

## SURVEY AND SUMMARY

# Master regulatory GATA transcription factors: mechanistic principles and emerging links to hematologic malignancies

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Received February 14, 2012; Revised March 13, 2012; Accepted March 14, 2012

### ABSTRACT

**Numerous examples exist of how disrupting the actions of physiological regulators of blood cell development yields hematologic malignancies. The master regulator of hematopoietic stem/progenitor cells GATA-2 was cloned almost 20 years ago, and elegant genetic analyses demonstrated its essential function to promote hematopoiesis. While certain GATA-2 target genes are implicated in leukemogenesis, only recently have definitive insights emerged linking GATA-2 to human hematologic pathophysiologies. These pathophysiologies include myelodysplastic syndrome, acute myeloid leukemia and an immunodeficiency syndrome with complex phenotypes including leukemia. As GATA-2 has a pivotal role in the etiology of human cancer, it is instructive to consider mechanisms underlying normal GATA factor function/regulation and how dissecting such mechanisms may reveal unique opportunities for thwarting GATA-2-dependent processes in a therapeutic context. This article highlights GATA factor mechanistic principles, with a heavy emphasis on GATA-1 and GATA-2 functions in the hematopoietic system, and new links between GATA-2 dysregulation and human pathophysiologies.**

### INTRODUCTION

Drilling into mechanisms governing the control of hemoglobin synthesis led to the discovery in the 1980s of a new class of transcription factors containing a highly

conserved Cys4 dual zinc finger DNA-binding module. These proteins were deemed GATA factors based on the nucleotide composition of their cognate DNA-binding motif (1). The discovery of GATA-1 was followed by the cloning of five additional mammalian GATA factors (GATA-2–6) (2–9). Historically, GATA-1, GATA-2 and GATA-3 are deemed the hematopoietic GATA factors (10), while GATA-4, GATA-5 and GATA-6 are termed the cardiac GATA factors (11,12). Extensive biological and genetic analyses have revealed exceptions to this generalization, including expression of the hematopoietic GATA factors in endothelium (9,13,14), breast and prostate (15,16) and neurons (17,18).

Loss-of-function analyses established the essential GATA-1 functions to promote erythrocyte, megakaryocyte, mast cell and eosinophil development (19–25) and GATA-3 functions to promote specific aspects of T-cell lymphopoiesis (26,27). GATA-2 is uniquely essential for the genesis and/or function of hematopoietic stem/progenitor cells (28–30). *Gata2*-null mouse embryos are severely anemic and die at approximately embryonic day (E) 10 (28). Despite the critical GATA-2 requirement for the formation of all lineages of blood cells, some primitive erythroblasts exist in *Gata2*-null embryos. The absence of these cells in *Gata1*<sup>-/-</sup>*Gata2*<sup>-/-</sup> compound mutants indicate that GATA-1 and GATA-2 can function redundantly in the genesis and/or survival of primitive erythroblasts (31). The GATA-2 requirement for the control of hematopoietic stem/progenitor cells is dose dependent, as *Gata2*<sup>+/-</sup> HSCs are functionally impaired, even though the mice are viable (30,32). GATA-2 overexpression in murine bone marrow is also inhibitory for hematopoiesis (33). While this result has potentially important pathophysiological implications, transcription factor overexpression studies are difficult to interpret, given ample opportunities for overexpressed

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proteins to aberrantly engage cellular regulatory factors. As GATA-2 is also expressed in endothelial cells, placenta, prostate, pituitary and select neurons, it will be instructive to compare GATA-2 mechanisms in hematopoietic versus non-hematopoietic systems, although this is currently a virgin territory. Clearly, many unanswered questions remain regarding cell type-specific GATA factor mechanisms and biological actions.

## GATA FACTOR MECHANISMS: FUNDAMENTAL PRINCIPLES

The purification and cloning of GATA-1 (34,35) ushered in studies that elucidated mechanistic principles governing GATA factor function (36). The zinc finger residing closest to the carboxy-terminus (C-finger) mediates sequence-specific DNA binding to WGATAR motifs (37,38), while the zinc finger proximal to the amino-terminus (N-finger) mediates an important protein-protein interaction with the nine zinc finger-containing coregulator Friend of GATA-1 (39–42). The N-finger may also stabilize DNA binding in certain contexts (43). Additional interactions involving the zinc fingers have been documented (44,45), including binding to the myeloid transcription factor PU.1 (46), the erythroid transcription factor ELKF (47) and the mediator complex component Med1 (48). Much less is known about the structural basis and biological implications of these interactions. The broad GATA-1 N-terminus enhances endogenous target gene activation in a context-dependent manner (49). Missense mutations in the N-terminus trigger the usage of an alternative translational start site, yielding a mutant that is strongly associated with the development of transient myeloproliferative disease and acute megakaryoblastic leukemia (50–52).

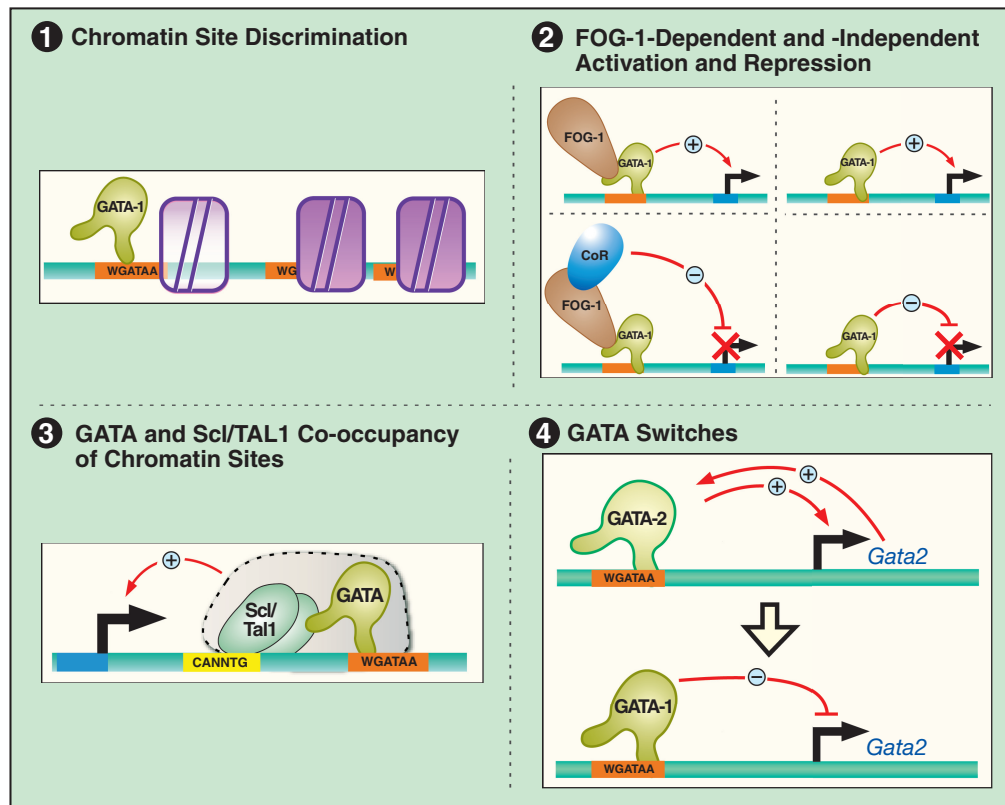
Despite approximately 7 million GATA motifs in the human genome, all capable of forming high-affinity complexes with GATA factors and naked DNA *in vitro*, GATA-1 and GATA-2 occupy only 0.1–1% of these motifs in erythroblasts, based on chromatin immunoprecipitation coupled with massively parallel sequencing and real-time PCR validation (14,53,54). While the molecular determinants for this exquisite discrimination are not fully understood (55), FOG-1 facilitates GATA-1 occupancy at a subset of chromatin sites (56,57). Genome-wide analysis of *cis*-elements residing at endogenous GATA-1 and GATA-2 occupancy sites led to refinement of the GATA consensus from WGATAR to WGATAA. However, the percent of total WGATAA motifs occupied remains very low. Beyond GATA motif sequence composition, the most rudimentary determinant of chromatin occupancy, diagnostic patterns of histone posttranslational modifications demarcate occupied versus unoccupied sites, both containing conserved GATA motifs (53,58–60) (Figure 1, Principle 1). In principle, the unique epigenetic signature of occupied sites may represent primed chromatin structures recognized by GATA-1 as a pivotal determinant of site selection. Alternatively, the signature may arise as a consequence of GATA-1 chromatin occupancy,

followed by recruitment of GATA-1 coregulators that modify chromatin surrounding the occupancy site.

GATA-1 chromatin occupancy leads to either activation or repression of target genes, both of which can be mediated by FOG-1 (36) (Figure 1, Principle 2). One mode of FOG-1 function involves interaction of its N-terminus with the NuRD chromatin remodeling complex (61–63), which can mediate both repression and activation. GATA-1 utilizes FOG-1 to induce higher order chromatin loops, based on chromosome conformation capture (3C) data (64–66). In principle, such loops can mediate activation or repression, dependent upon the physical relationship between the loop and functional features of a gene and the precise nature of the structure formed. GATA-1 also recruits the chromatin remodeler BRG1 to chromatin, which can mediate higher order looping (67–69).

Additional GATA-1 mechanisms exist, including FOG-1-independent activation and repression (41,70,71), although these mechanisms remain poorly understood. GATA-1 commonly co-localizes on chromatin with the stem cell leukemia/T-cell acute lymphocytic leukemia-1 (Scl/TAL1) protein (58,59,72), and the co-localization commonly correlates with transcriptional activity (54,58,72) (Figure 1, Principle 3). Scl/TAL1 is a master regulator of hematopoiesis that binds E-boxes and non-DNA-binding components including LMO2, LDB1, ETO2, and single-stranded DNA-binding proteins (73–79). In the context of naked DNA, optimal composite elements that support complex formation contain an E-box, a downstream GATA motif, and an 8-bp spacer (76). The 8-bp spacing is crucial for GATA-2-dependent enhancer activity in a transient transfection assay using cells expressing endogenous GATA-2 (58). However, GATA-1 and Scl/TAL1 also co-localize at certain chromatin sites lacking composite elements (59,72). Notably, the additional protein constituents of the complex modulate its transcriptional regulatory activity in a context-dependent manner (80) and are linked to the development and/or progression of human hematologic malignancies (81–84). Sophisticated ChIP-seq analyses in the HPC-7 multipotent hematopoietic cell line demonstrated that additional components co-localized with GATA-2 and Scl/TAL1 (85). This analysis revealed 1015 regions of 200 bp or less in which Scl/TAL1, LYL1, LMO2, GATA-2, ERG, FLI-1 and RUNX1 occupancy was detected. As each of these factors is likely to engage additional important partners, considerably more work is required to understand the structure/function of these higher order chromatin complexes containing multiple master regulators of hematopoiesis.

GATA factor interplay appears to be a common mechanism for controlling developmental processes (36,86). During the development of erythrocytes, GATA-1 displaces GATA-2 from chromatin sites at target genes, and this GATA switch (defined as an exchange of different GATA factors at a chromatin site) is tightly coupled to an altered transcriptional output (53,87–90) (Figure 1, Principle 4). GATA switches were first described at the *Gata2* locus, at which GATA-1 binding instigates repression, thus explaining the differential GATA-1 and GATA-2 expression pattern during



**Figure 1.** GATA factor mechanistic principles. The models depict mechanistic principles derived from studies of GATA-1 and GATA-2. While the fundamental nature of these principles is likely to be shared by other GATA factors, additional GATA factor-specific mechanistic permutations are expected. Principle 1: GATA factors occupy a very small percent of the WGATAA motifs in a genome (<1%), suggesting that crucial mechanisms exist that control the discrimination among these highly abundant motifs. However, such mechanisms are not firmly established. The model depicts the occlusion of select GATA motifs, thus creating an obligate requirement for chromatin remodeling/modification reactions to increase access of the WGATAA residues required for GATA factor binding and/or to selectively occlude the vast majority of sites. At certain sites, FOG-1 (56,57) and GATA-1 acetylation (95) enhance chromatin access. Presumably, a host of regulatory factors mediate the essential process of establishing/maintaining accessible and occluded sites. Principle 2: GATA factors activate and repress target genes via multiple mechanisms, including with or without FOG-1 (36). Presumably, this mechanistic diversity reflects the specific chromatin architecture at a genetic locus, the subnuclear environment in which the locus resides and the regulatory milieu characteristic of the specific environment. Principle 3: GATA-1 and GATA-2 commonly co-localize with Scl/TAL1, another master regulator of hematopoiesis (96), at chromatin sites. The model illustrates GATA factor and Scl/TAL1 occupancy of a composite element consisting of an E-box and a WGATAA motif, which was originally described by Wadman *et al.* (76). Similar to the description above, only a very small percentage of composite elements are occupied by GATA factors in cells (53,58). As co-localization does not require the E-box (72), there is much to be learned about the biochemical nature of the GATA factor and Scl/TAL1 interaction. However, the co-localization measured by ChIP often correlates with transcriptional activity (54,58,72). Principle 4: GATA switches are defined as a molecular transition in which one GATA factor replaces another from a chromatin site, which is often associated with an altered transcriptional output. The GATA switch depicted reflects that occurring at the *Gata2* locus during erythropoiesis, in which GATA-1 displaces GATA-2 from chromatin, which rapidly instigates repression (87). Context-dependent GATA switches may either activate or repress transcription and, in certain cases, may sustain the original transcriptional output (36).

erythropoiesis (36,87). GATA-1 utilizes FOG-1 to displace GATA-2 from chromatin (56). The capacity of FOG-1 to bind the NuRD complex is required for the GATA switch, as GATA switches were impaired in a knock-in mouse strain expressing FOG-1 defective in NuRD complex binding (62). Ectopic FOG-1 expression in mast cell progenitors induces a GATA switch in which GATA-1 replaces GATA-2 from the -2.8 kb GATA switch site of the *Gata2* locus, which was linked to *Gata2* repression and generation of erythroid, megakaryocytic and granulocytic progeny (97). During the differentiation of trophoblast giant cells, GATA-2 displaces GATA-3 at *Gata2*, which is associated with transcriptional activation (91). Though GATA switches have not been studied in many systems, it is attractive to

propose that they represent common devices to change transcriptional activity in diverse biological contexts.

Two aspects of the GATA switch paradigm merit careful consideration. First, erythroid GATA switches inform us that different GATA factors can exert qualitatively distinct functions through an identical chromatin site; one GATA factor mediates target gene activation, while the other confers repression or vice versa. Thus, while different GATA factors share certain biochemical attributes, including their highly conserved zinc finger module (92), intrinsic differences underlie the qualitatively distinct activities. A notable difference is the relative high and low stabilities of GATA-1 and GATA-2, respectively (93,94). As proteasome inhibition stabilizes GATA-2 and blocks GATA switches, the low stability appears to be an

important determinant of GATA switches (93). Another important implication of the GATA switch paradigm is that GATA switches and the requisite factors/signals that control the switches represent a novel tool to control developmental processes. Since certain non-hematopoietic cell types can express multiple GATA factors, it would not be surprising if the erythroid GATA switch mechanism were applicable to non-hematopoietic contexts. Despite major progress in elucidating GATA factor mechanistic principles, many questions remain unanswered regarding how cellular signaling pathways dynamically control GATA factor activities and GATA factor-dependent biological processes.

In summary, GATA factor mechanistic principles (Figure 1) include: (1) GATA factors target a small subset of chromatin sites containing a cis-element with the consensus sequence WGATAA; (2) GATA-1 activates or represses target genes in a FOG-1-dependent or -independent manner; (3) GATA-1 and GATA-2 commonly co-occupy chromatin sites with Scl/TAL1, and members of the Scl/TAL1 complex promote or suppress GATA factor-regulated transcription in a context-dependent manner; and (4) GATA switches can involve qualitatively distinct activities of different GATA factors through an identical chromatin site.

## REGULATING GATA FACTORS POSTTRANSLATIONALLY

While multiple posttranslational modifications are implicated in regulating GATA factor function, progress on defining the respective mechanisms does not seem to be commensurate with the level of activity in the field. Common themes have not emerged regarding how posttranscriptional mechanisms regulate different GATA factors. Furthermore, the precise impact of most posttranslational modifications on GATA factor activities, including chromatin occupancy, coregulator recruitment, GATA switches and higher order chromatin transitions at endogenous loci is unknown.

GATA-1 harbors seven serines that can be phosphorylated in cultured cells (98). Six of these serines (S26, S49, S72, S142, S178 and S187) reside in the N-terminal region, while another (S310) is near the C-finger. S72, S142 and S310 are conserved among multiple species. Whereas six serines in the N-terminal region are constitutively phosphorylated, S310 phosphorylation is elevated upon dimethyl sulfoxide (DMSO)-induced differentiation of mouse erythroleukemia (MEL) cells (98). Substitution of all seven serines with alanines does not affect GATA-1 binding to naked DNA or transactivation activity in a non-erythroid cell transient transfection assay (98). S310 resides in the region implicated in DNA bending, based on GATA-1 C-finger peptide binding to DNA (38), but S310 mutations do not affect DNA bending (98). Though mutation of S310 blocks fetal liver erythroid progenitor cell maturation (99), mice bearing alanine substitutions at S72, S142 and S310 exhibit a normal phenotype, save moderately decreased erythroid burst-forming unit (BFU-E) and erythroid

colony-forming unit (CFU-E) in bone marrow (100). Phosphorylation of these residues is therefore either not essential for murine erythropoiesis or undefined mechanisms compensate for loss of phosphorylation sites *in vivo*. Treatment of K562 cells with hemin, sodium butyrate (NaB) or N-acetylcysteine increases GATA-1 phosphorylation and enhances DNA binding *in vitro*, but the phosphorylated residues mediating this effect are unknown (101). Mitogen-activated protein kinase (MAPK)-mediated phosphorylation of S26 in interleukin 3 (IL-3)-dependent Ba/F3 hematopoietic cells increases expression of *E4bp4* and *Bcl-X<sub>L</sub>* survival genes in a transient transfection assay (102). Erythropoietin induces S310 phosphorylation via phosphatidylinositol 3-kinase (PI3K)/Akt (103), and this enhances expression of *TIMP-1*, which encodes tissue-inhibitor of metalloproteinase-1 (103). Since multiple kinases phosphorylate GATA-1, and GATA-1 phosphorylation is regulated in distinct contexts, it is attractive to consider how extracellular stimuli, such as hematopoietic cytokines, instigate cellular signaling mechanisms that orchestrate GATA factor function in physiological and pathophysiological states. However, the triple phosphorylation site knockin mouse described above did not reveal compelling insights in this regard.

IL-3 induces GATA-2 phosphorylation in hematopoietic progenitor cell lines, which is dependent upon MAPK. However, the phosphorylated residues were not described (104). In transiently transfected COS cells, GATA-2 phosphorylation does not affect reporter gene activity (104). Insulin treatment of HEK293 cells stimulates PI3-K/Akt signaling, which induces GATA-2 phosphorylation at serine 401 (105). Serine 401 phosphorylation was reported to impair nuclear translocation, based on overexpression of the mutant in HEK293 cells (105). In addition, naked DNA-binding studies suggested that serine 401 phosphorylation impairs GATA-2 DNA-binding activity (105). Additional work is required to discover the full ensemble of GATA-2 phosphorylation sites, relevant kinases and functional consequences of phosphorylation in distinct cell types *in vivo*.

Analogous to phosphorylation, posttranslational acetylation of the  $\epsilon$ -amino group of lysine represents a common mode of controlling protein structure/function (106–109). Acetylation of histone and non-histone proteins (110) is mediated by a host of histone acetyltransferases (HATs) or histone deacetylases (HDACs). Through recruitment to chromatin via binding *trans*-acting factors (111), HATs acetylate the N-terminal flexible tails of core histones in nucleosomes at specific genetic loci. Molecular consequences of histone acetylation include neutralizing the lysine positive charge, which reduces the histone affinity for DNA and increases *cis*-element accessibility to their cognate binding protein. Histone acetylation can also increase chromatin accessibility by opposing higher order chromatin folding (112). Finally, acetyl-lysine binds a protein module termed a bromodomain (113), thus creating a platform for protein recognition (114,115).

GATA factors contain multiple acetylation sites located predominantly within their zinc finger regions. The Adenovirus E1A-binding region of the HATs

CREB-binding protein (CBP) (116) and its paralog p300 (117) bind and acetylate the GATA-1 C-finger (118). Studies with the CBP/p300 inhibitor E1A provided evidence for an important role of CBP/p300 in erythroid maturation and gene regulation (118). Two lysine-rich motifs (amino acids 243–246 and 312–315) at the C-terminus of the GATA-1 zinc fingers are acetylated (119). GATA-1 acetylation facilitates transactivation in transient transfection assays (119) and promotes GATA-1 chromatin occupancy (95). Acetylated GATA-1 binds and recruits Bromodomain Protein 3 (BRD3) to chromatin (120). As a small molecule inhibitor that antagonizes this interaction reduces GATA-1 and BRD3 chromatin occupancy and decreases erythroid maturation of G1E-ER4 cells, it will be interesting to further explore the mechanistic and biological implications of this interaction. GATA-1 recruits CBP/p300 to chromatin sites, including the  $\beta$ -globin LCR and  $\beta$ major promoter, and presumably this underlies GATA-1-dependent induction of H3 and H4 acetylation at these sites (121–123).

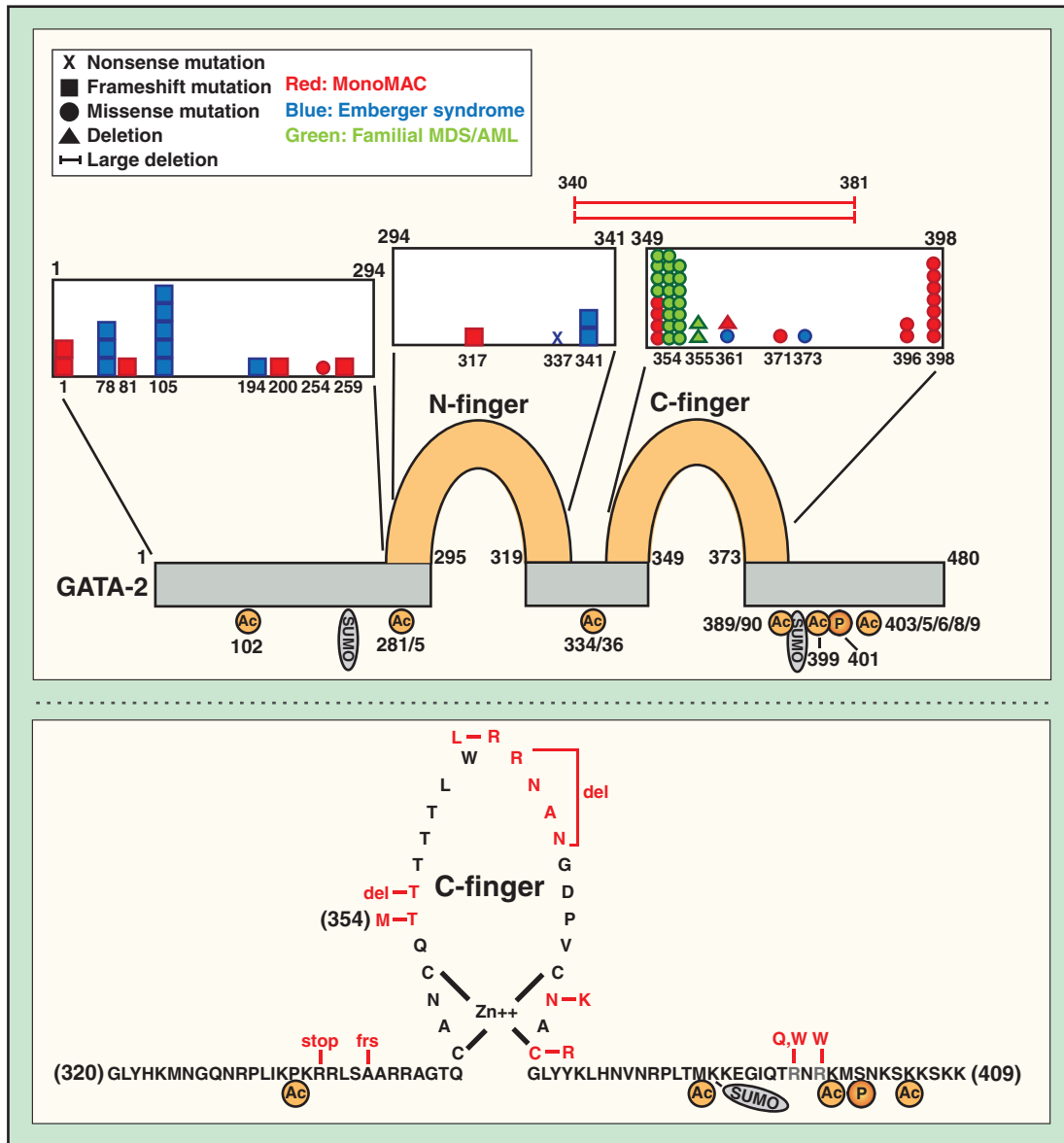
GATA-2 is acetylated at K102 within the N-terminal region and at multiple additional lysines within the zinc finger module including K281, 285, 334, 336, 389, 390, 399, 403, 405, 406, 408 and 409 (124) (Figure 2). p300-mediated acetylation of GATA-2 in hematopoietic cells enhances its DNA binding and transactivation activities in a transient transfection assay and inhibits GATA-2-mediated growth inhibition (124). A GATA-2 mutant lacking four lysine acetylation sites, C-terminal to the C-finger, was unable to rescue primitive erythropoiesis in GATA-2 morphant *Xenopus* tadpoles (125). In this system,  $\text{Ca}^{+2}$ -calmodulin-dependent kinase-4 signaling inhibits GATA-2 acetylation and function (125). Thus, signal-dependent control of GATA-2 acetylation appears to represent an important mode of regulating GATA-2 activity. HDAC3 and HDAC5, but not HDAC1, bind GATA-2, suppressing GATA-2 transactivation activity in HEK293T cells (126).

Certain posttranslational modifications involve the conjugation of small proteins, including ubiquitin and related small ubiquitin-related modifier (SUMO) proteins, to recipient proteins. The four vertebrate SUMO proteins are ~10 kDa and structurally resemble ubiquitin (127,128). While SUMO-2 and SUMO-3 share >90% sequence identity, SUMO-1 is only 50% identical to SUMO-2/3 (129). SUMO-4 has sequence similarity to SUMO-2, but endogenous SUMO-4 has not been detected (130). Sumoylation, which covalently links SUMO to a lysine within a target protein, is reversible and dynamically regulated (131). Sumoylation involves an enzymatic cascade, analogous to ubiquitination (132). The E1 activating enzyme Aos1-Uba2 forms a thioester bond with SUMO in an ATP-dependent reaction and subsequently transfers SUMO to the E2 conjugating enzyme Ubc9. An E3 ligase facilitates the transfer of SUMO to its substrate and an isopeptide bond is formed between the C-terminal glycine residue and the  $\epsilon$ -amino group of a lysine residue of the acceptor protein. Conjugating enzymes and SUMO-specific proteases regulate the level of sumoylation. Six mammalian sentrin/SUMO-specific protease (SENPs) homolog (SENPs1–3, SENPs5–7) have

been identified (133). Whereas polyubiquitination triggers proteasome-mediated proteolysis, sumoylation commonly controls protein–protein interactions by regulating the activity, localization and stability of target proteins, masking an existing binding site, occluding a site for a distinct modification or providing an interface for interaction with proteins containing a SUMO-interacting/binding motif (SIM/SBM) (134).

GATA-1, GATA-2 and GATA-4 sumoylation have been described (135–137). Though most sumoylation substrates contain the consensus motif  $\Psi$ KXE (138) ( $\Psi$ , large hydrophobic amino acid; X, any amino acid), some SUMO targets lack this consensus, and experimental analysis is required to determine whether a consensus is a *bona fide* sumoylation site *in vivo*. GATA-1 is sumoylated at K137, which is embedded in a sumoylation consensus, within the N-terminal region. The SUMO ligase PIASy can sumoylate K137 (136). Initial analyses using a transient transfection assay in non-erythroid cells and a *Xenopus* animal cap explant assay suggested that the K137R mutant and wild-type GATA-1 have similar activities (136). PIASy binds GATA-1 and was reported to repress GATA-1-mediated transactivation via a K137-independent mechanism in a transient transfection assay with overexpressed factors. In genetic complementation analysis in GATA-1-null erythroid precursor (G1E) cells expressing GATA-1 fused with an estrogen-receptor ligand-binding domain (ER-GATA-1) at near physiological levels, K137 sumoylation promotes GATA-1-mediated transcriptional regulation (both activation and repression) at a subset of endogenous GATA-1 target genes (139). SUMO-dependent genes are predominantly FOG-1-dependent targets. The GATA-1 V205G mutant, defective in FOG-1 binding, yields molecular phenotypes similar to the K137R mutant. Furthermore, SUMO- and FOG-1-dependent genes migrate away from the nuclear periphery upon GATA-1-induced erythroid maturation, while SUMO- and FOG-1-independent genes persist at the periphery (139). The use of tiled bacterial artificial chromosome probes revealed that sumoylation endows GATA-1 with the capacity to expel the  $\beta$ -globin locus from the nuclear periphery without inducing gross changes in the positioning of neighboring chromosomal regions (140). Given these mechanistic insights, it is of considerable interest to investigate how SUMO-specific proteases fit into the GATA factor regulatory circuitry. SENP1 knockout mice die from severe anemia between E13.5 and postnatal day 1 (141). SENP1 knockout mice exhibit hematopoietic defects in the fetal liver, which correlate with accumulation of sumoylated GATA-1, as well as hypoxia-inducible factor-1 $\alpha$ . As SENP1 desumoylates a broad spectrum of substrates, the hematopoietic defects presumably reflect the aggregate actions of this broad activity, presumably including FOG-1, which is sumoylated in erythroid cells (142).

GATA-2 interacts with PIASy in transfected COS cells, which preferentially conjugates SUMO-2 to GATA-2 (135). In a transient transfection assay in endothelial cells, PIASy suppresses GATA-2 transcriptional activity at the *endothelin-1* (*ET-1*) promoter. Whereas the repression requires the GATA-2-PIASy interaction, the



**Figure 2.** GATA-2 mutations in human hematologic disorders (143–146). Specific GATA-2 mutations in human patients are indicated, with details denoted in the legend. Each symbol represents a single patient with the particular mutation. The diagram of GATA-2 protein organization illustrates the N- and C-fingers, acetylation sites (124), the serine 401 phosphorylation site and two sumoylation consensus motifs. The diagram at the bottom illustrates the amino acid sequence composition of the C-finger and neighboring regions, with the positions of disease mutations highlighted. Stop, mutation that creates stop codon; frs, frameshift mutation; del, deletion.

PIASy RING-like domain with SUMO ligase activity is dispensable, indicating that PIASy regulates GATA factor activity independent of sumoylation. While GATA-2 contains two potential sumoylation sites (human amino acids 221–224 and 388–391) that conform to the consensus (Figure 2), the sumoylation site has not been described. Further analysis is required to elucidate the function of GATA-2 sumoylation at endogenous loci. GATA-4 is sumoylated at K366 in the C-terminal region (137). Based on the initial evidence for functional significance of at least certain GATA-1 and GATA-2 posttranslational modifications, it is attractive to propose that signal-dependent targeting of GATA factors represents a canonical mode of regulating hematopoiesis. By contrast

to well-established cytoplasmic to nuclear signaling paradigms, many questions remain unanswered regarding the nature of the signaling pathways that target GATA factors, the precise molecular consequences of the posttranslational modifications and how dysregulated signaling, often a hallmark of hematologic malignancies, influences GATA factor activity.

### HUMAN PATHOPHYSIOLOGIES CAUSED BY GATA-2 DYSREGULATION

Given the essential GATA-2 function to promote hematopoiesis, alterations in GATA-2 levels/activity would be

expected to initiate and/or promote the development of hematologic malignancies. However, until recently, only circumstantial evidence implicated GATA-2 in human cancers. Four related human disease syndromes harboring germline mutations in *GATA2* are associated with an increased incidence of myeloid neoplasia, either myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). Three of the four syndromes—Monocytopenia/*Mycobacterium avium* complex (MonoMAC) (143,147), Dendritic cell, monocyte, B and Natural Killer Lymphoid deficiency (DCML deficiency) (144) and Emberger's syndrome (145,148)—share the hallmark feature of immune dysfunction with an increased propensity to develop MDS or AML. The fourth, a familial MDS/AML, lacks the immune dysfunction and systemic symptomatology characteristic of the other cases (146). Though these entities appear to be rare, their study provides new insight into the role of *GATA2* in immune function and in the regulation of growth, maturation and apoptosis in the myeloid compartment.

Sequencing of candidate gene exons in the genomic DNA of pedigrees of familial MDS/AML revealed a T354M *GATA2* mutation in three pedigrees and a T355del in one pedigree (146). This analysis also revealed several pedigrees harboring mutations in *RUNX1* or *CEBPA*, disease genes for familial AML (149). Molecular modeling suggested that T354 and T355 stabilize the GATA-2 C-finger (146). The T354M mutant exhibits lower affinity for DNA, reduced transactivation activity and is less effective in synergizing with PU.1 to activate the CSF1R (*Fms*) promoter in a transient transfection assay (146). When tested for the ability to block ATRA-induced maturation and apoptosis of HL-60 cells, the T355del mutant acted as a null, while the T354M mutant blocked ATRA effects, consistent with expectations for a leukemogenic oncogene. Based on gene expression profiling in HL-60 cells, the T354M and T355del mutants appeared to be null alleles (146). Two additional pedigrees harboring the T354M mutation were recently reported (150,151). Further mechanistic analysis is required to rigorously analyze the function of these disease mutants at endogenous loci and in diverse cellular contexts.

Holland's group had previously described a syndrome of monocytopenia with susceptibility to opportunistic infections by the *Mycobacterium avium* complex, termed MonoMAC (143,147), which occurs in both sporadic and autosomal dominant familial form. The immune deficiencies were significant: patients suffered from disseminated cutaneous human papilloma virus infection, aspergillosis, histoplasmosis or cryptococcal meningitis. In addition, some suffered from pulmonary alveolar proteinosis, which is typically associated with macrophage dysfunction. Patients had markedly diminished circulating monocytes (10 cells per microliter, average), as well as B cells and natural killer (NK) cells; T cells were variable (147). Other causes typically associated with the particular spectrum of immune defect seen in these patients (e.g. HIV infection, IL12/IL23/IFN $\gamma$  or NF- $\kappa$ B dysfunction) were ruled out (147).

The frequency of myeloid neoplasia (MDS or AML) in the combined familial and sporadic cases of MonoMAC

was 50%; these were associated with trisomy 8, monosomy 7 and dicentric chromosome 6 (147). Given the *GATA2* mutations in familial MDS/AML (146), the authors investigated such mutations in the MonoMAC kindreds, 13 of the 16 they originally reported. This revealed frameshift mutations (G81fs, M1del290, D259fs and N317fs), a deletion spanning the N- and C-fingers (D340–381), a small deletion in the C-finger ( $\Delta$ 362–365) and missense mutations within the GATA-2 C-finger (T354M, N371K, R396W, R396Q, R398W) (143). In addition, one missense mutation occurred outside the finger region: P254L.

Bigley *et al.* (152) further characterized the immune deficiency in four patients with MonoMAC syndrome, referring to it as DCML deficiency. DCML is associated with the absence of granulocyte monocyte progenitors (GMPs) and common lymphoid progenitors (CLPs) (152). As expected, with long latency, DCML can lead to MDS (153,154). Exome sequencing revealed *GATA2* mutations in these four patients: a frame shift after amino acid 200; a T354M missense mutation; a R398W missense mutation and a D340–381 deletion spanning the C-terminal end of the N-finger, extending into the C-finger (144).

Emberger's syndrome is characterized by lymphedema with myelodysplasia progressing to AML, as well as immune dysfunction (widespread cutaneous warts and sensorineural deafness) (148). Whole-exome sequencing of three individuals with Emberger syndrome revealed *GATA2* mutations, and further analysis of additional cases of Emberger syndrome (four more sporadic cases and additional individuals from the two affected kindreds) revealed additional *GATA2* mutations (145). These mutations span from the N-terminus to the C-finger, and include five frame shift mutations, one nonsense mutation and two missense mutations within the C-finger (R361L and C373R) (145). Using a luciferase reporter bearing a GATA-2-responsive CD34 promoter construct transfected into HEK293 cells, they demonstrated that the R361L and C373R mutants have a decreased capacity to transactivate the reporter. However, the molecular basis for the defective activity was not established.

It is instructive to consider the mechanistic basis of the immune deficiency in MonoMAC/DCML deficiency and Emberger's syndrome. It is unlikely that the myeloid neoplasia in these patients yields immune dysfunction, since the spectrum of opportunistic infections is distinct from that seen in the neutropenia of MDS, but is similar to that seen in IRF8 deficiency and IFN $\gamma$ /IL-12 deficiency. There are several clues that through its interaction with PU.1 (155), GATA-2 plays an important role in monocyte/macrophage/dendritic cell development. First, GATA-2 controls phagocytosis by pulmonary alveolar macrophages (156), and pulmonary alveolar proteinosis is a feature of the MonoMAC syndrome. Second, with haploinsufficient GATA-2 mice, there is loss of lymphoid and monocytic cells with retention of granulocytes (32). Furthermore, there are specific defects in the granulocyte/macrophage progenitor pool (157). GATA-2 interacts with and represses PU.1 (158), which induces

*c-Fms* and *Flt3* expression (159,160). The common developmental origin for macrophage/dendritic cells and lymphoid cells (161) may tie together the deficiencies in B and NK cells seen in MonoMAC patients. In addition to PU.1, GATA-2 can bind C/EBP- $\alpha$  (162), a key regulator of granulopoiesis. Whether GATA-2 disease mutations affect this interaction, thereby altering C/EBP- $\alpha$  function, has not been addressed.

Regarding mechanisms underlying the pathogenic activities of mutant *GATA2* alleles, do the proteins function as null alleles? Dickinson *et al.* posit that the frameshift and 42 amino acid deletions act as null alleles, though N-terminal frameshifts in *GATA1* (associated with AML) can be translated through initiation at a downstream codon, resulting in the production of an N-terminally truncated mutant with reduced activity (50); whether this happens with GATA-2 frameshift mutations is not known. Strikingly, nearly all the point mutations identified in these four papers localize to the *GATA2* C-finger. Initial mechanistic analyses (146) suggested that the T354M and T355del alleles exhibit certain functional differences. While we propose that these mutations lead to haploinsufficiency, considerably more work is required to evaluate functional consequences of the mutations.

Are there links between the immune deficiency and MDS/AML? It was suggested that the increased incidence of MDS in patients with GATA-2 mutation-induced MonoMAC syndrome may result from defective regulation of HSC proliferation: in the face of systemic immune dysfunction and recurrent infection, the stem cell compartment is stimulated to proliferate, and since committed progenitors are also dysfunctional due to GATA-2 haploinsufficiency, this leads to chronic stress on the HSC compartment, HSC exhaustion and MDS (163). GATA-2 haploinsufficient mice have a decreased stem cell pool, with a higher percentage of quiescent LSK cells and increased apoptosis (32). The immune deficiency and MDS/AML may not be linked, since GATA-2 mutations alone, independent of immune dysfunction, may lead to MDS/AML (146). The presence of chromosomal aberrations (trisomy 8 and 21, monosomy 7 and dicentric chromosome 6 (147) suggest that additional events are needed for malignancy to develop and/or GATA-2 mutations increase the likelihood of these mutagenic events occurring. These points may explain the long latency for the development of this set of syndromes and the low penetrance of MDS/AML development. Alternatively, the long latency and variable penetrance may be due to the time required for subtle deregulation of hematopoiesis to segue to a full-blown malignancy.

GATA-2 expression can be upregulated in AML, and this portends a poor prognosis (164–167). By contrast, GATA-2 can be downregulated in murine retroviral transplant models of AML, and forced GATA-2 expression did not sustain leukemic cell growth (168). GATA2 has been identified as a common site of proviral insertional activation in AMLs occurring in retrovirally infected NUP98-HOXD13 transgenic mice (169). Zhang *et al.* (170) identified a gain-of-function GATA-2 mutation occurring in the accelerated phase (AP) and blast crisis

(BC) phase of CML (170). They analyzed 85 cases of CML in either accelerated phase or blast crisis for genetic alterations in any of 13 candidate genes of known importance to hematopoiesis or tumor progression. Eleven of these had mutations in Runx1 (AML1), while eight had a L359V mutation. Another case had a six amino acid internal deletion (D341–346). Both these reside in the C-finger. Kaplan–Meier-type analysis of patients with the L359V mutation showed a statistically significant shortening of time to disease progression. Though more work needs to be done to establish the mechanism, Zhang *et al.* provide initial evidence that the L359V mutant acts as a dominant-active allele: it appears to bind naked DNA more tightly; to bind to PU.1 more tightly, and to more strongly inhibit PU.1-mediated transactivation on target promoters; it had a modest inhibitory effect on vitamin D3 and all-trans retinoic acid differentiation of HL-60 cells and caused BCR-ABL-transduced primary hematopoietic cells to take on a more monocytic phenotype (170). Analysis of 688 DNA samples from patients with non-CML-AP/BC hematologic malignancies failed to identify sequence alterations in *GATA2*, emphasizing that the L359V mutation is specific to AP/BC phase of CML (171).

## CONCLUDING REMARKS

Despite the emergence of compelling mechanistic principles, knowledge of how cellular signaling pathways regulate GATA factor activity remains primitive. Taken together with the many unanswered questions regarding how GATA-2 coding region mutations dysregulate GATA-2 function, major efforts are required to construct a lucid picture of how physiological GATA-2 activity suppresses the development of MDS and leukemia. Elucidating the cell type-specific regulatory circuitry in which GATA-2 is embedded, and careful scrutiny of the dynamics of this circuitry, will reveal key regulatory components/nodes that offer opportunities for innovative and efficacious targeted interventions. Given the rapidity of progress vis-à-vis linking GATA-2 dysregulation to hematologic pathophysiology, and the ever-increasing ease in conducting whole-genome analyses, further rigorous explorations into this rich pipeline will almost certainly yield pivotal insights into the molecular basis of the human diseases discussed and more.

## FUNDING

National Institutes of Health (NIH) (DK68034, DK50107 to E.H.B., CA120313 to A.S.P.). H.-Y.L. was the recipient of an American Heart Association Predoctoral Fellowship. Funding for open access charge: NIH.

*Conflict of interest statement.* None declared.

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