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MINIREVIEW

GATA1 Function, a Paradigm for Transcription Factors in Hematopoiesis

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TRANSCRIPTIONAL CONTROL OF HEMATOPOIESIS

The development of mature blood cells of distinct lineages, from the hematopoietic stem cells (HSCs), involves a progressive restriction of differentiation potential and the establishment of lineage-specific gene expression profiles (Fig. 1). The establishment of these expression profiles relies on lineage-specific transcription factors to modulate the expression of their target genes. Therefore, hematopoiesis is an excellent model system to investigate how particular transcription factors influence the establishment of lineage-specific expression profiles and how their activity is regulated. In this review we focus on the present knowledge of the biological functions of the hematopoietic transcription factor GATA1. Many aspects of its function have been revealed since its first description in 1988. Yet many new questions have surfaced, and many old questions remain to be answered. Thus, GATA1 has been in the floodlight of modern biology as a paradigm for hematopoietic transcription factors in general and GATA factors in particular.

THE GATA FAMILY OF TRANSCRIPTION FACTORS

The GATA family consists of six transcription factors, GATA1 to GATA6. These transcription factors are categorized as a family due to the fact that they all bind to the DNA consensus sequence (A/T)GATA(A/G) by two characteristic C_4 (Cys-X_2-Cys-X_{17}-Cys-X_2-Cys) zinc-finger motifs specific to the GATA family (50, 60, 65, 126, 129). The DNA-binding regions are highly homologous between the GATA family members (Fig. 2). Outside these regions, the conservation between GATA factors is low (81, 129). Furthermore, the overall homologies for individual members are higher between species than between different members of the same species (129, 136).

The GATA family is divided into two subfamilies on the basis of the expression profiles of the individual transcription factors. GATA1, GATA2, and GATA3 belong to the hematopoietic subfamily, since they are expressed mainly in the hematopoietic system (Fig. 2) (121). The nonhematopoietic subfamily is composed of GATA4, GATA5, and GATA6, which are expressed in several tissues, including intestine, lung, and heart (70).

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GATA1: A BIRD IN THE HAND

GATA1, also known as NF-E1, NF-1, Ery-1 and GF-1, is the founding member of the GATA family of transcription factors. It was first identified as a protein with binding specificity to the β-*globin* 3′ enhancer (117) and cloned from a mouse erythroleukemia cell line cDNA expression library (105) and from chicken red cells (21). The human homologue was cloned soon after, and its localization was assigned to the X chromosome at position Xp21-11 (137). The mouse *GATA1* gene is also located on the X chromosome (137).

GATA1 is expressed in primitive and definitive erythroid cells (27, 55), megakaryocytes (61, 92), eosinophils (138), and mast cells (61) and in the Sertoli cells of the testis (46, 131). Several gene-targeting studies were performed to elucidate the importance of GATA1 function in these cells.

These studies have shown that GATA1 is essential for normal erythropoiesis. GATA1-deficient embryonic stem cells are able to contribute to all different tissues in chimeric mice, with the exception of the mature red blood cells (87). More detailed analysis of erythropoiesis in these chimeric mice revealed that GATA1 null erythroid cells fail to mature beyond the proerythroblast stage (86). In vitro differentiation of GATA1-deficient embryonic stem cells confirmed this arrest of both primitive and definitive erythropoiesis at the proerythroblast stage (120) and showed that the arrested precursors die by apoptosis (122). Not surprisingly, GATA1 null mouse embryos die from severe anemia between embryonic day 10.5 (E10.5) and E11.5 (27). GATA1 knockdown embryos (GATA1.05), which express only approximately 5% of the wild-type GATA1 levels, also show an arrest of the primitive erythropoiesis and die between E11.5 and E12.5 (100). Other GATA1 knockdown mice (GATA1 low) (63), which express about 20% of the wild-type GATA1 levels, show a somewhat milder phenotype. Despite the fact that the majority of GATA1-low mice die between E13.5 and E14.5 due to ineffective primitive and definitive erythroid differentiation, some are born alive (2% instead of the expected 25%) and a small number survive to adulthood. These mice are anemic at birth, but they recover from the anemia and show a normal life span. From the analysis of these different mouse models a direct relationship between the expression levels of GATA1 and the severity of the phenotype is evident.

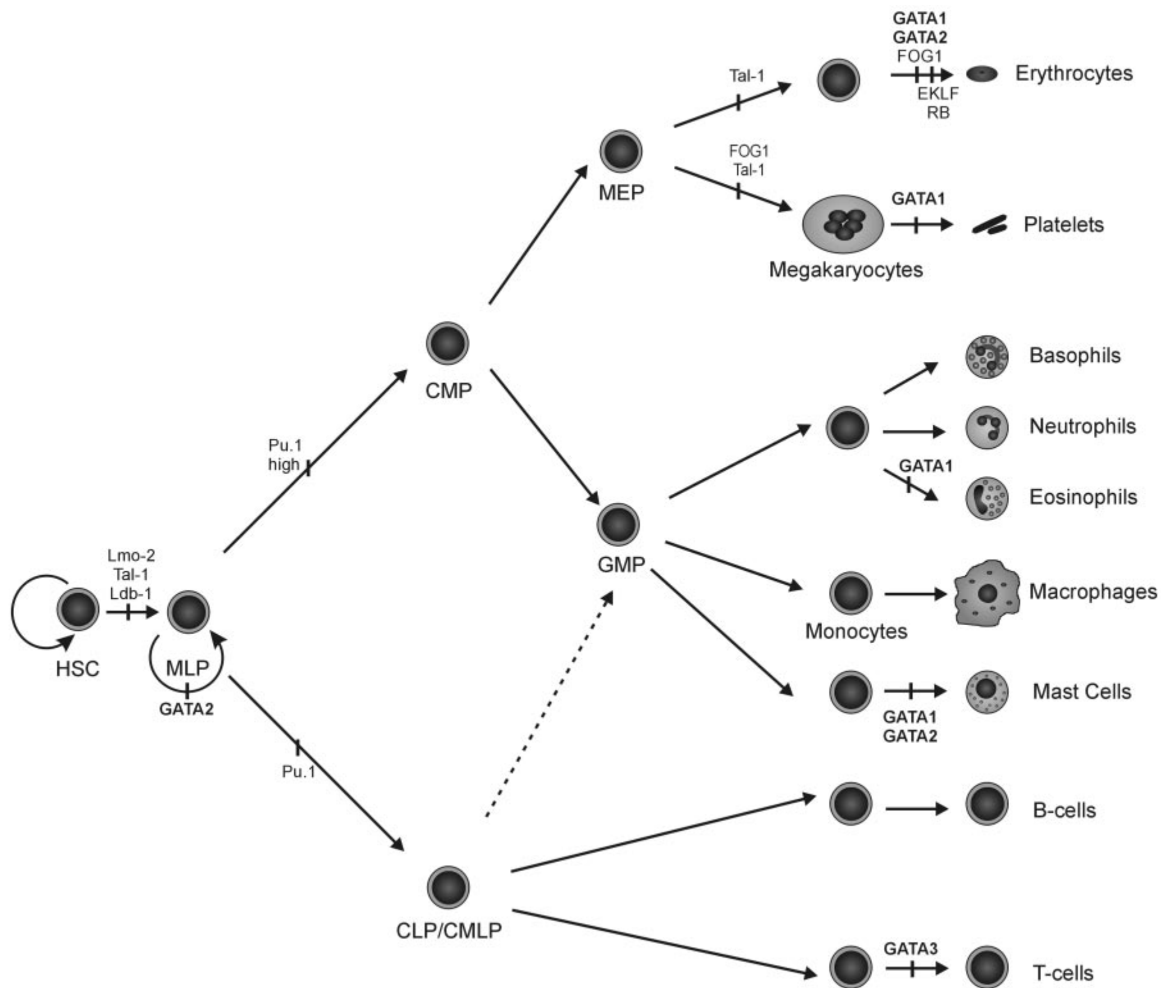

FIG. 1. The hematopoietic tree. Schematic representation of the main lineage commitment steps in hematopoiesis. The hematopoietic stem cell (HSC) is the basis of the hematopoietic hierarchy and gives rise to multilineage progenitors (MLP), which can differentiate into all the hematopoietic lineages. MLPs become lineage restricted to the lymphoid and myeloid lineages in the common lymphoid progenitor (CLP) and common myeloid progenitor (CMP), respectively. CLPs can give rise exclusively to B and T cells, while CMPs can give rise to megakaryocyte-erythrocyte progenitors (MEP) and granulocyte-monocyte progenitors (GMP). Alternatively, it is also believed that the first lineage commitment separates myeloid and erythroid potential, in the CMP, from myeloid-lymphoid potential, in the common myeloid lymphoid progenitor (CMLP). CMLPs can than further differentiate in B cells, T cells, and GMPs (dashed line). Hematopoietic GATA factors and GATA1 cofactors relevant for the development of particular hematopoietic lineages are indicated.

The analysis of a megakaryocyte-specific knockdown of GATA1 has revealed a critical role for this factor in megakaryocytic development (97). Absence of GATA1 in megakaryocytes leads to an increased proliferation and deficient maturation of megakaryocytic progenitors as well as reduced number of circulating platelets. The platelets produced are not fully functional and show an abnormal morphology (113).

GATA1 also plays an essential role in eosinophil development. The first evidence of the role of GATA1 in eosinophil development came from the observation that forced GATA1 expression in Myb-Ets-transformed chicken myeloblasts induced a reprogramming of these myeloblasts into cells resembling either transformed eosinophils or thromboblasts (52). Furthermore, the deletion of a double GATA site present in the *GATA1* promoter causes the selective loss of the eosinophilic lineage (132).

Mast cells are somewhat different from the majority of the hematopoietic cells. They originate in the HSC in the bone marrow, but the precursor cells migrate, through the blood, to connective or mucosal tissues, where they proliferate and differentiate into mature mast cells. GATA1 is abundant in the more mature mast cells, but it is almost undetectable in the bone marrow progenitors, suggesting a possible role in the terminal differentiation of mast cells (38). Also, it was noticed that with GATA1.05 heterozygous female mice, expressing 5% of the wild-type GATA1 levels in approximately 50% of the cells owing to the process of X inactivation, some mast cells show defective maturation (38). Final proof of the importance of GATA1 in mast cell maturation arose from the analysis of

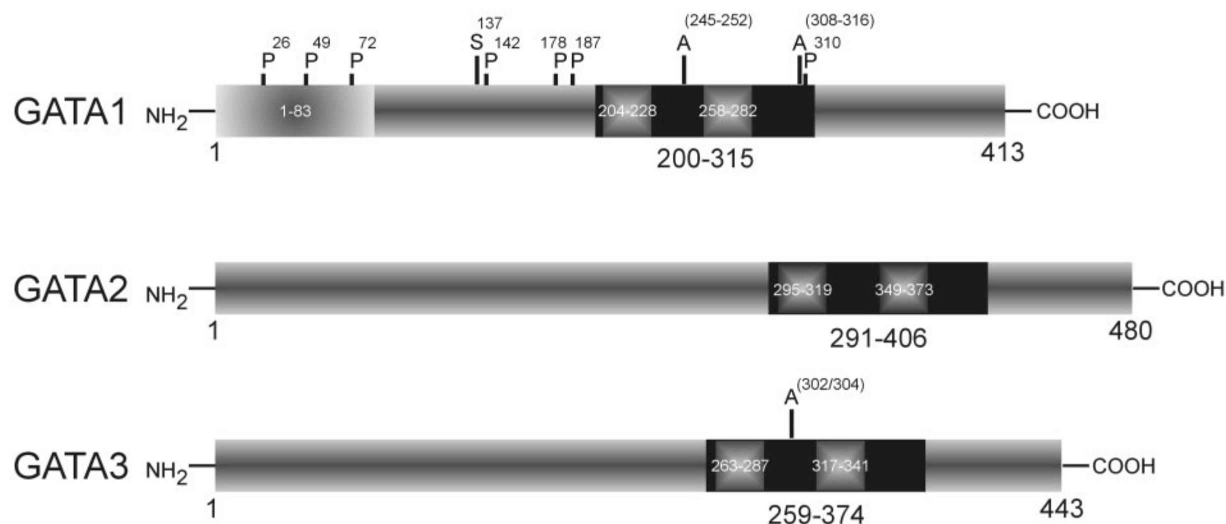

FIG. 2. The hematopoietic GATA transcription factors. A schematic representation of the mouse hematopoietic GATA proteins is shown. The highly conserved region comprising the zinc-finger domains is indicated in black; the regions between the zinc-chelating cysteines are highlighted. The N-terminal activation domain (aa 1 to 83 of GATA1) and the known sites modified by acetylation (A), phosphorylation (P), or SUMOylation (S) are indicated by numbers. Numbering starts at the first methionine of the proteins.

GATA1-low mice (67). Since some reach adulthood, a more detailed analysis of the mast cell phenotype could be performed. Indeed, connective tissues from GATA1-low mice contain large numbers of mast cell precursors but normal numbers of mature mast cells, with abnormal morphology. Many of these precursors die by apoptosis, which explains the normal numbers of mature cells. The defect observed is GATA1 specific, since forced expression of GATA1 rescues the maturation potential of these cells.

Outside the hematopoietic system, GATA1 is expressed in the Sertoli cells of the testis at critical stages of spermatogenesis (46, 131). However, Sertoli cell-specific deletion of *GATA1* does not result in an apparent phenotype (56).

Despite all the knowledge about the consequences of GATA1 absence in different hematopoietic lineages we are far from knowing the specific functions performed by this transcription factor in those cells. On the basis of the observation that GATA1 null erythroid cells undergo apoptosis, it as been suggested that GATA1 is directly involved in cell survival. Several lines of evidence support this theory: GATA1 activates transcription of the erythropoietin receptor (*EpoR*) (10), and Epo signaling is known to be important for the survival of erythroid progenitors (53). Furthermore, one of the known target genes of GATA1 is $Bcl\text{-}_{XL}$, a gene encoding an anti-apoptotic protein (32).

Another possible GATA1 function is the regulation of G_1/S cell cycle progression. Cell cycle control is of the utmost importance in hematopoietic differentiation, since progenitors must be able to proliferate to proceed through hematopoietic development, but for terminal differentiation to occur cells must exit the cell cycle (127). A variety of GATA1 target genes have been identified that are involved in cell cycle regulation or have known functions in proliferation and differentiation processes (93).

GATA1 has also been implicated in the reprogramming of hematopoietic precursors. Forced expression of GATA1 was shown to reprogram myeloblasts and $CD34^+$ bone marrow cells to develop into eosinophils (41, 52). Furthermore, forced expression of GATA1 reprograms granulocyte-monocyte progenitors (GMPs) to give rise to erythroid, eosinophilic, and basophile-like cells (40). By clone tracking the authors demonstrated that the GATA1 effect occurs at the cell commitment level and is not due to effects on clone selection. Another recent report (47) shows that ectopic GATA1 expression guides hematopoietic precursors to commitment to the erythrocyte-megakaryocytic lineage. It is not clear from these reports whether lineage reprogramming is a GATA1-specific characteristic or whether it is a general effect of the ectopic expression of lineage-specific transcription factors.

FUNCTIONAL DOMAINS OF GATA1

At least three functional domains have been identified within the GATA1 protein (Fig. 2): an N-terminal activation domain, the N-terminal zinc finger (N-finger), and the C-terminal zinc finger (C-finger). The C-finger is essential for GATA1 function, since it is responsible for the recognition of the GATA consensus sequence and consequent binding to DNA (Fig. 3) (60, 130). The importance of the N-finger to GATA1 function has been more difficult to define. Although early studies, in nonerythroid cells, indicated that the N-finger was not essential for GATA1-mediated transcriptional activation (60), it was later shown that this zinc finger plays a crucial role in GATA1's ability to induce terminal erythroid differentiation (123). The N-finger contributes to the stabilization and specificity of DNA binding (28, 60, 104, 126). The N-finger mediates the formation of complexes with cofactors. These interactions can involve only the N-finger, as is the case with FOG-1 (23), or occur in collaboration with the C-finger, for example, with Sp1 and EKLF (31, 66) and GATA1 itself (58). Early studies, using reporter assays in nonerythroid cells, showed that the most N-terminal 80 amino acids of the

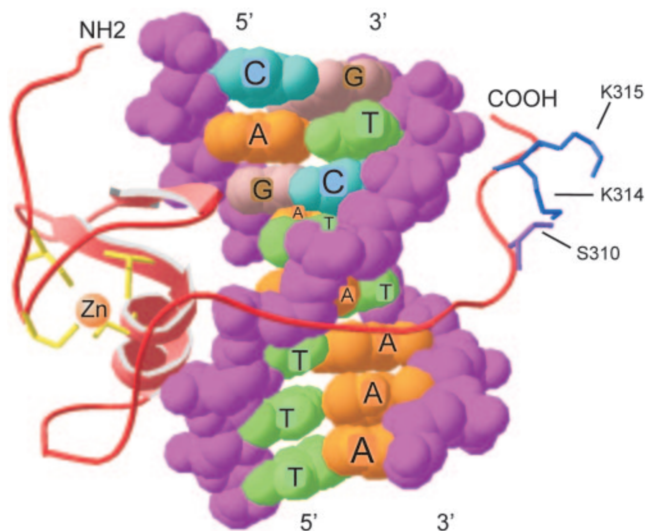

FIG. 3. Three-dimensional (3D) representation of the C-terminal finger of chicken GATA1 bound to DNA. The figure was prepared using the file 2GAT.pdb (101) and Swiss-pdb viewer software (http://www.expasy.org/spdbv/) (33). The 3D structure of the 66-aa peptide is displayed as a ribbon (red). The sequence of this peptide is >90% identical to residues 252 to 317 in human and mouse GATA1. Side chains of the four Cys residues (yellow) chelating the zinc atom (orange sphere), one residue of the phosphorylation site (S310; purple), and two residues of the critical acetylation site (K314 and K315; dark blue) are shown. Numbering is according to the homologous residues in human and mouse GATA1. DNA residues (5′-TTTATCTG-3′ and 5′-CAGATAAA-3′) are labeled and color coded. The C-terminal extension of the zinc-finger makes extensive contacts with the minor groove of the DNA (78). The side chains of S310, K314, and K315 point away from the DNA, suggesting that they might be accessible to other proteins when GATA1 is bound to DNA.

GATA1 protein are essential for its transcriptional activation activity (60). Surprisingly, in another study (123) this transactivation domain appeared to be dispensable for GATA1-mediated terminal erythroid differentiation.

To examine the function of each of the three GATA1 domains in a more robust way, Shimizu and colleagues (95) made use of transgenic rescue of GATA1.05 knockdown mice. By analysis of the offspring resulting from the crossing between GATA1.05 mice and transgenic mice expressing different GATA1 mutants, the requirements for the different functional domains were unraveled. In agreement with the previous reports, the C-finger was found to be indispensable for GATA1 function in both primitive and definitive erythropoiesis, but the N-finger was found to be necessary only for definitive erythropoiesis. Like the N-finger, the transactivation domain appeared to have different functions in primitive and definitive erythropoiesis. When expressed at levels higher than that of the endogenous GATA1, the transactivation domain mutant can sustain both primitive and definitive erythropoiesis, but when expressed at lower levels, definitive erythropoiesis is impaired. From these data it can be concluded that all three GATA1 domains are required for definitive erythropoiesis. For primitive erythropoiesis, GATA1 lacking either the N-terminal transactivation domain or the N-finger suffices. This demonstrates that the primitive and definitive erythroid lineages have distinct requirements for GATA1.

REGULATION OF GATA1 ACTIVITY

GATA1 activity in vivo is tightly regulated. Increasing GATA1 activity can lead to phenotypes as strong as embryonic lethality (see, e.g., reference 125). The activity of proteins can be regulated by a wide variety of mechanisms. The mechanisms thought to be involved in the regulation of GATA1 activity is discussed in this section.

Transcriptional regulation. The *GATA1* transcription unit is composed of two alternative untranslated first exons, IT and IE (46), and five translated exons, II to VI (106). Exon IT is primarily used in Sertoli cells of the testis, while exon IE is used in hematopoietic cells (Fig. 4) (46). The proteins expressed in hematopoietic and Sertoli cells are identical, since exon II harbors the translation start site. The two zinc-finger motifs are encoded separately in exons IV and V.

The testis promoter and exon IT are located 8 kb upstream of exon IE. Disruption of the erythroid promoter leads to an arrest in primitive erythropoiesis without affecting the expression from the testis promoter (100). Both testis- and erythroid-specific promoters contain GATA sites that are required for the proper functioning of the promoter (46, 80, 114), suggesting a possible feedback loop (106). Both *GATA1* promoters lack an obvious TATA box (37, 106).

DNase I hypersensitivity analysis of the *GATA1* locus in erythroid cells identified three main hypersensitive (HS) regions. HS1 is located between 3.9- and 2.6kb upstream of IE, HS2 corresponds to the region surrounding the IE promoter, and HS3 is localized in intron 1 (Fig. 4) (62, 110).

Transcription of the *GATA1* gene in different hematopoietic lineages has different regulatory sequence requirements. HS1, coinciding with the *GATA1* hematopoietic enhancer, can drive reporter gene transcription exclusively in primitive erythrocytes whereas together with intron 1 this element can drive expression of a reporter gene in both primitive and definitive erythroid cells (79). Furthermore, *GATA1* transcription in ery-

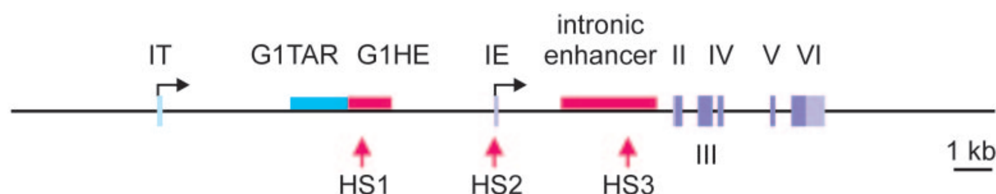

FIG. 4. The mouse *GATA1* locus. The exon-intron structure of the mouse *GATA1* gene is displayed, and the positions of known regulatory elements (bars) and hematopoietic DNase I HS sites (red arrows) (36, 63) are shown. Translated sequences are in dark purple. IT, testis-specific exon 1; IE, hematopoietic-specific exon 1; G1TAR, *GATA1* testis-activating region; G1HE, *GATA1* hematopoietic enhancer.

throid and megakaryocytic cells has different sequence requirements within HS1 (97, 114). Expression in both lineages is dependent on the presence of an intact GATA site, but megakaryocytic expression requires the 3′ end of HS1, which is dispensable for erythroid expression. Surprisingly, deletion of HS1 does not affect the expression of *GATA1* in eosinophils, suggesting that the intron 1 enhancer/HS3 is essential for this function (36).

Translational regulation. GATA1 possesses an alternative translation initiation site located at methionine 84 (8). Translation from this site gives rise to a 40-kDa protein, GATA1s, which lacks 83 amino acids at the N-terminal region, i.e., the N-terminal transactivation domain (Fig. 2). GATA1s can be detected in MEL and K562 cells as well as in mouse tissues, but its expression level is much lower than that of full-length GATA1. The GATA1s protein shows normal DNA binding activity but a reduced transactivation potential, which is in agreement with the reported role of the N terminus as a transactivation domain (60).

Although GATA1 mutants lacking the N-terminal transactivation domain can rescue the GATA1.05 knockdown phenotype when expressed at high levels (95), it can be concluded that GATA1s is not fully functional and therefore is unable to drive terminal erythroid and megakaryocytic differentiation when expressed at normal levels.

Posttranslational regulation. (i) Acetylation. GATA1 can be acetylated both in vitro and in vivo by the ubiquitously expressed acetyltransferases P300 (6) and CREB-binding protein (CBP) (43). Mouse GATA1 is acetylated at two conserved lysine-rich motifs (amino acids 245 to 252 and 308 to 316) localized just C terminal from each of the zinc fingers (Fig. 2 and 3). These motifs are conserved among members of the GATA family and between different species.

The functional importance of GATA1 acetylation is not clear. The interaction between GATA1 and P300/CBP and, consequently, acetylation of the transcription factor appears to stimulate its transcriptional activity (5, 6). Boyes et al. reported that in chicken GATA1, acetylation increased DNA-binding activity (6), but Hung et al. did not see this effect with mouse GATA1 (43). Acetylation of GATA1 appears to be required for the in vitro differentiation of the GATA1 null cell line G1E (43). Furthermore, an acetylation mutant of GATA1 was severely impaired in its ability to rescue the phenotype of the GATA1 mutation *vlad tepes* in the zebra fish, which was attributed to the reduced ability of the acetylation mutant to self-associate (76).

(ii) Phosphorylation. GATA1 can be phosphorylated at seven serine residues (16). Six of these residues (S26, S49, S72, S142, S178, and S187), situated at the N terminus of the protein, are phosphorylated in uninduced MEL cells. The seventh serine (S310), which is located near the DNA-binding domain (Fig. 3), only becomes phosphorylated upon dimethyl sulfoxide induction of the MEL cells. This suggests a possible role of phosphorylation in both DNA binding and transcriptional activity of the protein. Surprisingly, substitution of the serines for alanines did not have any consequence in GATA1 DNA binding or transcriptional activity (16). Another somewhat contradictory report shows that the phosphorylation of GATA1, in induced K562 cells, increases DNA binding (84). The same report confirms that such an increase in DNA binding does not occur in induced MEL cells and suggests that GATA1 is already phosphorylated in uninduced MEL cells whereas in K562 cells GATA1 becomes phosphorylated upon induction, which leads to an increased DNA binding activity of the protein. Recently, it has been demonstrated that mitogen-activated protein kinase signaling has a role in the control of GATA1 phosphorylation and that GATA1 is phosphorylated in response to cytokine-induced signaling in factor-dependent hemopoietic progenitor cells (103). This study identified extracellular signal-regulated kinase as an in vivo GATA1 kinase.

(iii) SUMOylation. The small ubiquitin-related modifier (SUMO) is a ubiquitin-like peptide that can be ligated to a lysine residue of the target protein. A number of transcription factors can be modified by SUMOylation (111). This reversible modification is thought to be associated with transcriptional repression (94). The consensus site for SUMOylation (I/L/V)KXE is present at K137 of mouse GATA1 (Fig. 2). This motif can be modified by SUMOylation through the action of the SUMO ligase PIASy (13). The functional significance of this modification remains unknown.

Protein degradation. Another possible regulatory mechanism for protein activity in general is degradation. One hypothesis is that levels of GATA1 activity must be high at early stages of erythroid differentiation but must be downregulated for terminal erythroid differentiation to occur (125). This implies that protein degradation is a potentially important regulatory mechanism for GATA1 function.

GATA1 degradation via caspase-mediated cleavage has been reported previously (18). This study shows that activation of caspases, via death receptors, leads to an arrest in terminal erythroid differentiation. The authors attributed the differentiation arrest to a decrease in GATA1 protein levels due to caspase-mediated cleavage, since expression of a caspase-resistant GATA1 mutant, but not that of wild-type GATA1, restored erythroid differentiation.

From the observation that the Fas death receptor is expressed throughout erythroid differentiation but its ligand, FasL, is only expressed in the more mature erythroblasts a model emerges in which mature erythroblasts participate in a negative-feedback loop to attenuate differentiation of earlier erythroid progenitors.

Intriguingly, we have shown that overexpression of GATA1 in erythroid cells, both in vitro (127) and in vivo (125), inhibits erythroid differentiation. On the basis of these observations a slightly different model can be envisaged: GATA1 degradation by caspases leads to a reduction of GATA1 levels at late stages of erythroid differentiation, thereby allowing terminal differentiation (Fig. 5). We have found that in the presence of high levels of GATA1, erythroid cells fail to differentiate but, surprisingly, that if wild-type erythroid cells are present, the overexpressing cells can differentiate normally (125). Further analysis showed that differentiating erythroid cells can signal to GATA1-overexpressing erythroid cells, which are normally blocked in differentiation, to terminally differentiate (35). This might involve activation of death receptors present in differentiating cells by ligands produced by terminally differentiating erythroid cells, promoting caspase-mediated cleavage of GATA1. Such a homotypic signaling mechanism could ensure that the production of mature erythrocytes is in keeping with demand, because the abundance of terminally differentiating

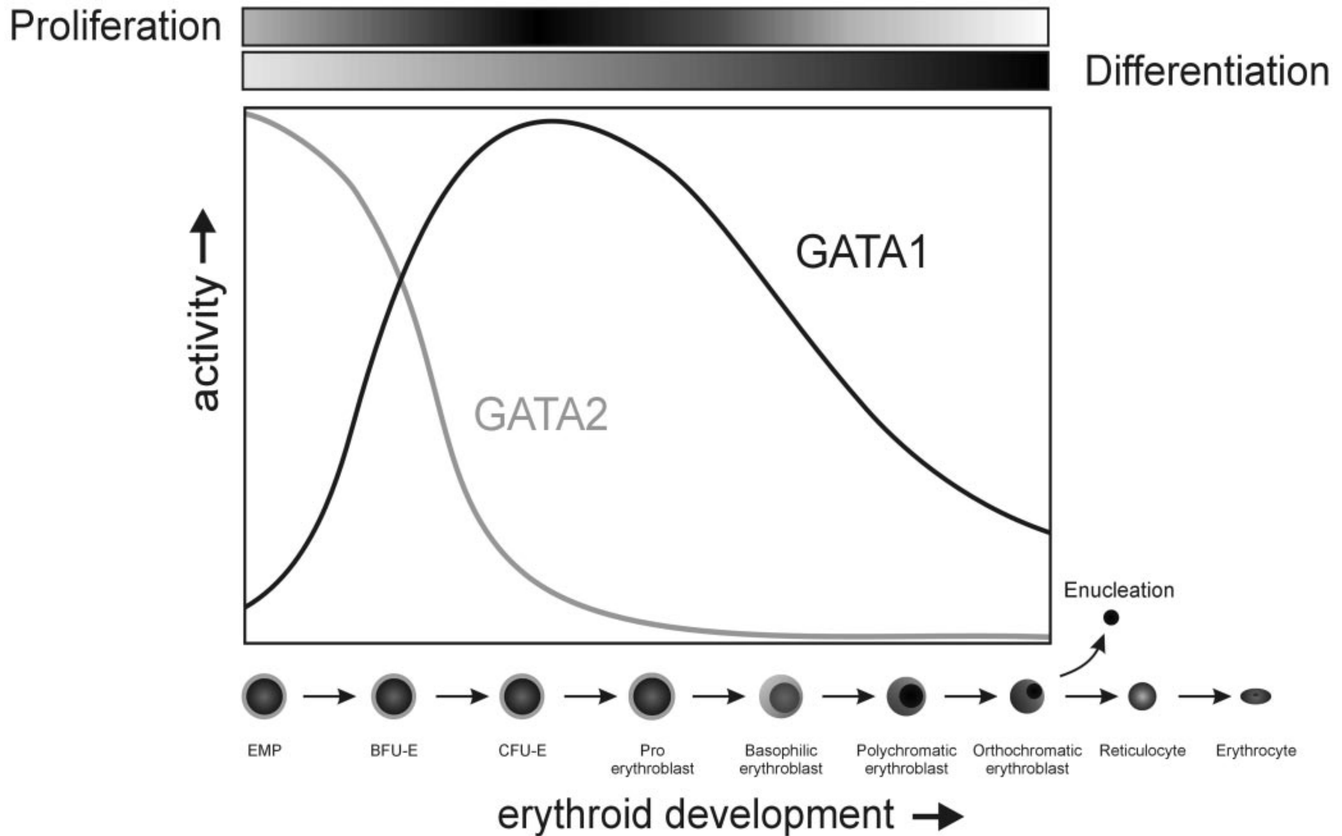

FIG. 5. Model for the dynamic regulation of GATA1 and GATA2 activity during erythropoiesis. GATA2 is expressed at high levels in early erythroid progenitors. When GATA1 is activated, GATA2 is repressed and GATA1 levels increase, possibly through cross-talk between GATA1 and GATA2. During terminal erythroid differentiation, GATA1 is downregulated via an autonomous cell signaling mechanism that might involve death receptors and/or ligands.

cells would show an inverse correlation with the expansion of erythroid progenitors.

PROTEIN-PROTEIN INTERACTIONS

GATA1 is now known to interact with a variety of proteins, either cofactors or transcription factors. These interactions play important roles in hematopoiesis, since they lead to transcriptional activation or repression of GATA1 target genes. The most important interactions between GATA1 and other proteins known to date are reviewed in this section.

GATA1. It has been shown that GATA1 can self-associate in vitro (15), both in solution and when bound to DNA. Both the C-finger and the N-finger can independently associate with full-length GATA1 protein, since the interaction is mediated by N-finger–C-finger contacts (58). GATA1 dimerization may play an important role in the regulation of promoters containing multiple GATA sites, since mutation of particular residues in the finger regions reduces the GATA1 transactivation potential in reporter assays (58). Furthermore, GATA1 dimerization was shown to be important for the positive regulation of the GATA1 promoter in zebra fish (76).

Other potential functions for GATA1 dimerization may be to establish contact between promoters and enhancers and to recruit chromatin-remodeling complexes.

Friend of GATA1 (FOG, FOG-1, or ZFPM1). FOG-1, a protein containing nine widely spaced zinc fingers, was identified in a yeast two-hybrid screening as a GATA1 cofactor (108). It binds to the N-terminal zinc finger of GATA1 mainly via its zinc finger 6 (23), although fingers 1, 5, and 9 also contribute to the binding (24). FOG-1 is coexpressed with GATA1 in the erythroid and megakaryocytic lineages and cooperates with GATA1 during erythroid and megakaryocytic differentiation (108).

The phenotype of FOG-1 null mice closely resembles the GATA1 knockout phenotype. Mutant mice of both lineages die during midgestation from severe anemia caused by a defect in primitive and definitive erythropoiesis, suggesting that FOG-1 is essential for GATA1 function. In contrast to GATA1 deficiency, however, loss of FOG-1 leads to a complete elimination of the megakaryocytic lineage, revealing a GATA1-independent role of FOG-1 in megakaryopoiesis (Fig. 1) (107). Definitive proof that the FOG-1/GATA1 interaction is essential for GATA1 function during erythroid differentiation was obtained by the analysis of GATA1 mutants defective in FOG-1 binding and subsequent identification of compensatory mutations in the FOG-1 (14). Erythroid precursors expressing GATA1 mutants unable to bind FOG-1 fail to differentiate, but this phenotype is rescued by the expression of the FOG-1 mutants that can bind these GATA1 mutant proteins.

Although it has no apparent DNA-binding activity, FOG-1 is known to differentially modulate GATA1 activity depending on the promoter context. It synergizes with GATA1 in the activation of certain promoters (108) while repressing GATA1-mediated activation of other promoters (24). A recent paper suggests that FOG-1 is rapidly induced by GATA1 in erythroid cells (124). This induction is independent of protein synthesis. Together with the observation that GATA-1 binds to the *FOG-1* locus in vivo at a putative enhancer, these data suggest that the *FOG-1* gene is activated directly by GATA1. In contrast, protein synthesis appeared to be required for the activation of β-*globin* transcription. Thus, a model emerges in which GATA1 first induces FOG-1, after which both factors cooperate in the activation of the β-*globin* locus.

RB. The tumor suppressor protein retinoblastoma (RB) plays important roles in many stages of the differentiation process, including regulation of progenitor proliferation, terminal cell cycle exit, induction of tissue-specific gene expression, and protection from apoptosis (57). Mice deficient for RB are embryonically lethal and show neuronal and erythroid defects (Fig. 1) (48, 54). An intrinsic role for RB in erythropoiesis is further supported through the use of use of an in vitro erythroid differentiation culture system (11). GATA1 has been shown to bind RB. Furthermore, GATA1 overexpression in MEL cells leads to RB inactivation through hyperphosphorylation via an as-yet-unknown mechanism (127).

Krüppel-like factors. GATA1 can physically interact and functionally synergize with Sp1 and erythroid Krüppel-like factors (EKLF or KLF1) (31, 66), two transcription factors belonging to the SP/KLF family of transcription factors (reviewed in reference 89).

Sp1 is a ubiquitously expressed transcription factor essential for early embryonic development. Sp1 null embryos die around E9.5 and show a broad range of abnormalities, but transcription of embryonic *globin* genes is activated (59). In contrast, EKLF is an erythroid-specific transcription factor (69). EKLF null mice are embryonic lethal due to a defect in definitive erythropoiesis (Fig. 1) (77, 85). These embryos succumb to fatal anemia owing to a defect in hemoglobin accumulation, explained by the EKLF requirement for β-*globin* expression (77, 85).

The fact that Sp1 and EKLF can recognize GC and/or CACC motifs, which are found in the close proximity to GATA motifs in several promoters, enhancers, and locus control regions (LCRs), suggests a functional cooperation between these proteins. GATA1 was shown to bind the zinc-finger domain of Sp1 and EKLF mainly via its C-finger, and reporter assays demonstrated that GATA1 transcriptional activity can be synergistically increased by these interactions (31, 66). The interaction between GATA1 and either Sp1 or EKLF is dependent on the presence of the promoter (31), suggesting discrete roles for these two factors in the regulation of erythroid-specific genes. *EKLF* is also a GATA1 target gene: three GATA binding sites were identified in the *EKLF* promoter, and one of them appears to be crucial for initiation of transcription (17). Moreover, forced expression of GATA1 can activate the *EKLF* promoter in nonerythroid cells, and EKLF expression is downregulated in the absence of GATA1 (125) and restored upon its reintroduction (96).

Interaction between GATA1 and Sp1 or EKLF may play a crucial role in, for example, bringing regulatory elements such as enhancers and LCRs in close proximity to promoters by promoting the formation of DNA loops. Recently, the formation of a complex containing the β-*globin* LCR and the promoters of the actively transcribed β-*globin* gene, the active chromatin hub (ACH), has been demonstrated previously (9, 102). Furthermore, the presence of the transcription factor EKLF is crucial for the formation of the ACH (19). It is conceivable that GATA1 in conjunction with EKLF also plays a role in the formation of the ACH.

Lmo2/Ldb1/Tal-1/E2A. GATA1 is found in a complex together with Lmo2, Ldb1, Tal-1, and E2A that can activate transcription from promoters containing an E-box (CANNTG consensus sequence) and a GATA binding site separated by nine nucleotides (116). The GATA-E-box is present in the promoters of several genes (1, 12, 116), suggesting an important role for this motif. In the hematopoietic system, Tal-1 expression, driven by promoter Ia, is restricted to erythroid, megakaryocytic, and mast cell lineages (71a). The similarities between Tal-1 and GATA1 expression patterns and the presence of GATA consensus sequences in the *Tal-1* promoter Ia suggested regulation of Tal-1 expression by GATA1. More detailed analysis of the promoter requirements for Tal-1 expression confirmed that GATA1 could activate its transcription (53a).

GATA1 interacts directly with Lmo2, but not with Tal-1, in erythroid cells (82, 83). These authors also showed that GATA1 and Tal-1, in the presence of Lmo2, synergistically activate transcription of reporter genes (83). From these data, a model was proposed in which GATA1 and the Tal-1-E2A complex bind DNA (109, 115). Lmo2 makes the bridge between the transcription factors, either as a single molecule or by homodimerization. The role of such a complex in hematopoiesis is not known, but, considering the similar functions of the proteins in hematopoiesis, it is likely that this complex is of extreme importance (Fig. 1) (68, 72, 118). This complex binds to the GATA-E-box motif in the upstream regulatory sequence (HS1) (Fig. 4) of the *GATA1* gene, and the integrity of the GATA binding site is crucial for binding. However, the functional importance of these interactions remains unknown (114).

PU.1. PU.1 is a member of the *Ets* family of transcription factors required for the development of the lymphoid and granulocytic-monocytic lineages (Fig. 1). Expression of PU.1 and GATA1 appears to be mutually exclusive, suggesting an antagonistic effect of these two transcription factors.

Several lines of evidence indicate that GATA1 and PU.1 functionally antagonize each other via direct physical interaction (74, 91, 134). PU.1 and GATA1 interaction takes place via the DNA-binding domains of both proteins (91, 134), but the mechanisms by which these transcription factors antagonize each other are quite distinct. GATA1 inhibits PU.1 by preventing it from interacting with its cofactor c-Jun (134), while PU.1 inhibits GATA1 by preventing its binding to DNA (135).

P300/CBP. P300 and CBP are ubiquitously expressed proteins with histone acetyltransferase properties, known to interact with a large number of transcription factors. The mechanism by which P300 and CBP intervene in transcription regulation is not clear, and several models have been proposed. Binding of P300/CBP to transcription factors can be a way to

recruit histone acetyltransferase to the vicinity of nucleosomes and induce an open chromatin configuration, thus stimulating transcription. CBP and P300 can also serve as a bridging molecule between components of the general transcription machinery and enhanceosome complexes (4). Furthermore, P300 and CBP are known to be responsible for the acetylation of transcription factors which may have a direct effect on their function (reviewed in reference 112). Indeed, mice homozygous for point mutations in the KIX domain of p300, disrupting the binding surface for the transcription factors c-Myb and CREB, display multilineage defects in hematopoiesis (49). It remains to be determined whether the interactions between GATA1 and CBP/P300 are of similar importance.

GATA1 binds P300 (6) and CBP (43) both in vitro and in vivo, and P300 and CBP acetylate GATA1 (Fig. 2 and 3) (6, 43). The involvement of P300/CBP in the recruitment of other transcription factors, cofactors, or chromatin-remodeling complexes to regulatory sequences bound by GATA1 may be envisaged.

PIASy. The SUMO ligase PIASy interacts with GATA1 through recognition of amino-acid residues 136 to 139 (LKTE) of mouse GATA1 (Fig. 2) (13). The functional consequences of these interactions, and of SUMO modification of GATA1, are unknown.

GATA1 TARGET GENES

GATA sequences are quite abundant in the genome, and GATA consensus sequences can be found in the promoters of many genes. An increasing number of GATA1 target genes are being identified using functional assays and, more recently, high-throughput gene expression analysis afforded by DNA microarrays. In addition to those discussed in the section Protein-protein Interactions, a short overview of the most relevant of the growing number of known GATA1 target genes is given.

α- and β-globins. GATA1 was first identified by its interaction with the β-*globin* gene enhancer and was soon shown to bind to multiple regulatory regions in both the α- and β-*globin* loci (22, 60, 117). Despite the absence of GATA1, GATA1 null erythroid cells are still able to produce hemoglobin, suggesting that GATA1 does not play a critical role in the transcription of the *globin* genes (120). Another possibility is that GATA1 is replaced by GATA2, which is known to be upregulated in GATA1 null cells, since GATA binding sites are essential for expression of β-LCR-driven transgenes (88).

Heme biosynthesis enzymes. Hemoglobin, present in large quantities in erythrocytes, is the protein responsible for the transport of oxygen and carbon dioxide throughout the body. Hemoglobin is a tetrameric protein composed of two α-like and two β-like *globin* chains. Each *globin* chain carries a heme group, a ring-shaped molecule containing a central iron atom, which can reversibly bind oxygen. Heme is synthesized from glycin and succinyl coenzyme A via a series of steps involving the action of eight enzymes (reviewed in reference 90). Several of the genes encoding the enzymes involved in heme biosynthesis (*ALA-S*, *ALA-D*, *PBG-D*) are known GATA1 target genes (81, 93). The GATA1 target gene *ABCme*, encoding a mitochondrial transporter enzyme, is thought to mediate mitochondrial transport functions related to heme biosynthesis (96), emphasizing the important and broad function of GATA1 in erythroid cells.

Epo and EpoR. Erythropoietin (Epo) is the major growth factor for erythroid cells. Epo interacts with the Epo receptor (EpoR), a cell surface receptor expressed in erythroid, megakaryocytic, and mast cells, triggering signaling cascades leading to the proliferation, differentiation, and survival of erythroid progenitors (reviewed in reference 53).

GATA1 was first reported to be involved in the regulation of the *EpoR* gene by Zon and colleagues (139). These authors showed that GATA1 could specifically bind and transactivate the *EpoR* promoter. A second report (10) also shows that GATA1 is expressed prior to the EpoR but that its expression is strongly enhanced by EpoR-mediated signals. Surprisingly, Weiss and colleagues (120) showed that *EpoR* is normally transcribed in GATA1 null erythroblasts, strongly suggesting a role for GATA2 in EpoR expression in early erythroid precursors.

In contrast to *EpoR* regulation results, GATA proteins negatively regulate *Epo* expression by binding to GATA sites in its promoter (44, 45).

Bcl-X_L. It has been hypothesized that GATA1, in collaboration with Epo, can act as a survival factor during erythroid differentiation. The mechanism by which that is accomplished is not known, but Bcl-x, a member of the Bcl2 gene family, is a good candidate to mediate survival during erythroid development. Due to alternative splicing the *Bcl-X* gene can produce two distinct mRNAs: Bcl-X_L, a larger mRNA that codes for a protein with antiapoptotic properties, and Bcl-X_S, a smaller mRNA that, surprisingly, codes for a proapoptotic protein. Bcl-X_L expression increases in late stages of erythroid differentiation and appears to be dependent on the presence of Epo (30, 71, 98).

On the basis of these observations, Gregory and colleagues (32) analyzed the expression of Bcl-X_L during erythroid differentiation in G1E GATA1 null erythroid progenitors rescued by the expression of GATA1. These data show that Bcl-X_L is upregulated in a GATA1-dependent manner during erythroid differentiation. This suggests that GATA1, together with Epo, prevents apoptosis in differentiating erythroid cells by promoting the expression of antiapoptotic proteins such as Bcl-X_L. A direct interaction between GATA1 and the *BCL-X* promoter has yet to be demonstrated.

Hematopoietic transcription factors. (i) GATA2. GATA2 is an important regulator of hematopoiesis, its downregulation being crucial for hematopoietic differentiation. The first clue about a possible regulation of the *GATA2* gene by GATA1 was the observation that GATA2 is upregulated in the absence of GATA1 (120). Further analysis of GATA1-regulated genes consistently identified *GATA2* as being repressed by GATA1 (93, 96), but the mechanism through which repression is achieved remains unknown.

In a recent study (29), GATA1 was reported to bind to a region 2.4 kb upstream of the *GATA2* 1G promoter. The same report also shows that GATA2, together with CBP, can bind to the same regions as GATA1 in its absence and that displacement of GATA2 by GATA1 is the cause of repression (Fig. 5). This result suggests a mechanism by which GATA1 directly represses GATA2: GATA2, when bound to the kb −2.8 site of its own locus, recruits CBP to this region, leading to histone

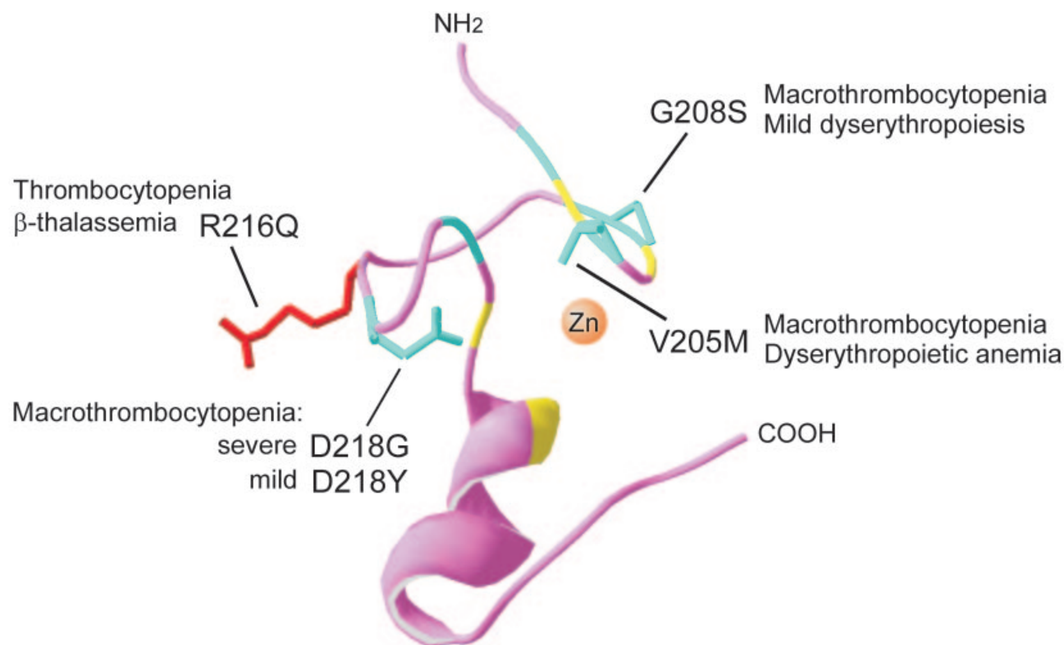

FIG. 6. Mutations in the N-terminal finger of GATA1 causing human disease. The figure was prepared using the file 1GNF.pdb (51) and Swiss-pdb viewer software (http://www.expasy.org/spdbv/) (33). The 3D structure of A201 to P240 of human and mouse GATA1 is displayed as a ribbon (pink); the zinc atom is displayed as an orange sphere. The positions of the zinc-chelating Cys residues on the ribbon are indicated in yellow; the positions of residues involved in the interaction with FOG-1 are in blue (23). Side chains are shown for the residues mutated in patients. Blue, the mutations V205 M (75), G208S (64), D218G (25), and D218Y (26) interfere with FOG-1 binding; red, the mutation R216Q (133) interferes with DNA binding.

acetylation and, consequently, activation of transcription. GATA2 displacement by GATA1 leads to a loss of CBP and the establishment of a closed chromatin configuration. The displacement of GATA2 by GATA1 during erythroid differentiation has also been reported to occur at the α-*globin* locus (2); in this case, expression of the α-*globin* genes is activated after binding of GATA1.

(ii) MaFK and p45 NF-E2. In addition to the results seen with other megakaryocyte-specific genes, the expression of the transcription factors MafK and p45 NF-E2 is significantly decreased in megakaryocytes expressing an N-finger mutant of GATA1 (V205G) and in GATA1-deficient megakaryocytes (96, 116). p45 NF-E2 p45 and small Maf factors are critical for terminal differentiation of megakaryocytes (71, 100). This suggests that the attenuated expression of the essential transcription factors NF-E2 p45 and MafK is a major cause of the megakaryocytic phenotype of GATA1 mutations.

Cell cycle core components and proliferation-related genes. Overexpression studies have assigned a function to GATA1 in G_1/S cell cycle progression (20, 127). Recently, Rylski and colleagues (93) have reported the identification of core cell cycle components as target genes of GATA1. The GATA1 null erythroid cell line G1E can be induced to differentiate by reintroduction of GATA1 (123). The authors used this model system to analyze the influence of GATA1 on the transcription of genes involved in activation and inhibition of cell cycle progression. GATA1 appeared to repress the expression of core cell cycle proteins such as *Cyclin D2* and *Cdk6* and activate transcription of cell cycle progression inhibitors such as *p18*^{INK4c} and *p27*^{Kip1}. Furthermore, GATA1 induced expression of growth inhibitors, including Btg2, *Hipk2*, *JunB*, and *Crep*, and downregulated the expression of genes with mitogenic properties such as *Myc*, *Myb*, and *Nab2*.

The experiments described above were not able to distinguish between a direct GATA1 target gene and genes that are differentially expressed due to secondary effects. To clarify this issue, the authors performed a more detailed analysis of the interactions between Myc and GATA1 and showed that GATA1 represses transcription of the *Myc* gene by directly binding to its promoter. Surprisingly, forced expression of Myc inhibited the GATA1-induced cell cycle arrest but not erythroid maturation. This suggests that Myc may be involved in the transcriptional control of cell cycle genes such as *Cyclin D2* and *p18*^{INK4c} and *p27*^{Kip1} but does not interfere with the control of genes involved in erythroid differentiation.

GATA1 MUTATIONS IN HUMAN DISEASE

Mutations in the N-terminal transactivation domain and the N-finger of GATA1 have been linked to human disease (Fig. 2 and 6). Acquired mutations in GATA1 are a hallmark of the transient myeloproliferative disorder (TMD) that occurs in ~10% of newborn children with constitutional trisomy 21 (Down syndrome) (119). In ~20% of the TMD cases, this is followed by Down syndrome-related acute megakaryocytic leukemia (DS-AMKL) later in life. The large majority of the mutations found introduce a premature stop codon in the N-terminal transactivation domain of GATA1, but splice site mutations also occur (reviewed in reference 34). These mutations result in the exclusive translation of the GATA1s iso-

form, lacking the N-terminal transactivation domain (Fig. 2). This truncated GATA1 protein has diminished transactivation potential in in vitro assays (119). Investigations of the GATA1.05 knockdown mutation in the mouse have demonstrated that reduced GATA1 activity prevents differentiation of the precursor cells but allows their survival. Interestingly, female mice heterozygous for the knockdown mutation develop a myeloproliferative disorder at high frequency (99). Together, these data strongly suggest that reduced GATA1 activity is an early event in the pathogenesis of DS-AMKL, allowing the expansion of TMD blast cells from which DS-AMKL develops after the apparent spontaneous remission.

Missense mutations in the N-finger of GATA1 have been found in patients with X-linked thrombocytopenia and anemia (3, 25, 26, 64, 75, 133). The majority of these mutations affect the FOG 1 interaction surface of the N-finger (Fig. 6), adversely affecting the binding of FOG-1 to the N-finger mutants (3, 25, 26, 64, 75, 133). This further emphasizes the importance of the FOG-1–GATA1 interaction. One mutation, R216Q, displays normal FOG-1 interaction. Compared to wild-type GATA1 results, this mutant binds with comparable affinity to single GATA sites but with decreased affinity to palindromic sites (133). This indicates that the DNA binding properties of the N-finger contribute to the overall function of GATA1. The severity of disease depends on the particular mutation: D218G results in macrothrombocytopenia and mild dyserythropoietic features but no marked anemia, while D218Y is a more severe mutation resulting in deep macrothrombocytopenia, marked anemia, and early mortality (26). These phenotypic differences correlate well with the stronger loss of affinity of the D218Y mutant for FOG1 binding compared with that seen with the D218G mutant.

Thus far, only a few mutations in GATA1 causing human disease have been described. This likely reflects the lethality of mutations affecting the function of GATA1 more severely, as can be deduced from the studies in the mouse. Nevertheless, the mutations in the N-terminal transactivation domain and N-finger have revealed novel and sometimes unexpected aspects of GATA1 function, opening up new directions for future research such as the elucidation of the molecular mechanisms leading to leukemogenesis in Down syndrome.

GATA1: THE FUTURE LOOKS BRIGHT

The interactions with cofactors and/or regulatory complexes are important parameters in transcription factor regulation. A variety of proteins have already been identified as interaction partners of the GATA transcription factors. These proteins include other transcription factors, non-DNA binding cofactors, chromatin-remodeling factors, and proteins involved in cell cycle regulation. The interactions with each of these proteins were identified individually; such procedures are laborious and overall reveal only a small amount of information. Recently a new one-step purification technique for isolation of protein complexes was developed. This technique consists in tagging the protein of interest with a small peptide that is specifically recognized by the *Escherichia coli* biotin ligase BirA (17). The system enables one-step purification of the in vivo biotinylated protein and its interacting partners through binding to streptavidin-coated beads. Purified proteins can then identified by mass spectrometry. By use of this method several new GATA1-interacting proteins were identified, in addition to previously described interaction partners (P. Rodriguez, E. Bonte, J. Krijgsveld, K. Kolodziej, B. Guyot, A. J. R. Heck, P. Vyas, E. de Boer, F. Grosveld, and J. Strouboulis, submitted for publication). The interacting proteins are components of well-characterized complexes involved not only in transcriptional activation but also in repression. Genome-wide chromatin immunoprecipitation analysis will help to clarify the function of these distinct complexes at GATA1 target loci during erythroid development (2, 7). In addition, it will be interesting to isolate complexes with, for example, GATA1 mutants defective in particular posttranslational modifications. The comparison between complexes identified with wild-type and mutant GATA1 proteins may reveal specific functions of a particular posttranscriptional modification. Because there are many more elusive aspects of GATA1 biology, we expect that GATA1 will indeed remain in the floodlight of modern biology, as a paradigm for hematopoietic transcription factors in general and GATA factors in particular.

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