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Regulation of GATA1 Gene Expression

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GATA1 is one of the most fascinating transcription factors for biologists. It regulates many haematopoietic genes and interacts with a number of other transcription factors. Its functions are mostly conserved among vertebrates. Upon disruption of the GATA1 gene, mice show a drastic bloodless phenotype. GATA1 knockdown mice are predisposed to tumour development, the extent of which varies depending on the level of knockdown. When GATA1 is overexpressed, certain cells face an altered cell fate. These observations suggest the importance of regulation on the expression level and timing of GATA1. Deciphering such regulation is the key to understanding the commitment and differentiation of blood cells. There are so many splendid reviews describing GATA1 as a transcription factor. We therefore focus our topics on GATA1 gene regulation in this review.

Key words: cell differentiation, gene expression level, haematopoiesis, lineage determination, transcription factor.

Abbreviations: BFU-E, burst-forming units-erythroid; CFU-E, colony-forming units-erythroid; ChIP, chromatin immunoprecipitation; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; EEP, early erythroid progenitor; ES, embryonic stem; G1HE, GATA1 gene haematopoietic enhancer; G1HRD, GATA1 haematopoietic regulatory domain; G1TAR, GATA1 testis activation region; GMP, granulocyte/monocyte progenitor; HSC, haematopoietic stem cell; KLF, Krüppel-like factor; LCR, locus control region; LEP, late erythroid progenitor; MEP, megakaryocyte/erythrocyte lineage-restricted progenitor; ProE, proerythroblast.

GATA1 is a member of the GATA family of transcription factors, which bind to the consensus sequence WGATAR by two characteristic zinc fingers (*1*, *2*). Since strong binding sites for GATA1 are widely distributed throughout the promoters and enhancers of the globins and other erythroid-specific genes, GATA1 is believed to be a key regulator of erythropoiesis. Indeed, several gene-targeting studies were performed to elucidate the importance of GATA1 functions in the differentiation of erythroid and some myeloid lineages. GATA1 deficient embryonic stem (ES) cells gave rise to primitive and definitive erythroid progenitors that arrested at the proerythroblast stage *in vitro* and failed to contribute to red blood cell formation in chimeric mice. GATA1-null mice showed complete ablation of primitive and definitive erythropoiesis, resulting from the arrested maturation and apoptosis of erythroid progenitors, and died from severe anaemia between embryonic days 10.5 (E10.5) and E11.5. GATA1 is critical not only for erythroid cells, but also terminal maturation of megakaryocytes, the early stage of eosinophil differentiation and the late stage of mast cell differentiation.

GATA1 has been found throughout vertebrates and its specific expression in haematopoietic cells is strikingly conserved (*3*, *4*). This implies that the roles of GATA1 in haematopoiesis are conserved among vertebrates. Indeed, a bloodless mutant fish, *vlad tepes*, was shown to result from a nonsense mutation in the zebrafish GATA1 gene. Natural mutations in the human GATA1 gene have also been described in several disease syndromes (*5*–*7*), including dyserythropoietic anaemia, thrombocytopenia, thalassemia, erythropoietic porphyria, grey platelet syndrome and Down syndrome-related acute leukaemia.

In the haematopoietic system, the other GATA family proteins GATA2 and GATA3 exist and play important roles in haematopoiesis (*8*, *9*). GATA2 is crucial for the development and function of multipotent haematopoietic progenitors, while GATA3 regulates lymphopoiesis. Analyses of gene-disrupted mice have indicated that GATA1, 2 and 3 perform distinct actions in controlling haematopoiesis, with some redundancy (*10*). These three vertebrate GATA factors seem to have evolved from a single ancestor by gene pluralization (*3*), which has enabled vertebrates to develop a complex haematopoietic system. Such complexity in haematopoiesis is considered to have arisen from divergence in the protein structures of each GATA factor and from accompanying gene pluralization leading to diversity in gene expression profiles. It is yet unknown whether divergence in protein structure or gene pluralization has been more important. However, we have some interesting findings related to this mystery. We have demonstrated that GATA1 knockout or knockdown defective mice can be rescued by supplying either GATA1, 2 or 3 under the control of

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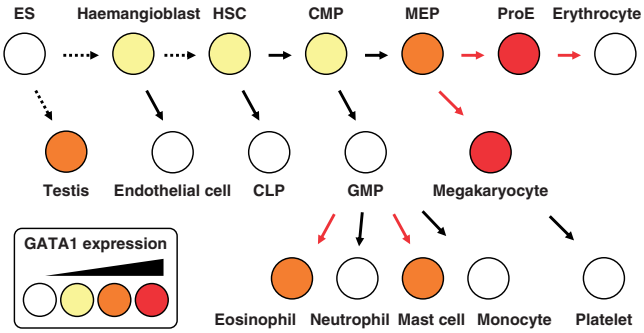

Fig. 1. **Model for the dynamic regulation of GATA1 during haematopoiesis.** The relative expression levels of the mouse GATA1 gene are indicated by various colors as shown in the box. Red arrows indicate the stages of differentiation which are inhibited when the expression levels of GATA1 are genetically altered in mice. Note that the expression levels in the testis, eosinophil and mast cell are imprecise. CLP, common lymphoid progenitor; CMP, common myeloid progenitor; ES, embryonic stem; HSC, haematopoietic stem cell; MEP, megakaryocyte/erythrocyte lineage-restricted progenitor; ProE, proerythroblast.

a GATA1 gene regulatory unit (*11*, *12*). This surprising result indicates that GATA2 and GATA3 can replace GATA1 to bring about conventional regulation of GATA1 target genes and underpins the minor importance of structural differences among the haematopoietic GATA factors in this respect. Interestingly, expression of GATA1, 2 or 3 by β-globin regulatory elements failed to rescue GATA1-null mice, even though these elements actually represent one of the strongest promoters in erythroid cells (*12*). Therefore, regulation of GATA1 gene expression may be regarded as intricate and that precise regulation of the GATA1 gene may be critical for its physiological roles.

EXPRESSION PROFILE OF THE GATA1 GENE

The expression profile of the GATA1 gene has been studied extensively (Fig. 1) (*2*, *13*). GATA1 is expressed within the haematopoietic system, in primitive and definitive erythroid cells, megakaryocytes, eosinophils and mast cells, and in Sertoli cells of the testis. The structure and haematopoietic-expression of GATA1 are conserved in lower vertebrates, such as chicken (*14*), *Xenopus* (*15*) and zebrafish (*16*). Interestingly, analysis of haematopoietic progenitors purified using cell-surface markers and flow cytometry revealed that GATA1 is expressed in haematopoietic stem cells (HSC), common myeloid progenitors (CMP) and megakaryocyte/erythrocyte lineage-restricted progenitors (MEP) and at extremely low levels, except in MEP (*17*, *18*). The low GATA1 expression level in these progenitors is supposed to be important for multilineage priming (*19*). A base level of GATA1 was also observed in haemangioblasts, but not in ES cells (*20*, *21*).

The dynamic change occurring in GATA1 expression alongside erythroid differentiation was analysed using bone marrow cells from *GATA1*-GFP transgenic mice (*22*). Erythroid progenitors were separated into six consecutive stages based on the expression levels of cell-surface markers and GFP. The first stage progenitor fraction corresponds to HSC and CMP and expresses an extremely low level of GATA1. In the second stage, there is a medium level of expression and this early erythroid progenitor (EEP) fraction contains MEP and burst-forming units-erythroid (BFU-E). The third stage, the late erythroid progenitor (LEP) stage, corresponds to colony-forming units-erythroid (CFU-E) and exhibits the highest expression level. Following the LEP stage, expression levels of GATA1 gradually decrease to medium in the fourth proerythroblast stage, to low in the fifth basophilic and polychromatic erythroblast stage, and finally to extremely low in the sixth orthochromatic erythroblast stage. Since fluctuations in the levels of GATA1 cause disorganized erythropoiesis, as described in the next paragraph, this dynamic change in GATA1 expression must be critical, especially in the erythroid lineage.

IMPORTANCE OF CONTROLLED GATA1 EXPRESSION LEVELS

An altered expression level of GATA1 takes place in various ways during erythropoietic differentiation. Interestingly, each target gene of GATA1 has a different sensitivity to the expression level of GATA1. This was shown in analyses using GATA1-null cells with an estrogen receptor-tagged GATA1 transgene whose activity can be controlled by estrogen (*23*). Similar results were obtained from comparative studies of cells derived from GATA1 knockout and GATA1 knockdown mice. Knockdown GATA1.05 mice, in which a neo-cassette was inserted in the GATA1 promoter region, showed only 5% of the wild-type GATA1 mRNA expression level (*24*). Both GATA1.05 and GATA1-null cells showed a reduced expression of $p16^{INK4A}$, a cyclin-dependent kinase inhibitor, while only GATA1-null cells lost the expression of the antiapoptotic gene Bcl-X_L (*25*, *26*). Consequently, GATA1.05 cells had an accelerated cell cycle progression, while GATA1-null cells died by apoptosis (*26*). These results indicate that only 5% of the normal GATA1 level is sufficient for erythroid cells to express Bcl-X_L, but not $p16^{INK4A}$, and that the expression profiles of GATA1 target genes change upon a subtle alteration in the GATA1 expression level.

The importance of controlled levels of GATA1 gene expression is obvious from the phenotypical differences observed between GATA1-null and GATA1 knockdown mice. GATA1.05 embryos died from severe anaemia between E11.5 and E12.5, which is 1 day later than found with GATA1-null mice. As a result of elevated cell cycle progression without apoptosis, female GATA1 gene knockdown mice frequently suffered from acute leukaemia, which was not observed in GATA1-null mice (*27*). $GATA1^{low}$ mice, in which the neo-cassette was replaced with an upstream enhancer of the GATA1 gene, expressed about 20% of the wild-type GATA1 levels (*28*). These mice showed a somewhat milder phenotype compared with GATA1-null and GATA1.05 mice. Not only a reduction, but also an elevation in GATA1 expression caused defects in erythropoiesis. Whyatt *et al*. (*29*) demonstrated that overexpressing

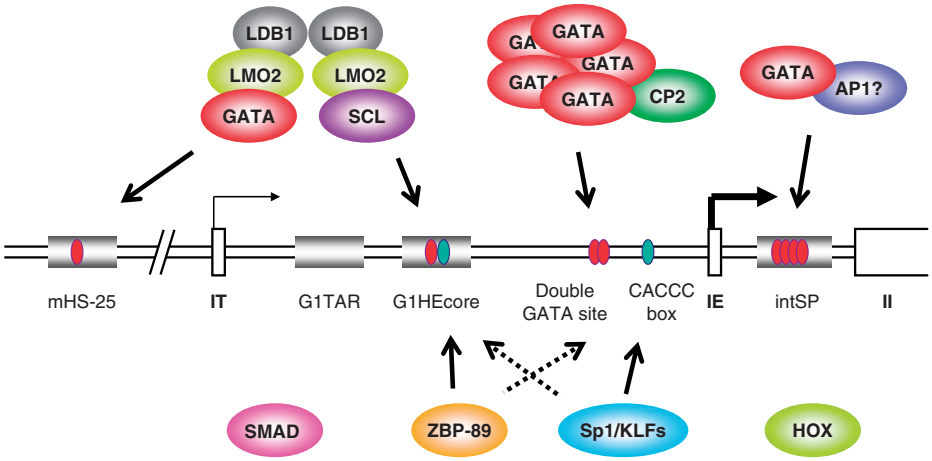

Fig. 2. ***Cis-* and *trans*-acting factors controlling mouse GATA1 transcription.** The mouse GATA1 gene locus is displayed and the positions of known *cis*-regulatory regions are shown. Large ovals indicate transcription factors involved in the regulation of GATA1 gene expression. Small red and turquoise ovals denote GATA sites and CACCC boxes, respectively.

of GATA1 in erythroid cells inhibited their differentiation and led to lethal anaemia. These observations indicate that precise regulation of the expression level of GATA1 at each progenitor stage is critical for erythroid cell development.

GATA1 expression is not only vital for erythropoiesis, but also important for other haematopoietic lineages. GATA1^{low} mice died between E13.5 and E14.5 due to ineffective primitive and definitive erythroid differentiation, but some were born alive and a small number survived to adulthood. Interestingly, these mice were anaemic at birth, yet recovered from the anaemia while remaining thrombocytopenic all their lives (*30*). In the GATA1^{low} model, GATA1 was expressed in erythroid cells, but barely present in megakaryocytes, explaining the different sensitivity to GATA1 knockdown between erythroid and megakaryocyte lineages. Similarly, GATA1^{low} mice also showed defects in mast cell differentiation (*31*). These observations suggest that GATA1 has roles in the several steps of haematopoiesis, and that regulatory mechanisms of gene expression differ among each step. Indeed, a recent report by Ghinassi *et al*. (*32*) demonstrated that the reduced levels of GATA1 gene expression in GATA1^{low} mice varied among haematopoietic progenitors.

The significance of GATA1 expression in determining haematopoietic cell fate was also shown in gene knockout, knockdown and overexpression studies. GATA1-null proerythroblast cells acquired the ability to differentiate into macrophages, neutrophils or mast cells (*33*). Similarly, GATA1 gene knockdown in zebrafish embryos resulted in a marked expansion of granulocyte and monocyte gene expression (*34*, *35*). Elevated GATA1 expression generated excess erythroid cells in mouse (*36*), *Xenopus* (*37*) and zebrafish (*38*). GATA1 overexpression also reprogrammed myeloid cells to undergo erythroid and megakaryocytic (thrombocytic) differentiation (*39*, *40*). Reprogramming to the eosinophil lineage was shown by forced GATA1 expression in various models (*39*, *41*, *42*). All these data make it evident that the expression level of GATA1 is one of the crucial criteria for lineage determination.

cis-REGULATORY ELEMENTS FOR CONTROLLING GATA1 TRANSCRIPTION

All vertebrate GATA1 genes are composed of an untranslated first exon(s) and five translated exons (Fig. 2) (*43*, *44*). Interestingly, mouse and rat GATA1 genes contain an additional testis-specific first exon IT upstream of the main haematopoietic-specific first exon IE (*45*, *46*). In this paragraph, we mainly discuss the *cis*-regulatory elements in the mouse GATA1 gene for controlling GATA1 transcription from the IE exon.

The transcriptional regulation of GATA1 in haematopoietic cells is coordinated by several *cis*-regulatory regions located 5′ to the IE exon and in the first intron (Fig. 2) (*43*, *44*). In early studies, *cis*-regulatory regions required for the haematopoietic expression of GATA1 were studied using MEL and K562 cells. These studies concluded that the promoter regions containing the two conserved elements, a CACCC box and a double GATA site, were necessary and sufficient for the expression of mouse and human GATA1 genes. This seemed likely since the presence and importance of these two sites were also shown in the chicken and zebrafish GATA1 genes. Another conserved property of vertebrate GATA1 promoters is the lack of TATA boxes and the existence of multiple transcription start sites. Surprisingly, reporter genes including the GATA1 promoter did not express in transgenic mice, but became functional when the upstream haematopoietic enhancer G1HE was added. Therefore, the condition in cultured cells does not reflect *in vivo* regulation of the GATA1 gene properly and thus transgenic animals have taken the place of cultured cells for analysing *cis*-regulatory regions of GATA1.

Double GATA Site—A double GATA site near the erythroid-specific promoter was found to be an enhancer of the mouse *GATA1* gene in MEL cells (*47*) and in K562 cells (*48*). The double GATA site is also present in the chicken *GATA1* promoter and is critical for its

activity in chicken erythroid cells (*49*). Footprinting of this sequence *in vivo* in mouse erythroid cells or *in vitro* using extracts from chicken erythroid cells implied the existence of a conserved specific binding activity in erythroid cells (*47*, *50*). The double GATA site is also critical for *in vivo* reporter gene expression in transgenic mice (*51*) and zebrafish (*52*, *53*). Analysis of stable transgenic zebrafish showed that the site is indispensable for maintenance or late expression, but not for initiation or early expression of a *GATA1*-GFP reporter gene (*54*). Surprisingly, mutation of this double GATA site in the endogenous locus did not abrogate GATA1 gene expression in erythroid cells, but instead eliminated the eosinophil lineage (*55*). Erythropoiesis was slightly abnormal in these animals, while platelets and mast cells appeared normal. These data suggest that the double GATA site carries functionally redundant elements and is not essential for every haematopoietic expression of the GATA1 gene.

CACCC Box—CACCC boxes are typically found in the promoter regions of GATA1 genes in a diversity of vertebrate species. These sites contributed significantly to GATA1 promoter activity in transfection assays and were occupied in the endogenous GATA1 gene locus in erythroid cells (*47*). The importance of the CACCC box was demonstrated in transgenic mice and zebrafish (*51*, *52*). Unlike the double GATA site, the CACCC box is essential for the initiation of GATA1 gene expression, since mutation or deletion of the CACCC box completely disrupted the promoter activity of GATA1 in zebrafish embryos (*52*).

G1HE Core—A transgene incorporating a 2.6-kbp region of the mouse GATA1 promoter, including both the double GATA site and the CACCC box, was expressed infrequently in mouse erythroid and other haematopoietic cells, while the addition of an upstream 1.3-kbp sequence greatly enhanced its expression. The region from 3.9-kbp upstream of the IE exon through the first intron is sufficient for directing transgene expression in primitive and definitive erythroid cells, as well as in megakaryocytes and eosinophils (*22*, *56*, *57*). This region is therefore referred to as the GATA1 haematopoietic regulatory domain (G1HRD) and the upstream 1.3-kbp enhancer sequence is called the GATA1 gene haematopoietic enhancer (G1HE). G1HE was marked by a tissue-specific chromatin hypersensitive site and histone H3/H4 hyperacetylation in erythroid cells (*57–59*). Furthermore, *in vivo* footprinting assays revealed that it was indeed occupied in erythroid cells (*58*).

Deletion analysis showed that a mere 55-bp core region was sufficient to account for the erythroid activity of G1HE, whereas a somewhat larger 144-bp region was required for megakaryocyte expression (*60*, *61*). The core region of G1HE contains a GATA box which is indispensable for reporter gene expression in both erythroid cells and megakaryocytes (*58*, *60*, *61*). For reporter gene expression in megakaryocytes, a CACCC box is required in addition to the GATA box (*62*).

To examine whether or not G1HE is necessary for endogenous GATA1 gene expression, a 7-kbp region of genomic DNA, including the whole of G1HE, was deleted in embryonic stem cells (*30*, *63*). Surprisingly, this deletion virtually abrogated megakaryocyte GATA1 gene expression, with the expression in red cells and eosinophils being unaffected, and mice were viable and not anaemic. This suggests that G1HE plays a unique non-redundant role in expression in megakaryocytes, and that other elements in the GATA1 gene locus must compensate for loss of G1HE function in red cells. Interestingly, deletion of G1HE was associated with a striking loss of the domain enriched with hyperacetylated histone H3 in megakaryocytes, suggesting that G1HE is required to maintain histone acetylation at the GATA1 gene locus (*62*).

intSP—The promoter region and upstream G1HE sequence are sufficient for expression in primitive erythroid cells, while sequences in the first intron are additionally required for efficient expression in definitive erythroid cells (*56*). The importance of the first intron was also suggested by the existence of a tissue-specific chromatin hypersensitive site in that region (*57*, *58*). The activity of the first intron has also been shown in zebrafish and chicken GATA1 genes (*50*, *53*). It is not required for inducing zebrafish GATA1 expression in early stage embryos, but is necessary for maintaining expression in mature erythroid cells.

Deletion analyses revealed that a region near the middle of the mouse GATA1 first intron (320-bp, intSP) was necessary for expression in definitive erythroid cells in transgenic mice (*51*) and in stably transfected erythroid cells (*64*). The intSP contains GATA and AP1 repeats. It is important to note, however, that intSP does not fully account for the activity of the intact first intron in transgenic mice.

G1TAR—The developmental activation and spermatogenic stage-specific repression of the endogenous mouse GATA1 gene can be recapitulated, largely, by a genomic fragment containing a 1.5-kbp upstream region of the IT exon together with a 4-kbp region of the testis-specific first intron (*65*). A 623-bp region in the first intron appears to be indispensable for testis-specific expression and was named the GATA1 testis activation region (G1TAR) (*65*). Interestingly, G1TAR lies just upstream of G1HE and these two regions act specifically on different promoters, the IT and IE promoters, respectively. This implies that these two regions contribute to the formation of a locus control region (LCR) to separate two transcription units. The testis-specific exon and promoter are not present in non-rodent animals, such as human and zebrafish (*53*, *59*). In addition, a Sertoli cell-specific knockout of the GATA1 gene in mouse revealed that GATA1 expression in Sertoli cells is not essential for testis development or spermatogenesis (*66*). Further studies are required to elucidate the importance of the G1TAR.

mHS-25/hHS+14—Transgenic reporter genes under the control of G1HRD recapitulated proper temporal and spatial transcription of the endogenous GATA1 gene, including the response to acute haemolytic anaemia (*67*). In addition, transgenic GATA1 expression under the control of G1HRD rescued GATA1-null and GATA1.05 mice from lethal dyserythropoiesis (*11*, *12*), suggesting that G1HRD could support GATA1 gene expression at a physiological level sufficient for erythropoiesis.

However, several problems remain: (1) the expression of reporter genes in mast cells has not been observed (*57*); (2) some type of GATA1-positive immature erythroid progenitors does not express the reporter gene (*22*); (3) deletion of G1HE from the genomic GATA1 locus does not affect GATA1 gene expression in red cells or eosinophils (*30*, *63*); (4) G1HE-like sequences have not been found in genomic databases of non-mammals, such as zebrafish and chicken (http://www.ensembl.org/index.html); (5) G1HRD is often subject to position effects and also prone to some type of age-related epigenetic silencing (*22*, *56*, *57*). These observations suggest that a LCR, regulatory elements for some specific haematopoietic cells, and a region compensating for G1HE functions are located outside this 8-kbp region of G1HRD.

To address these issues, Valverde-Garduno *et al*. (*59*) systematically mapped the positions of DNase I hypersensitive sites, the acetylation status of histone H3/H4, and *in vivo* binding of transcription factors over ~100-kbp flanking the mouse GATA1 gene. Consequently, they identified a region 25-kbp upstream of the IE exon (mHS-25) that was hypersensitive to DNase I and enriched for acetylated histone H3/H4 in erythroid cells. mHS-25 contains two GATA sites and four E boxes, although its functions require interpretation. The human GATA1 gene also carries a similar region, although it is located 14-kbp downstream of the IE exon (hHS+14). Analyses of the functions of these regions will be worthwhile in the near future.

trans-ACTING FACTORS FOR CONTROLLING GATA1 TRANSCRIPTION

Compared with *cis*-regulatory regions, we have a poor understanding of *trans*-acting factors controlling GATA1 transcription, even in erythroid cells. We know that regulation of the GATA1 gene is not so simple, as redundancy of upstream signals and the existence of feedback regulation add complexity. In this paragraph, we illustrate some candidates for the *trans*-acting factors, mostly those for erythroid expression, and present our current model (Fig. 2).

The results of DNase I hypersensitivity analyses and reporter gene assays suggest that factors binding to the double GATA site, CACCC box, the G1HE core and intSP are important in erythroid cells. Since critical elements in the G1HE core and intSP have been identified as GATA sites and/or CACCC boxes, factors binding to these two sites must be participating in GATA1 gene regulation.

GATA Factors and Associating Proteins—Reporter analyses using mice and cells revealed that key *cis*-regulatory elements in the G1HE core and intSP are GATA sequences, as described earlier. This and the importance of the double GATA site signify that GATA factors must play main roles in regulating GATA1 expression.

Tsai *et al*. (*47*) proposed that positive feedback regulation by GATA1 contributes to its increased expression throughout the maturation of haematopoietic cells and to maintaining the state of differentiation. This is plausible because chromatin immunoprecipitation (ChIP) analysis revealed that GATA1 binds to G1HE, the double GATA site and the first intron of mouse and human GATA1 genes in erythroid cells (*51*, *59*). Forced expression of GATA1 activated the chicken GATA1 promoter (*50*). Similarly, expression of a transgenic GATA1 reporter gene was upregulated by overexpression of GATA1 in zebrafish embryos (*53*). This up-regulation depends on the double GATA site, as was shown in stable transgenic zebrafish (*54*). These findings suggest that GATA1 positively regulates its own gene expression. It is a certainty that GATA1 is unable to regulate initiation of its own gene expression in GATA1-negative progenitors, yet GATA1 may self-regulate at later stages in haematopoiesis. Indeed, in GATA1 mutant zebrafish, levels of GATA1 gene expression were normal in early embryos, but decreased in later stages (*68*). Likewise, in CMP or MEP populations isolated from $GATA1^{low}$ mice, the expression levels of GATA1 were comparable with those from wild-type mice. In contrast, GATA1 expression was 10-fold lower in $GATA1^{low}$ megakaryocyte progenitors compared to their wild-type counterparts (*18*).

Consideration of GATA1 autoregulation makes it easier to explain the reduction in GATA1 expression in the later stage of erythropoiesis in wild-type mice. One plausible explanation of the down-regulation of GATA1 expression is a collapse of GATA1 autoregulation due to GATA1 protein degradation. It has been reported that caspase 3 provokes specific degradation of GATA1 protein in erythroblast cells and that this protein degradation is usually prevented by the chaperone protein Hsp70 whose nuclear localization is induced by erythropoietin (*69*, *70*). Degradation of GATA1 protein may result in down-regulation of GATA1 autoregulation, followed by reduced GATA1 expression.

Considering GATA1 autoregulation also accounts for the effects of GATA1 competitive factors, such as PU.1, which do not seem to act directly at the GATA1 locus to affect GATA1 gene expression. PU.1 is a member of the Ets family of transcription factors required for the development of lymphoid and granulocytic–monocytic lineages. Several lines of evidence indicate that PU.1 functionally antagonizes GATA1 activity through direct physical interaction (*71*). In zebrafish embryos, gene knockdown of PU.1 resulted in ectopic expression of GATA1 in presumptive myeloid cells (*34*). These observations suggest that PU.1 hinders the binding of GATA1 protein to the GATA1 gene locus, thus inhibiting autoregulation, and as a result reduces the expression of GATA1 indirectly. It was, however, shown that PU.1 inhibits the erythroid program by binding to GATA1 protein on DNA and creating a repressive chromatin structure (*72*). It is possible that PU.1 also regulates the GATA1 gene in a straight line.

GATA2 also seems important for controlling GATA1 expression, since it is expressed in HSC and early progenitors of erythroid cells, mast cells and megakaryocytes prior to GATA1 (*43*). The GATA1 promoter can drive the expression of a reporter gene in GATA1-null cells at the proerythroblast stage, when GATA1 expression is observed to be highest in wild-type cells during erythropoiesis and when GATA1 autoregulation should

function (*57*). Since GATA2 expression is upregulated in these cells (*73*), it is possible that GATA2 compensates for GATA1 in the regulation of GATA1 gene expression. However, the effect of GATA2 on GATA1 gene regulation is controversial. The GATA1 gene was induced by forced expression of GATA2 in some cells (*74*, *75*), but not in others (*76*, *77*). In GATA2-null cells, GATA1 expression was downregulated in blast cells (*75*), but unchanged in differentiated cells (*78*). This probably means that GATA2 is not essential for inducing the GATA1 gene, but plays some roles in its expression in stem cells and early progenitors. The simple idea is that GATA1 expression is regulated redundantly by both GATA factors, but mainly by GATA2 in an early stage and by GATA1 later on. Anyhow, the effects of GATA2 on each *cis*-regulatory region remain to be elucidated.

Non-redundant functions of the double GATA site, the G1HE core, and intSP have been demonstrated using an artificial minigene, in which these three elements plus the CACCC box were linked together into a 979-bp sequence (*51*). Deletion of any of these regions resulted in a great reduction in promoter activity, suggesting that different transcription factor complexes associate with each region. Indeed, ChIP analysis revealed that SCL, LMO2, LDB1 and E2A proteins bind to G1HE, but not to the double GATA site or to the first intron in erythroid cells (*59*). Interestingly, all three elements were demonstrated to be bound by GATA1 protein *in vivo*. It is plausible to hypothesize that different GATA1 complexes are recruited to each element.

A pentameric complex composed of GATA1, LMO2, LDB1, SCL and E2A was shown to activate transcription from promoters containing an E-box and a GATA-binding site (*79*). G1HE contains two consensus E-boxes, although mutation of these E-boxes did not abrogate reporter gene expression (*60*, *61*). However, it is feasible to think that SCL plays critical roles in GATA1 expression through G1HE, because SCL can be recruited to *cis*-regulatory regions in the absence of direct DNA binding (*80*) and binding of SCL to G1HE is GATA1 dependent, as shown in $GATA1^{low}$ mice (*62*). Recently, novel members associating with this pentameric complex, such as ETO2, MTGR1 and CDK9, were identified and shown to dissociate from the complex after late erythroid differentiation, implying a dynamic regulation of the complex during haematopoiesis (*81*).

A GATA1 homo-oligomeric complex was shown to be required for GATA1 gene expression in zebrafish embryos (*54*). The double GATA site is required for the formation of this GATA1 homo-oligomeric complex. The importance of GATA1 self-association was also demonstrated in mice (*82*). It is unknown whether or not other proteins are included in the GATA1 homo-oligomeric complex. One such candidate is CP2, which is a ubiquitously expressed transcription factor belonging to the *Drosophila* grainyhead-like gene family. Bosè *et al*. (*83*) showed that CP2 binds to a CP2-binding site near the double GATA site of the mouse GATA1 gene, and that mutations in the CP2-binding site greatly impaired promoter activity in transient transfection assays with K562 cells. ChIP experiments revealed that CP2 is bound *in vivo* to the regulatory elements of the GATA1 gene and is bound simultaneously to GATA1. Although no CP2-binding site was found around the double GATA site in the zebrafish gene, it is possible that CP2 is included in this complex, since GATA1 and CP2 can physically interact even in the absence of DNA.

Sp1/KLF Proteins—Members of the Sp1/Krüppel-like factor (KLF) family bind to CACCC boxes (*84*). Among them, some KLF proteins are candidates for serving as GATA1 expression regulators. The best candidate is KLF1/EKLF, since it is expressed abundantly in erythroid cells and is required for both primitive and definitive erythropoiesis (*85*). However, the GATA1 gene is normally expressed in KLF1-null mice (*86*) and thus it is plausible that other KLF proteins transactivate the GATA1 gene redundantly. Interestingly, KLF2-related BiKLF or Neptune in zebrafish and *Xenopus*, respectively, are expressed in presumptive erythroid cells earlier than the GATA1 gene (*87*, *88*). Knockdown of BiKLF in zebrafish embryos reduced the expression of GATA1 (*89*), suggesting that BiKLF regulates GATA1 expression. Similarly, mouse KLF2/LKLF was reported to be essential for primitive erythropoiesis and regulates the GATA1 gene *in vivo* (*90*). Furthermore, KLF6/CPBP was also shown to be required for primitive erythropoiesis in mice (*91*). It is likely that members of the Sp1/KLF family that are expressed in presumptive erythroid cells regulate GATA1 gene expression redundantly.

Factors binding to the CACCC box in the G1HE core, which is required for GATA1 expression in macrophages, might also be members of the Sp1/KLF family. Indeed, Krüppel-related transcription factor ZBP-89 was shown to bind to the CACCC box in the G1HE core by ChIP analysis and gel-shift analysis using magakaryoblast extracts (*62*). ZBP-89 is not an authentic member of the Sp1/KLF family, but has Krüppel-related zinc fingers and is able to interact with GATA1 protein. Interestingly, gene knockdown of zebrafish ZBP-89 reduced GATA1 expression and disrupted haematopoiesis (*92*). Zebrafish ZBP-89 is expressed in haematopoietic tissues and in a region of the brain. Guyot *et al*. (*62*) showed that ZBP-89 also binds *in vivo* to the proximal promoter region in which the CACCC box is included. These results indicate that ZBP-89 may participate in GATA1 expression in megakaryotic cells, as well as in erythroblast cells. The relationship between ZBP-89 and other Sp1/KLF proteins should be elucidated in the future.

It is possible that some members of the Sp1/KLF family negatively regulate GATA1 gene expression. Hu *et al*. (*93*) analysed the roles of nine Sp1/KLF proteins in erythroid cells using systematic gene knockdown analyses with siRNAs. The results testified that Sp1 and KLF8 play negative roles in erythroid differentiation, suggesting a complex and coordinated regulation by Sp1/KLF proteins via CACCC boxes.

Other Factors—Other factors unrelated to GATA or CACCC sites may control GATA gene expression, albeit the lack of information for such binding sites in the GATA1 gene locus. A homeobox transcription factor HOXA10 is expressed in the most primitive haematopoietic cells. Overexpression of HOXA10 in mice downregulated GATA1 expression and blocked erythropoiesis and megakaryocyte development (*94*).

Consensus sequences for HOXA10 binding exist in the first intron, but not in the promoter, of the mouse GATA1 gene. Gene expression of GATA1, but not GATA2 or SCL, was induced in BMP-treated embryonic bodies (*95*). The roles of BMP in blood formation were revealed in *Xenopus* and zebrafish (*96*, *97*). It is possible that SMAD1, a downstream regulator of the BMP pathway, transactivates the GATA1 gene. Several Smad consensus sites are present in GATA1 promoters.

PERSPECTIVE

The mechanisms of GATA1 gene expression have been studied for more than 16 years. Various *cis*-regulatory regions were identified in many kinds of cells, organisms and stages. However, only two critical elements, the GATA site and the CACCC box, have come to light (Fig. 2). Surprisingly, this conclusion is almost the same as in the first report in which the isolation of the GATA1 promoter was described (*47*). It is possible that some critical elements have not yet been identified, but more likely the key to unlocking the mystery of GATA1 gene regulation is hidden within the GATA site and the CACCC box. It was demonstrated that the GATA2 promoter is regulated positively by GATA2 protein itself initially before switching to negative regulation by GATA1 (*8*, *9*). This regulation is called the 'GATA switch'. In the same way, 'switching' GATA and/or Sp1/KLF proteins during haematopoiesis may control temporal and spatial GATA1 gene expression. Understanding how complexes containing these proteins organize epigenetic regulation of the GATA gene locus is intriguing.

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