Cloning and Analysis of the Thrombopoietin-induced Megakaryocyte-specific Glycoprotein VI Promoter and Its Regulation by GATA-1, Fli-1, and Sp1*

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**RESULTS**

**Identification of Differentially Expressed Genes in UT-7/TPO Cells following Stimulation with Thrombopoietin—UT-7/TPO cells resemble mature megakaryocytes and require TPO for growth and survival (4). To identify TPO-regulated genes, we have used the technique of RDA to isolate cDNAs corresponding to genes that are expressed at higher levels in TPO-stimulated UT-7/TPO cells than in cells starved of this cytokine. A selection of the cDNAs isolated in this way, including those corresponding to genes that are expressed at higher levels in TPO-stimulated UT-7/TPO cells than in cells starved of this cytokine, were further characterized by Northern Blotting (5). The Northern Blot analyses indicated that cDNA probes of similar specific activities had been used.

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**Expressions of Plasmids**—Expression plasmids for Sp1 (human Sp1 cloned into pEF-Box and pJur (murine GATA-1 cloned into pBluescript KS+).
To roughly map the region(s) necessary for high level expression of GPVI in UT-7/TPO cells, an initial series of GPVI promoter constructs were created bearing large deletions from the 5'-end of the promoter. As can be seen in Fig. 2B, deletion of the region from -694 to -462 reduced luciferase levels by ~30% (compare rows 2 and 3), but further deletion from -462 to -315 resulted in luciferase levels comparable with the full-length construct GPVI–694 suggesting positive and negative regulatory regions, respectively. However, the deletion of the region from -315 to -118 (GPVI–118) drastically reduced luciferase levels to around 10% of the GPVI–694 or GPVI–315 activities (compare columns 5 to columns 2 and 4).

The removal of upstream sites in the 5'-deletion series described above may mask potential effects of binding motifs further downstream. For example, because expression of the GPVI–118 construct was only 10% of the full-length promoter, the effect of further deletions may not be observed. Therefore, we created a second deletion series in which segments of the GPVI promoter were removed, leaving upstream elements intact (Fig. 2B, rows 6–8). The deletion from -605 to -462 (GPVIdel–605/-462) resulted in a modest (~30%) decrease in promoter activity (compare row 6 to row 2). The second deletion (GPVIdel–315/-118), removing a 193-nt sequence between -315 and -118 but otherwise leaving the remaining 0.5-kb promoter region intact, resulted in a dramatic (94%) decrease in luciferase expression to levels similar to the GPVI–118 construct (compare row 7 to rows 2 and 5). Finally, deletion of a region proximal to the transcription start site, from -118 to -27, also affected GPVI promoter activity, reducing luciferase levels to 20% of the full-length construct GPVI–694 (compare row 8 to row 2).

Previous studies with several MK-specific promoters have identified negative regulatory regions, which when deleted result in expression of promoter constructs in nonmegakaryocytic cell lines (15, 16). To determine whether GPVI is similarly regulated, we tested the deletion constructs in the nonhematopoietic HeLa cell line. None of the GPVI constructs were expressed at significant levels (Fig. 2C) suggesting that no such regions exist in the GPVI promoter. The two regions, -315 to -118 and -118 to -27, which when deleted, caused significantly reduced (16- and 5-fold, respectively) luciferase expression compared with GPVI–694 in UT-7/TPO cells were then analyzed in more detail.

**Fine Mapping of the -315 to -118 GPVI Region**—A second series of GPVI 5'-deletion mutants were generated to further define the region(s) necessary for maximal expression. As luciferase levels driven by the GPVI–315 construct were comparable with that of the longer GPVI–694 plasmid, all future
Fig. 2. Analysis of the GPVI promoter. A, nucleotide sequence flanking the GPVI coding sequence (uppercase) with the numbers corresponding to the transcription start site (+1) as defined by Ref. 14. This sequence has been submitted to GenBankTM (AF521646). The sites of the various deletions are indicated with short vertical lines and numbers above the sequence. The sequences corresponding to the EMSA probes (S227, G177, and E48) are underlined. The position of Sp1, GATA, and Ets binding sites investigated in this study are shown with arrows. B, effect of GPVI promoter deletions (1.5 µg of DNA) on transcriptional activity in UT-7/TPO cells. Luciferase levels were measured 48 h after transfection and are shown relative to the activity driven by the SV40 promoter (SV40-luci). C, HeLa cells were also transfected with the GPVI-luciferase constructs (0.2 µg of DNA). D, fine mapping of the GPVI −315 to −118 promoter region to identify motifs involved in GPVI expression in UT-7/TPO cells. Luciferase levels were measured 48 h after transfection and activities were compared with the GPVI −315 construct.
transfection experiments show luciferase levels relative to this shorter construct.

Fig. 2D shows the results of a typical experiment when these constructs were transfected into UT-7/TPO cells. The deletions from −315 to −235 had only a modest effect on the promoter activity (compare rows 2–4 to row 1). However, the deletion of a region from −235 to −210 within the GPVI promoter, including a potential Sp1 binding site (Sp1227), reduced the activity of the promoter by ~60% (compare row 5 to row 4). Luciferase levels were further reduced (3-fold, row 6 compared with row 5) with the deletion of sequences from −210 to −159. This region contains a consensus GATA site at −177 (Fig. 2A, GATA177). The similar luciferase activities of GPVI−159 and GPVI−118 suggest that removing an additional 41 nucleotides between −159 and −118 had no apparent effect on residual GPVI promoter activity.

**Gel Mobility Shift Assays Identify Proteins in UT-7/TPO Nuclear Extracts Binding to Sp1, GATA, and Ets Sites within the GPVI Promoter**—Our deletion analyses using transient transfection assays had identified three regulatory regions within the GPVI promoter: −235 to −210, −210 to −159, and −118 to −27. Presumably, transcription factors present in UT-7/TPO cells could bind to sites within these regions and modulate the transcriptional activity of the GPVI promoter. The sequence of these regions were analyzed for consensus binding sites using Transfac (bioinformatics.weizmann.ac.il/transfac) and Motif (motif.genome.ad.jp) data bases. A number of potential sites were identified including Sp1227, GATA177, and Ets48 sites. Therefore, we performed gel mobility shift experiments with various probes derived from these regions to determine whether protein factors expressed in UT-7/TPO cells could bind to these sites.

Fig. 3A shows the EMSA analysis of the first of these regions: the probe S227 corresponds to the region −231 to −209 within the GPVI promoter fragment, encompassing the potential Sp1 binding site at −227 (see Fig. 2A). Incubation of S227 with nuclear extracts from UT-7/TPO cells resulted in the formation of two distinct protein complexes (Fig. 3A, lane 1). One of these (labeled ns) was a nonspecific complex as it could be competed away using nonrelated sequences (data not shown). The second lower mobility complex (labeled s1) is shifted by an Sp1 monoclonal antibody (lane 2). Moreover, mutation of the Sp1227 site (Smut227, lane 3) prevents the formation of this s1 complex. Therefore, it appears that Sp1 is both present and able to bind the Sp1227 site within the GPVI promoter in UT-7/TPO cells.

The second region we analyzed, −210 to −159, included a potential GATA binding site at −177 (GATA177). GATA-1 is highly expressed in UT-7/TPO cells (see Fig. 5A), and GATA sites have been shown to play an important role in the regulation of a number of MK-specific genes. Thus, we first used recombinant GATA-1-NC protein (truncated GATA-1 corresponding to the DNA-binding domain2) to determine whether GATA-1 could bind to the GPVI promoter fragment G177 (from −195 to −163, Fig. 2A). Fig. 3B shows that GATA-1-NC does bind to G177 (lane 1) and that mutation of the GATA177 site prevents GATA-1-NC binding to Gmut177 (lane 2). Next, we determined whether UT-7/TPO nuclear extracts contained proteins that interact with G177, and in particular, to the GATA177 site, and these results are also shown in Fig. 3B, lanes 3–6. A major complex, labeled g1, could be competed away with a GATA consensus oligonucleotide (lane 4), whereas the addition of an excess of either Gmut177 (the probe with mutated GATA177 site, lane 5), or a nonrelated sequence (lane 6), had no effect on the formation of this complex. There is also a reduction in the intensity of a faster migrating complex labeled ns in two of the samples (lanes 4 and 6 compared with lanes 3 and 5).

However, as an oligonucleotide containing no GATA binding site (Non-Rel, lane 6) competed as effectively as the GATA consensus oligonucleotide, we concluded that this complex was not GATA site-specific. Therefore, we have shown that a GATA site-binding complex is present in UT-7/TPO cells and that it is able to bind to the G177 fragment within the −210 to −159 region of the GPVI promoter.

The third promoter region, −122 to −27, included an Ets
motif at −48 (Ets48). Ets factors have also been implicated in the regulation of many MK-specific promoters. As the Ets factor Fli-1 is present in both MK and platelets (17), we tested whether recombinant Fli-1 protein could bind to a fragment, E48 (see Fig. 2A), corresponding to −69 to −32 of the GPVI promoter and encompassing the potential Fli-1/Ets binding site. Fig. 3C shows the results of these gel retardation experiments: recombinant Fli-1/Ets protein (truncated Fli-1 including the DNA-binding Ets domain) does bind to the GPVI E48 probe (lane 1) and mutation of the Ets48 site disrupts Fli-1 binding (lane 2). This figure also shows the complexes formed when nuclear extracts from UT-7/TPO cells are incubated with either the E48 or Emut48 probes (lanes 3 and 4, respectively). Two low mobility complexes in lane 3, labeled e1 and e2, are of particular interest as their formation requires an intact Ets48 site. Although the intensity of a faster migrating complex binding to the Emut48 probe was also decreased slightly (lane 4 labeled ns), this complex was not Ets site-specific as when a related probe was used (overlapping E48, Ets site mutated) the intensity of this complex increased with respect to the wild-type sequence (data not shown). Together, these EMSA results show that proteins present in UT-7/TPO nuclear extracts bind to the Ets48 site within the −122 to −27 GPVI promoter region and that the Ets factor Fli-1 can bind to this site.

Functional Importance of the Sp1227, GATA177, and Ets48 Sites—To assess the functional importance of the Sp1227, GATA177, and Ets48 sites identified in the EMSA analysis above, we used site-directed mutagenesis to alter the sites within the GPVI−315 luciferase reporter construct to those corresponding to the mutated probes used in the EMSA studies. The effect of each of these mutations on GPVI promoter activity in transiently transfected UT-7/TPO cells are shown in Fig. 4A. Mutation of the Ets48 site resulted in luciferase levels ~5-fold lower than the wild type GPVI−315 construct (compare column 3 to column 4), indicating that the Ets48 site plays an important role in GPVI promoter activity. The GATA177 mutation (GPVI−315Gmut177) also had a dramatic effect on GPVI promoter activity as luciferase levels were reduced 16-fold compared with the wild type promoter (compare column 2 to column 4). Finally, mutation of the Sp1227 site (GPVI−315Smut227) decreased luciferase levels by 40% (compare column 1 to column 4).

GATA-1, Fli-1, and Sp1 trans-activate the GPVI−315 Construct in Nonmegakaryocytic Cell Lines—Because of the importance of the Sp1227, GATA177, and Ets48 sites for GPVI promoter activity, and the presence of Sp1, GATA-1, and Fli-1 in MKs, we decided to determine whether these factors could trans-activate the GPVI promoter in transient transfection assays. Neither GATA-1 nor Fli-1 are expressed in HeLa cells and luciferase levels driven by the GPVI−315 construct are barely higher than the promoterless control (Fig. 2C, rows 4 and 9). However, co-transfection of a Fli-1 expression plasmid with the GPVI−315 reporter construct resulted in a dose-dependent increase in luciferase levels, at least 9-fold above basal levels (GPVI−315 alone; Fig. 4B, columns 2–4, compared with column 1). Interestingly, the GATA-1 expression plasmid alone had only a modest effect on GPVI−315 activity: luciferase levels were 2–3-fold higher than GPVI−315 alone (Fig. 4B, columns 5–7 compared with column 2). This was somewhat surprising as the GATA177 mutation reduced GPVI promoter activity in UT-7/TPO cells by greater than 90% (Fig. 4A, column 2 compared with column 4). This suggested that activation of the GPVI promoter by GATA-1 might require an additional factor(s) not present in HeLa cells. In another study, we have found that GATA-1 and Fli-1 can physically interact and their association is involved in the synergistic activation of two other MK-specific promoters, GPIX and GPIba. Therefore, we co-transfected HeLa cells with the GPVI−315 reporter construct and both GATA-1 and Fli-1 expression vectors (Fig. 4B, columns 8–10). The resulting luciferase levels were greater than additive suggesting that GATA-1 and Fli-1 may synergize to regulate GPVI expression, perhaps through their physical interaction or the recruitment of additional factors (see “Discussion”).

The GPVI−315 construct was also cotransfected with an Sp1 expression vector. As can be seen in Fig. 4C, Sp1 had a dramatic effect on GPVI−315 expression and resulted in luciferase levels 30-fold higher than the reporter alone. Thus, GATA-1, Fli-1, and Sp1 can activate the MK-specific GPVI promoter.

Expression of GPVI, GATA-1, and Fli-1 in Cell Lines with Erythroid and Megakaryocytic Characteristics—To determine whether GPVI expression correlates with the endogenous expression of GATA-1, Fli-1, and Sp1, we performed Northern blot hybridization of mRNAs from four erythro-megakaryocytic cell lines: Dami, K562, UT-7/TPO, and UT-7/EPO Mpl. Sp1 is ubiquitously expressed so was not included in this analysis. As
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FIG. 5. GPVI mRNA is only detected in cell lines expressing both Fli-1 and GATA-1. A, Northern blot analysis of Dami (lane 1), K562 (lane 2), UT-7/TPO (lane 3), and UT-7/EPO Mpl (lane 4) cells. Each lane contains 3 μg of poly(A) + RNA. The membrane was sequentially hybridized and then stripped of each of the indicated probes (GPVI, Fli-1, GATA-1, and GAPDH). B, the level of GPVI, Fli-1, and GATA-1 expression in K562 cell lines stably transfected with control plasmid pRES-eGFP (K562-GFP, lane 1) or Fli-1 expression plasmid pRES-Fli1-eGFP (K562-Fli, lane 2) were also assessed by Northern blot analysis. Hybridization with a GAPDH probe was used as a control for equivalent loading of samples (3 μg of poly(A) + RNA).

can be seen in Fig. 5A, GATA-1 is expressed in all four cell lines. However, Fli-1 mRNA can only be detected in Dami and UT-7/TPO cells (lanes 1 and 3, respectively), and it is these same two cell lines that express GPVI. The similarity in GAPDH levels indicates that equivalent amounts of poly(A) + RNA were loaded in each lane. Therefore, all three of these factors are expressed in cell lines expressing GPVI, further supporting a role for Sp1, GATA-1, and Fli-1 in GPVI regulation.

Overexpression of Fli-1 in K562 Cells Results in Expression of the Endogenous GPVI Gene—Although GATA-1 and Fli-1 had been shown to regulate GPVI-315 in transient assays in a non-MK cell line (Fig. 4B), chromosomal context and cellular environment play essential roles in gene regulation. Therefore, we assessed the importance of GATA-1 and Fli-1 on the expression of the endogenous GPVI. The cell line K562, which has some MK features but is generally thought to represent erythroid cells, expresses GATA-1 but expresses little or no Fli-1 or GPVI (Fig. 5A, lane 2). We have established the clonal cell lines K562-GFP and K562-Fli by transfecting K562 cells with either the control vector pRES-eGFP or the Fli-1 expression vector pRES-Fli1-eGFP/Fli-1, respectively.2 Stably transfected cells were then selected that were G418-resistant and expressed high levels of green fluorescent protein (GFP) as determined by fluorescence-activated cell sorter analysis (data not shown). Overexpression of Fli-1 in the K562-Fli cell line was confirmed by Northern blotting (Fig. 5B, lane 2). Both of the cell lines chosen for Northern analysis exhibited similar levels of GFP and GFP expression was maintained even after extensive cell passaging.

The levels of GATA-1 and GPVI mRNAs in the cell lines K562-GFP and K562-Fli were then analyzed by Northern hybridization (Fig. 5B). The similar intensity of GAPDH bands indicate equal amounts of mRNAs were loaded. Overexpression of Fli-1 did not appear to effect GATA-1 expression as similar mRNA levels are seen in both cell lines. Importantly, although no GPVI mRNA was detected in the K562-GFP cell line (lane 1), GPVI was expressed in K562-Fli cells (lane 2). Therefore, overexpression of Fli-1 in K562 cells resulted in expression of the endogenous GPVI gene, providing further evidence that Fli-1 in combination with GATA-1 and Sp1 may play a role in GPVI regulation.

Expression of GATA-1, Fli-1, and Sp1 in UT-7/TPO Cells Stimulated with Thrombopoietin—Our experiments to date have shown that TPO stimulation of UT-7/TPO cells results in the increased expression of GPVI; GATA, Ets, and Sp1 sites in the promoter play an important role in GPVI expression in UT-7/TPO cells maintained in TPO; and that GATA-1, Fli-1, and Sp1 can bind to the GPVI promoter. Therefore, we were interested in determining whether the increase in GPVI expression in TPO-stimulated UT-7/TPO cells is because of the TPO-induced expression of GATA-1, Fli-1, or Sp1. As can be seen in Fig. 5A, lane 3, GPVI mRNA can readily be detected in UT-7/TPO cells maintained in TPO. However, following 16 h starvation of TPO, GPVI mRNA levels are negligible (Figs. 1B and 6, “0” time point) but increase to near maximal levels within 4 h of TPO stimulation. Fig. 6 shows the levels of GATA-1, Fli-1, and Sp1 mRNAs in TPO-starved and -stimulated UT-7/TPO cells. The level of expression of all three of these factors are maintained throughout the time course of this experiment. Therefore, although our experiments have demonstrated a critical role for GATA-1, Fli-1, and Sp1 in regulating constitutive expression of the GPVI promoter, the role that these factors play in TPO-induced expression of GPVI is unclear (see “Discussion”).

DISCUSSION

Thrombopoietin supports hematopoietic stem cell survival, stimulates progenitors committed to various lineages, causes megakaryocyte progenitors to proliferate, and induces the expression of surface proteins necessary for platelet function. To identify TPO-responsive genes, we used the RDA technique and this resulted in the cloning of a number of different cDNAs. Several of these correspond to genes encoding proteins involved in signaling, such as the hematopoietic-specific G protein Gz16 (18), Stat5b, the Stat5 target genes SOCS-3 and Pim-1 (19), and CalDAG-GEF-1 (Map4K). Genes encoding enzymes such
as phosphoenolpyruvate carboxykinase, which is involved in gluconeogenesis, and cystathionine β synthase were also identified. Heterogeneous nuclear ribonucleoprotein D (or AUF1) was also cloned and this gene encodes a protein that binds to short lived mRNAs and is involved in regulating expression of genes such as c-myc, c-jun, and c-fos (20). Although preliminary experiments suggested that the expression of these genes did increase following TPO stimulation of UT-7/TPO cells (Fig. 1A), their regulation is beyond the scope of this investigation and they were not pursued further. However, the isolation of GPVI and Pim-1 by RDA attest to the success of this technique as both GPVI and Pim-1 are indeed regulated by TPO (this report and Ref. 11).

GPVI, a collagen receptor specifically expressed on the surface of MKs and platelets, plays an important role in limiting blood loss. It is noncovalently associated with the Fc receptor γ chain, the signaling subunit of the complex. Upon injury of the vessel wall, collagen fibers are exposed that are highly thrombogenic. Initially, the platelets adhere to the collagen via integrin αIIbβ3, and this is followed by platelet activation involving GPVI. Defective collagen-induced responses in a patient lacking GPVI testify to the importance of GPVI in this process (21). In addition, the level of collagen receptors on platelets may predispose individuals to either excessive bleeding (low collagen receptor levels) or coronary heart disease and strokes (high levels), and genetic screening of patients with a family history of these diseases for GPVI polymorphisms could aid the planning of treatment strategies (22). It is also possible that GPVI will prove to be a therapeutic target as mice treated with an anti-GPVI antibody prior to collagen and adenalin infusion were protected from lethal thrombus formation (23).

Because of the clinical importance of GPVI and its restricted expression to MKs and platelets, we chose to examine the regulation of this gene. Northern blot analysis confirmed that the abundance of GPVI mRNA increased in UT-7/TPO cells stimulated with TPO. Two transcripts were detected, a major 2-kb mRNA and a less abundant message of ~3.6 kb. These two transcripts may represent alternatively spliced mRNAs, the utilization of an alternative promoter, or differences in the 3′-untranslated region. Three GPVI isoforms and differences in the 3′-regions have been discussed in another study (14). GPVI levels have previously been shown to be higher in mature MKs than in immature cells (24) and to increase in megakaryoblastic cell lines HEL and CMK induced to differentiate with phorbol 12-myristate 13-acetate (13). However, the factors controlling the regulation of GPVI expression have not been investigated.

Several groups reported the cloning of the GPVI gene from mice and humans and have shown that its expression is restricted to embryonic liver, megakaryocytes, and platelets (12, 14, 25). The major transcription start site has been mapped to an adenosine 29 nucleotides upstream of the coding region (14). In the present study, we have cloned a 694-bp region 5′-flanking the GPVI gene and have demonstrated that it includes the regulatory regions necessary for MK-specific expression. Our promoter deletion analysis has resulted in the identification of three regions (~235 to ~210, ~210 to ~159, and ~118 to ~27), which are important for the positive regulation of GPVI expression in UT-7/TPO cells. The binding of proteins present in UT-7/TPO nuclear extracts to Sp1227, GATA177, and Ets48 sites within these three regions was determined by EMSA. As Sp1, GATA-1, and Fli-1 are expressed in MKs and platelets, we used an Sp1 antibody and recombinant GATA-1 and Fli-1 proteins to demonstrate that these proteins can bind to the GPVI Sp1227, GATA177, and Ets48 motifs, respectively. The functional importance of these three sites was demonstrated by transient transfection of UT-7/TPO cells with GPVI-reporter constructs; the targeted disruption of any one of the sites reduced GPVI promoter activity. Mutation of GATA177 decreased the GPVI promoter activity in UT-7/TPO cells by ~94%. The disruption of Sp1227 and Ets48 sites decreased the GPVI promoter activity by 40 and 80%, respectively. In addition, co-transfection of Sp1, GATA-1, and Fli-1 expression plasmids with the GPVI-reporter construct increased luciferase levels between 2- and 30-fold. We found that GPVI is expressed only in the cell lines that express all three of these factors. Furthermore, the overexpression of Fli-1 in K562 cells (which express endogenous GATA-1 and Sp1) results in the expression of the endogenous GPVI, whereas, GPVI mRNA was not detected in K562 cells in the absence of Fli-1. Taken together, these data strongly suggest that Fli-1, GATA-1, and Sp1 play a role in regulating GPVI expression. However, the role if any, of these three factors in the induction of GPVI expression by TPO is unclear as their mRNA levels are constant throughout the time course of TPO induction. It is possible that these factors are subject to post-translational modifications or that additional factors that are regulated by TPO may bind to Fli-1, GATA-1, and/or Sp1 and augment their activity. The precedent for this is that although the TPO-responsive element within another MK-specific promoter, GPIX, has been mapped to an Ets site, and Fli-1 had been shown to bind to this site, a difference in Fli-1-binding activity before and after TPO induction could not be demonstrated (3). Future work could include the mapping of TPO-responsive elements within the GPVI promoter.

The analysis of cis-acting regulatory sequences in megakaryocyte-specific promoters has advanced our understanding of the control of differentiation and maturation in this lineage. A common theme to emerge from these studies is the presence of functional GATA and Ets elements in most MK-expressed genes (26-29). An excellent example of the importance of GATA sites comes from the study of a patient suffering from Bernard-Soulier syndrome, a congenital bleeding disorder caused by insufficient expression of the GPIb-IX-V complex on MK and platelets (30). The cause was a mutation mapped to a GATA site in the GPIbβ promoter, and the corresponding mutation in a GPIbβ-reporter construct was shown to reduce expression by 84%.

GATA-1 is the founding member of a family of DNA-binding zinc finger proteins that play crucial roles in cell development (for a review, see Ref. 31). Although initial studies focused on the importance of GATA-1 in the erythroid lineage, such as its involvement in the regulation of the globin genes (32), the selective knockout of GATA-1 in the megakaryocytes of mice revealed that GATA-1 also plays a key role in megakaryocyte maturation and platelet production (33). In addition, a number of GATA-1 mutations have now been identified in humans suffering from X-linked thrombocytopenia (34, 35). However, the control of gene expression in megakaryocytes is not achieved through GATA-1 alone. Both cell-restricted and ubiquitous factors act in combination to enable the flexibility necessary for cell- and temporal-specific gene expression. Some of these factors, such as Fli-1, bind to distinct sequences in the regulatory regions of MK genes (17), whereas others such as FOG-1 do not appear to contact DNA directly but modulate gene expression through their interaction with other factors (36, 37).

Fli-1, a member of the Ets family of winged helix-turn-helix proteins, has been implicated in the normal development of megakaryocytes. Inactivation of the Flit-1 gene in mice results in embryonic lethality because of hemorrhaging caused by vascular development abnormalities, but these mice also were found to have small, undifferentiated MK progenitors (38, 39). The cooperative action of GATA-1 and Fli-1 during megakaryo-
poiesis is supported by the observed similarities between the GATA-1-null and Fli-1-null megakaryocyte abnormalities, the presence of functional GATA and Ets sites in the majority of MK gene promoters, and the demonstration that these two factors can directly interact.2

The GPVI promoter lacks a TATA box (14). Sp1 has frequently been shown to be involved in enhancing transcription of TATA-less promoters (40) and these include the α2 integrin and GPIIb gene promoters expressed in MKs (16, 41). The α2 integrin, together with the β1 subunit, form another MK collagen receptor. A reduction in α2β1 receptors on MKs and platelets is associated with two single-base polymorphisms, C→T and C→G, found in the general population (42). The T→C and G→T substitutions have been shown to decrease binding of Sp1 to two adjacent sites and to reduce α2 gene transcription (42, 43). Interestingly, there appears to be a correlation between α2β1 subunit expression and the presence of functional GATA and Ets sites in the majority of MK promoters (16, 2062). GPIIb/IIIa and GPIb/IX genes was also suggestive that α2β1 subunit expression was dependent on the involvement of Sp1 in the transcriptional synergy we observe between GATA-1 and Fli-1. Therefore, it is possible that Sp1, GATA-1, and Fli-1 binding to the Sp1, GATA, and Ets cis-elements interact with GATA-1 (44). Therefore, it is possible that Sp1, α2β1, and α2 gene transcription (22) and the involvement of Sp1 in the expression of both α2 and GPVI genes was also suggestive that these two receptors may be coordinately regulated.

Recently, we have found that Fli-1 can bind to GATA-1 and together these factors synergistically activate the expression of two other MK genes GPIX and GPIba.2 Sp1 is known to physically interact with GATA-1 (44). Therefore, it is possible that Sp1, GATA-1, and Fli-1 binding to the Sp1, GATA, and Ets sites within the GPVI promoter are able interact directly with each other, and these interactions may be important for the correct regulation of the GPVI promoter. For example, physical interaction between GATA-1 and Fli-1 may be important for the transcriptional synergy we observe between GATA-1 and Fli-1 on the GPVI promoter. MK-specific expression of GPVI promoter are able interact directly with GATA-1 and Fli-1 on the GPVI promoter. MK-specific expression of GPVI promoter. MK-specific expression of GPVI promoter are able interact directly with GATA-1 and Fli-1 on the GPVI promoter. MK-specific expression of GPVI promoter. MK-specific expression of GPVI promoter. MK-specific expression of GPVI promoter. MK-specific expression of GPVI promoter. MK-specific expression of GPVI promoter. MK-specific expression of GPVI promoter. MK-specific expression of GPVI promoter. MK-specific expression of GPVI promoter.