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Review

Regulation of the transcription factor GATA-1 at the gene and protein level

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Abstract. GATA-1, an important hematopoietic transcription factor, plays a critical role in differentiation and maturation of erythroid and megakaryocytic cell lines. GATA-1 appears to serve as a factor for virtually all characterized erythroid and megakaryocytic-expressed genes. Thus, defining the mechanisms by which the GATA-1 gene and protein are regulated should provide important clues regarding the establishment of erythroid and megakaryocytic programs of gene expression in committed cells and their maintenance thereafter in maturing precursors. This review focuses on the regulation of GATA-1 expression and elucidates the regulation of GATA-1 at the gene and protein levels. Such research is expected to provide insights into the mechanisms involved in hematopoietic commitment.

Key words. GATA-1; regulation; erythroid; megakaryocytic; development.

Introduction

Members of the GATA family display distinct but overlapping patterns of expression in primitive and definitive erythoid cells, and also in nonhematopoietic tissues [1]. This family is characterized by recognition of the W(A/T)GATAR(A/G) motif. Moreover, their DNA-binding domains include highly conserved homologous zinc fingers Cys-X_2-Cys-X_{17}-Cys-X_2-Cys [2]. In vertebrates, this domain is composed of two similar zinc fingers, whereas nonvertebrate members include the two-fingered *Caenorhabditis elegans* protein elt-1 and several single-finger fungal factors which also bind GATA motifs.

There are six members in this family: GATA-1, GATA-2, and GATA-3 are essential hematopoietic transacting factors, while GATA-4, GATA-5, and GATA-6 are regulators of heart, lung, and gut cell development. GATA-1, the founding member of the family, was first identified as an erythroid nuclear protein (named Eryfl) in chicken erythroid cells by Evans et al. [2]. It is expressed at high levels in mature erthroid cells, mast cells, and megakaryocytes, and at lower levels in multipotent progenitor cells, and in Sertoli cells of testis in young mice [3]. Gene-targeting experiments showed that GATA-1 is essential for the maturation of both erythroid and megakaryocytic precursors. In GATA-1-negative erythroid precursors, cellular maturation is accompanied by apoptosis [4, 5], whereas in megakaryocytic precursors, deficiency is associated with profound hyperproliferation [6]. Thus, GATA-1 controls aspects of cellular growth and death. To date, the GATA consensus sequence has been found in various regulatory regions: promoters of genes expressed in erythroid, megakaryocytic, mast, and endothelial cells; globin gene enhancer and T cell receptor α- and δ-chain gene enhancers (regulated by GATA-3), and β-globin locus control regions (LCRs). It has been shown to participate in the transcriptional regulation of virtually all erythroid and megakaryocytic-expressed genes examined [6, 7].

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Since GATA-1 appears to regulate many erythroid and megakaryocytic-expressed genes, defining the mechanisms by which the GATA-1 gene and protein are regulated should provide important clues regarding the establishment of erythroid programs of gene expression in committed cells and their maintenance thereafter in maturing precursors.

Cis-elements of the GATA-1 gene

The GATA gene is located on the X chromosome. The murine GATA-1 gene comprises six exons distributed over 8 kb. Exon I is noncoding and followed by a relatively large intron of 4.3 kb. The initiator codon for the mature protein is contained in exon II. The two homologous zinc finger domains of the protein are encoded separately in exons IV and V [8] (fig.1).

Cis-elements in the GATA-1 gene promoter

GATA-1 is expressed from two alternative promoters or noncoding first exons, designated IT or IE. The distal (IT) promoter specifies the expression of the GATA-1 gene in Sertoli cells [9], whereas the proximal (IE) promoter, located between the IT exon and the common coding exons, directs GATA-1 gene expression in the hematopoietic lineages [8].

Transient transfection experiments in murine erythroleukemic cells but not 3T3 fibroblasts showed that the isolated 900-bp promoter 5′ to exon IE retains the cell specificity exhibited by the intact gene, suggesting that the promoter region contributes substantially to the cell specificity of GATA-1 expression. Full promoter activity requires the presence of two proximal CACCC box sequences separated by 17 bp and an upstream, double GATA motif that binds a single GATA-1 molecule in an asymmetric fashion [8].

Deletion experiments showed that a palindromic GATA sequence (GATApal) in the GATA-1 gene promoter, composed of one complete [(A/T)GATA(A/G)] and one partial (GAT) canonical motif, is important for positive regulation of GATA-1 expression in erythroid cells. The evolutionary conservation of the two sequences implies that they have a significant and specific function [10, 11].

Based on the above, there should be a positive feedback loop mediated through GATA-1. However, experiments in which the transgene was introduced into a GATA-1 deficiency environment showed that GATA-1 is not strictly required either for activation or maintenance of GATA-1 gene expression in vivo. This finding argues against positive auto-regulation by GATA-1 in the simplest sense, but it does not exclude the positive action of other GATA factors, such as GATA-2 [12].

Surprisingly, more recent data have demonstrated a specific increase in the expression of the distal (but not the proximal) GATA-1 transcripts during in vivo and in vitro differentiation of primary erythroid cells. The increase is associated with other known erythroid-specific modifications of gene expression, such as an increase in the expression of EKLF, SCL, and NFE2, and also with the preceeding down-regulation of GATA-2, EpoR, and Myb. This indicates that the increase in the levels of the distal GATA-1 transcripts may be part of the coordinate regulation of erythroid-specific genes in the terminal erythroid differentiation program [13].

Other active elements in the GATA-1 gene

DNase I hypersensitivity mapping was performed with nuclei of erythroid and nonerythroid cells. Several regions of hypersensitivity were detected in mouse erythroid, but not 3T3, cell chromatin. These include a region of strong hypersensitivity upstream of IE exon (HS I), sites surrounding the IE exon, including the proximal promoter (HS II), and a region within the intron downstream of the IE exon (HS III) [12].

In transgenic experiments, transgenes with 2.7 kb of promoter sequence are expressed infrequently and only within adult (definitive) erythroid cells. Inclusion of a region covering a strong hypersensitive site (HS I) markedly enhances the frequency of expressing transgenic lines and activates expression in primitive erythroid cells. The other two sites, however, are not critical [12]. In more recent experiments, the upstream-activing element (UAE) region located at 3.9–2.6 kb 5′ of the IE exon of

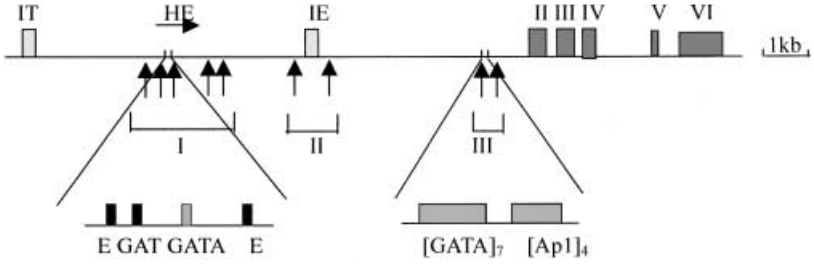

Figure 1. GATA-1 gene and its important cis-element. Boxes represent the exons and the motifs in this gene. The arrows represent hypersensitivity (HS) sites.

the GATA-1 gene was found to be necessary to recapitulate gene expression in both the primitive and definitive erythroid lineages. In transfection analyses, this region activates a reporter gene from an artificial promoter in a position- and orientation-independent manner, indicating that this region functions as the GATA-1 hematopoietic enhancer (G1HE). However, when analyzed in transgenic embryos in vivo, G1HE activity is orientation dependent and also requires the presence of the endogenous GATA-1 gene promoter [14].

Further analysis has shown that the G1HE contains a 149-bp core region which is critical for GATA-1 gene expression in both primitive and definitive erythroid cells. Expression in megakaryocytes requires additional sequences from G1HE. This core region contains one GATA, one GAT, and two E boxes. Mutational analyses revealed that only the GATA box is critical for gene regulatory activity. Furthermore, GATA-1, GATA-2, and GATA-3 all bind to the consensus GATA box of G1HE. In doing so, they may serve as anchoring factors, as well as contributing to the formation of the high-molecular-weight protein complex which regulates GATA-1 gene expression [14, 15].

Moreover, deletion of an extended region spanning intron 1 and exon IEb also inhibited transcription in mice of β-galactosidase reporter constructs containing intact UAE and exon IEa promoter domains. This observation, together with preliminary characterizations of a hypersensitive site within intron 1 and S1 nuclease mapping of transcription from exon IEb, prompted current investigations of intron 1 structure, its role in directing high-level erythroid-specific transcription, and its apparent effects on transcription from initiation sites in exon IEa and IEb. In recent experiments, elements within intron 1 (a 900-bp region immediately 5′ to exon IEb) were shown to markedly affect its erythroid-restricted transcription. For transiently as well as stably transfected reporter constructs, this central subdomain of intron 1 was shown to be necessary and sufficient for high-level transcription in erythroid cells, whereas in the parental myeloid FDCER cells, low-level transcription is unaffected by intron 1 deletions. This region contains a repeated consensus for GATA and Ap1 elements which are footprinted in vivo. Deletion of intronic GATA and Ap1 motifs abrogated the activity of G6.8-pEGFP (a full-length 6.8-kb GATA-1 gene construct fused with pEGFP) by 43 and 56%, respectively, and the above-mentioned 1800-bp region of intron 1 per se is transcribed at rates uniformly greater than G6.8-pEGFP. Mechanistically, this might involve effects on transcription initiated at exon IEb, IEa, or both. Deletion of this region inhibits overall reporter gene expression from exon II about ten fold, while levels of transcription derived from exon IEb in erythroid cells account for only 10% of total levels. Thus transcription from both exon IEa and IEb depends upon the intactness of intron 1. Within the GATA-1 gene, whether the integrity of intron 1 supports transcript stability, transport, and/or initiation is under active investigation (as is the possible lineage specificity of this effect) [16].

The above results demonstrate that GATA boxes have been found in several important sites in the GATA-1 gene. Thus, a network of GATA factors and binding sites may regulate GATA-1 gene expression in hematopoietic cells. The findings also suggest that GATA-1 and/or other factors work as key regulators in this network through direct interaction with the cis-acting GATA boxes in multiple regulatory regions. This network seems to be the molecular basis for the erythroid cell-specific expression of the GATA-1 gene.

Negative element in the GATA-1 gene of zebrafish

Transient assays showed that the expression of GATA-1 of zebrafish in the notochord is apparently suppressed through an interaction between a negative regulatory element in the GATA-1 promoter and notochord-derived negative factors [17]. Although the zebrafish GATA-1 gene sequence has diverged substantially from those of the mouse and human GATA-1 genes [14], this is the first report describing a negative regulatory mechanism for GATA-1 gene expression.

Target DNA sequence

The target DNA sequence can also regulate GATA-1 factor activity. GATA-1 does not stimulate transcription through all high-affinity GATA-binding sites. Specifically, a double site with a nonconsensus GATC recognition sequence does not support transactivation when placed on a reporter gene in a position from which canonical binding sites activate. In other cases, increasing the affinity of the GATA-binding site in a reporter gene reduces transactivation by GATA-1. Therefore GATA-1 activity can be modulated by the sequence content of its DNA-binding site, and is not necessarily directly related to binding affinity. Distinctly structured binding sites could have gene-specific functions. Interactions of GATA-1 with DNA may induce conformational changes in the protein that alter the interface between GATA-1 and auxiliary factors or change the potency of its transactivition domains by repositioning them. The precise conformation of the transcription factor complex formed could be critical to activation. Many cofactors of GATA-1, including members of the FOG family of proteins, interact directly with the zinc fingers, and the conformation of GATA-1 on DNA could determine which regions are available for these interactions [18].

Moreover, solution nuclear magnetic resonance (NMR) studies of the complex formed between the C-terminal finger of GATA-1 and its cognate DNA sequence indicate

that the DNA is bent by an overall angle of about 15° [19]. By using a series of full-length GATA-1, double zinc finger, and single C-terminal finger constructs, GATA-1 was shown to bend DNA by about 24°, irrespective of the DNA-binding site. N- and C-terminal fingers of GATA-1 are proposed to adopt different orientations when bound to different cognate DNA sites [20].

Modification of the transcription factor GATA-1

Acetylation

Modification of histones, DNA-binding proteins found in chromatin, by addition of acetyl groups occurs to a greater degree when histones are associated with transcriptionally active DNA. CREB-binding protein (CBP) and its close relative p300 are acetyltransferase and serve as coactivators for a variety of transcription factors involved in growth control and differentiation. CBP associates with GATA-1 in vitro and in vivo and markedly stimulates its activity. Expression of the adenovirus oncoprotein E1A, which interferes with the action of CBP, blocks the effects of CBP on GATA-1 activity, inhibits erythroid differentiation, and reduces the expression of several GATA-1-dependent genes [21].

Boyes et al. [22] pinpointed the positions of the acetylated sites by radioactive sequencing and mass spectroscopy. They found that all acetylated lysines were within the DNA-binding region of GATA-1. Acetylation significantly increased the amount of GATA-1 bound to DNA and altered the mobility of GATA-1-DNA complexes, suggesting a conformational change in GATA-1. Acetylation of GATA-1 in vivo directly stimulates GATA-1-dependent transcription. Mutagenesis of important acetylated residues showed that there is a relationship between the acetylation and in vivo function of GATA-1. Acetylation of transcription factors may alter interactions between these factors and DNA, and may be an integral part of transcription and differentiation processes [22].

In contrast to the findings of Boyes et al. [22], Hung et al. [23] reported that CBP acetylates GATA-1 at two highly conserved lysine-rich motifs present at the C-terminal tails of the two zinc fingers both in vivo and in vitro. They found that acetylation of GATA-1 did not affect its ability to bind DNA and the substitutions or deletions in these motifs did not affect DNA binding of mammalian cell-expressed GATA-1. These discrepancies might be the result of differences between the chicken and mouse GATA-1 genes and between CBP and p300 used in their respective experiments. They also found that mutations in the C-terminal acetylation motif, but not in the N-terminal motif, reduced the binding to CBP and diminished the response to CBP in transient transfection assays. Moreover, they examined the function of the acetylation sites in an assay that measured GATA-1 activity by its ability to trigger terminal differentiation when introduced into the GATA-1-deficient erythroid cell line G1E. This assay revealed that mutations in either motif reduce GATA-1 function without affecting its ability to bind DNA. These results indicate that the acetylated motifs are of biological importance independent of a role in DNA binding [23].

Phosphorylation

Phosphorylation plays a critical role in modulating the activity of transcription factors in vivo. It is a post-translational modification capable of producing rapid modulations of protein activity in response to changes in metabolic activity, environmental conditions, or hormonal signals [24].

The phosphorylation sites of GATA-1 have been mapped in COS cells and confirmed in uninduced and DMSO-induced MEL cells. Six serine residues at the N terminus are phosphorylated in uninduced MEL cells and a seventh, near the C-terminal boundary of the DNA-binding domain, becomes phosphorylated after induction [25]. However, no role for phosphorylation of GATA-1 influencing DNA binding or transcriptional activation was found [25]. Conversely, the DNA binding of baculovirus-expressed human GATA-1 was reported to be sensitive to treatment by alkaline phosphatase, and the phosphotryptic peptide composition is similar to that of GATA-1 purified from K562 cells [26]. More recent data have shown that the level of GATA-1 phosphorylation increases after the induction of K562, but not MEL cells where GATA-1 is already highly phosphorylated. Furthermore, phosphorylation increases the binding affinity of GATA-1 for a canonical binding site. The important differences between the assays used in each case have yet to be identified [27].

Self-association of GATA-1 factor

GATA-1 contains a DNA-binding domain composed of two adjacent homologous zinc fingers. The C-terminal finger of GATA-1 alone is capable of independent binding to the GATA recognition sequence. Previously, the role of the N finger (NF) was known only to increase the stability of the GATA-1-DNA complex and influence the specificity of binding. More recently, physiologically relevant experiments investigating the ability of GATA-1 to induce erythroid maturation have revealed a critical role for this finger: the N finger is required for terminal erythroid differentiation [28].

Crossley et al. [29] have shown that GATA-1 self-association can be observed in solution and in whole-cell extracts and that the zinc finger region of the molecule is sufficient to mediate this interaction. Because a mutant

form of GATA-1, which is defective in DNA binding, superactivates a promoter containing bound GATA-1, the physical interaction appears to influence transcription. Moreover, the minimal domain required for GATA-1 self-association was localized to 40 amino acid residues within the C-terminal zinc finger region [29].

Mackay et al. [30] focused on the NF to investigate its involvement in GATA-1 self-association. They demonstrated that this domain does not homodimerize but instead makes intermolecular contacts with the C-finger (CF). This suggests that GATA dimers are maintained by reciprocal NF-CF contacts. Deletion analysis identified a 25-residue region, C-terminal to the core NF domain, which is sufficient for the interaction with intact GATA-1. A similar subdomain exists C-terminal to the CF [30]. It is interesting to compare the latter results with previous work centered on the CF. Like the NF, the CF is able to interact with GATA-1. Nevertheless, the relevant subdomains within the CF revealed the presence of two distinct, overlapping domains. One subdomain appears analogous to the tail subdomain mapped in the NF in the later experiment, in that it also lies immediate downstream of the zinc-binding region. The second subdomain centers around the actual zinc-binding region of the CF. This result may be understood in light of the model of an antiparallel association, whereby the two subdomains of the CF interact with different regions of GATA-1; that is to say, the CF tail may interact with the NF, while the CF contacts the NF tail. Consistent with this, disruption of both intermolecular interactions is required to abolish dimerization, while the mutation of a single contact region reduces the interaction affinity to a lesser degree [30].

Moreover, self-association is substantially reduced when both NF and CF subdomain are disrupted by mutation. Mutations that impair GATA-1 self-association also interfere with its ability to activate transcription in transfection studies [30].

However, other researchers have proposed that for similar intramolecular interactions to occur, the fingers must be capable of motion relative to one another through the 22 amino acids linking them. This linking region of GATA-1 has recently been shown by NMR to have little secondary structure and is consequently unlikely to independently restrict such relative movement of the fingers. Therefore the interference seen with GATA-1 likely reflects an association between regions of the protein contained within the two zinc fingers, as determined by their relative orientation when bound to DNA [31].

Overall, the exact biological role of GATA-1 self-association is not certain, but there are a number of appealing possibilities. The most simple effect of self-association is to increase the local concentration of GATA-1 and presumably, therefore, to increase its potency as a transcriptional activator. Additionally, self-association between molecules of GATA-1 bound at distant sites may mediate the formation of chromatin loops and the establishment of regions of active chromatin. Finally, numerous erythroid promoters and enhancers contain multiple GATA elements, and self-association of GATA-1 may play a specific role in regulating this subset of genes.

Recently, Trainor et al. [18] demonstrated that the NF and adjacent linker region can alter the binding specificity of the CF sufficiently to prevent it from recognizing some consensus GATA sequences. Together with the previous experiments that the NF of GATA-1 participates in DNA binding, these results show that the GATA zinc fingers within a single molecule can cooperate to form a binding domain with specificity distinct from that of the individual fingers, and that each finger strongly influences the binding of the other. So the NF of GATA-1 can not only stablize binding, but can also disrupt binding, or modify the DNA-binding specificity of the CF, depending on the consensus DNA-binding site [18].

Interaction of GATA-1 with other transcription factors

Protein-protein interactions play significant roles in the control of gene expression. Lineage-specific gene expression programs are widely assumed to be established and maintained by both positive and negative interactions between transcription factors expressed in different compartments of the hematopoietic system. As an important transcription factor in hematopoiesis, GATA-1 has been found to interact with other factors to coregulate developmental processes.

Sp1 and EKLF

Merika and Orkin [32] provided the first evidence for functionally important protein-protein interactions involved in erythroid cell-specific expression: they showed that GATA-1 interacts with two Krüppel family factors, the ubiquitous protein Sp1 and the erythroid-restricted factor EKLF, which recognize GC and/or GT/CACC motifs; these interactions are mediated through their respective DNA-binding domains. Evidence was provided that GATA-1 activates transcription in a synergistic fashion with Sp1 and EKLF. In addition, the formation of GATA-1-Sp1 complexes was demonstrated in vivo by the ability of Sp1 to recruit GATA-1 to a promoter in the absence of GATA-binding sites. Based on the fact that GC or GT/CACC are often found in association with GATA motifs in promoters, enhancers, and LCR cores of erythroid genes, together with experiments that GATA-1 and Sp1 synergize at a distance in constructs designed to mimic the architecture of globin LCR and downstream globin promoters, the interactions may aid in the formation or

stabilization of chromosomal loops between distant LCR and promoter elements [32].

Friend of GATA-1

Using the conserved zinc finger DNA-binding domain of GATA-1 in the yeast two-hybrid system, Tsang et al. [33] identified a novel, multitype zinc finger protein, friend of GATA-1 (FOG-1). FOG-1 is coexpressed with GATA-1 during embryonic development and in erythroid and megakaryocytic cells.

Shortly after the isolation of FOG-1, a related *Drosophila* protein, U-shaped, was isolated and shown to interact physically with the *Drosophila* GATA factor, Pannier [34]. Shortly thereafter, a new mammalian FOG family member, human FOG-2, was cloned [35]. Unlike FOG-1, which is only expressed in erythroid and megakaryocytic cells, FOG-2 appears to be widely expressed, predominantly in heart and brain. The three FOG proteins are diverse in sequence but their individual zinc finger regions share considerable homology [33, 35].

FOG proteins contain multiple zinc fingers (FOG-1 and Ush have nine, FOG-2 has eight) and four of these appear to be classical TFIII-like Cys-Cys:His-His fingers and five have a more unusual Cys-Cys:His-Cys configuration [33–35].

First, only the sixth finger of FOG-1, whose configuration is CCHH, was found to interact specially with the NF but not the CF of GATA-1 [33]. Using a scanning substitution strategy, the key residues within the GATA-1 NF' which are required for FOG binding, are found in a 32-amino acid peptide encompassing the zinc-binding region of the NF [36]. Then, Fox et al. [37] showed that fingers 1, 5, and 9 of FOG-1 (CCHH configuration) also interact with the NF of GATA-1, and that FOG-2 and Ush also contain multiple GATA-interacting fingers. Furthermore, each interacting finger of FOG-1 contributes to its ability to modulate GATA-1 activity. This result suggests that the duplication of the finger domains during evolution has enhanced the ability of FOG family members to modulate GATA protein activity; this phenomenon also raises the possibility that FOG proteins may bind multiple GATA proteins and be involved in bridging between GATA proteins bound at distant sites in the control regions of particular genes [37].

Transfection experiments in mammalian cells have demonstrated the synergistic activation of transcription from the hematopoietic-specific p45 NF-E2 regulatory region by mFOG-1 and GATA-1 [33]. In addition to its function as a coactivator, there is evidence that mFOG-1 may be involved in negatively regulating gene expression from certain promoters. The same result was confirmed in the experiment with hFOG-2 [35]. This can be explained by the involvement of mCtBP, one member of the corepressor family, since mCtBP can associate with a repression domain in FOG-1, this physical interaction contributing to repression of GATA-1 [35, 36]. Deconinck et al. [38] confirmed that *Xenopus* FOG (most similar to FOG-1) can act as a repressor of red blood cells in vivo, in part through interaction with CtBP. However, when using the EKLF promoter, FOG-1 can repress the GATA-mediated activation in a dose-dependent way, implying that FOG protein can also directly repress the activition of GATA-1 [37].

Though the exact role of the interaction of GATA-1 and FOG proteins is unknown, the interaction of GATA-1 and FOG-1 is clearly very important in both megakarycyte and erythroid development, and many animal models demonstrate this function. This was also confirmed by Nichols et al. [39]. They showed that the V205M mutation abrogates the interaction between GATA-1 and FOG-1, inhibiting the ability of GATA-1 to rescue erythroid differentiation in an erythroid cell line deficient for GATA-1. This amino acid substitution results in family dyserythropoietic anemia [39].

PU.1

PU.1 is a transcription factor with a winged helix-turn-helix-type DNA-binding domain that is a member of the Ets family of proteins and is expressed specifically in myeloid and B-lymphoid cells of the hematopoietic system, and which acts as an important transcription factor in the development of these cells [40].

Restoration of terminal differentiation in the mouse MEL cells requires a decrease in the level of PU.1, indicating that PU.1 can block erythroid differentiation, a feature that had already been identified by transfection experiments. This effect of PU.1 on erythroid differentiation is also seen in other cell culture models. So there should be some mechanisms that down-regulate the expression of PU.1 or inhibit its function. Rekhtman et al. [41] were the first to indicate that GATA-1 interacts directly with PU.1 both in vivo and in vitro, and the interaction requires intact DNA-binding domains in both proteins. The interaction leads to repression of GATA-1-mediated transcriptional activation. Furthermore, they showed that PU.1 and GATA-1 oppose the biological activities of the other in both normal and leukemic erythroid cells. This suggests that PU.1 and GATA-1 can antagonize the actions of each other by binding to one another and altering some aspect of their functions as transcription factors [41]. In fact both the N and C terminal of PU.1 can interact with the C terminal of GATA-1. Nerlov et al. [42] localized these interaction sites to two small regions: (β 3/β 4) no longer than 12 amino acids within the PU.1 C-terminal DNA-binding (ETS) domain, 70 amino acids within the N-terminal of PU.1. They then examined the ability of GATA-1 to repress the expression and function of PU.1. They found that GATA-1 is capable of suppressing the myeloid

phenotype without interfering with PU.1 gene expression, instead inhibiting the activity of the PU.1 protein in a dose-independent manner. This inhibition is independent of the ability of GATA-1 to bind DNA, suggesting that it is mediated by protein-protein interaction. Moreover, they found that the PU.1 DNA-binding domain (ETS), rather than the transactivation domain, is the target for GATA-1-mediated repression [42].

Meanwhile, Zhang et al. [43] reported that in both K562 and G1ER cell lines [the GATA-1 null erythroid cell line G1E transduced with a GATA-1-estrogen receptor (ER) fusion gene, which is directly dependent on induction of the GATA-1 fusion protein to effect erythroid maturation], using an electrophoretic mobility shift assay with purified proteins, the N-terminal 70 amino acids of PU.1, but not the C terminus of PU.1, can specifically block GATA-1 DNA binding. This is in contrast with Nerlov's view. Based on this and previous work, they hypothesized a reasonable mechanism by which PU.1 represses GATA-1 function through an interaction with the CF of GATA-1. This results in inhibition of GATA-1 DNA binding, but is independent of the NF which binds the FOG. This differs from the mechanism by which GATA-1 represses PU.1 function, in which GATA-1 interaction with PU.1 blocks the ability of the PU.1 coactivitor c-jun to bind to the same small region that GATA-1 binds but does not inhibit PU.1 DNA binding [43].

Other factors

The ER has also been demonstrated to interact with GATA-1, and the experiment showed that the interaction reduces the number of erythroid progenitor cells in a ligand-dependent manner in primary human bone marrow cultures [44]. GATA proteins have also been reported to associate with the LIM domain protein Rbtn2/LMO2, presumably through the finger domain [45]. Blobel et al. [21] found that CBP can also cooperate with GATA-1 to regulate erythroid differentiation: CBP acts to stimulate the transcriptional activation by GATA-1. Finally, GATA-1 has been shown to interact with c-Myb, as a result of which, c-Myb inhibits DNA-binding activities of GATA-1 in a dose-dependent manner [46].

Regulation by caspase-mediated cleavage

Death receptors are a family of surface molecules that trigger caspase activation and apoptosis in a variety of cell types [47–49]. De Maria et al. [50] showed that immature erythroid cells express several death receptors (e.g., CD95, TNF, DR4, DR5) whose ligands are produced by mature erythroblasts.

Exposure of erythroid progenitors to mature erythroblasts or death receptor ligands results in caspase-mediated degradation of the transcription factor GATA-1. A dramatic decrease in GATA-1 expression is observed in erythroblasts treated for 2 days with anti-CD95 in the absence of zVAD (the caspase inhibitor). Both in vitro and in vivo, human GATA-1 is targeted by caspase and the cleavage position is located at an aspartic acid at position 73. Expression of a caspase-resistant GATA-1 mutant, but not of the wild-type gene, completely restored erythroid expansion and differentiation following the triggering of death receptors, indicating that there is regulatory feedback between mature and immature erythroblasts through caspase-mediated cleavage of GATA-1. Thus, caspase-mediated cleavage of GATA-1 may represent an important negative control mechanism in erythropoiesis [50].

Perspectives

It is interesting that the expression level of GATA-1 is not the same between cell lines and differs at developmental stages of hematopoiesis: it is first expressed at low levels in multipotential hematopoietic progenitor cells and is then up-regulated during erythroid maturation and down-regulated during myeloid differentiation. The expression level of GATA-1 is important: reduced GATA-1 levels inhibit proerythroblast differentiation [51]. MEL cells overexpressing GATA-1 under the control of the erythroid-specific human LCR linked to the β-globin promoter fail to activate the expression of differentiation markers in response to the chemical inducer dimethylsulfoxide and do not undergo differentiation-associated proliferative arrest [52]. ES cell clones overexpressing GATA-1 also generate erythroid clones that are inhibited in terminal differentiation [52]. Whyatt et al. [53] also showed that overexpression of GATA-1 in erythroid cells inhibits their differentiation, leading to a lethal anemia. Using chromosome X inactivation of a GATA-1 transgene and chimeric animals, they showed that this defect is intrinsic to erythroid cells, but not cell nonautonomous. On this basis, they proposed an alternative mechanism in which a signal originating from wild-type erythroid cells restores normal differentiation to cells overexpressing GATA-1 in vivo [53].

The relative expression level of GATA-2 is also interesting. There is evidence that GATA-2 levels may be related to the different expression levels at various development stages. GATA-2 is essential in the earliest stages of hematopoiesis [7], and it is expressed at a high level during the primitive erythroid stage and at a low level during the definitive erythroid stage [54]. This is the opposite to the behavior of GATA-1. Increased GATA-1 expression downregulates expression of GATA-2, while increased GATA-2 has no effect on GATA-1 expression [55]. The overexpression of GATA-2 will block erythroid

differentiation and promote megakaryocytic differentiation [55]. Overall, the relative levels of both GATA-1 and GATA-2 at different stages through hematopoiesis are critical in the regulation of lineage specificity. An attractive model (fig. 2) proposes that GATA-2 binds to the GATA motif in the early stage of differentiation, whereas GATA-1 replaces GATA-2 in the late stage. This model is supported by the evidence that GATA-2 can partially rescue GATA-1 deficiency, and the G1HE is active in a GATA-1 knockdown environment, and that GATA-2 can bind to the GATA box in G1HE [14]. Concerning GATA-1 levels in different cell lines, Vyas et al. [15] have shown that there are different sequence requirements for expression in erythroid and megakaryocytic cells: the entire HS I region is required for expression in megakaryocytes, while only the 5′ 62 base pairs are needed for erythroid-specific reporter expression. Moreover, GATA-2 may be involved in this regulation process since its expression level differs in the two cell types. Regulation of the stage- and lineage-restricted expression of GATA-1 is not clear, and the cis-elements of the gene itself or the level/kind of other factors may play the pivotal role.

For GATA-1 dose-dependent regulation, several proposals have been put forward: multiple GATA boxes within the gene, inter-self-association of this factor, and the ability of monomolecular FOG to interact with GATA-1 through its multifingers. In fact, at the level of the GATA-1 gene, the exact role and the mechanism of the multiple GATA boxes in the gene are unknown. Moreover, whether the integrity of intron 1 is required to support transcription stability, transport, and/or initiation is under active investigation. In addition, the transcriptional relationship between the distal and proximal promoters of the GATA-1 gene requires more detailed study. Overall identification of cis-elements that organize the lineage specificity of GATA-1 gene expression and elucidation of the intricate relationships among the factors interacting with these elements are the focus of future research. At the level of the protein, although the self-association of GATA-1 has been investigated, its role and mechanism of action are not known. Furthermore, different transcription factors participate in the regulation of hematopoietic development and maturation, so the protein-protein interactions attract the attention of scientists. Given the complex interactions of many factors, though several factors such as FOG and PU.1 have been investigated, much more research needs to be done.

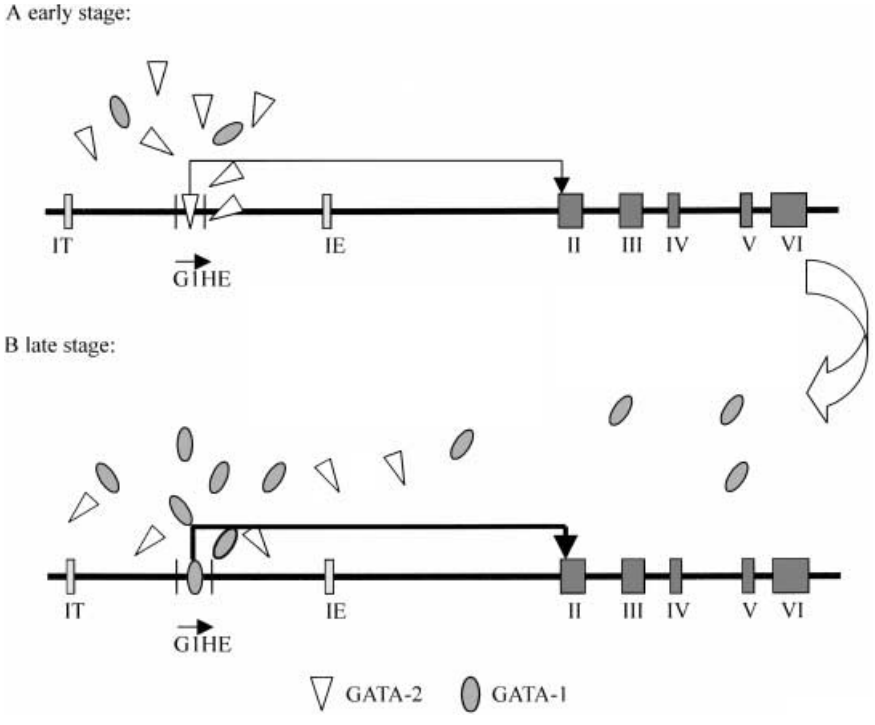

Figure 2. The mechanism of GATA-1 stage-restricted expression. Boxes represent exons, the arrows represent the role of enhancment and the thickness of the arrows expresses the degree of enhancment. The G1HE region is the active element. (*A*) During the early stage, GATA-2 is expressed highly and binds to G1HE to activate the expression of GATA-1. At this time, levels of GATA-1 are low, and it does not bind to G1HE. (*B*) During the late stage, levels of GATA-1 rise. It can compete with GATA-2, and the affinity of GATA-1-G1HE may be higher, so that GATA-1 replaces GATA-2. The enhancing ability may also be higher, so GATA-1 expression also rises.

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