for this pathology are unknown, and this symptom has not been reported in fucosylation-deficient CDG-IIc patients. It is interesting to note that the development of IBD in mouse models requires the participation of bacterial flora (Kosiewicz et al., 2001), and that the types of bacteria colonizing the intestine influence the glycosylation status of the intestinal epithelial cells (Hooper and Gordon, 2001). Some of the pathology may simply result from an imbalance of fucose metabolizing organisms in the gastrointestinal tract, since these organisms metabolize most of the fucose given to normal mice (Bocci and Winzler, 1969). Therefore, loss of fucose-catabolizing microbes might change the circulating levels of fucose in null animals. Unfortunately, the authors did not determine plasma fucose levels in the mice.

Selectin ligand synthesis in FX null mice requires exogenous fucose, and without it, nulls have a 25-fold increase in circulating leukocytes. Adding fucose dramatically decreases neutrophils within a few hours, and they are replaced by P-selectin-binding cells. Somewhat later, E-selectin binding also appears. The same dramatic disappearance of neutrophils and sequential appearance of P- and E-selectins is also seen in CDG-IIc patients given fucose (Marquardt et al., 1999). The preferential reexpression of P-selectin binding may occur because effective

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*Abbreviation used in this paper: CDG-IIc, congenital disorder of glycosylation II-c; dpc, days postcoitum; M/E, myeloid to erythroid.

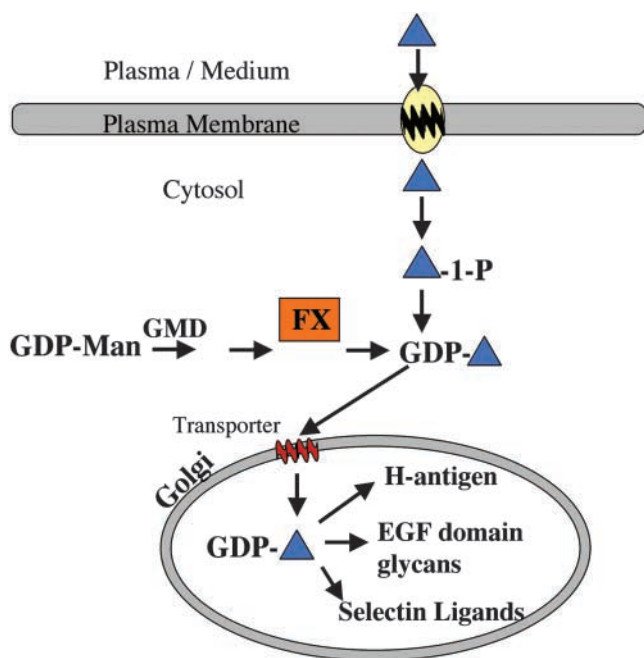


Figure 1. Fucose metabolism in mammalian cells. Fucose (blue triangle) is activated to GDP-Fucose in two ways. One is by conversion of GDP-Mannose via a two enzyme pathway (GMD and FX). Knocking out this pathway forces cells to rely on the other pathway where fucose enters the cell through a putative plasma membrane transporter, is converted to Fuc-1-P, and then to GDP-Fuc in the cytosol. A Golgi transporter delivers the donor to Golgi fucosyl transferases for synthesis of various glycoconjugates.

binding to the P-selectin counter receptor, PSGL-1, requires only a single fucosylated oligosaccharide.

Peripheral neutrophils drop within a few hours after giving fucose, primarily due to increased turnover of circulating cells that synthesize fucosylated counter receptors. In addition, and quite unexpectedly, fucosylated glycans also control neutrophil proliferation. The ratio of myeloid to erythroid (M/E) cells in the bone marrow is more than twofold higher than normal in FX null mice without fucose. Mature CFU-GM⁺ myeloid cells are also more than twofold higher. They normalize within 48 h on fucose, whereas erythroid precursors and other pluripotent progenitors are not altered. The M/E ratio of cells in the bone marrow takes another 48–96 h to normalize, suggesting that fucosylated glycans regulate granulocyte production. The mechanism is unknown, but this is another example of glycan-mediated cell signaling controlling cell proliferation and death (Lowe, 2001).

Withholding fucose again has the opposite effects: selectin ligands disappear, neutrophils increase, and bone marrow M/E ratio becomes abnormal again. The effects are more gradual than when adding fucose, since turnover provides a variable trickle of fucose for salvage.

The “fucose faucet” may provide a way to analyze when and where fucosylated glycans are critical for Notch signaling pathways involving lymphoid development, hematopoiesis, somitogenesis, and epithelial cell differentiation.

The modifier gene(s) permitting the survival of viable null males may give insight into the fucosylation pathways. Mice probably do not catabolize fucose, so improved fucose utilization through fucose kinase, GDP-fucose pyrophosphory-

lase, the Golgi GDP-fucose transporter, or putative plasma membrane fucose transporter (Wiese et al., 1994) may be important. The recent identification of 14 human facilitated diffusion monosaccharide transporter genes is far ahead of their functional analysis (Joost and Thorens, 2001). Most of the encoded proteins are assumed to transport glucose, but their conserved domain structure and divergent amino acid sequences give no clues about their monosaccharide specificity. Some mouse orthologues of the human transporters will probably carry fucose.

These studies show that, whereas glucose can generate all sugars for glycoconjugate synthesis, it is not the only source. A few CDG patients (Freeze, 2001) and FX mice show that dietary or salvaged sugars can make substantial contributions. Direct utilization of other sugars might be important in less acute situations. The controversial use of glucosamine to treat osteoarthritis (McAlindon et al., 2000) and N-acetylglucosamine for colitis (Salvatore et al., 2000) may supplement normally minor biosynthetic pathways in chronically stressed cells.

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