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PU.1 and partners: regulation of haematopoietic stem cell fate in normal and malignant haematopoiesis

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Abstract

During normal haematopoiesis, cell development and differentiation programs are accomplished by switching 'on' and 'off' specific set of genes. Specificity of gene expression is primarily achieved by combinatorial control, *i.e.* through physical and functional interactions among several transcription factors that form sequence-specific multiprotein complexes on regulatory regions (gene promoters and enhancers). Such combinatorial gene switches permit flexibility of regulation and allow numerous developmental decisions to be taken with a limited number of regulators. The haematopoietic-specific Ets family transcription factor PU.1 regulates many lymphoid- and myeloid-specific gene promoters and enhancers by interacting with multiple proteins during haematopoietic development. Such protein–protein interactions regulate DNA binding, subcellular localization, target gene selection and transcriptional activity of PU.1 itself in response to diverse signals including cytokines, growth factors, antigen and cellular stresses. Specific domains of PU.1 interact with many protein motifs such as bHLH, bZipper, zinc fingers and paired domain for regulating its activity. This review focuses on important protein–protein interactions of PU.1 that play a crucial role in regulation of normal as well as malignant haematopoiesis. Precise delineation of PU.1 protein-partner interacting interface may provide an improved insight of the molecular mechanisms underlying haematopoietic stem cell fate regulation. Its interactions with some proteins could be targeted to modulate the aberrant signalling pathways for reversing the malignant phenotype and to control the generation of specific haematopoietic progeny for treatment of haematopoietic disorders.

Keywords: haematopoiesis ● protein–protein interaction ● transcription factor ● co-activator ● co-repressor

Introduction

Haematopoiesis is a continuous and stepwise controlled process in which the pluripotent haematopoietic stem cells (HSCs) undergo differentiation to produce all mature blood cell lineages, which comprise the effector cells of both innate and acquired immunity. These cells fulfil specific roles in the host defence against invading pathogens and in the maintenance of homeostasis [1]. The regulation of haematopoiesis is essential for the replenishment of distinct blood cell types that include B and T lymphocytes;

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erythrocytes; megakaryocytes/platelets; basophils/mast cells; eosinophils; neutrophils/granulocytes and macrophages/ monocytes [2]. The haematopoietic development is under stringent control from extracellular and intracellular stimuli that result in the activation of specific downstream signalling cascades. The integration of these complex but finely tuned regulatory pathways provides almost unlimited possibilities for haematopoietic regulation and results in an elaborate fail-safe mechanism for controlling gene expression and an overall cellular haematopoietic response.

Central players in this process are sequence-specific transcription factors that 'program' precursor cells to express growth factor receptors, which mediate proliferation and differentiation signals produced by the microenvironment [3]. The mature blood cell types acquire their characteristic properties as a result of coordinated, lineage-specific gene expression at appropriate stages of lineage commitment, proliferation, terminal differentiation and functional maturation. Therefore, haematopoietic development is largely regulated by the activities of haematopoietic transcription factors that select genes to be activated and generate a highly specific response that is determined by the precise arrangement of DNA-binding sites and the diversity of protein–protein interactions [4].

Rather than being controlled by a single master regulator, lineage-specific gene expression depends on the combination of factors that bind in overlapping functional domains. Gene transcription requires the assembly of RNA polymerase II with a multiprotein pre-initiation complex at specific DNA sequences including the TATA box in a promoter. Interactions of general transcription factors with basal promoter elements are generally essential for basal transcription but not sufficient to modulate its rate. Therefore, the activation or repression of gene transcription takes place when several transcription factors bind to their cognate sequences on the lineage-specific promoters and stimulate or inhibit transcription through protein–protein interactions with the basal transcription machinery [5]. Based on bacterial gene regulation models, it has been proposed that protein interactions between the individual enhancer-bound factors serve to promote cooperative assembly on the DNA [6]. Recent observations, however, demonstrate that major functions of typical transcription factors do not require DNA binding, suggesting that due to their protein interaction potential, transcription factors carry information beyond the stabilization of DNA contacts [7]. In addition, transcription factors interact with other trans-regulatory proteins such as co-activators, co-repressors, chromatin remodelling factors and/or bridging factors to form gene regulatory complexes. For instance, in some cases interaction between transcription factors is required to modify chromatin in such a way that a previously silent gene may become accessible to the activation machinery. Once the chromatin is favourably restructured and the gene 'prepared' for expression, the transcription machinery needs to be recruited.

Among various transcription factors, the ETS factors are known to play a significant role in the regulation (activation or repression) of genes associated with pathogen and tumour defence. Especially, transcription factor PU.1 appears to have the greatest impact on immunity, primarily through its control of immune cell (myeloid and B-lymphoid) development. The deregulated PU.1 function severely impairs haematopoietic development. Its complete disruption causes embryonic and/or newborn lethality in mice [8, 9] whereas its knockout leads to a profound deficit in the development of neutrophils/monocytes and B cells, followed by an impairment in T- and natural killer (NK) cells but megakaryocyte/ erythroid (Meg/E) development remains intact [10, 11]. The over-expression of PU.1 in erythroid cells blocks differentiation and promotes erythroleukemic transformation, whereas its reduced expression in mice has been associated with the development of myeloid leukaemia [12, 13]. Therefore, in both erythroid and myeloid lineages, abnormally high or low levels of PU.1, respectively, contribute to the development of leukaemia, and restoration of normal PU.1 expression drives differentiation. Furthermore, heterozygous mutations in PU.1 have been reported in some patients with acute myeloid leukaemia (AML) [14].

Thus PU.1 is a multi-faceted protein that controls numerous normal and pathogenic functions within the haematopoietic system. These pleiotropic functions are attributed to its complex and dynamic expression pattern during haematopoiesis. All of its functional domains are associated with interactive protein partners. Such protein–protein interactions direct PU.1 and other transcription factors to act on promoters/enhancers in order to increase or decrease transcription of its target genes. Therefore, a detailed characterization of the molecular partnerships formed by PU.1 is the key to a profound understanding of its function in normal and malignant haematopoiesis, which will certainly facilitate the development of rationally targeted therapies for the treatment of haematological malignancies. In the following review, we have discussed the importance and mechanism of PU.1's interaction with structurally unrelated proteins that are crucial for PU.1-mediated functions such as lineage commitment and differentiation of HSCs during normal haematopoiesis. Some PU.1-protein interactions that are known to disrupt normal HSC differentiation and lead to a malignant phenotype have also been discussed here.

Transcription factor PU.1: expression distribution and function

Transcription factors of the ETS family have been studied as the key regulators of genes involved in haematopoietic cell growth and immune response. Their functional versatility emerges from their interaction with other structurally unrelated transcription factors. Indeed, this combinatorial control is the characteristic property of ETS family members [15]. PU.1 (Spi-1), a member of this family, is encoded by proto-oncogene *sfpi-1* and is expressed exclusively in haematopoietic cells, with high levels detected in monocytic, granulocytic and B lymphoid lineages [16–20]. Lower levels of PU.1 are found in mature erythrocytes [21], whereas it is not expressed in mature T cells [22]. It regulates a large number of genes of the myeloid and lymphoid lineages. In myeloid lineages, the putative PU.1 target genes include G-SCF receptor [23], GM-CSF receptor [24] and M-CSF receptor [25]. Besides, PU.1 also
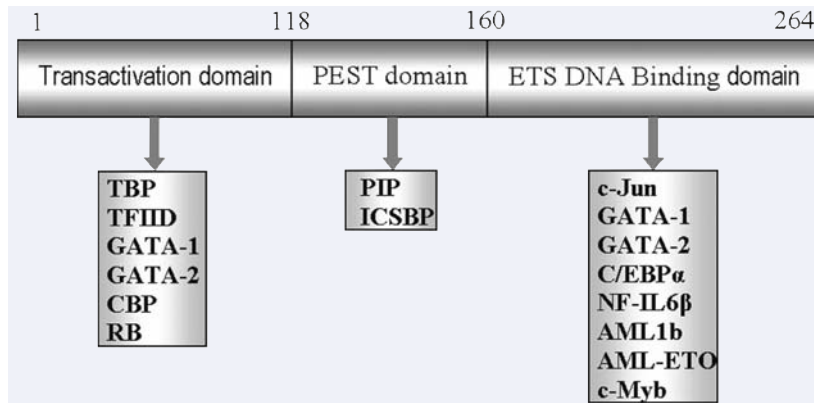
Fig. 1 Functional domains of PU.1 protein along with their interacting proteins. The N-terminus Transactivation domain interacts with TFIID, TBP, GATA-1, GATA-2, CBP and retinoblastoma protein. The C-terminus ETS domain codes for a DNA-binding domain that recognizes the sequence 5′-GGAA-3′. The ETS domain interacts with c-Jun, c-Myb, GATA-1, GATA-2, C/EBP-α, NFIL6-β, AML-1b, AML-ETO, etc. The PEST domain interacts with PIP (PU.1 interacting partner) and ICSBP. The phosphorylation at Ser148 is essential for this interaction.

transactivates other genes involved in the acquisition of a mature monocyte phenotype. This allows monocytes/ macrophages to carry out their immunological functions following an activation signal. For instance, PU.1 controls the expression of genes encoding FcγRI [26] and FcγRIIIA [27], the high- and low-affinity receptors, respectively, for the constant region of immunoglobulin G (IgG). Once expressed on the cell surface, these molecules allow the macrophage to recognize and phagocytose IgG-opsonized bacteria. PU.1 also induces the expression of the adhesion molecules CD11b [28], CD18 [29] and CD14 [25] during myeloid differentiation. CD18 is the β chain of integrins and, when associated with CD11b, they both constitute the membrane glycoprotein Mac-1, which mediates the adhesion of monocytes to endothelium and subsequent diapedesis, and the phagocytosis of complement-opsonized particles. CD14 is a membrane glycoprotein that specifically binds to lipopolysaccharide, a structural component of the bacterial wall. This recognition is a crucial step in triggering the microbactericidal function of the macrophage. Likewise, PU.1 mediates the expression of Scavenger receptors type I and II [30], which are maximally expressed during the terminal differentiation of monocytes to macrophages and are involved in the capture and subsequent degradation of proteins that have been chemically modified at inflammation sites.

A common feature of all these genes is the absence of defined TATA boxes in their promoters; they instead have a functional PU.1 binding site located upstream of the transcriptional start site at a position corresponding to that of a TATA box, accompanied by a cluster of binding sites specific for other ubiquitous regulators such as Sp1, AML-1, CAAT/enhancer binding protein (C/EBP) and core binding factor (CBF) family members [31, 32]. Because PU.1, through its N-terminal *trans*activation domain (TAD), can bind to the TATA binding protein (TBP) and a set of TATA associated factors (TAFs) *in vitro*, it can activate almost all myeloid promoters by recruiting TBP and subsequently other components of the basal transcriptional machinery. Evidently, mutation of a PU.1 binding site located just upstream of the transcriptional start site of the FcγRIB gene abrogates myeloid expression and replacement of this mutant site with a TATA box can re-establish myeloid expression indicating the presence of a positive feedback mechanism in the regulation of the PU.1 promoter [33].

In case of the B-lymphoid genes such as Ig heavy chain (μ) [34], Ig light chains (κ and λ) [35, 36], mb- (Ig α) [37], B29 (Ig β) [38] and Ig-J chain [33], the binding of PU.1 to the enhancer regions induces the bending of DNA that facilitates the binding of other transcription factors to their respective binding sites, thus forming a synergistic multiprotein-complex to augment transcription.

Functional domains of PU.1 protein

The cDNA sequence analysis revealed that PU.1 gene has an open reading frame of 816 bases, which encodes a protein of 272 or 266 amino acids, depending upon the initiation codon for translation being used. The human homologue (264 amino acids), having five exons and four introns, exhibits a sequence identity of 85% with mouse PU.1 and is located on chromosome 11, region p11.22 [39]. The PU.1 protein comprises three distinct functional domains, through which it executes both DNA binding as well as transactivation activity by interacting with various other structurally unrelated transcriptional regulators (Fig. 1). The evolutionarily conserved DNA binding domain (also known as ETS domain) is confined to the C-terminus of PU.1 and mediates its binding to DNA as well as interaction with a number of other proteins such as c-Jun, GATA-1, GATA-2, C/EBP-α and -β and AML-1 [40–42]. The N-terminus harbours a TAD that contains three acidic amino acid-rich regions and a glutamine-rich region. The TAD allows its interaction with the basal transcription factors such as TFIID and TBP (TBP) as well as other tissue-specific factors including retinoblastoma protein, GATA-1 and GATA-2, glucocorticoid receptor and heat shock protein (HSP90) [31, 43]. In addition, PEST domain sandwiched between the above two domains is essential for many protein–protein interactions and owes its name to an abundance of proline (P), glutamic acid (E), serine (S) and threonine (T) residues. This domain interacts with interferon regulatory factors (IRFs), including Pip/NF-EM5 and interferon consensus sequence binding protein (ICSBP) [44–46].

Structure of PU.1 ETS domain and its binding to DNA

The ETS domain (or DNA binding domain) of PU.1 recognizes a purine-rich core sequence (PU box) 5′-GAGGAA-3′ and binds as a monomer. In the crystal structure of PU.1-ETS domain-DNA complex, Kodandpani *et al.* showed that the ETS domain assumes a tight globular structure formed by three α-helices and a four-stranded anti-parallel β-sheet. It contacts DNA through a novel loop-helix-loop structure, quite similar to α + β (winged) helix-turn-helix motif. It binds to the 5′-GGAA-3′ core sequence in the major groove *via* the α_3-recognition helix, whereas the two loops on both sides contact the phosphate backbone in the minor groove. The first loop consists of β-strands 3 and 4 (the wing), whereas the second is formed by the turn between α-helices 2 and 3 [47]. DNA in the PU.1 complex is uniformly curved by approx. 8° towards the major groove without distinct kinks [48].

In case of myeloid promoters, the binding of PU.1 close to the transcription initiation site may facilitate the recruitment of TFIID complex and promote the binding of adjoining transcription factors. Binding of TFIID to the promoter region is the first step of the assembly process that forms transcription pre-initiation complex. Even though PU.1 may make the initial contact with TFIID, down-stream transcriptional factors are likely to help stabilize the TFIID complex through TAF interactions. The PU.1-initiated cooperative stabilization of TFIID may be sufficient to evoke myeloid gene-specific expression [49].

Furthermore, PU.1 serves as an architectural transcription factor for Ig light and heavy chain enhancers. In this capacity, the binding of PU.1 induces bending of DNA that promotes the binding of other transcription factors to nearby regulatory elements, thus forming a higher-order protein-enhancer complex. The PU.1-induced bending of DNA and the subsequent protein–protein interactions among other components of the multiprotein-enhancer complexes repositions the transactivation region of one or more of the other adjoining transcription factors, such as Pip, c-Jun and c-Fos, to augment transcription [50].

PU.1 gene regulation

The PU.1 gene expression is tightly regulated within the haematopoietic system, such that the relative expression level of PU.1 determines whether cells differentiate into macrophages, granulocytes or lymphocytes. As HSCs and multipotential progenitors differentiate, PU.1 is maintained or up-regulated in some lineages and down-regulated in others. For example, PU.1 is expressed in common myeloid progenitor (CMP); the level of its expression increases in granulocyte–monocyte progenitor (GMP) and their progeny (granulocytes and monocytes) but decreases in megakaryocyte–erythrocyte progenitors and their derivatives (megakaryocytes and erythroid cells) [18–20, 51, 52]. Failure to down-regulate PU.1 in erythroid cells leads to a block in erythroid

development and leukaemia. Similarly, PU.1 is expressed in common lymphoid progenitor and maintained in B cells but down-regulated in T cells, and forced expression of PU.1 in early T cells can inhibit development of mature T-cell types [52–54]. Finally, 'knockdown' mice in which PU.1 expression in the bone marrow was decreased to 20% of wild-type levels all develop a block in macrophage and B-cell development, genomic instability and AML [13]. All of these studies indicate that control of PU.1 levels is critical for normal development and that dysregulation can lead to erythroid or myeloid leukaemia.

Previous *in vitro* transient transfection studies demonstrated that myeloid-specific activity is confined to a 334 bp promoter sequence extending from approximately 170 bp upstream of the major transcription start sites to 180 bp 5′ untranslated region. The major functional sites confined within this region include the Octamer (Oct-1) site at –54 bp, the Sp1 site at –39 bp, the PU.1 site at +20 bp and also a GATA-binding site at –15 bp. Among these, the PU.1 binding site plays a crucial role in the expression of the PU.1 gene itself in myeloid cells [18, 20, 55–57]. PU.1 is expressed at low levels in the undifferentiated $CD34^+$/$CD38^-$ HSCs, prior to the up-regulation of PU.1 mRNA during myeloid development [20, 58]. The transcription factors including Oct-1, Sp1, GATA-1 and Spi-B are expressed in undifferentiated HSCs and mediate initial activation of PU.1. In addition to the activation by Oct-1 and Sp1 in myeloid and B cells, Spi-B has also been shown to bind *in vitro* and activate PU.1 in transfected HeLa cells [20, 59].

However, transgenic mice containing only the PU.1 promoter failed to express reporter genes *in vivo*, whereas a murine PU.1 P1 clone including the entire PU.1 gene locus and 35 kb each of 5′ and 3′ flanking sequences expressed exogenous PU.1 RNA in a manner similar to that of endogenous PU.1 in terms of both expression levels and cell-type specificity [60]. These data suggested that distal regulatory elements were necessary for expression of PU.1 *in vivo*. Subsequent DNase I hypersensitivity assays identified an element 14 kb upstream of the gene which conferred reporter gene activity more than 100-fold higher than that seen with the PU.1 promoter only in stably transfected myeloid lines [60]. These results strongly suggested that an element necessary for proper *in vivo* expression is located within this kb-14 region. Utilizing a combination of *in vivo* and *in vitro* assays, a PU.1 binding site was identified in the proximal (3′) conserved region of the kb-14 upstream regulatory element (URE). A mutation which abolished binding by PU.1 led to a loss of function of the URE in myeloid cells. These results also suggest that PU.1 might be positively regulated by PU.1 itself, and there could be two positive autoregulatory sites in the PU.1 gene: one in the kb-14 URE and one in the promoter region [61].

In addition, it has been reported that signalling by Notch1 transmembrane receptor up-regulates PU.1 gene expression and induces myeloid differentiation. Activated Notch1 directly increases PU.1 RNA levels, leading to a high concentration of PU.1 protein, which has been shown to direct myeloid differentiation. Thus Notch1 acts as an extrinsic regulator of myeloid commitment and PU.1 acts as a specific direct target gene of Notch1 [62].

Table 1 PU.1 interacting proteins

Name of the protein	PU.1's domain of interaction	Function	References
c-Jun	β_3/β_4 region	Co-activator	[40]
NF-IL6 β (C/EBP-δ)	ETS domain	Synergistic activation	[43]
GATA-1	β_3/β_4 region and TAD	Mutual repression	[41]
GATA-2	β_3/β_4 region and TAD	Cooperative as well mutual antagonism	[124]
C/EBP-α	β_3/β_4 region	Antagonistic	[106]
c-Myb	ETS domain	Cooperative	[63]
CBP	TAD	Synergistic	[81]
AML-1B	TAD and ETS domain	Synergistic	[32]
AML-1/ETO	β_3/β_4 region	Antagonistic	[114]
NF-EM5/ Pip/IRF-4	PEST domain	Synergistic	[118]
ICSBP/IRF-8	PEST domain	Cooperative	[125]

Fig. 2 PU.1 interacts through its c-terminal ETS domain with the leucine zipper domain of NF-IL6β. Both the proteins simultaneously bind to DNA during complex formation and the transcriptional synergy results from each protein independently influencing components of basal transcriptional complex.

PU.1-interacting proteins

Despite the importance of PU.1, it is not a lone determinant of immune cell development. A variety of proteins have been identified as interaction partners of PU.1. These proteins include other transcription factors, non-DNA binding cofactors, chromatin-remodelling factors and proteins involved in cell cycle regulation. The activation of gene expression mediated by transcription factor PU.1 is generally associated with synergistic interactions with other transcriptional regulators. For example, PU.1 has been shown to cooperate with NF-IL6β (C/EBP-δ) [43], c-Myb and C/EBP-α [63], c-Jun and c-Fos [40].

However, the regulation of transcription factor activity is not only restricted to synergistic combinatorial associations but negative interactions between transcription factors also play a critical role in the control of haematopoiesis. It has been presumed that transcription factors play a key role in the induction of differentiation events and the process of haematopoietic lineage commitment through antagonistic regulation of alternate lineage-specific factors [64]. For example, a negative cross-talk between PU.1 and GATA-1 plays a significant role in erythro-myeloid lineage commitment [65]. A list of PU.1-interacting proteins has been shown in Table 1.

Both activators and repressors may function by interacting with components of basal transcriptional apparatus, leading to modulation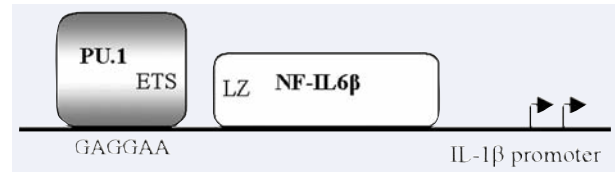 of transcription initiation. The function of an activator may be to recruit the basal transcriptional machinery to a promoter or to induce conformational changes in the complex either at the pre-initiation or elongation steps. However, the role of repressors is not well defined. They may act by specifically interfering, through their repressor domain, with the assembly of the transcriptional machinery (active repression), or they may mediate repression by quenching activators or co-activators (passive repression). Both activators and repressors require co-activator and co-repressors, respectively. These co-factors may function as bridging molecules between the transcription factors and the basal transcriptional machinery, facilitating or inhibiting recruitment to the promoter [66].

NF-IL6β (C/EBP-δ)

NF-IL6β is a leucine zipper transcription factor that belongs to the family of C/EBP proteins. It is a potent transactivator protein implicated in inflammatory responses [67]. Homozygous mutation of the NF-IL6β gene gives rise to mice deficient in macrophage bactericidal and tumoricidal activities. PU.1 and NF-IL6β synergistically cooperate to activate transcription [43]. It has been found that potential PU.1 and NF-IL6β sites are present in some naturally occurring promoters, including the IL-6 and RANTES gene promoters. These genes are responsive to a number of signalling pathways or to inflammatory agents in a variety of tissues, including those that express PU.1. Maximal interaction between them requires PU.1 carboxyl-terminal ETS domain and an intact NF-IL6β leucine zipper domain (Fig. 2). Although both can simultaneously bind to DNA to yield a complex containing both proteins, there is no considerable alteration in the kinetics or affinity of protein–DNA interaction. Instead the transcriptional synergy results from each protein independently influencing components of basal transcriptional complex. Furthermore, because maximal interaction between PU.1 and NF-IL6β requires sequences near their respective DNA binding domains, it is likely that their physical association can inhibit DNA binding by either protein. The deletion of carboxyl-terminal 28 amino acid residues of PU.1 caused a dramatic loss of its interaction with NF-IL6β [43].

A related transcription factor, C/EBP-β (NF-IL6) has also been shown to interact with PU.1 during the transcriptional regulation of IL-1β gene and other monocyte-specific genes. The two factors

strongly cooperate on IL-1β core promoter (−59/+12) in the absence of direct C/EBP-β binding to DNA. Transient transfection assays, using mutated IL-1β core promoters showed that PU.1, but not NF-IL6 binding site is absolutely required for functional cooperativity. Furthermore, the NF-IL6 transactivation domain is functionally indispensable and more critical than that of PU.1 [67, 68].

C-Jun

c-Jun belongs to the bZIP (basic leucine zipper) group of DNA binding proteins and is a component of AP-1 transcription factor complexes [69]. It forms homodimers or can heterodimerize with other Jun family members or with other bZIP proteins. It plays a significant role in monocytic differentiation [70]. c-Jun mRNA is up-regulated upon monocytic differentiation of bipotential myeloid cell lines [71, 72]. c-Jun enhances the ability of PU.1 to transactivate the human monocyte-specific M-CSF receptor promoter even when it does not bind to the M-CSF receptor promoter (as it does not contain any consensus AP-1 binding sites) [25]. In contrast, the macrosialin promoter and macrophage scavenger receptor promoter contain PU.1 and AP-1 binding sites, which are critical for their monocyte-specific expression [30, 73].

Because c-Jun physically binds to PU.1 at the β3/β4 region of its ETS domain and functionally activates it independently of JNK phosphorylation, it was concluded that c-Jun acts as a JNK-independent co-activator of PU.1 (Fig. 3). The transactivation domain of PU.1 (amino acids 1–118) is necessary for the basal transactivation of the M-CSF receptor promoter by PU.1. However, the mutants that lack the transactivation domain cannot interact with c-Jun [40]. It has been shown that during 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced monocytic differentiation of U937 cells, the mRNA expression of c-Jun and M-CSF receptor increases [74]. It has been demonstrated that the growth factors or other signals activate the Ras pathway, which in turn increases the c-Jun expression in monocytic progenitors. Increased c-Jun expression then activates PU.1, resulting in increased M-CSF receptor expression and hence increased survival, proliferation and differentiation of the monocytic lineage. In c-Jun-deficient F9 cells there is no effect of Ras on PU.1, whereas Ras enhances the ability of PU.1 to transactivate the M-CSF receptor promoter in c-Jun-containing CV-1 cells [75].

Usually, c-Jun forms heterodimers with c-Fos in AP-1 transcription factor complexes [69, 70]. However, c-Fos does not cooperate with c-Jun in its co-activator function. In contrast, c-Fos completely blocks the co-activation of PU.1 by c-Jun (Fig. 3). Because c-Fos does not physically bind to PU.1, it might compete with PU.1 for the binding partner c-Jun.

CBP

CREB (cAMP response element binding protein) binding protein (CBP) serves as a co-activator for a variety of transcription factors
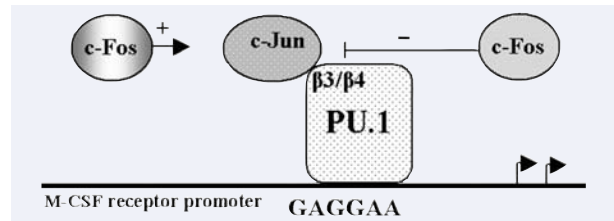

Fig. 3 c-Jun acts as an important co-activator of transcription factor PU.1 during the gene regulation of various myeloid gene promoters such as M-CSF receptor promoter and macrosialin promoter. c-Jun physically interacts with the β3/β4 region in the ETS domain of PU.1 and functionally activates it. Generally, c-Fos heterodimerizes with c-Jun but it blocks the coactivation of PU.1 by c-Jun.

involved in growth and differentiation. CBP and the related p300 function by bridging between sequence-specific transcriptional activators and general transcription factors of the basal transcription machinery [76] (Fig. 4). They are believed to enhance transcription by targeted acetylation of specific chromatin domains (amino acid lysine in histones) with their intrinsic histone acetyltransferase activities [77]. The acetylation and deacetylation of histones are thought to be the key machinery of transcriptional activation and repression. Histone acetylation weakens the interaction of histones with DNA and induces alterations in nucleosome structure. This enhances the accessibility of targeted promoters to components of the transcription machinery, thereby increasing transcription.

GST binding assays revealed that a portion of the transcriptional activation domain of PU.1 (amino acids 74–122) directly interacts with the region spanning residues 1283–1915 of CBP (Fig. 4). CBP enhances PU.1-mediated transcription of multimerized PU-box luciferase reporter constructs, suggesting that CBP acts as a co-activator for the transcription factor PU.1. Because the amount of CBP is limited in cells, CBP may mediate positive and negative cross-talk between PU.1 and other transcription factors in the process of haematopoietic cell differentiation [78].

CBP binds and stimulates the activity of erythroid-specific transcription factor GATA-1 by acetylating its two highly conserved lysine-rich motifs near each of the two zinc fingers [79]. Mutations in the acetylation sites impair the ability of GATA-1 to trigger differentiation of erythroid cells, suggesting that acetylation is important for GATA-1 function *in vivo* [80]. Hong *et al.* have shown that PU.1 inhibits CBP-mediated acetylation of GATA-1 *in vitro* and *in vivo*, which is one of the mechanisms by which PU.1 blocks erythroid lineage commitment. During differentiation of murine erythroleukaemia (MEL) cells there is an inverse correlation between PU.1 levels and CBP-acetyl transferase activity [81]. Structure-function analysis of PU.1 *in vitro* and *in vivo* suggests that both the activation domain and the ETS domain are required for full inhibitory activity towards CBP and p300.

It was shown through gel shift experiments using extracts from MEL and MEL-PU.1 cells that there is no detectable difference in GATA-1 DNA-binding activity before and after differentiation despite significant changes in PU.1 protein levels. Thus inhibition of GATA-1

Fig. 4 CBP/p300 functions by bridging between sequence-specific transcriptional activators and general transcription factors of the basal transcription machinery. CBP/p300 enhances transcription by targeted acetylation of specific chromatin domains with their intrinsic histone acetyltransferase activities. The region of CBP spanning residues 1283–1915 interacts with a portion of the TAD of PU.1 (aa residues 74–122) directly and acts as its co-activator. CBP also binds and stimulates the activity of erythroid-specific transcription factor GATA-1 by acetylating its two highly conserved lysine-rich motifs near each of the two zinc fingers.
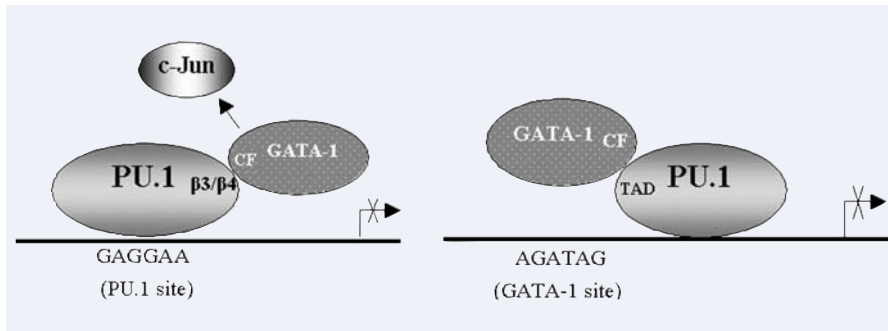

Fig. 5 Cross-antagonism between PU.1 and GATA-1. GATA-1 represses PU.1 function by interacting through its c-terminal zinc finger with the β_3/β_4 region of PU.1 and displacing its co activator c-Jun. On the other hand, PU.1 represses GATA-1 function by interacting through its transactivation domain with the c-terminal zinc finger of GATA-1 and thereby inhibiting its binding to the cognate DNA sequence.

activity results from inhibition of its co-activator (*i.e.* CBP) rather than from blocking its DNA contacts. Moreover, it has been reported previously that inhibition of GATA-1 DNA binding requires very high concentrations of PU.1 [82, 83]. Furthermore, CBP acetyltransferase activity has been reported to increase during MEL cell differentiation as PU.1 levels decline and is inhibited by sustained PU.1 expression [81], suggesting the importance of a regulated balance between CBP and PU.1 during erythroid cell differentiation.

GATA-1

GATA-1 is a key erythroid transcription factor required for the development of normal erythroid and megakaryocytic lineages in which it regulates the expression of many specific genes, such as, α- and β-globin genes [84, 85], erythropoietin receptor [86], erythroid Kruppel-like factor [87], α-spectrin [88], platelet factor 4 [89], glycoprotein IIb [90] and so on. GATA-1 also plays an essential role in eosinophil development. GATA-1 null mouse embryos die from severe anaemia between embryonic day E10.5 and E11.5 [91].

Antagonism between GATA-1 and PU.1

Several lines of evidence suggest that GATA-1 and PU.1 functionally antagonize each other through direct physical interaction *via* the DNA-binding domains of both proteins [41, 42, 65]. But the mechanisms by which these transcription factors antagonize each other are quite distinct. Zhang *et al.* demonstrated that the N-terminal TAD of PU.1 physically interacts with the conserved C-terminal zinc

finger of GATA-1 and blocks its DNA-binding ability *in vitro* and *in vivo*, thereby repressing GATA-1-mediated transcription of its target genes [83] (Fig. 5). Both the DNA binding and transactivation domains of PU.1 are required for inhibition of GATA-1-dependent transcription. In *Xenopus* embryos, ectopic expression of PU.1 blocks erythropoiesis during normal development. Introduction of exogenous GATA-1 can trigger *Xenopus* embryos to resume differentiation and undergo terminal cell division in order to lose their tumorigenicity [41]. Thus, the stoichiometry of these two mutually antagonistic transcription factors is important not only during normal erythroid development but also during leukemogenesis [92]. On the contrary, GATA-1 represses the transcriptional activity of PU.1 by blocking the binding of its co-activator c-Jun to the β_3/β_4 region in its DNA-binding domain [40, 65] (Fig. 5). The carboxy-terminal zinc finger of GATA-1 interacts with the β_3/β_4 region of PU.1, and disrupts the PU.1/c-Jun interaction in a competitive manner, thereby blocking c-Jun from co-activating PU.1 [41].

In many acute leukaemias and some lymphomas, aberrant differentiation is a major feature of the malignant phenotype that often results from a single genetic alteration and hence provides a site-specific target for therapy. For example, deregulation of PU.1 in erythroid precursors can cause erythroleukaemias in mice. Differentiation induction of haematological malignant cells as well as normal HSC differentiation is mediated by a stochiometric ratio of transcription factors GATA-1 and PU.1 [41], which broadly determine lineage and cooperate with more specific transcription factors such as RAR receptor and CEBP family members [59, 93, 94]. Erythropoietin-induced erythroid differentiation of leukemic cells is associated with GATA-1 induction and down-regulation of

PU.1 and other transcription factors that direct myeloid lineage during normal differentiation. Furthermore, PU.1 and GATA-1 both potentially exhibit autoregulation, whereas at the same time interactions between these two mediate inhibition of each other's function. Therefore, the reciprocal inhibition and autoregulation of these two regulators provides a mechanism for lineage choice during early blood stem cell and progenitor cell differentiation into either myeloid or erythroid cells [65, 83].

Synergistic interaction between PU.1 and GATA-1

In contrast to the functional antagonism reported for PU.1 and GATA-1 for the activation of the M-CSFR promoter and various erythroid genes, these factors have also been shown to cooperate synergistically in the activation of the eosinophil MBP-P2 (major basic protein) promoter. Key combinatorial interactions of GATA-1, PU.1 and C/EBP-δ isoforms mediate either synergy or antagonism of eosinophil gene transcription during myeloid development and terminal differentiation [95].

The synergistic effect of PU.1 on GATA-1-mediated transactivation is mediated by a conformational change in GATA-1 when it binds through both of its C- and N-terminal zinc fingers to the two canonical non-overlapping tandem GATA-binding sites present in the MBP-P2 promoter. Involvement of the N-terminal zinc finger by binding to the other GATA site in the MBP-P2 promoter might trigger a conformational change leading to decreased DNA binding activity of the C-terminal zinc finger and a decrease in transactivating activity for the β-globin silencer [96]. A low amount of PU.1 may interact differentially or preferentially with the N-terminal zinc finger of GATA-1 and prevent this decrease in transactivating potential; thus accounting for PU.1/GATA-1 synergy in transactivation of the MBP-P2 promoter.

On the contrary, expression of a high amount of PU.1 could lead to interactions with both zinc fingers, thus interfering with the DNA binding activity of GATA-1 and abrogating the synergy. Of note, PU.1 has been shown to interact with both the C- and N-terminal zinc fingers of GATA-1 [41]. Only the C-terminal zinc finger of GATA-1 is capable of independently binding to a GATA consensus site and stimulating transcription, whereas the N-terminal finger shows no independent DNA binding activity but can modify binding specificity (stabilize or disrupt binding) at some naturally occurring dual GATA sites that have been shown to be critical for gene expression [97–99].

Interestingly, C/EBP family members C/EBP-ε and C/EBP-β, were found to antagonize both GATA-1 transactivation and GATA-1/PU.1 synergistic activation of the eosinophil MBP-P2 promoter, as well as PU.1-mediated transactivation of PU.1 target genes/promoters such as the M-CSFR, suggesting a potent repressor function for this isoform in myeloid development [95].

C/EBP-α

C/EBP-α (CAAT/EBP), a bZip transcription factor, regulates not only a variety of hepatocyte and adipocyte genes, but several myeloid-specific genes as well [100]. For example, it regulates M-CSF receptor, G-CSF receptor and GM-CSF receptor α promoters. C/EBP-α expression is prominent in immature myeloid cells [24, 101]. C/EBP-α-null mice lack the entire granulocyte lineage but develop normal monocytes [102].

Combinations of inhibition and autoregulation involving PU.1 and C/EBP-α have been hypothesized to mediate the decision of myeloid progenitors to differentiate into either granulocytes or monocytes [103, 104]. The haematopoietic progenitors require PU.1 to initiate monocyte differentiation and C/EBP-α to initiate granulopoiesis. Laslo *et al.* have shown that nonlinear positive feedback regulates differentiation of CMPs into macrophages or neutrophils [105]. Earlier it was suggested that the relative difference in expression of the transcription factors PU.1 and C/EBP-α regulates the differentiation of CMPs [103]. They found that macrophage differentiation is favoured when the level of PU.1 is higher than that of C/EBP-α, whereas neutrophil differentiation is favoured when the level of C/EBP-α is higher than that of PU.1. Thus a mutual corepression was thought to exist between PU.1 and C/EBP-α that drives CMPs towards one fate or the other. Each factor has been shown to synergize on various promoters, including M-CSF receptor promoter and neutrophil elastase (NE) promoter [24, 63]. Each is expressed in a bipotential myeloid cell; C/EBP-α is capable of functionally blocking the PU.1 protein, and this interference is mediated through interaction between the β_3/β_4 region of the PU.1 DNA-binding domain and the leucine zipper in the DNA-binding domain of C/EBP-α, which in turn displaces PU.1's co-activator c-Jun [106, 107].

Additionally, a mutual corepression was found between Egr-2/Nab-2, a complex of genes activated by PU.1, and Gfi-1, a gene activated by C/EBP-α. Because both Egr-2 and Gfi-1 are known to promote the expression of genes specific to macrophages and neutrophils, respectively, their corepression may indeed be the basis of a positive feedback loop that promotes and stabilizes a particular cell fate during CMP differentiation. For instance, when PU.1 and C/EBP-α are expressed at low levels as is the case in undifferentiated CMPs, positive feedback between Egr-2/Nab-2 and Gfi-1 is sufficiently weak such that neither is amplified and the mixed-lineage stage persists. In contrast, when PU.1 is expressed at a much higher level than C/EBP-α, the mixed-lineage state of gene expression is resolved and the system is monostable, promoting differentiation of the CMP into a macrophage (or, if the ratio is reversed, into a neutrophil) [105]. C/EBP-α also functionally interacts with the activation domain of PU.1, which might disrupt possible protein–protein interactions important for the PU.1-induced differentiation program. One such candidate is CBP/p300, a co-activator of PU.1 [78], which binds to the transactivation domain of PU.1. This would further block PU.1 activity to induce dendritic cell formation and enhance the capacity of C/EBP-α to induce granulocytes. Interestingly, C/EBP-α is also known to cooperate with PU.1 in controlling the expression of the GM-CSF receptor [108]. The GM-CSF receptor α promoter contains functional PU.1-specific binding site between positions -53 and -41. A C/EBP-α site is located upstream of the PU.1 site between positions -70 and -54. Point mutations of either the PU.1 site or of the

C/EBP-α site that abolish the binding of the respective factors result in a significant decrease of GM-CSF receptor α promoter activity in myelomonocytic cells.

c-Myb

c-Myb is predominantly expressed in immature cells of haematopoietic lineages and mice lacking the c-Myb gene have defective foetal haematopoiesis [109]. C-Myb has been implicated in the activation of mammalian myeloid genes, both alone and cooperatively with C/EBP-α and/or PU.1. There are potential C/EBP-, c-Myb- and PU.1-binding sites in the azurocidin and myeloblastin genes. The presence of functional sites matching the c-Myb, C/EBP and PU.1 consensus sequences in the murine myeloperoxidase gene [110, 111] indicates that cooperation between these factors is relevant to the activation of a substantial number of early myeloid genes. In most experiments, the activation seen with C/EBP-α, c-Myb and PU.1 was equivalent to the multiplication of the individual activations obtained with these factors. This interaction has been termed as 'cooperative' instead of synergistic because synergism exists when a factor combination produces much more than a multiplicative effect [112].

A 91-bp NE promoter region contains three evolutionarily conserved *cis* elements, which are essential for activation of the promoter in differentiating 32Dcl3 myeloid cells. These elements bound c-Myb (at 249), C/EBP-α (at 257) and PU.1 (at 282) (Fig. 6). In NIH 3T3 cells, the NE promoter was activated by c-Myb, C/EBP-α and PU.1, *via* their respective binding sites. Cooperative activation was seen by any combination of c-Myb, C/EBP-α and PU.1, including all three together, again *via* their DNA-binding sites. In CV-1 cells, but not in NIH 3T3 cells, cooperation between Myb and C/EBP-α depended on the integrity of the PU.1-binding site [78].

AML-1

AML-1 is a member of the CBF or polyoma EBP (PEBP2) family [113]. During monocytic commitment and differentiation, AML-1B regulates the myeloid-specific expression of M-CSF receptor in synergistic association with C/EBP-α and PU.1 by forming a ternary complex on DNA [32] (Fig. 7).

AML-1 and PU.1 interact physically and this interaction occurs primarily through their DNA binding domains. AML-1 synergizes with PU.1 to activate the M-CSF receptor promoter and this activity requires DNA-binding sites for both factors and regions contained within the transactivation domains of AML-1 and PU.1. The deletion of the activation domain of either PU.1 or C/EBP-α abrogates synergy with AML-1B. Although AML-1 and PU.1 exhibit a relatively weak synergistic activation of the promoter, but because the effect seen in the presence of both PU.1 and AML-1 is more than additive, the interaction is defined as synergistic [32].

Detailed analysis of the physical and functional interaction of AML-1 with PU.1 and C/EBP-α has revealed that the proteins
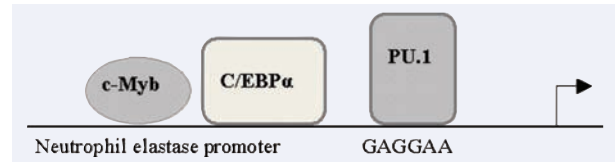

Fig. 6 The activation of neutrophil elastase gene is regulated by the cooperative interaction between c-Myb, C/EBP-α and PU.1 through their respective DNA binding domains.
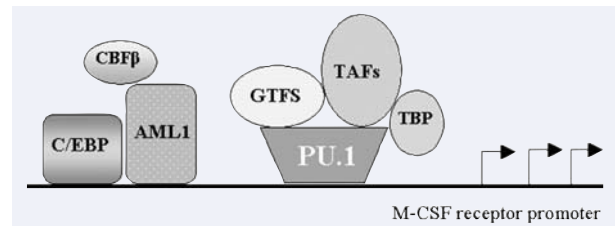

Fig. 7 The M-CSF receptor gene is regulated by transcription factor PU.1 in combination with C/EBP-α and AML-1B. PU.1, C/EBP-α and AML-1B interact through their respective DNA binding domains and transactivation domains. AML-1 exhibits cooperative DNA binding with C/EBP-α but not with PU.1. The co-activator CBF-β serves to amplify the activation by increasing transcription efficiency.

contact one another through their DNA-binding domains and that AML-1 exhibits cooperative DNA binding with C/EBP-α but not with PU.1. This difference in DNA-binding abilities may explain, in part, the differences observed in synergistic activation. Furthermore, the activation domains of all three factors are required for synergistic activation, and the region of AML-1 required for synergy with PU.1 is distinct from that required for synergy with C/EBP-α. These observations present the possibility that synergistic activation is mediated by secondary proteins contacted through the activation domains of AML-1, C/EBP-α and PU.1.

PU.1, C/EBP-α and AML-1 form a transcriptional unit or a primary complex, on the DNA and this primary complex makes multiple and specific contacts with a second, perhaps ubiquitous, complex composed of co-activators. The role of the DNA-binding proteins is to confer tissue-specific, temporal regulation, whereas the co-activators (such as CBF-β) serve to amplify the activation by increasing transcription efficiency [32].

AML-1/ETO

PU.1 plays a major role in leukemogenesis as suggested by the heterozygous PU.1 mutations reported in some patients with AML [14]. However, PU.1 was not found to be mutated in AML patients with translocation t(8; 21), which indicates that distinct pathways of inactivation of PU.1 might be occurring in t(8; 21). The fusion protein AML-1/ETO physically interacts with PU.1 and thereby
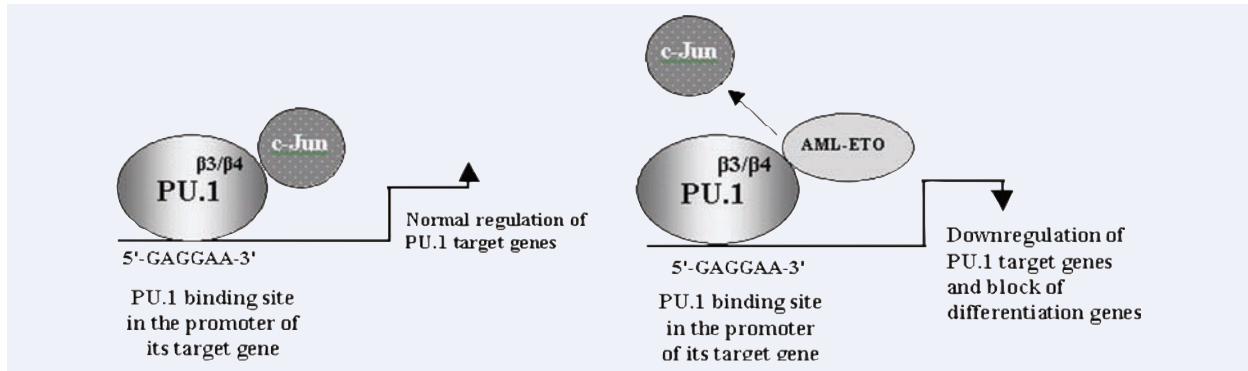
Fig. 8 The fusion protein AML-1/ETO inhibits the function of PU.1 by displacing its co-activator c-Jun from its β_3/β_4 region.

causes inactivation of the transcriptional activity of PU.1 by displacing its co-activator c-Jun [114] (Fig. 8).

AML-1/ETO down-regulates the transcriptional activity of PU.1 in myeloid cells and physically interacts at the β_3/β_4 region in the DNA-binding domain of PU.1. The competitive protein–protein interaction experiments with *in vitro*-translated proteins indicated that AML-1/ETO disrupts PU.1/c-Jun interaction in a competitive manner, thus blocking c-Jun from co-activating PU.1, a mechanism similar to GATA-1 and C/EBP-α. The physical interaction between AML-1/ETO and PU.1 did not abolish the DNA-binding capacity of PU.1, although AML-1/ETO interacts with the PU.1 DNA-binding domain. It has been shown in the crystal structure of PU.1 that its β_3/β_4 region does not interact with DNA, but is exposed to the solvent [47]. This structural ability allows PU.1 to retain its DNA binding though being functionally repressed. Interaction of PU.1 with AML-1/ETO and subsequent suppression of PU.1 target genes might contribute to the phenotypic changes seen in t(8; 21).

Because the expression of AML-1 and PU.1 genes was observed even in a leukemic condition or in presence of AML-1/ETO, like in Kasumi-1 cells, it was suggested that the presence of AML-1/ETO does not completely repress the expression levels of these genes, but may block their functions by protein–protein interactions. Overexpression of PU.1 in t(8; 21)-Kasumi-1 cells directs their differentiation towards the monocytic lineage by overcoming the functional block imposed by AML-1/ETO. PU.1 and C/EBP-α are important factors for myeloid differentiation and AML-1/ETO down-regulating these two factors could be an important step towards leukaemia. This also suggests the possibility of using these two factors independently or in combination for therapy of t(8; 21) myeloid leukaemias. Expression of AML-1/ETO fusion protein in the myeloid progenitor cell line 32Dcl3 prevents G-CSF-induced granulocytic differentiation [115].

Pip/NF-EM5/IRF-4

Pip/NF-EM5/IRF-4, a member of IRF family, is a lymphoid-restricted transcription factor that is recruited to a composite
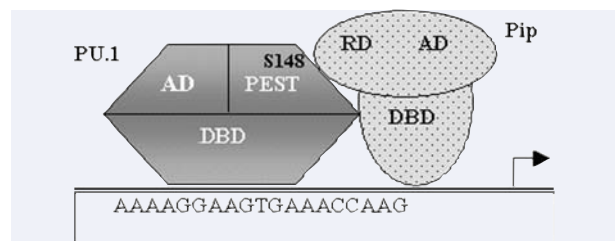

Fig. 9 PU.1 recruits PIP (PU.1 interacting partner) by binding with its regulatory domain through its PEST domain. For PU.1's interaction with Pip, phosphorylation of Ser148 residue is essential. RD, regulatory domain; AD, activation domain; DBD, DNA binding domain.

element (IRF/ETS-binding site) within Ig light chain enhancers through a specific interaction with PU.1 protein [116, 117]. The formation of the ternary complex requires both protein–protein and protein–DNA interactions. A model was proposed for PU.1/Pip ternary complex. In this two-step recruitment mechanism, PU.1/Pip interaction is DNA template-directed and involves two distinct protein–protein interaction surfaces: (*i*) the ETS (PU.1) and IRF (Pip) DNA binding domains and (*ii*) the phosphorylated PEST domain of PU.1 and a putative α helix in Pip [118, 119] (Fig. 9). Specifically, the first step involves an interaction between the DNA binding domains of PU.1 and Pip. In the second step, phosphorylation of PU.1 at Ser 148 results in a conformational change in Pip (either before or after interaction with Pip) allowing cooperative DNA binding [117]. This leads to the alteration or unmasking of the Pip auto-inhibitory c-terminal DNA binding domain, enabling Pip recruitment to DNA. Protein–protein contacts that occur between the two DNA binding domains may then contribute to additional specificity and stability to the ternary complex. The recent molecular modelling study of the PU.1-Pip DNA binding domain complex reveals that the DNA recognition helix α_3 of PU.1 Ets domain might interact with Pip wHTH (winged helix-turn-helix) domain to allow cooperative DNA binding. Another highly homologous IRF family member, IRF-8/ICSBP, competes with Pip/IRF-4 for binding to PU.1 on the enhancer element
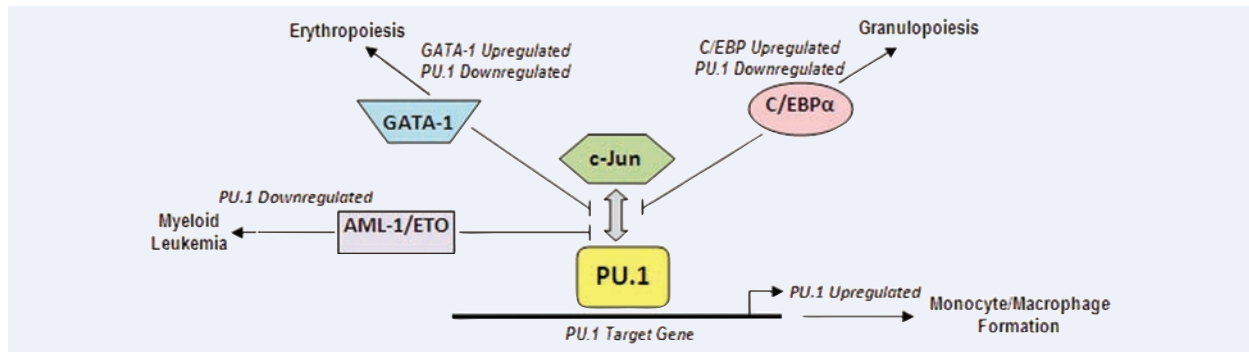
Fig. 10 PU.1-protein interactions play a vital role in HSC fate determination. Co-activator c-Jun interacts with the β_3/β_4 region of PU.1 and enhances its transcriptional ability that in turn leads to increased formation of monocyte/macrophages. When the relative concentration of GATA-1 is higher, it inhibits the PU.1-c-Jun interaction by displacing c-Jun from its binding site and thus blocking the formation of monocytes. The up-regulated activity of GATA-1 in turn leads to enhanced erythropoiesis. Similarly, transcription factor C/EBP-α also inhibits PU.1-c-Jun interaction and leads to the formation of granulocytes. Additionally, a fusion protein AML-1/ETO also represses PU.1 by inhibiting its interaction with co-activator c-Jun, leading to maturation arrest of myeloblasts and thus causing myeloid leukaemia.

and thus represses the transactivation activity of the complex [46]. Although IRF-4/Pip is known to respond to any extrinsic stimuli, IRF-8/ICSBP is strongly induced by interferon [120]. Thus, interferon largely controls the presence of IRF-8/ICSBP in the ternary complex on the enhancer.

PU.1-protein interactions and HSC fate determination

The development of different lineages of blood cells is triggered not only by a combination of particular sets of transcription factors but also by their levels relative to one another. The successive steps in the maturation of haematopoietic progenitor cells are thought to involve the up-regulation of factors promoting a particular lineage and the repression of factors promoting alternate lineages. For example, both PU.1 and GATA-1 direct lineage commitment not only by transactivating their individual genetic targets but also by disrupting the functions of opposing transcription factors. When the concentration of GATA-1 is relatively high, it represses PU.1's function by inhibiting PU.1's interaction with its co-activator c-Jun, which leads to a reduction in myelopoiesis and a concomitant increase in erythropoiesis because of increased GATA-1 activity. Likewise an antagonism exists between PU.1 and C/EBP-α whereby the relative concentration of each transcription factor is important for differentiating GMPs into either monocytic or granulocytic cells. A higher concentration of C/EBP-α also down-regulates PU.1 by inhibiting its interaction with c-Jun and as C/EBP-α is up-regulated, GMPs are directed towards granulopoiesis. Furthermore, a fusion protein AML-1/ETO is also known to inhibit PU.1's activity again by inhibiting its interaction with co-activator c-Jun. This in turn leads to a maturation arrest of myeloblasts ultimately causing myeloid leukaemia. Taken together, the above three proteins, namely GATA-1, C/EBP-α and AML-1/ETO, down-regulate PU.1 by displacing its co-activator c-Jun thereby modulating HSC fate in altogether different directions, *i.e.* erythrocytes, granulocytes and myeloid leukaemia, respectively (Fig. 10). This suggests that PU.1-protein interactions certainly play a significant role in HSC lineage commitment. A slow fluctuation or cycling of gene activity tends to maintain cells in a stable state, while also priming them to differentiate when conditions are right. Progenitor cells commit to one of their potential paths and do not switch to different lineages or revert to less differentiated progenitor cell types. Such stability is certainly important for immune cells, which receive a transient lineage-specific differentiation stimulus in the bone marrow and otherwise stay undifferentiated in the absence of this stimulus in the blood stream or other tissues [121].

Concluding remarks

Although PU.1 is one of the most intensively studied of all mammalian transcription factors, there is still much that we do not understand about the mechanisms through which it regulates gene expression. Understanding how PU.1 controls immune cell development could have important medical applications. Once we understand the regulatory circuitry that controls immune cell development we could exploit that knowledge to develop treatments that direct progenitor cells to differentiate into desired cell types for therapeutic purposes. Also many cancers of the immune system involve interruption of blood cell differentiation. Therapeutic targeting of protein–protein interactions that have been identified as mediators of transcriptional repression that block haematopoietic differentiation should be further explored.

For example, in myeloid leukaemia cells that express an AML-1/ETO fusion protein, PU.1 activity is reduced, which is characterized by maturation arrest of myeloblasts [114]. Likewise, the expression of the PML-RAR-α fusion protein in acute promyelocytic leukaemia (APL) reduces PU.1 expression [122]. Disruption of PU.1's interaction with repressor proteins AML-1/ETO and PML-RAR-α can restore PU.1 expression in AML and APL cells thereby inducing monocytic and neutrophil differentiation respectively. Similarly, GATA-1-mediated repression of PU.1 blocks myeloid differentiation. Therefore, abolishing protein–protein interaction between PU.1 and GATA-1 in myeloid cells would lead to an increase in PU.1's activity and hence immune cell differentiation. Therefore, it is a challenge for the future to determine the extent to which dysfunction of these transcription factor antagonisms contributes to human leukaemias and how this knowledge can be exploited to benefit cancer therapy.

Further studies are required to delineate the precise interactions of transcription factor PU.1 with basal transcriptional machinery, co-repressors or co-activators and other bridging proteins that collectively are critical for lympho-myeloid transcriptional responses. Efforts are needed to determine precise characterization of its association with different protein partners and to determine how these interactions influence target gene selection and transcription activation or repression. Overlap between specific protein–protein interactions may provide a mechanism to control the diverse functions of this factor. As it is important to correlate specific physical interactions with physiological processes, more sensitive methods need to be developed for characterization of transient interactions and for direct assessment of the biological consequences of specific interactions. Crystallographic structure determination of multiple protein complexes will tremendously help in understanding the combinatorial control of PU.1-mediated transcription. Ultimately, the protein–protein interface may provide a unique target for intervention, thus, providing a novel approach for blocking aberrant signalling pathways or reversing the malignant phenotype associated with oncogenic transcription factors [123].

Acknowledgements

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