Published in final edited form as:

Crit Rev Oncol Hematol. 2006 August ; 59(2): 85–97. doi:10.1016/j.critrevonc.2006.02.002.

The growth factor independence-1 transcription factor: New functions and new insights[★]

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Abstract

The growth factor independence-1 (Gfi1) transcription factor is required for proper development of neuroendocrine cells, sensory neurons, and blood. Patients with mutations in Gfi1 exhibit severe congenital neutropenia (SCN) or non-immune chronic idiopathic neutropenia of adults. Gfi1 was initially described as an oncoprotein that mediates tumor progression in a mouse model of leukemia; however, recent data suggest that Gfi1 may act as either an oncogene or an anti-proliferative tumor suppressor gene depending on the cell type. Here we review the latest literature on Gfi1, and emphasize its role in the hematopoietic, sensory and neuroendocrine systems.

Keywords

Growth factor independence-1; Notch; Ash1; T lymphopoiesis; Hematopoiesis; Peripheral nervous system; Pulmonary neuroendocrine cells; Oncogene; Tumor suppressor

1. Clinical relevance

Growth factor independence-1 (Gfi1) is a transcriptional repressor protein that plays important biological roles in hematopoietic and neuronal cell development [1–4]. Specifically, mice deleted for Gfi1 not only lack neutrophils [2,3], but have defective hematopoietic stem cell maintenance [5,6], and developmental deficits in sensory neurons [7,8] and neuroendocrine cells [9]. Gfi1 mutations in humans were recently shown to correlate to severe congenital neutropenia (SCN) and non-immune chronic idiopathic neutropenia of adults (NI-CINA) [10]. Interestingly, Gfi1 is also expressed and is oncogenic in human small cell lung cancer (SCLC), the deadliest neuroendocrine tumor [9]. Thus, understanding the biological and biochemical activities of Gfi1 may provide a unique opportunity to understand these human diseases.

[★]This work was partially supported by NIH grants CA112405, CA105152 and HL079574, and by a Leukemia and Lymphoma Society Scholar Award.

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2. Gfi1 mediates tumor progression as a frequent oncogenic target of the Moloney murine leukemia virus

The Moloney murine leukemia virus (MoMLV) mediates oncogenic function through insertion mutagenesis of cellular proto-oncogenes and tumor suppressors. MoMLV infection leads to aggressive leukemias which accumulate proviral insertions and progress from interleukin-2 (IL2)-dependent to IL2-independent growth [11]. Growth factor independence-1 (*Gfi1*) was first identified as the gene activated by a MoMLV proviral insertion site (*gfi-1*) in a screen for MoMLV-target genes capable of inducing progression of rat T cell leukemias to IL2-independent growth [12]. Activating MoMLV insertions in the *gfi-1* locus mapped to the promoter of the affected *Gfi1* gene, and result in long terminal repeat (LTR)-driven overexpression of an unaltered *Gfi1* open reading frame [12]. *Gfi1* overexpression does not induce IL2 expression. Instead, IL2-dependent cell lines transduced with *Gfi1*-expressing retroviral vectors either frequently gave rise to IL2-independent cell lines [12], or showed an impaired ability to maintain cell cycle checkpoints upon IL2 withdrawal [13,14]. Thus, in the context of the in vitro screen, *Gfi1* overexpression is the effector of MoMLV insertion at this locus.

Naturally occurring IL2-independent MoMLV tumors also display insertions at *gfi-1* [12]. In fact, murine retroviruses that cause T and B cell leukemias upregulate *Gfi1* by occupying multiple common proviral insertion loci in rodent genomes [12,15–17]. Leukemias in mice transgenic for *Myc* or *Pim* oncoprotein overexpression and infected with MoMLV display frequent MoMLV proviral integrations in the *pal-1* common integration locus [15,18], the extra integration site-1 (*eis-1*) locus, and the T cell Moloney integration site (*tmi-1*) locus [15]. The common mink-cell focus-forming (MCF) virus insertion locus, *evi-5*, in AKXD mouse T cell leukemias, maps 18 kilobases upstream of *Gfi1* [19]. Notably, *tmi-1* and *evi-5* loci are different names for the same locus [16], and the gene disrupted by MoMLV insertion encodes a ubiquitously expressed gene (*Evi5*) which is homologous to the *Tre 2* oncogene. However, it is not known if *Evi5* is itself involved in leukemogenesis or if proviral insertions within the *Evi5* locus simply serve to enhance *Gfi1* expression [17]. Each of the MoMLV insertion loci *gfi-1*, *eis-1*, *evi-5/tmi-1*, and *pal-1* are distinct chromosomal locations surrounding the *Gfi1* gene, and proviral occupancy in any of these loci results in the induction of *Gfi1* overexpression in leukemias [14,15]. A majority of MoMLV-induced T cell leukemias have integrations in one of the *gfi-1*, *eis-1*, *evi-5/tmi-1*, and *pal-1* loci (mouse retroviral tagged cancer gene database, <http://rtcgd.ncifcrf.gov/> [20]). Interestingly, retroviral insertions in these loci are also associated with MoMLV-induced marginal zone B cell lymphomas [21], and murine acquired immunodeficiency syndrome (MAIDS) virus-induced lymphomas [17]. In sharp contrast, proviral occupancy of these loci is extremely rare in myeloid tumors [20]. Thus, the oncogenic function of *Gfi1* overexpression may be restricted to lymphocytes.

Gfi1 is a tumor progression oncoprotein. MoMLV insertion mediated overexpression of *Gfi1* suggests that simply deregulating expression of *Gfi1* is oncogenic; however, overexpression of *Gfi1* alone is not a potent initiating oncogenic event. Mice transgenic for *Gfi1* expression do not show an unusual incidence of leukemia [22,23]. Moreover, while chromosomal irregularities in a variety of human cancers map to 1p22, the location of *Gfi1* [24,25], no mutations that directly affect *Gfi1* have been identified. To confirm a role for *Gfi1* in MoMLV leukemogenesis, mice transgenic for *Gfi1* overexpression were infected with MoMLV. While Moloney virus infection of wild type mice leads to leukemia in about 6 months, *Gfi1*-transgenic mice were leukemic in one-third the time [14]. Thus, while *Gfi1* overexpression is a significant mechanism utilized during MoMLV-induced leukemogenesis, *Gfi1* oncogenic function is only unleashed upon transformation that is initiated by other oncogenes. These data are in agreement with the identification of *Gfi1* as a tumor progression oncoprotein.

Clues to the oncogenic action of Gfi1 lie in the pattern of insertion sites within MoMLV leukemias. At least three groups of MoMLV-target genes can be specified from the common proviral insertion sites in transgenic mice: the Pim family of serine-threonine kinases, the Myc family transcription factors, and a group consisting of *Bmi1*, *Gfi1*, and *Gfi1b* [26]. Gfi1 and Gfi1b are encoded by different genes, but are both transcription factors with nearly identical transcription-repression domains and zinc-finger DNA-binding motifs [27] (Fig. 1). However, insertions that activate *Gfi1b* expression are exceedingly rare [27]. It is currently unclear whether Gfi1b is a poor oncogene. Given that Gfi1b transgenic mice show a mild acceleration of naturally occurring lymphomagenesis [22], it is more likely that local chromatin and/or potentially tumor suppressive genes surrounding *Gfi1b* make it an infrequent MoMLV target. In contrast to either Gfi1 or Gfi1b, overexpression of *Bmi1* alone induces leukemia in mice [28]. Unlike members of the Myc or Pim oncogenic groups, *Bmi1* and *Gfi1/Gfi1b* are not structurally related. Bmi1 (B-cell-specific Moloney murine leukemia virus insertion site-1) is a nuclear protein with an amino terminal Cys/His metal binding RING-zinc-finger motif and a helix-turn-helix-turn-helix-turn DNA binding motif. Bmi1 is a member of the polycomb family of negative transcriptional regulators of both homeotic genes [29–31], and the p16^{ink4a}/p19^{arf} tumor suppressor locus [32]. Proviral insertions in the *gfi-1*, *eis-1*, *evi-5*, *tmi-1*, and *pal-1* loci show very little overlap with integrations activating Bmi1 overexpression, suggesting that the activation of the *Bmi1* and *Gfi1* genes are mutually exclusive. Thus, Bmi1 and Gfi1 may act on identical or complementary targets to perform the same cellular function [15].

Both Gfi1 and Bmi1 cooperate with Myc oncogenesis [16,33]. Overexpression of the Myc proteins induces cell cycle progression and apoptosis [34]. Activation of Myc overexpression is a central mechanism of MoMLV leukemogenesis [20]. Notably, both Gfi1 and Bmi1 can induce both cell cycle progression and cell survival [33,35,36], which may explain why they cooperate with Myc-induced oncogenesis. While Bmi1 cooperation with Myc may rely on transcriptional repression of the p16^{ink4a}/p19^{arf} tumor suppressor locus [33], there is no evidence for Gfi1 regulation of these genes.

3. Gfi1 is a transcriptional repressor

Gfi1 is a DNA-binding protein with six C₂H₂-type, C-terminal zinc-finger motifs that bind the DNA sequence TAAATCAC(A/T)GCA [12,37]. Position- and orientation-independent active transcriptional repression activity by Gfi1 depends on a 20 amino acid N-terminal repressor domain that is coincident with a nuclear localization motif [13] (Fig. 1). The amino acid sequence of this domain is nearly identical in Gfi1B, and similar in the insulinoma-associated protein IA-1, the homeobox protein Gsh-1, the Ovo-like proteins, and all the vertebrate members of the Snail/Slug family of mesoderm-determining transcription factors (*Snail* + Gfi1 = SNAG domain) [13] (Table 1). Deletion or mutation of the first seven amino acids in the Gfi1 or Gfi1B SNAG domain destroys the repression activity of either protein [13,27]. Notably, mutation of the proline at position 2 to alanine (P2A) in Gfi1 significantly impaired repression function without disturbing nuclear localization [13]. In contrast to wild-type Gfi1, the P2A mutant fails to mediate IL-2 independent growth of MoMLV leukemias [13]. Similarly, the transcriptional repressor activity of the SNAG domain in Snail has been confirmed [38]. While Snail represses the promoter of the *E-cadherin* gene, deletion of the first seven amino acids of Snail, or a P2A-mutant Snail, failed to affect *E-cadherin* transcription [38]. Thus, for both Gfi1 and Snail, SNAG transcription repression function and oncogenic biology appear to be linked. Moreover, the Gfi1 SNAG domain is the prototype of a novel family of evolutionarily conserved repressor domains that operate in multiple cell lineages and are critical mediators of oncogenic function in both lymphoid and solid tumors (Table 1).

The SNAG domain mediates repression function, but is constrained by positional effects. While structure-function analyses of Gfi1, Gfi1b and Snail confirm SNAG activity [13,27,38], fusion proteins containing the SNAG domain of either the Scratch or IA-1 transcription factors did not validate SNAG domain activity [39,40]. A fusion protein consisting of the Gfi1 SNAG domain, the DNA binding domain of the PAX3 transcription factor and the hormone-binding domain of the estrogen receptor (SNAG/PAX3/ER) displayed hormone-inducible transcriptional repression of PAX3 target genes, as well as inhibition of growth and survival of PAX3-FKHR oncoprotein-expressing rhabdomyosarcomas [41]. Similarly, fusion of the Gfi1 amino terminus to the amino terminus of the LexA DNA-binding domain transformed LexA into a transcriptional repressor [13]. However, fusion of the same Gfi1 amino terminus to the carboxy terminus of LexA did not yield a repressor protein (Grimes, unpublished data). Fusion of immuno-epitope-tags to the amino-terminal SNAG domain of Gfi1 also significantly impairs repressor function (Grimes, unpublished data). Given the sensitivity of the proline at position 2 in the SNAG domain, it may not be surprising that the SNAG domain often fails to function in contexts other than the amino terminus. Since the Scratch and IA-1 SNAG domains were fused to the carboxy-terminus of a DNA binding protein [39,40], the function of these SNAG domains remains to be determined.

Gfi1 mediates reversible transcriptional repression through recruitment of the eight 21 corepressor (ETO), histone deacetylase (HDAC) enzymes and the G9a histone lysine methyltransferase. The SNAG/PAX3/ER fusion protein described above represses transcription of PAX3 target genes in a hormone-inducible manner, but these genes were quickly reactivated upon withdrawal of the inducing hormone [41]. SNAG-mediated repression is therefore, mediated by reversible chromatin changes, such as local histone deacetylation, and is unlikely to induce long-term silencing of target genes. The ETO family of proteins bind to the zinc fingers of Gfi1 and Gfi1b, and a subset of both ETO and Gfi1 protein are attached to the nuclear matrix [42]. Both ETO and Gfi1 coimmunoprecipitate with HDAC1, HDAC2 and HDAC3 [43,42], and Gfi1 transcriptional repression is significantly impaired by the HDAC inhibitor Trichostatin A [42]. It is possible that the SNAG domain tethers a transcriptional corepressor that mediates the chromatin changes that precede HDAC recruitment, or stabilizes the ETO/HDAC interactions. Indeed, recent data indicate that Gfi1 interacts with the histone lysine methyltransferase G9a and recruits G9a and HDAC1 to its target genes in order to repress transcription through histone H3-K9 dimethylation. The methylation of H3-K9 accumulates after Gfi1 binding and may be required for recruitment of HDAC1 [102]. The relationship between ETO, HDACs and G9a in Gfi1-mediated development and oncogenesis remains to be determined.

4. Gfi1 regulates hematopoietic stem cell (HSC) proliferation

In strict pathogen-free animal colonies, the inability of *Gfi1*^{-/-} HSC to sustain long term hematopoiesis appears to be the dominant defect resulting in a short life span. *Gfi1*^{-/-} mice show a dramatic decrease in Kit⁺Lin⁻Sca⁺ (KLS)Flt3⁺ HSC [5], and die within a few months after birth due to bone marrow failure [5,6]. Transplantation of large numbers of *Gfi1*^{-/-} bone marrow or purified KLS HSC is capable of reconstitution of irradiated mice and sustained hematopoiesis for over 3 months. The same *Gfi1*^{-/-} bone marrow cells are apparently impaired in competitive engraftment, even when excess numbers of *Gfi1*^{-/-} bone marrow cells are used in relation to wild type marrow [5,6]. The failure of *Gfi1*^{-/-} HSC is due to intrinsic HSC defects and not due to an altered microenvironment or homing ability of transplanted cells. *Gfi1*^{-/-} HSC can home properly after transplantation as indicated by the short term reconstitution ability [5,6]. Since wild type bone marrow efficiently reconstitutes lethally irradiated *Gfi1*^{-/-} mice, a defect in microenvironment due to Gfi1 absence can be excluded [6]. Moreover, chimeras generated with *Gfi1*^{-/-} embryonic cells (ES) and wild type blastocysts demonstrated that the progeny of the *Gfi1*^{-/-} ES cells are initially found throughout the developing

hematopoietic system [5]; however, 2-month-old chimeras specifically lack bone marrow cells from *Gfi1*^{-/-} ES cells. These data indicate that an intrinsic defect in early stem cell function results in a failure to sustain hematopoiesis in the chimeric animals [5].

Gfi1 is an inhibitor of HSC proliferation. HSC must balance self renewal with the production of proliferating daughter cells. Analysis of gene expression profiles of HSC as they proliferate and return to quiescence in vivo revealed that *Gfi1* and *Gfi1b* steady state mRNA levels are depressed during proliferation and are dramatically induced when the cells return to quiescence [44]. While loss of *Gfi1b* is embryonic lethal due to a failure in erythropoiesis [45], *Gfi1b*^{-/-} fetal liver HSC competitively repopulate and serial transplant [5]. Thus, the defect in *Gfi1*^{-/-} HSC is unique to Gfi1. Cell cycle analysis of *Gfi1*^{-/-} HSC showed marked differences when compared to wild type HSC. Significantly more *Gfi1*^{-/-} HSC actively proliferate in comparison to wild type HSC [5,6]. The excessive proliferation of *Gfi1*^{-/-} HSC most likely exhausts the rare HSC pool and thus compromises their hematopoietic function. In sharp contrast to the stem/progenitor cells, the thymocytes of the *Gfi1*^{-/-} mouse show a mild decrease in cell cycle progression [5]. While the latter result is consistent with the oncogenic activity of Gfi1 in T cells, together these findings establish contrasting roles for Gfi1 in proliferation depending on the cell type and the developmental stage.

Several cell cycle genes have been suggested as Gfi1 targets including E2F5, E2F6 and p21^{waf1} [27,46]. Analysis of these genes in the KLS FLT3⁻ *Gfi1*^{-/-} HSC showed transcriptional down regulation of the cell cycle inhibitor p21^{waf1} in one case [5]. However, analysis of total KLS *Gfi1*^{-/-} HSC did not also show transcriptional effects on p21^{waf1}. Instead, the level of p21^{waf1} protein was significantly lower in *Gfi1*^{-/-} bone marrow lysates [6]. The difference in the steady state levels of p21^{waf1} mRNA might be explained by a lack of KLS FLT3⁺ cells in the knockout animals, which would skew the Lin⁻ populations. Interestingly, p21^{waf1} is a target gene for repression by Gfi1b [27]. Gfi1 regulates the transcription of Gfi1b [47] and vice versa [48,49] (elaborated below). In agreement with this, *Gfi1b* steady state mRNA levels were increased two-fold in *Gfi1*^{-/-} marrow [5]. Therefore, it is possible that the defect in *Gfi1*^{-/-} HSC is partly through induction of Gfi1b, which represses p21^{waf1}. Since p21^{waf1} has been shown to regulate HSC self renewal [50], the deregulated expression of p21^{waf1} may partially explain the increased proliferative ability of the *Gfi1*^{-/-} HSC [5,6].

5. Gfi1 controls lymphopoiesis

Gfi1 plays a unique role in T cell activation. In normal activated primary T lymphocytes, Gfi1 expression is induced considerably after that of IL2 [12]; suggesting a role for Gfi1 in transducing IL2-receptor signaling. Intriguingly, putative Gfi1 binding sites are found in the promoters of several genes encoding cytokine or cytokine receptors implicated in T cell activation [37]. The signal transducer and activator of transcription-3 (STAT3) transcription factor conveys signals from the IL-6 receptor to stimulate T cell proliferation upon antigenic stimulation [51,52]. Protein inhibitor of activated STAT3 (PIAS3) directly binds activated STAT3 and blocks STAT3-induced transcriptional activation [53]. Forced expression of Gfi1 augments IL-6-dependent T cell proliferation, perhaps through binding and sequestering PIAS3 [54]. However, no differences in STAT3 dependent electrophoretic mobility shift could be detected between control and Gfi1-overexpressing nuclear extracts [54]. Constitutively activated STAT3 is an oncoprotein [55]. Consequently, a portion of the oncogenic effect of Gfi1 may result from its role as a positive regulator of signal transduction through STAT3 [54].

Independent of IL-6, transgenic overexpression of Gfi1 in T cells increased antigen-induced T cell entry into S phase [22,54]. High level Gfi1 expression in Jurkat T cell lines induced an increase in the proliferation rate and a reduction in levels of p21^{waf1}, p27^{Kip1} and Rb1 compared

to control cell lines. Enforced Gfi1 expression reduced activation-induced apoptosis and phorbol ester-generated G₁ arrest in Jurkat T cells [36]. Thus, Gfi1 may control survival and cell cycle checkpoints during T cell proliferation.

In activated naïve T cells, Gfi1 expression is increased by IL-4 in a STAT6-dependent process. IL-4 drives the differentiation and expansion of activated cells to a T-helper type 2 (Th2) phenotype. Retroviral vector mediated expression of Gfi1 in activated CD4⁺ cells resulted in preferential expansion of Th2 cells through enhancement of proliferation and reduction of apoptosis [56]. Similar to oncogenic studies [13], expression of a P2A SNAG-domain-mutant Gfi1 had no effect [56] indicating that the biological effects of Gfi1 in this context is SNAG domain dependent. Gfi1 is not induced in quiescent CD4⁺ cells by IL-4, and Gfi1 alone cannot drive Th2 differentiation. Another IL4/STAT6 target, the Gata3 transcription factor, controls Th2 differentiation. Together, GATA3 and Gfi1 dramatically synergize to expand and polarize activated CD4⁺ cells [56].

Gfi1 is required for early T cell development. Transgenic mice constitutively expressing Gfi1 or Pim1 in T cells reveal the involvement of both proteins in T lymphopoiesis. CD4⁻CD8⁻ (double negative; DN) thymocytes that productively rearrange the β -chain of the T cell receptor are selected for expansion and differentiation to CD4⁺CD8⁺ (double positive; DP) cells. Forced overexpression of Gfi1 interferes with the selection of β -chain-expressing cells, and therefore, blocks pre-T cell development. In contrast, Pim1 promotes β selection. Moreover, forced Pim1 expression can rescue the Gfi1-induced block in Gfi1/Pim1 bitransgenic mice [57]. Notably, both proteins also collaborate in MoMLV transformation of T cells. These data indicate that there may be a desirable stoichiometry between Pim and Gfi1 in order to facilitate normal T cell development.

Like Gfi1 transgenics, Gfi1 deficient mice exhibit diminished thymic cellularity. *Gfi1*^{-/-} defects result in blocked differentiation and proliferation as well as increased cell death of early thymic DN cells [4]. Within the double negative thymocyte pool, the transition between DN1 to DN2 marks a significant commitment to the T cell lineage. Notably, the level of *Gfi1* mRNA increases by a log from DN1 to DN2 [22]. Thus, *Gfi1*^{-/-} thymocytes may have difficulty committing to the earliest stages of the T lymphopoiesis program. However, some cells escape this bottleneck and differentiate. In fact, *Gfi1*^{-/-} DP thymocytes appear skewed towards development of CD8⁺ cells [4]. An increase in CD8⁺ cells also points to a critical role for Gfi1 in the CD4/CD8 lineage decision. Indeed, in mice which report the transcriptional activity of the *Gfi1* promoter via knockin of the green fluorescent protein (GFP), the GFP signal in DP cells is highest in CD4^{hi}CD8^{lo} cells, which are poised to commit to either the CD4⁺ or CD8⁺ lineage [49].

Both naïve CD4⁺ and CD8⁺ T cells are dependent on IL-7 survival signals. IL-7 is limiting for T cell homeostasis. It was recently shown that IL-7 and other pro-survival cytokines repress IL7R α expression in these cells as a possible mechanism to maximize the number of T cells receiving IL-7 stimulation [58]. Gfi1 is involved in transducing this signal in CD8⁺ T cells. *Gfi1*^{-/-} CD8⁺ T cells, but not CD4⁺ T cells, have increased expression of IL7R α . Restoration of Gfi1 expression in *Gfi1*^{-/-} CD8⁺ T cells reduces IL7R α expression. The IL7R α gene has two potential Gfi1 binding sites, and chromatin immunoprecipitation experiments demonstrate Gfi1 binding to the IL7R α gene in living cells [58]. It is not clear why the IL-7-induced repression of the IL7R α gene in CD4⁺ cells does not require Gfi1.

Gfi1B is a homolog of Gfi1 with a 97% identical zinc finger DNA binding domain, although the homology of the remainder of the protein is limited to the N-terminal SNAG repressor domain. Gfi1B functions as a transcriptional repressor specific for a consensus DNA binding site virtually indistinguishable from that of Gfi1 [27]. While Gfi1 is expressed primarily in the

bone marrow and thymus, Gfi1B is found predominately in splenocytes and in the bone marrow. One defined target of Gfi1B repression is *p21^{waf1}*, a cyclin-dependent kinase inhibitor with a Gfi1/1B binding sites in the promoter [27]. Overexpression of Gfi1B inhibits both IL-6-induced G₁ arrest and differentiation of the M1 murine myelomonocytic cell line [27]. Both Gfi1 and Gfi1B are expressed in T cell precursors; however, Gfi1B expression is up-regulated concurrent with T cell activation [22]. As stated earlier, enforced Gfi1 expression promotes T cell activation. In contrast, over-expression of Gfi1B reduces T cell activation [22]. Transgenic mice expressing Gfi1B in T cells display an increased number of CD4⁺ cells and severely reduced number of CD8⁺ thymocytes. Most probably, a striking reduction of IL7R α expression in Gfi1B transgenic thymocytes reduces the survival of developing CD8⁺ thymocytes. Notably, the defect in generating CD8⁺ thymocytes can be rescued by the expression of Bcl2, a pro-survival IL-7R target [22]. These data compliment the above mentioned data indicating an increase in CD8⁺ cells in *Gfi1*^{-/-} mice, which additionally show an increase in IL7R α expression. Thus, repression of IL7R α expression in Gfi1b transgenics reduced the generation of CD8⁺ thymocytes; whereas deletion of Gfi1 increased IL7R α expression and the generation of CD8⁺ thymocytes.

Gfi1 is autoregulated in lymphoid, but not myeloid cells. Putative Gfi1/1B consensus binding sites are found in homologous regions of the human, mouse and rat *Gfi1* promoters. Chromatin immunoprecipitation (ChIP) assays demonstrate that Gfi1 binds to its own promoter in vivo, and EMSA indicates multiple functional Gfi1 binding sites in the Gfi1 promoter. Ectopic expression of Gfi1 downregulates endogenous Gfi1 in murine thymocytes and transfected Jurkat T cells, but not in the myelo-monocytic U937 cell line [48]. Co-transfection with *Gfi1* promoter/reporter constructs and a Gfi1 expression vector indicate that Gfi1 directly binds and represses the Gfi1 promoter. In the Gfi1B transgenic thymocytes described above, *Gfi1* mRNA is undetectable. In fact, Gfi1B was also found to repress endogenous *Gfi1*. Moreover, Gfi1b binds to the same binding sites in the *Gfi1* promoter as Gfi1, and represses the *Gfi1* promoter. Thus, utilizing the same promoter binding sites, Gfi1 regulates the *Gfi1* locus, and Gfi1B also potentially represses Gfi1 expression [48]. In support of this hypothesis, expression of a T cell targeted Gfi1 transgene in a *Gfi1*/GFP knock-in mouse lead to down-regulation of the *Gfi1*/GFP allele [49], as did expression of a Gfi1B transgene [47]. Interestingly, the *Gfi1B* promoter contains two adjacent Gfi1/Gfi1B binding sites that can be bound by both Gfi1 and Gfi1B [47]. In conclusion, both Gfi1 and Gfi1B are autoregulated and can cross regulate the other locus. To add complexity, the autoregulatory mechanisms appear to be cell type specific.

6. Gfi1 is essential for myelopoiesis, neutrophil differentiation and function

Blood cells with specialized functions, such as neutrophils, are derived from multipotent hematopoietic stem cells by a process that is tightly controlled by transcription factors [59]. Failure to control hematopoietic stem cell fate decisions leads to neutropenia. Bone marrow myeloid progenitors normally switch between default monocytic differentiation, and granulopoiesis [60]. When monocyte production increases, neutrophil production decreases, and vice versa. *Gfi1* deficient mice lack mature neutrophils and are especially susceptible to infections with Gram-positive bacteria. Interestingly, examination of the blood and the bone marrow of *Gfi1*^{-/-} mice identified the accumulation of an undifferentiated immature cell population that express both granulocyte (Gr1⁺) and macrophage (Mac1⁺) markers; an indicator of a developmental block at the promyelocyte stage [2,3]. Both respiratory burst and phagocytic activity were not altered in *Gfi1*^{-/-} cells; however, like immature promyelocytes, the abnormal *Gfi1*^{-/-} cells express neutrophil primary granule proteins. A lack of secondary or tertiary granule proteins indicates that they are not mature [2]. Thus, *Gfi1*^{-/-} mice completely lack neutrophils.

The developmental defects in neutrophil differentiation in *Gfi1*^{-/-} mice are cell autonomous. Neither in vivo granulocyte colony-stimulating factor (G-CSF) administration nor ex vivo cytokine treatment in colony formation assays was sufficient for differentiation and the production of *Gfi1*^{-/-} granulocytes [2,3]. G-CSF administration increased the accumulation of the abnormal Gr1⁺Mac1⁺ promyelocytes [2]. However, *Gfi1*^{-/-} sorted granulocyte-monocyte progenitor (GMP) cells that were transduced with a Gfi1-expressing retrovirus vector could differentiate to neutrophils in response to G-CSF stimulation [2]. The rescue of the defect by reintroduction of Gfi1 in GMP also suggests that the developmental block to granulopoiesis occurs at or after this point.

Gfi1 is an essential transcription factor in the network of factors regulating granulopoiesis. Gfi1 has been shown to bind the promoters of several genes including CCAAT/enhancer-binding protein α (C/EBP α), C/EBP ϵ , and PU.1 [46]. Both C/EBP α and C/EBP ϵ are required for myelopoiesis, as deletion of either gene results in the absence of neutrophils and eosinophils [61,62]. Germline PU.1 deletion results in multiple defects including failure to produce granulocyte progenitors [63,64]. Northern analysis of RNA from *Gfi1*^{-/-} whole bone marrow revealed reduced C/EBP ϵ and increased PU.1 and C/EBP α mRNA steady state levels in total bone marrow [2]. Whether these changes reflect a direct effect of Gfi1 loss or an accumulation of abnormal promyelocytes remains to be determined. Interestingly, treatment of CD34⁺ cells with the thalidomide derivative CC-4047 inhibits erythroid colony formation and enhances the frequency of myeloid colonies. Microarray analysis of CC-4047 treated CD34⁺ cells showed induction of Gfi1 along with the transcription factors C/EBP α , C/EBP δ , C/EBP ϵ and PU.1 [65]. While Gfi1 may not be critically required for the expression of C/EBP α , C/EBP δ , C/EBP ϵ and PU.1, their co-regulation and possible modulation by Gfi1 suggest that Gfi1 may work with these factors to regulate granulopoiesis.

Gfi1 may regulate genes that are important for neutrophil function. The promoters of several genes encoding proteinases expressed in the myelomonocytic lineages such as azurocidin, and α_1 -antitrypsin were shown by chromatin immunoprecipitation to be bound by Gfi1 [46]. Moreover, Gfi1 binds to the promoter of IL8 and its receptor [46]. Both IL8 and its receptor are important for neutrophil activation and migration from peripheral blood into tissues [66]. Ultimately, characterization of these putative Gfi1 target genes will define the significance of Gfi1 regulation in the context of defined function.

Gfi1 is an essential regulator of human neutrophil development and function. A major proteinase of primary neutrophil granules is the neutrophil elastase encoded by *Ela2*. Mutations in *Ela2* are a major cause of human severe congenital neutropenia [67,68]. Screening of SCN patients without *Ela2* mutations identified mutations in *Gfi1* that were associated with neutropenia. The *Gfi1* mutant SCN patients were similar to *Gfi1*^{-/-} mice in terms of their responsiveness to G-CSF, monocytosis, lymphopenia and the presence of an abnormal population of immature myeloid cells with both neutrophil and monocytic features. Importantly, the patients only had one mutant *Gfi1* allele, suggesting that the mutant protein functions as a dominant negative. In contrast, murine *Gfi1*^{+/-} heterozygotes appear to be normal [2,3]. Moreover, the heterozygous mutations in humans do not seem to induce defects associated with the inner ear, growth retardation and runting that are observed in *Gfi1*^{-/-} mice [2,3,10]. It is possible that tissue specific autoregulation of Gfi1 may result in compensation by the wild type allele in non-myeloid tissues [48].

Gfi1 mutations in the fifth and sixth zinc finger have been identified in some SCN and non-immune chronic idiopathic neutropenic (NI-CINA) patients. The mutation in zinc finger five is a substitution of serine for the asparagine at position 382 (N382S) [10]. This mutation is critical because zinc finger five is involved in sequence-specific DNA binding [37]. The Asparagine at this position is hypothesized to contact an adenine in the "AATC" core of the

consensus Gfi1 DNA-binding site [37]. The N382S mutation apparently abolishes proper DNA binding to this residue and thus subsequently disrupts Gfi1 transcriptional repression. The mutation in zinc finger six is a substitution of arginine for the lysine at position 403 (K403R). The disease associated with the K403R mutation is less severe than the disease associated with N382S zinc finger five mutation in SCN patients. Zinc finger six is dispensable for DNA binding [37]; however, K403R mutants were impaired in transcriptional repression assays with synthetic reporter constructs [10]. The decreased transcriptional repression and the phenotype associated with the mutation in zinc finger six suggests a role for this zinc finger in either the DNA binding or protein–protein interactions important for the transcriptional activity of Gfi1. It is also possible that K403 is important as a site of modification such as sumoylation or ubiquitination, and the K403R mutation prevents such modification. This change could affect the overall stability of Gfi1, or affect its interactions with the DNA or other proteins [10].

The defects in Gfi1 repression associated with the SCN mutations may help elucidate the mechanisms underlining the pathology of human neutropenic diseases. As mentioned earlier, in human myelomonocytic cell lines *Ela2* is bound by Gfi1 [46]. Therefore, it is possible that a lack of efficient Gfi1 repression of the *Ela2* gene results in overexpression of *Ela2* in human SCN. Overproduction of neutrophil elastase could lead to mistargeting to cellular compartments other than the neutrophil granules, subsequently disrupting or altering its normal function [10]. Characterizing Gfi1 mutations and defining both their function and gene regulation will provide a valuable insight to the mechanisms and the pathways involved in neutrophil function and development.

Gfi1 is dispensable for macrophage development but required for activated macrophage function. *Gfi1*^{-/-} mice accumulate abnormal promyelocytes with monocytic characteristics. Hock et al. [2] proposed that Gfi1 function is required to antagonize monocyte/macrophage differentiation during myelopoiesis. Therefore, Gfi1 most probably functions early in progenitor cell differentiation. Indeed, *Gfi1*^{-/-} progenitors favor macrophage production upon cytokine stimulation, again suggesting a role for Gfi1 in antagonizing macrophage differentiation [2,3]. Likewise, Gfi1 expression is absent in mature macrophages. However, Gfi1 expression in macrophages is upregulated upon endotoxin-mediated activation, suggesting a requirement for macrophage function. Lipopolysaccharide (LPS) stimulation of *Gfi1*^{-/-} macrophages resulted in increased levels of pro-inflammatory cytokine production (TNFα, IL-1β and IL10) when compared to wild type activated macrophages. These data suggest that Gfi1 modulates macrophage function through the regulation of cytokine production. Activated *Gfi1*^{-/-} macrophages also show increased sensitivity to lower doses of LPS compared to wild type controls, implying an inherent defect in *Gfi1*^{-/-} macrophages. Therefore, the increased sensitivity and cytokine production could be responsible for exaggerated inflammatory responses observed in the *Gfi1*^{-/-} mice followed by a toxic shock [3].

Gfi1 is essential for dendritic cell development and function. Lack of Gfi1 expression appears to have profound effects on dendritic cell (DC) development and function. *Gfi1*^{-/-} mice have reduced numbers of myeloid and lymphoid DC and increased epidermal Langerhans cells. The DC in *Gfi1*^{-/-} mice showed defective maturation and, similar to activated *Gfi1*^{-/-} macrophages [3], increased cytokine production upon stimulation [69]. Progenitor cells from *Gfi1*^{-/-} mice were unable to differentiate into DC and instead differentiated into macrophages, supporting the hypothesis that Gfi1 antagonizes macrophage specification. The *Gfi1*^{-/-} block to DC development is associated with decreased STAT3 activation. Rathinam et al. [69] suggested that Gfi1 control over STAT3 signaling through interaction with PIAS3 and regulation of SOCS genes might be essential for DC development. STAT3 has been described as an important mediator of DC differentiation [70]. The complex hierarchial molecular network

controlling DC development, maturation and function is relatively unknown. Thus, the requirement for *Gfi1* in the generation of DC should provide invaluable insights.

7. The role of *Gfi1* in the peripheral nervous system (PNS)

Gfi1 is orthologous to *Drosophila senseless* and *C. elegans Pag3*. Both *Senseless* and *Pag3* are essential for nervous system development and specification of sensory organs [1,71–74]. *Drosophila* peripheral nervous system development has been studied extensively and its conserved pathways may help to explain mammalian development. PNS development in *Drosophila* is regulated by proneural basic helix-loop-helix (bHLH) transcription factors *atonal*, *scute*, *amos* and *achaete* [1] (Fig. 2). These proneural bHLH transcription factors dimerize with *daughterless* proteins to bind E-box consensus DNA sequences and transactivate target genes. Proneural bHLH proteins act in direct antagonism to the Notch pathway. Conversely, activation of Notch signaling induces enhancer-of-split; a bHLH transcriptional repressor which antagonizes proneural bHLH factor expression and function. Proper function of proneural genes is required for the expression of *senseless*, which encodes a zinc-finger transcription factor (*Senseless*) [1]. PNS development requires *senseless*. Embryos lacking *senseless* specify PNS cells, but most of these cells die through apoptosis. In contrast, forced expression of *Senseless* is sufficient to mediate PNS development [1]. Thus, *Senseless* enforces the activity of the *achaete* and *scute* proneural bHLH transcription factors and antagonizes the activity of the Notch signaling pathway. Orthologous proteins for each member of this pathway can be found in mammalian neuronal and neuroendocrine systems.

Gfi1^{-/-} mice are deaf. *Gfi1*^{-/-} mice show behavioral defects consistent with hearing loss and inner ear defects due to morphological and organizational defects of the inner hair cells and an eventual reduction in the numbers of cochlear neurons [7]. Examination of *Gfi1* expression in late stage embryos revealed specific expression of *Gfi1* mRNA and protein in the inner ear hair cells [7]. Cochlear neurons have *Gfi1* mRNA but not detectable *Gfi1* protein [7]. Initially, *Gfi1*^{-/-} hair cells are specified but morphologically appear abnormal. In addition to their morphological defects, *Gfi1*^{-/-} hair cells are disorganized, with aberrant cellular patterns both in the vestibule and cochlea as well as improper innervation [7]. The organizational and morphological defects, along with altered expression of neuronal markers in *Gfi1*^{-/-} inner hair cells, represent a developmental block and emphasize a requirement for *Gfi1*. Furthermore, *Gfi1*^{-/-} hair cells, at least in the cochlea, undergo rapid degeneration and are completely lost prior to or soon after birth [7]. This is very similar to *Senseless*^{-/-} fruit fly PNS cells, which die of apoptosis shortly after they are specified [1]. While *Gfi1*^{-/-} cochlear hair cells apoptose, the vestibular hair cells stay unorganized but do not disappear, suggesting a different site-specific requirement for *Gfi1* function [7]. Following cochlear hair cell loss, *Gfi1*^{-/-} cochlear neurons undergo a dramatic reduction in numbers by 5 months of age. It is likely that cochlear neuronal degeneration is a secondary effect to hair cell loss, as trophic support from hair cells is required for maintaining the neurons [7].

Inner ear hair cell specification requires bHLH and *Gfi1* coexpression. The expression pattern of the *Gfi1* protein was similar to *Math1*, the mammalian orthologue of the *Drosophila* bHLH protein *atonal*. Both *Gfi1* and *Math1* are coexpressed in inner ear hair cells. *Math1* is necessary and sufficient for hair cell development and specification in vertebrates [75,76]. Based on the *Drosophila* model discussed above (Fig. 2) and the interplay between *Senseless* and *atonal* proteins, *Math1* was suggested to be necessary for *Gfi1* expression and vice versa. However, examination of *Gfi1*^{-/-} mice indicates no obvious effects on *Math1* expression. Therefore, in contrast to the observation in the fruit fly, *Math1* expression is independent of *Gfi1*. Moreover, *Math1* mutant mice do not exhibit altered *Gfi1* mRNA expression, but there was a drastic reduction or even complete absence of *Gfi1* protein expression in the otic epithelia. Thus, either *Math1* is required for *Gfi1* regulation on the translational level or the cells which express *Gfi1*

are absent [7]. Since *Gfi1* mRNA is present in *Math1* null hair cells, an upstream regulator of *Gfi1* expression other than *Math1* is suggested. In fact, the class IV Pou domain transcription factor, *Pou4f3*, an essential factor in the development of all hair cells in human and mouse, controls *Gfi1* expression in hair cells [77–79].

The interplay between bHLH factors, class IV Pou domain containing factors and *Gfi1* exists not only in inner ear hair cells but also in the mammalian retina [7,8,79]. *Math5* is another mammalian orthologue of the *Drosophila atonal*. *Math5* is essential, but not sufficient, for the differentiation of most retinal ganglion cells [8]. The fruit fly *atonal* bHLH protein is involved in the development of the R8 cells of the retina and acts in concert with *Senseless* in a positive feedback loop [72]. However, in the mammalian system *Gfi1* appears to be downstream of *Math5*, as *Gfi1* expression is detected after *Math5* expression is turned off. *Brn3b*, also known as *Pou4f2*, is another member of the Pou domain family. *Gfi1* expression is absent in the *Brn3b*^{-/-} retina, indicating that *Gfi1* is downstream of *Brn3b*. It is possible that Pou domain factors regulate *Gfi1* expression in presumptive neurons to potentiate the action of bHLH factors [8].

The *Gfi1* protein is expressed in specialized sensory cells of the PNS such as the hair cells of the inner ear, the eye, the presumptive Merkel cells, and rare pulmonary neuroendocrine cells [7,9,79]. Pulmonary neuroendocrine cells (PNEC) are a minor cell population of the airway epithelium that are present as single cells or in clusters referred to as neuroepithelial bodies (NEB). PNEC secrete amines and peptides that act to regulate vascular and bronchial tone [80]. NEB are highly innervated and are believed to act as intrapulmonary chemoreceptors. NEB are sensitive to hypoxia and perform several suggested “corpuscular” functions such as nociceptive signal transmission and mechanosensation [81–85]. *Gfi1* expression in the PNEC persists both during embryonic development and in adult mice [9]. *Ash1* (also referred to as *hAsh1* or *ASCL1*, mouse homolog = *Mash1*) is the mammalian orthologue of *Drosophila achaete* and *scute* encoded proneural bHLH proteins. *Ash1* is critical for murine PNEC development [86]. Analysis of bHLH *Ash1* expression in *Gfi1*^{-/-} mice showed no change in the number or the level of *Ash1* expressing PNEC. Therefore, similar to the relationship of *Gfi1* and bHLH in the hair cells of the inner ear and the retinal ganglion neurons, and unlike the *Drosophila* model, *Gfi1* is not required for *Ash1* expression in PNEC [1,7–9,72,79]. However, *Gfi1*^{-/-} pulmonary *Ash1*-expressing cells showed a marked reduction in the expression of the PNEC neuroendocrine markers calcitonin gene related peptide (CGRP) and synaptophysin. These data indicate that, similar to the hair cells in the inner ear, PNEC were specified by *Ash1* in the absence of *Gfi1*, but differentiation and proper maturation were impaired [9].

8. *Gfi1* is a small cell lung carcinoma (SCLC) oncoprotein

Lung tumors with a neuroendocrine phenotype include SCLCs, large cell neuroendocrine carcinomas (LCNE), and classical and atypical carcinoid tumors [87]. SCLC are aggressive lung tumors which are strongly associated with smoking, and for which there is no cure [87, 88]. SCLC account for 15–20% of all human lung cancers [88]. The neuroendocrine phenotype of SCLC involves the production of neuropeptides which stimulate transformation and tumor growth through autocrine signaling [89]. Although direct mutations in neuroendocrine pathway genes have not been discovered, the neuroendocrine phenotype in SCLC is well accepted as the driving force behind SCLC paracrine and autocrine stimulation [86,90]. While it is clear that NE growth factors secreted by SCLC participate in tumorigenesis and tumor maintenance, the genetic and biochemical pathways underlying NE differentiation are not well understood. Subsequently, attempts at clinical intervention rescue few SCLC patients beyond 5 years [87].

Similar to PNEC, pulmonary neuroendocrine tumors such as SCLC express Ash1 bHLH proteins [91,92]. Human SCLC secrete neuropeptides that stimulate proliferation and transformation in autocrine and paracrine stimulatory loops [89]. The neuroendocrine phenotype is essential in maintaining these loops [86,90]. Gfi1 is expressed in murine PNEC and, like Ash1 and neuroendocrine markers, is expressed in cell lines and primary human biopsies of lung neuroendocrine tumors [9]. Gfi1 coexpression with Ash1 and neuroendocrine markers that are downstream targets of Ash1 in PNEC, as well as in human primary biopsy specimens, strongly suggest its involvement in the maintenance of the neuroendocrine phenotype of neuroendocrine lung tumors [9]. Indeed, forced Gfi1 expression in SCLC cell lines potentiated tumor formation in nude mice xenografts, suggesting that Gfi1 acts as an oncogene in SCLC [9].

It should be noted that *Drosophila* developmental cascades have illustrated “gatekeeper” pathways which form the molecular basis of our understanding of colorectal carcinoma (Wnt/beta catenin) and basal cell carcinoma (Hedgehog/Gli) [93]. These pathways are conserved between humans and *Drosophila*, and function in pattern formation in the *Drosophila* embryo. Moreover, they have both positive (oncogenes) and negative acting (tumor suppressor) components that regulate each other and terminate in the regulation of oncogenic transcription factors [93]. The illustrated conservation between *Drosophila* sensory organ precursor development and SCLC oncogenesis contains many of these features.

The mechanism through which Gfi1 enhances SCLC xenograft growth is unknown. It is possible that the sole oncogenic action of Gfi1 in SCLC is to maintain the Ash1/neuroendocrine phenotype, and that in non-neuroendocrine cells this activity is tumor suppressive. In the *Drosophila* model of sensory organ precursor development (Fig. 2), Notch signaling prevents cells from adopting a neuronal fate; whereas bHLH activity with Senseless antagonizes Notch signaling to induce sensory organ precursors. The orthologous proteins are active in human SCLC, which rely on Ash1 and neuroendocrine factors for survival and cell cycle progression. The non-neuroendocrine cells of the lung form Non-SCLC tumors. NSCLC express the Notch pathway target gene HES1 [86] and may thus be considered similar to the *Drosophila* cells that fail to become the sensory organ precursors. In fact, forced expression of an activated Notch protein in human neuroendocrine SCLC cell lines causes a G₁ block in cell cycle progression and correlates to destabilization of Ash1 [94,95]. In a similar manner, forced expression of Gfi1 in non-neuroendocrine NSCLC cell lines results in G₁ cell cycle arrest. Moreover, while SCLC tumors show the highest level of Gfi1 expression and have a poor prognosis, the few human biopsies from patients with NSCLC that express Gfi1 show a trend towards better prognosis and longer survival (Gilks and Grimes, unpublished data). These data suggest that Notch and Ash1/Gfi1 play opposing roles in neuroendocrine versus non-neuroendocrine lung tumors. Each may be oncogenic or suppressive depending on the commitment of the cell to the neuroendocrine phenotype. Given the reduced cell cycle progression of *Gfi1*^{-/-} thymocytes but increased cell cycle progression of *Gfi1*^{-/-} bone marrow stem/progenitor cells, it is enticing to speculate that another Notch versus Gfi1 axis is at work in blood cells. Notably, Notch can regulate commitment to the T cell lineage, and stem cell self renewal [96]. However, it is likely that non-neuroendocrine pathway targets of Gfi1 are involved.

Repression of non-neuroendocrine pathway targets of Gfi1 in different tissues might also yield opposing biological outcomes. Non-neuroendocrine pathway targets might also be functional in the oncogenic action of Gfi1 in SCLC lines. 25-Hydroxyvitamin D 1- α hydrolase (CYP27B1) is one non-neuroendocrine target of Gfi1 in epithelial tumors [97]. In prostate cancer, 1,25-dihydroxyvitamin D (1,25D) is hypothesized to have potential cancer preventative capacity due to its anti-proliferative and pro-differentiation actions [98–100]. CYP27B1 regulates local 1,25D levels, and CYP27B1 is substantially repressed in prostate cancers

[101]. Interestingly, Gfi1 is induced in prostate cancer (Gilks and Grimes, unpublished data) and Gfi1 binds to and represses the CYP27B1 promoter [97]. Gfi1 may therefore, be involved in epithelial tumor progression by focally repressing 1,25D levels. Tissues other than the prostate may be more or less sensitive to 1,25D antiproliferative action. Thus, like the neuroendocrine phenotype, the biological effect of CYP27B1 downregulation may be dependent on the target tissue.

Finally, it appears that Gfi1 can act as an oncogene in T-lymphocytes, SCLC and prostate cancer but is anti-proliferative and perhaps a tumor suppressor in hematopoietic stem cells, myeloid progenitors and NSCLC (Fig. 3). These observations advocate for common mechanistic pathways through which Gfi1 functions to exert its oncogenic or anti-proliferative activities, and suggest that examination of genes regulated by Gfi1 in each system should uncover common targets.

Biography

H. Leighton Grimes received his doctorate in molecular pathology and immunology from the University of Florida School of Medicine. He received postdoctoral training at the Fox Chase Cancer Center, where he was part of the team that first identified and characterized the Gfi1 and Gfi1b oncoproteins. He is currently a tenured associate professor in pediatrics at the Cincinnati Children's Hospital Medical Center, and his scientific work focuses on the role of Gfi1 in hematopoiesis, leukemogenesis and lung tumorigenesis.

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Fig. 1. Schematic representation of the transcriptional repressors Gfi1 and Gfi1B. Both Gfi1 and Gfi1B bind to the consensus-DNA binding site 5'-AATCNNG-3' with C₂H₂ type zinc-fingers on target genes. Once bound to DNA, Gfi1 and Gfi1B repress transcription through the N-terminal SNAG repressor domain.

A model for drosophila neurogenesis

Fig. 2.

Senseless and Gfi1 interaction with proneural basic-helix-loop-helix (bHLH) proteins in fly and human neurogenesis. (1) bHLH transcription factors of the *achaete-scute* complex (AS-C) regulate the development of the fly peripheral nervous system but are antagonized by Notch signaling. (2) AS-C encoded proteins are tissue specific, but bind the ubiquitous *daughterless* encoded proteins. These proneural bHLH dimers bind DNA sequences known as an E box (CANNTG) to transactivate the expression of *senseless*. (3) AS-C factors require Senseless for PNS development, because Senseless is a positive regulator of AS-C/Da function, probably through direct repression of a repressor of AS-C activity. (4) Senseless synergizes with the proneural bHLH factors to transactivate genes (including *senseless*), resulting in a positive-feed-back loop. Once critical levels of functional proneural bHLH dimers and Senseless are reached, Notch signals are antagonized within the cell. (5) Subsequent to inhibition of Notch signaling, neural cell fate is fixed. A similar sequence of events is proposed in human neurogenesis with orthologous proteins.

Fig. 3.

Gfi1 may function as either an oncogene or a tumor suppressor. Biological processes corresponding to either function are listed and discussed further in the text.

Table I

SNAG domain containing family of proteins from representative species

SPECIES	PROTEIN	1	5	10	15	20
<i>Homo sapiens</i> *	Gfi1	MPRSFLVKS	SKKAHSYH	QPRSP		
<i>Homo sapiens</i>	GfiB	MPRSFLVKS	SKKAHTYH	QPRVQ		
<i>Gallus gallus</i>	ZFP	MPRSFLVKS	SKKAHTYH	QHRFV		
<i>Xenopus laevis</i>	Znt544-prov P	MPRSFLVKS	KKKTHTYN	QHRVY		
<i>Homo sapiens</i>	SNAI1	MPRSFLVR	KPSDPN	RKPNYSE		
<i>Homo sapiens</i>	SNAI2	MPRSFLVKK	HFNASKK	PNYSE		
<i>Gallus gallus</i>	Sna1 like P	MPRSFLVKK	HFSASKK	PNYSE		
<i>Danio rerio</i>	Sna1	MPRSFLVKK	YFTSKR	PNYSEL		
<i>Danio rerio</i>	Sna1 2	MPRSFLVK	KYFTNKK	PNYSEL		
<i>Xenopus laevis</i>	xSna1	MPRSFLVKK	HFSASKK	PNYSE		
<i>Xenopus laevis</i>	xSna2	MPRSFLVKK	HFNASKK	PNYGE		
<i>Nematostella vectensis</i>	Sna1 ZFP-A	MPRSFLVK	KTCDDKK	ALLRNG		
<i>Nematostella vectensis</i>	Sna1 ZFP-B	MPRSFLVK	TKTERC	SHFDSPI		
<i>Branchiostoma Floridae</i>	Sna1	MPRSFLKK	KKLHHA	AKKPRFD		
<i>Homo sapiens</i> **	Slug	MPRSFLVKK	HFNASKK	PNYSE		
<i>Gallus gallus</i>	Slug P	MPRSFLVKK	HFNSSKK	PNYSE		
<i>Danio rerio</i>	Zgc:92564	MPRSFLVKK	HFNAAKK	PNYSE		
<i>Xenopus laevis</i>	ZFTF Slug β	MPRSFLVKK	HFNNTTK	PNYGE		
<i>Homo sapiens</i>	Scratch 1	MPRSFLVKK	VKLDAF	SSADLE		
<i>Homo sapiens</i>	Scratch 2	MPRSFLVKK	IKGDG	QC	SGVP	
<i>Danio rerio</i>	Scratch-a	MPRSFLVKK	IKLDD	FSSSPVS		
<i>Mus Musculus</i>	Smuc	MPRSFLVK	THSSHR	VPNYGKL		
<i>Tetraodon nigroviridis</i>	(unnamed)	MPRSFLVK	AKRGSS	GGWQWKE		
<i>Lytechinus variegatus</i>	ZFTF	MPRSFLVKK	NAKQA	ASGLKKL		
<i>Mus Musculus</i>	Gsh1	MPRSFLVDS	LVLRE	ASDKKAP		
<i>Danio rerio</i>	P for MGC: 110817	MPRSFLVDS	LLIREN	SEKGE		
<i>Homo sapiens</i>	INSM1	MPRGFLV	KRSKK	STPVSYRVR		
<i>Tetraodon nigroviridis</i>	(unnamed)	MPRGFLV	KRNK	KANAVSYRVR		

SPECIES	PROTEIN	1	5	10	15	20
<i>Homo sapiens</i> [#]	OVO-like 1 Binding P	MPRAFLVKKPCVSTCKRNWSE				
<i>Homo sapiens</i>	ZFP 339	MPKVFLVKRRSLGVSRSWDE				
<i>Tetraodon nigroviridis</i>	(unnamed)	MPRAFLVKKANVSPGKRNWSE				
<i>Xenopus tropicalis</i>	MGC89597 P	MPRAFLVKRRSPPPVIRNWDE				

ZFTF, zinc finger transcription factor; ZFP, zinc finger protein; P, protein.

* *Mus musculus*, *Rattus norvegicus*, *Canis familiaris*, and *Danio rerio* have identical N-terminal 20 amino acids to *Homo sapiens* Gfi1.

** *Mus Musculus* and *Rattus norvegicus* have identical N-terminal 20 amino acids to *Homo sapiens* Slug.

Mus musculus has identical N-terminal 20 amino acids to *Homo sapiens* OVO-like 1 binding P.