Published in final edited form as:

Sci Signal. ; 5(207): pe1. doi:10.1126/scisignal.2002789.

A Sweet Spot in the FGFR Signal Transduction Pathway

Amin S. Ghabrial*

Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania

Abstract

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 the fly 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 like heparin; modified lipids, such as glycosylphosphatidylinositol, 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-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 FGFR: Heartless, which is required for mesoderm migration, and Breathless, which is required for tracheal migration (17-20).

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 HSPG synthesis, 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. They discovered that mutations in *nesthocker* inactivate a hexosamine biosynthetic pathway enzyme, phosphoacetylglucosamine mutase

*Corresponding author. ghabrial@mail.med.upenn.edu.

(PGM3), and block mesodermal and tracheal development by a mechanism that is independent of the requirement for UDP-GlcNAc in the synthesis of heparin-sulfated proteoglycans. 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 gene expression of the candidate genes were eliminated (maternal and zygotic *nst* loss of function embryos are referred to as *nst* embryos hereafter). This approach was used because previous zygotic 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 (22). In contrast to *nst*, loss of function mutations in *mummy* (*mmv*, 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, 23). Perhaps more surprising, the *mmv* tracheal defects are quite different from those found in *sulfateless*, *sugarless*, or *nst* mutants. Indeed, the *mmv* 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 *mmv* 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. First, they determined that mutations in *mmv* 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 *mmv* expression had ~50% of wild-type amounts at a similar developmental stage. The strong contribution of maternal *mmv* (6) likely 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 *mmv* mutants (24).] 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 appeared normal in *nst* embryos, the authors sought to identify the glycosylation process that was affected in *nst* mutant embryos. No changes were detected in GPI modification of proteins (as indicated by assessment of membrane association of GPI-linked 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 to 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, 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) (25-27). 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 (28). This experiment, then, strongly suggests that Sms is the FGFR pathway component that requires O-GlcNAcylation.

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. 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 homozygous animals, likely due to a large maternal contribution of *sxc* gene products.

It remained to be determined whether Sms was itself O-GlcNAcylated, or if 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 (29). 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.

References

1. Dennis JW, Lau KS, Demetriou M, Nabi IR. Adaptive regulation at the cell surface by N-glycosylation. *Traffic*. 2009; 10:1569–1578. [PubMed: 19761541]
2. Eisenhaber B, Maurer-Stroh S, Novatchkova M, Schneider G, Eisenhaber F. Enzymes and auxiliary factors for GPI lipid anchor biosynthesis and post-translational transfer to proteins. *Bioessays*. 2003; 25:367–385. [PubMed: 12655644]
3. Merzendorfer H. The cellular basis of chitin synthesis in fungi and insects: common principles and differences. *Eur J Cell Biol*. 2011; 90:759–769. [PubMed: 21700357]
4. Zachara NE, Hart GW. Cell signaling, the essential role of O-GlcNAc! *Biochim Biophys Acta*. 2006; 1761:599–617. [PubMed: 16781888]
5. Boehmelt G, Wakeham A, Elia A, Sasaki T, Plyte S, Potter J, Yang Y, Tsang E, Ruland J, Iscove NN, Dennis JW, Mak TW. Decreased UDP-GlcNAc levels abrogate proliferation control in EMeg32-deficient cells. *EMBO J*. 2000; 19:5092–5104. [PubMed: 11013212]

6. Araujo SJ, Aslam H, Tear G, Casanova J. mummy/cystic encodes an enzyme required for chitin and glycan synthesis, involved in trachea, embryonic cuticle and CNS development--analysis of its role in *Drosophila* tracheal morphogenesis. *Dev Biol.* 2005; 288:179–193. [PubMed: 16277981]
7. Devine WP, Lubarsky B, Shaw K, Luschnig S, Messina L, Krasnow MA. Requirement for chitin biosynthesis in epithelial tube morphogenesis. *Proc Natl Acad Sci U S A.* 2005; 102:17014–17019. [PubMed: 16287975]
8. Luschnig S, Batz T, Armbruster K, Krasnow MA. serpentine and vermiform encode matrix proteins with chitin binding and deacetylation domains that limit tracheal tube length in *Drosophila*. *Curr Biol.* 2006; 16:186–194. [PubMed: 16431371]
9. Moussian B, Soding J, Schwarz H, Nusslein-Volhard C. Retroactive, a membrane-anchored extracellular protein related to vertebrate snake neurotoxin-like proteins, is required for cuticle organization in the larva of *Drosophila melanogaster*. *Dev Dyn.* 2005; 233:1056–1063. [PubMed: 15844167]
10. Moussian B, Tang E, Tonning A, Helms S, Schwarz H, Nusslein-Volhard C, Uv AE. *Drosophila* Knickkopf and Retroactive are needed for epithelial tube growth and cuticle differentiation through their specific requirement for chitin filament organization. *Development.* 2006; 133:163–171. [PubMed: 16339194]
11. Tonning A, Helms S, Schwarz H, Uv AE, Moussian B. Hormonal regulation of mummy is needed for apical extracellular matrix formation and epithelial morphogenesis in *Drosophila*. *Development.* 2006; 133:331–341. [PubMed: 16368930]
12. Tonning A, Hemphala J, Tang E, Nannmark U, Samakovlis C, Uv A. A transient luminal chitinous matrix is required to model epithelial tube diameter in the *Drosophila* trachea. *Dev Cell.* 2005; 9:423–430. [PubMed: 16139230]
13. Wang S, Jayaram SA, Hemphala J, Senti KA, Tsarouhas V, Jin H, Samakovlis C. Septate-junction-dependent luminal deposition of chitin deacetylases restricts tube elongation in the *Drosophila* trachea. *Curr Biol.* 2006; 16:180–185. [PubMed: 16431370]
14. The I, Bellaiche Y, Perrimon N. Hedgehog movement is regulated through tout velu-dependent synthesis of a heparan sulfate proteoglycan. *Mol Cell.* 1999; 4:633–639. [PubMed: 10549295]
15. Tsuda M, Kamimura K, Nakato H, Archer M, Staatz W, Fox B, Humphrey M, Olson S, Futch T, Kaluza V, Siegfried E, Stam L, Selleck SB. The cell-surface proteoglycan Dally regulates Wingless signalling in *Drosophila*. *Nature.* 1999; 400:276–280. [PubMed: 10421371]
16. Lin X, Buff EM, Perrimon N, Michelson AM. Heparan sulfate proteoglycans are essential for FGF receptor signaling during *Drosophila* embryonic development. *Development.* 1999; 126:3715–3723. [PubMed: 10433902]
17. Shishido E, Ono N, Kojima T, Saigo K. Requirements of DFR1/Heartless, a mesoderm-specific *Drosophila* FGF-receptor, for the formation of heart, visceral and somatic muscles, and ensheathing of longitudinal axon tracts in CNS. *Development.* 1997; 124:2119–2128. [PubMed: 9187139]
18. Gisselbrecht S, Skeath JB, Doe CQ, Michelson AM. heartless encodes a fibroblast growth factor receptor (DFR1/DFGF-R2) involved in the directional migration of early mesodermal cells in the *Drosophila* embryo. *Genes Dev.* 1996; 10:3003–3017. [PubMed: 8957001]
19. Beiman M, Shilo BZ, Volk T. Heartless, a *Drosophila* FGF receptor homolog, is essential for cell migration and establishment of several mesodermal lineages. *Genes Dev.* 1996; 10:2993–3002. [PubMed: 8957000]
20. Klambt C, Glazer L, Shilo BZ. breathless, a *Drosophila* FGF receptor homolog, is essential for migration of tracheal and specific midline glial cells. *Genes Dev.* 1992; 6:1668–1678. [PubMed: 1325393]
21. Klagsbrun M. The affinity of fibroblast growth factors (FGFs) for heparin; FGF-heparan sulfate interactions in cells and extracellular matrix. *Curr Opin Cell Biol.* 1990; 2:857–863. [PubMed: 2083085]
22. Gryzik T, Muller HA. FGF8-like1 and FGF8-like2 encode putative ligands of the FGF receptor Htl and are required for mesoderm migration in the *Drosophila* gastrula. *Curr Biol.* 2004; 14:659–667. [PubMed: 15084280]

23. Schimmelpfeng K, Strunk M, Stork T, Klambt C. Mummy encodes an UDP-N-acetylglucosamine-diphosphorylase and is required during *Drosophila* dorsal closure and nervous system development. *Mech Dev.* 2006; 123:487–499. [PubMed: 16793242]
24. Ostrowski S, Dierick HA, Bejsovec A. Genetic control of cuticle formation during embryonic development of *Drosophila melanogaster*. *Genetics.* 2002; 161:171–182. [PubMed: 12019232]
25. Vincent S, Wilson R, Coelho C, Affolter M, Leptin M. The *Drosophila* protein Dof is specifically required for FGF signaling. *Mol Cell.* 1998; 2:515–525. [PubMed: 9809073]
26. Michelson AM, Gisselbrecht S, Buff E, Skeath JB. Heartbroken is a specific downstream mediator of FGF receptor signalling in *Drosophila*. *Development.* 1998; 125:4379–4389. [PubMed: 9778498]
27. Imam F, Sutherland D, Huang W, Krasnow MA. stumps, a *Drosophila* gene required for fibroblast growth factor (FGF)-directed migrations of tracheal and mesodermal cells. *Genetics.* 1999; 152:307–318. [PubMed: 10224263]
28. Dossbach C, Rock S, Affolter M. Specificity of FGF signaling in cell migration in *Drosophila*. *Development.* 2001; 128:4563–4572. [PubMed: 11714681]
29. Zachara NE, Hart GW, Cole RN, Gao Y. Detection and analysis of proteins modified by O-linked N-acetylglucosamine. *Curr Protoc Mol Biol.* 2002; Chapter 17(Unit 17):16.

Fig. 1.

The hexosamine biosynthetic pathway and its dependent processes. Glucose and 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; red, 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, while the phenotype of the other genes is for loss of of zygotic gene products alone. GFAT, glutamine:fructose-6-phosphate amidotransferase.