DEVELOPMENT

A Sweet Spot in the FGFR Signal Transduction Pathway

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The hexosamine biosynthetic pathway, whose end product is UDP-N acetylglucosamine (UDP-GlcNAc), lies at the base of cellular glycosylation pathways, including glycosylation of lipids, formation of heparin sulfated proteoglycans, and N- and O-linked glycosylation of proteins. Forward genetic studies in *Drosophila* have revealed that mutations in genes encoding different enzymes of the hexosamine biosynthetic pathway result in reduction of UDP-GlcNAc to different extents, with a consequent disruption of distinct glycosylation pathways and developmental processes. A maternal and zygotic loss-of-function screen has identified mutations in *nesthocker* (*nst*), which encodes an enzyme in the hexosamine biosynthetic pathway. Embryos lacking maternal and zygotic *nst* gene products show defective O-GlcNAcylation of a fibroblast growth factor receptor (FGFR)–specific adaptor protein, which impairs FGFR-dependent migration of mesodermal and tracheal cells.

Sugar molecules in several different forms play essential roles during animal development. Examples include simple polymers such as chitin and glycosaminoglycans such as heparin; modified lipids, such as glycosylphosphatidylinositol (GPI), which act as membrane anchors for glypiated proteins; and N- and O-linked sugar moieties in glycoproteins (*1*–*4*). The hexosamine biosynthetic pathway supplies the UDP-N acetylglucosamine (UDP-GlcNAc), which is the basic building block for all of these end products (Fig. 1). Mutations in genes encoding enzymes in the hexosamine biosynthetic pathway cause early embryonic death in mice (*5*) and lead to defects in organogenesis during *Drosophila* development. In fly embryos defective for either the synthesis or the modification of the GlcNAc polymer chitin, the morphogenesis of ectodermally derived tracheal tubes is defective (*6*–*13*). Fly embryos lacking heparin-sulfated proteoglycans show defects in Wingless and Hedgehog signaling pathways (*14*, *15*), as well as in the migration of the mesoderm and of the tracheal epithelium (*16*). Strikingly, each of these migration processes in the fly is regulated by a different fibroblast growth factor receptor (FGFR): Heartless, which is required for mesoderm migration, and Breathless, which is required for tracheal migration (*17*–*20*).

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The importance of sugar modifications in FGF signaling has long been appreciated. Indeed, the affinity of FGFs for the highly sulfated glycosaminoglycan heparin was a key to their efficient biochemical purification, and heparin potentiates and stabilizes the activity of FGFs (*21*). Heparin-sulfated proteoglycans also play a role in FGF signaling. They have been suggested to act as co-receptors for FGFs, and genetic evidence from *Drosophila* shows that mutations in *sulfateless* and *sugarless*, which are required for synthesis of heparin-sulfated proteoglycans, eliminate FGFR signaling and block migration of mesodermal and tracheal cells (*16*). It is to this rich literature that Mariappa and colleagues now add evidence of an additional requirement for UDP-GlcNAc downstream of the FGFRs, independent of the requirement for UDP-GlcNAc in the synthesis of heparin-sulfated proteoglycans. They discovered that mutations in *nesthocker* (*nst*) inactivate a hexosamine biosynthetic pathway enzyme, phosphoaceylglucosamine mutase (PGM3), and block mesodermal and tracheal development (*22*). Indeed, in *nst* mutants synthesis of heparin-sulfated proteoglycans appeared to be unimpaired at the time of tracheal and mesodermal migration, with no discernable Wingless or Hedgehog signaling defects.

The identification of a role for *nst* in FGFR signaling came from a genetic screen for mesoderm migration mutants in which maternal and zygotic expression of the candidate genes were eliminated (maternal and zygotic *nst* loss-of-function embryos are referred to as *nst* embryos hereafter) (*22*). This approach was used because previous loss-of-function studies had identified few genes whose zygotic expression is essential for early FGFR dependent processes, thus implying that the contribution of maternal mRNAs and proteins for many of the factors required in FGFR pathways is sufficient to drive mesodermal and tracheal migration (*23*). In contrast to *nst*, loss-of-function mutations in *mummy* (*mmy*; also known as *cystic* or *cabrio*)—which encodes UDP-GlcNAc pyrophosphorylase (UAP), the final enzyme in the hexosamine biosynthetic pathway—cause a zygotic defect in tracheal morphogenesis (but not in mesoderm development) (*6*, *7*, *24*). Perhaps more surprising, the *mmy* tracheal defects are quite different from those found in *sulfateless*, *sugarless*, or *nst* mutants. Indeed, the *mmy* phenotype more closely resembles that seen with mutations in *krotzkopf verkehrt* (*kkv*), the chitin synthase enzyme, suggesting that UDP-GlcNAc first becomes limiting for chitin synthesis in *mmy* zygotic mutants. These data posed two mysteries: (i) why loss-of-function mutations in different enzymes in the same core metabolic pathway would have different phenotypes, and (ii) what UDP-GlcNAc–requiring process is essential for FGFR signaling downstream of the receptors?

Mariappa and colleagues managed to get a handle on both of these questions (*22*). First, they determined that mutations in *mmy* and *nst* differentially affected the amount of UDP-N-acetylhexosamines (UDP-HexNAc; includes UDP-GlcNAc, UDP-GalNAc, and others) remaining in mutant embryos. In embryos lacking maternal and zygotic expression of *nst*, UDP-HexNAc amounts were reduced to ~20% of wild type, whereas embryos lacking zygotic *mmy* expression had ~50% of wild-type amounts at a similar developmental stage. The strong contribution of maternal *mmy* (*6*) probably accounts for the higher amount of UDP-HexNAc and for the ability of FGFR signaling to proceed unhindered, with later tracheal tube morphogenesis defects reflecting a requirement for large amounts of UDP-GlcNAc in the synthesis of chitin. [It should be noted that all chitin-secreting epithelia in the fly embryo, not just the tracheal system, are affected in mutants that lack chitin (*25*).] At ~20% of wild-type amounts, defects in FGFR-dependent cell migration occurred (and masked any later defects in tube morphogenesis).

Having established that synthesis and activity of heparin-sulfated proteoglycans

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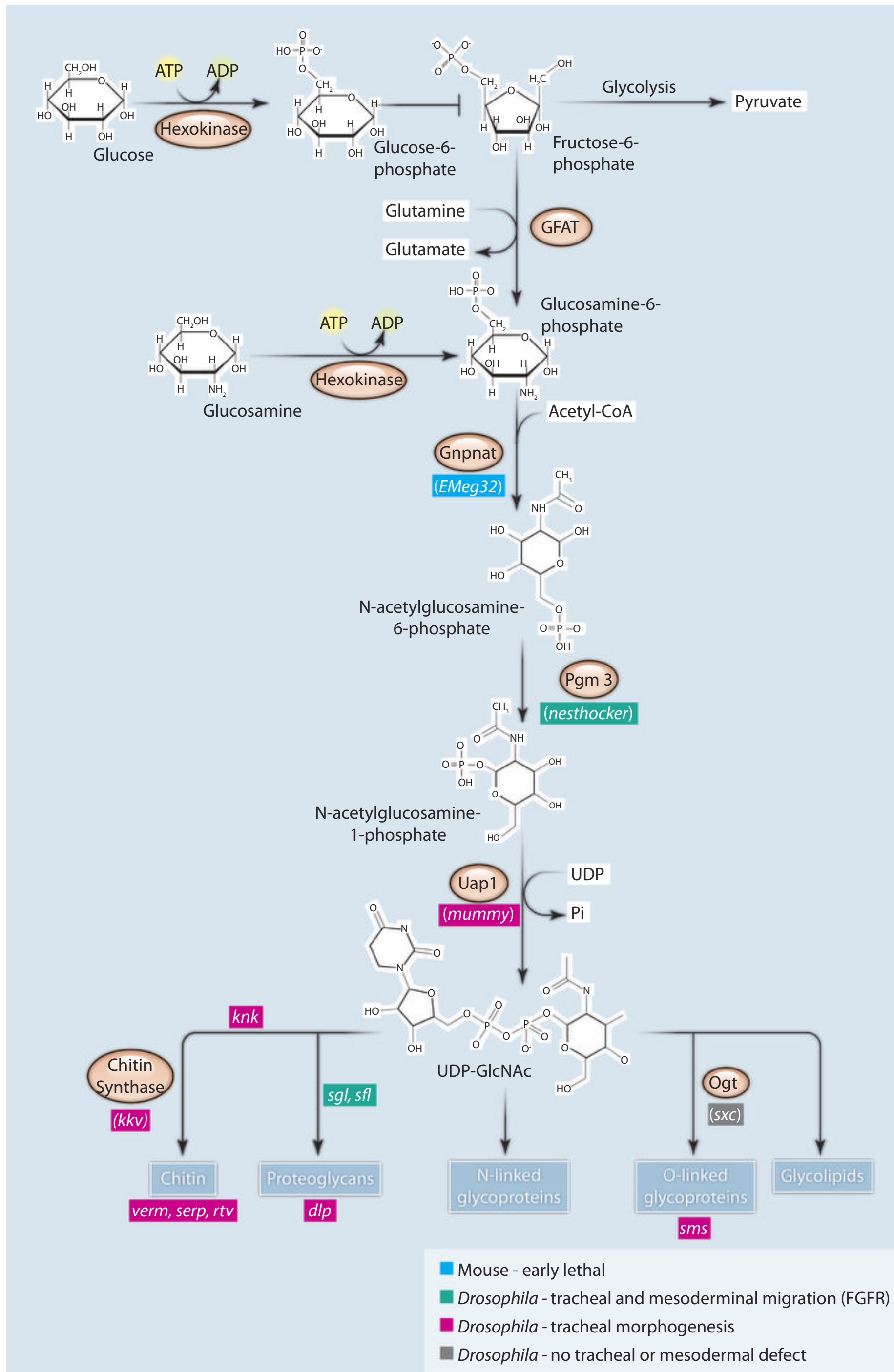

Fig. 1. The hexosamine biosynthetic pathway and its dependent processes. Glucose and acetyl–coenzyme A (acetyl-CoA) are consumed in the biosynthesis of UDP-GlcNAc, which is directly required for the formation of chitin and for the glycosylation of various proteins and lipids. Mutations in genes encoding hexosamine biosynthetic pathway enzymes and in some UDP-GlcNAc–requiring processes have been described in flies and in mice. The loss-of-function phenotypes are indicated by color: blue, early lethal; green, failure in FGFR signaling-dependent cell migrations; magenta, defective tracheal tubulogenesis; gray, no embryonic defect described, but later defect in homeotic gene expression. The phenotype of *nst* reported here is for the combined loss of maternal and zygotic *nst* gene products, whereas the phenotype of the other genes is for loss of zygotic gene products alone. ATP, adenosine 5´-triphosphate; ADP, adenosine 5´-diphosphate; GFAT, glutamine:fructose-6-phosphate amidotransferase.

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appeared normal in *nst* embryos, the authors sought to identify the glycosylation process that was affected in the mutant embryos (*22*). No changes were detected in GPI modification of proteins [as indicated by assessment of membrane association of GPI-linked green fluorescent protein (GFP) in *nst* embryos] or global protein glycosylation patterns (as indicated by lectins specific for N- or O-linked glycans). Immunohistochemistry for terminal O-GlcNAc moieties on proteins showed a reduced signal in *nst* embryos as compared with wild–type embryos, suggesting that O-GlcNAcylation was the UDP-GlcNAc–dependent modification that was inhibited in *nst* embryos, a result that was confirmed by Western blotting. Because the *nst* effect on FGFR signaling was independent of heparin-sulfated proteoglycans, the authors next sought to determine whether signaling was blocked upstream or downstream of FGFRs. In *nst* embryos, tissue-specific expression of wild-type Nst in FGFR-containing cells was sufficient to rescue the migration defect, indicating that O-GlcNAcylation of the FGFs in the signal-sending cells was not required for migration. They next tested whether activation of the FGFR Heartless, by using a constitutively active form of the receptor, could rescue mesoderm migration in *nst* embryos. Although the constitutively active form of Heartless rescued mesoderm migration in embryos deficient in Heartless, it could not rescue migration in *nst* embryos, suggesting that the defect in FGFR signaling lay downstream of the receptor. Moreover, Mariappa and colleagues found that a chimeric receptor in which the extracellular (and ligand-binding) domain of Heartless was combined with the intracellular kinase domain of the receptor tyrosine kinase Torso, strongly activated mitogen-activated protein kinase (MAPK) in *nst* embryos. Like Torso, the FGFRs are receptor tyrosine kinases, and signal through the canonical receptor tyrosine kinase signaling pathway, diverging from other receptor tyrosine kinases in only a single respect: use of an essential adaptor protein officially named Stumps (Sms), but which is usually called Downstream of FGFR (Dof) and is also known as Heartbroken (Hbk) (*26–28*). Previous studies with chimeric receptors have shown that Sms is dispensable if the kinase domain of another receptor tyrosine kinase is substituted for that of FGFR (*29*). This experiment, then, strongly suggests that Sms is the FGFR pathway component that requires O-GlcNAcylation.

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To confirm the requirement for O-GlcNAcylation in FGF signaling, the authors asked whether inhibition of O-GlcNAcase (OGA, which removes O-GlcNAc modifications) by injection of a chemical inhibitor, or transgene-based overexpression of O-GlcNAc transferase (OGT, which catalyzes O-linked GlcNAcylation) could suppress the *nst* mutant phenotype (*22*). Both treatments substantially ameliorated the *nst* developmental defects. Mutations in OGT [*super sex combs* (*sxc*)] were also examined but did not exhibit migration defects in homzygous animals, probably because of a large maternal contribution of *sxc* gene products.

It remained to be determined whether Sms was itself O-GlcNAcylated or whether the requirement was indirect, as would be the case if a Sms-regulating protein required modification. Because endogenous amounts of Sms are low, Mariappa and colleagues used succinylated wheat germ agglutinin (sWGA) to detect O-linked glycosylation of Sms in cultured *Drosophila* S2 cells. Whereas WGA recognizes sialic acid as well as GlcNAc, sWGA is considered specific for O-GlcNAc (*30*). Knockdown of OGT reduced the amount of Sms precipitated by sWGA, whereas overexpression of OGT or chemical inhibition of OGA increased the amount of sWGA-bound Sms. Thus, O-GlcNAcylation of Sms appears to be essential for FGFR signal transduction. In the future, it will be fascinating to learn how modification of Sms alters its biochemistry, and whether such modification is constitutive or subject to physiological or developmental regulation.

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