



Regulation of GATA1 levels in erythropoiesis

| | |
|-------------------------------|--|
| Journal: | <i>IUBMB Life</i> |
| Manuscript ID | TBMB-19-0671-WJW.R1 |
| Wiley - Manuscript type: | Special Issue |
| Date Submitted by the Author: | 09-Oct-2019 |
| Complete List of Authors: | Gutierrez, Laura; Instituto de Investigación Sanitaria del Principado de Asturias; Universidad de Oviedo, Department of Medicine Caballero, Noemi; Instituto de Investigación Sanitaria del Principado de Asturias Fernández-Calleja, Luis ; Instituto de Investigación Sanitaria del Principado de Asturias Karkoulia, Elena; IMBB Strouboulis, John; King's College London, School of Cancer and Pharmaceutical Sciences |
| Keywords: | transcription factors, transcriptional regulation, erythrocytes, protein function |
| | |

SCHOLARONE™
Manuscripts

Regulation of GATA1 levels in erythropoiesis

Laura Gutiérrez^{1,2*}, Noemí Caballero¹, Luis Fernández-Calleja¹, Elena Karkoulia³, John Strouboulis^{4*}

1 Platelet Research Lab, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain

2 Dept. of Medicine, Universidad de Oviedo, Spain

3 Institute of Molecular Biology and Biotechnology, Foundation of Research & Technology Hellas, Heraklion, Crete, Greece

4 Cancer Comprehensive Center, School of Cancer and Pharmaceutical Sciences, King's College London, United Kingdom

** Corresponding authors: gutierrezglaura@uniovi.es, john.strouboulis@kcl.ac.uk*

Abstract

GATA1 is considered as the “master” transcription factor in erythropoiesis. It regulates at the transcriptional level all aspects of erythroid maturation and function, as revealed by gene knockout studies in mice and by genome-wide occupancies in erythroid cells. The GATA1 protein contains two zinc finger domains and an N-terminal activation domain. *GATA1* translation results in the production of the full-length protein and of a shorter variant (GATA1s) lacking the N-terminal activation domain, which is functionally deficient in supporting erythropoiesis. GATA1 protein abundance is highly regulated in erythroid cells at different levels, including transcription, mRNA translation, posttranslational modifications and protein degradation, in a differentiation-stage specific manner. Maintaining high GATA1 protein levels is essential in the early stages of erythroid maturation, whereas downregulating GATA1 protein levels is a necessary step in terminal erythroid differentiation. The importance of maintaining proper GATA1 protein homeostasis in erythropoiesis is demonstrated by the fact that both GATA1 loss and its overexpression, result in lethal anemia. Importantly, alterations in any of those GATA1 regulatory checkpoints has been recognized as an important cause of hematological disorders such as dyserythropoiesis (with or without thrombocytopenia), β -thalassemia, Diamond Blackfan anemia, myelodysplasia or leukemia. In this review, we provide an overview of the multi-level regulation of GATA1 protein homeostasis in erythropoiesis and of its deregulation in hematological disease.

Introduction

Cell lineage specification and differentiation is a complex process that must be tightly regulated through cell-type specific transcriptional programs. These programs are regulated by master transcription factors which play essential roles in any given cell lineage to successfully commit and differentiate with the appropriate temporal and spatial coordinates and at the exact demanded rate, thus assuring homeostatic balance in the organismal tissue composition [1]. Hematopoiesis has long served as an excellent model system for studying the molecular principles governing transcription factor functions in regulating cell lineage commitment and differentiation. The diversity of mature blood cells derives from a common progenitor, the hematopoietic stem cell (HSC), which undergoes a series of commitment and differentiation steps in response to specific stimuli in specialized tissue microenvironments (e.g. bone marrow, thymus etc.), to generate not only the circulating blood cells (white blood cells, red blood cells and platelets), but also the immune and non-immune organ resident immune cells [2-4]. Amongst the circulating blood cells, the highly specialized, oxygen-carrying, enucleated erythroid (red blood) cells must be produced daily at high rates, according to a very specialized and well characterized cell differentiation program, which includes progressive size reduction, cell cycle arrest, massive synthesis of globin chains and enucleation [5-7]. In this respect, GATA1 has been acknowledged as the master transcriptional regulator of erythropoiesis, controlling all aspects of differentiation, survival and cell cycle arrest [8, 9]. Maintaining GATA1 protein homeostasis has been shown to be a highly regulated process in erythropoiesis, with deregulation being a major factor in ineffective erythropoiesis or leukemic transformation in hematological disease. In this review we comprehensively discuss GATA1 functions in erythroid differentiation and, further, we focus on the regulation of GATA1 abundance in physiological and pathological erythropoiesis.

GATA Transcription Factors

GATA1 belongs to the GATA family of transcription factors which comprises six members: GATA1 to GATA6. The GATA family of transcription factors plays an important role in the regulation of several biological processes, such as embryonic development, cell growth, cell differentiation and tissue morphogenesis [10]. All members of the GATA family of transcription factors recognize and bind to a specific DNA motif, (A/T)GATA(A/G) (WGATAR), through two highly conserved Cys4-type zinc finger regions. This DNA consensus sequence is evolutionarily conserved in fungi, plants, invertebrates and vertebrates, such as zebrafish, *Xenopus*, chicken and mammals, further supporting the notion that GATA family members play important roles in the regulation of crucial cellular processes [11]. In mammals, GATA1, GATA2 and GATA3

1
2
3 have important roles primarily, but not exclusively, within the hematopoietic system [12,
4 13] and GATA4, GATA5, and GATA6 drive the differentiation of mesoderm and
5 endoderm derived tissues, thus regulating diverse developmental processes within the
6 cardiac, gastrointestinal, endocrine and gonadal systems [10]. The binding of GATA
7 transcription factors to the WGATAR sequence motif depends highly on the C-terminal
8 zinc finger (C-ZnF), whilst the N-terminal zinc finger (N-ZnF) domain may bind to sites
9 containing additional canonical, non-canonical (GATC), or palindromic GATA motifs, or
10 may strengthen binding through interaction with other surrounding DNA motifs, mainly in
11 partnership with other transcriptional co-factors [10, 14]. GATA transcription factor
12 tissue/lineage specificity is afforded by the transactivation domains surrounding the zinc
13 finger domains, the homology of which is weaker amongst the members of the GATA
14 transcription factor family [9]. The global sequence landscape at each specific locus will
15 determine the transcriptional activity and the higher order transcription factor complexes
16 involved in its regulation. GATA1, the founding member of the GATA family of
17 transcription factors, was initially shown to be essential for the erythroid/megakaryocytic
18 lineages and subsequently in eosinophils/basophils, mast cells and in dendritic cells [15-
19 19]. However, it is the study of GATA1 in erythropoiesis that has provided the most
20 valuable insight into GATA1 functions and regulation.
21
22
23
24
25
26
27
28
29
30
31
32

33 **GATA1 functions in erythropoiesis**

34 GATA1 is considered the “master” transcription factor of erythropoiesis as it
35 transcriptionally regulates all aspects of erythroid maturation and function [9]. The
36 GATA1 protein has three main functional domains: a N-terminal activation domain (N-
37 TAD) and two homologous ZnF domains in the C-terminal half of the protein. The C-ZnF
38 binds to DNA and the N-ZnF binds to the GATA1 main cofactor FOG-1 (Friend of GATA1)
39 and modulates the affinity of GATA1 for binding to complex and palindromic sites *in vitro*.
40 Translation of the *GATA1* mRNA produces two isoforms: the full-length protein and a
41 shorter variant (GATA1s), translated from codon 84 within the third exon. GATA1s lacks
42 the N-TAD and results in a protein with aberrant functional properties [20, 21]. The *Gata1*
43 gene knockout in the mouse results in embryonic lethality at around day E10.5-E11.5
44 due to severe anemia, with *Gata1*-null cells undergoing apoptosis at the proerythroblastic
45 stage [22-24]. The conditional erythroid knockout in adult mice causes aplastic anemia,
46 revealing its essential role in both steady-state and stress erythropoiesis [25].
47
48
49
50
51
52
53
54
55
56

57 Genome-wide occupancy studies (ChIPseq) have confirmed that all known erythroid
58 genes are regulated by GATA1 [26-29]. In addition, GATA1 is critical for the positive
59 regulation of genes involved in cell cycle regulation, such as *GF11B* [30], *CDC6* [31, 32]
60

1
2
3 and p21 (*Cdkn1a*)[33]. GATA1 also activates genes important for cell survival, such as
4 Bcl-2 (*Bcl2*) and Bcl-xL (*Bcl2l1*) [34, 35]. Furthermore, GATA1 has also been described
5 as a regulator of restrictive lineage specification through the repression of myeloid genes
6 in the erythroid lineage. Repressed targets of GATA1 include *Gata2*, the *Myb* and *Myc*
7 oncogenes and PU.1 (*Spi1*)[36]. Another important feature of GATA1 in the erythroid
8 lineage differentiation, is its role as a chromatin bookmark as it has been shown that
9 GATA1 binding to certain gene loci is preserved through mitosis, assuring the immediate
10 expression of a given set of genes after resumption of cell cycling [37].
11
12
13
14
15
16

17 GATA1 transcription functions in erythropoiesis are mediated by the formation of
18 activating and repressive transcription complexes, through GATA1 interactions with a
19 number of other transcription factors, co-factors and chromatin remodeling and
20 modification proteins and complexes [8]. The best studied GATA1 interactions are with
21 FOG-1 (Friend of GATA1), the SCL/TAL1/E2A/LDB1/LMO2 complex and with PU.1
22 (SPI1). FOG-1 interacts with GATA1 through the latter's N-ZnF in the erythroid and
23 megakaryocytic lineages, with their interaction being essential for differentiation [38, 39].
24 Genetic studies in mice and the identification of disease-causing mutations in humans,
25 confirmed the importance *in vivo* of the GATA1/FOG-1 interaction in erythropoiesis [40,
26 41]. GATA1/FOG-1 mediated repression involves interactions with the NuRD
27 (Nucleosome Remodeling and Deacetylating) complex, which is contacted directly by
28 FOG-1 [42, 43].
29
30
31
32
33
34
35
36
37

38 Another critical GATA1 transcription complex in erythropoiesis is the so-called
39 pentameric complex with the SCL/TAL1/E2A/LDB1/LMO2 factors [44]. The pentameric
40 complex binds GATA-E-box motifs comprised of a GATA consensus sequence located
41 proximally (7-9 bp) to an E-box CANNTG consensus motif bound by SCL/TAL1 [44].
42 These motifs occur primarily in erythroid specific genes that become activated by the
43 pentameric complex [45, 46]. LMO2 serves as the bridge between SCL/TAL1 and
44 GATA1 [44], whereas LDB1 associates with LMO2 and is capable of self-association
45 (dimerization) through its N-terminus domain [47]. This dimerization allows LDB1-
46 mediated long-range DNA looping to activate transcription, as occurs, for example, in the
47 β -globin locus [48, 49]. GATA1 has also been reported to interact in erythroid cells with
48 other transcription factors such as KLF1, SP1 and GFI1B [50-52], as well as with
49 chromatin remodeling factors such as ACF/WCRF [42], BRG1 [53] and pRB/E2F [54].
50 Lastly, GATA1 interacts with the CBP/p300 histone acetyltransferase which mediates
51 acetylation of the GATA1 ZnFs, modulating its DNA binding properties and/or protein
52 stability (see below) [55, 56].
53
54
55
56
57
58
59
60

***Cis* regulation of GATA1 gene expression**

GATA1 is located on the X chromosome in mouse and human and is comprised of 6 exons distributed over 8 Kb [57]. Exon I is noncoding, exon II contains the starting codon for the full length GATA1 protein and exons IV and V code for the two ZnF domains. The *Gata1* gene has been largely studied in mice. It comprises two tissue-specific promoters: the distal (IT) promoter drives expression of the *Gata1* gene in Sertoli cells in the testis, whereas the proximal (IE) promoter located 8kb downstream of the IT promoter and 5' of exon II, directs *Gata1* expression in hematopoietic cells [58]. The transcriptional regulation of *Gata1* in hematopoietic cells is coordinated by *cis*-regulatory regions clustered within three DNase I hypersensitive (HS) sites [59, 60]. HS1 is located between 3.9 and 2.6kb upstream of IE; HS2 corresponds to the putative region of the *Gata1* IE promoter and contains a double palindromic GATA site and CACCC boxes and HS3 is located in intron I. The regulated expression of *Gata1* throughout hematopoiesis involves the differential contribution of these *cis*-regulatory elements. Thus, in the megakaryocytic lineage, *Gata1* expression requires the extended (317 bp) HS1 region, whereas for erythroid expression only the first 62bp of HS1 are necessary [61, 62]. In eosinophils, *Gata1* expression is independent of the HS1 region [63], although it does require the double GATA motif that is present in the HS2 region [16]. Whereas utilization of the isolated HS sites in gene expression constructs confers normal *Gata1* expression in committed hematopoietic cells, physiological repression of *Gata1* in the HSC compartment requires the interspaced sequences between the HS sites [64, 65].

GATA1 expression in hematopoiesis

From earlier progenitors towards the terminally mature erythroid cell, *GATA1* expression levels fluctuate. Most of the studies have been performed in murine model systems, while more recent studies tackling this aspect using single-cell approaches have been performed both in mouse and human models. HSCs and common myeloid progenitors (CMP) express very low levels of *Gata1* mRNA ([65] and references therein). *Gata1* expression increases moderately in early erythroid progenitors and burst-forming units-erythroid (BFU-E). High *GATA1* expression levels have been shown in colony-forming units-erythroid (CFU-E): at this stage *Gata1* activates the expression of the erythropoietin receptor (EpoR) and, in turn, EpoR mediated signals enhance the GATA1 transcriptional program in erythroid progenitor cells, as has been shown *in vitro* [34, 66-68]. After this point, expression levels of *Gata1* gradually decrease from the proerythroblast stage as differentiation proceeds through the basophilic, polychromatic and orthochromatic erythroblast stages [65]. In summary, *GATA1* mRNA is expressed at low levels in early

1
2
3 progenitors, its expression levels reach a maximum prior to the proerythroblast stage
4 and gradually declines towards the final maturation of erythroid cells, as has been shown
5 in mice and human model systems [65, 69, 70]. GATA1 protein levels follow this pattern,
6 and decrease towards terminal maturation, as was shown in mouse erythroid progenitors
7 using a hanging-drop culture method, and comparing the levels of GATA1 protein in the
8 same number of cells amongst successive stages of differentiation [71]. Studies using
9 intracellular flow cytometry or flow cytometry analysis of bone marrow progenitors of
10 GATA1-reporter transgenic mice, clearly supported this notion (Figure 1 and [70]).
11
12
13
14
15
16

17 Recent studies at the single cell level have also provided renewed insight as to GATA1
18 protein expression in human erythropoiesis, from hematopoietic stem and progenitor
19 cells (HSPCs) to terminally differentiated erythroid cells. In one study, GATA1 protein
20 could be detected at low levels in a subset of HSPCs, gradually increasing as cells
21 differentiate, reaching high levels in lineage committed erythroid cells [72]. Similarly, in
22 another study, GATA1 protein was clearly detectable at the CMP stage and gradually
23 increased with cell commitment and differentiation through the CFU-E, proerythroblast
24 and basophilic erythroblast and orthochromatic erythroblast stages [73]. Previously, it
25 has been shown that GATA1 protein levels decline at the very last stages of terminal
26 erythroid differentiation [74, 75], in what appears to be a regulated event necessary for
27 terminal erythroid maturation (see below).
28
29
30
31
32
33
34
35
36

37 **Regulation of GATA1 mRNA translation**

38
39
40 The translation of the *GATA1* mRNA in erythroid cells gives rise to the production of two
41 protein isoforms as a result of the utilization of two ATG start codons: one in exon II which
42 gives rise to full length *GATA1* encoding a 413 amino acid protein and a second ATG in
43 exon III which gives rise to a shorter isoform (*GATA1s* [76, 77]) lacking the N terminal-
44 most 83 amino acids, which span a so-called transactivation domain [78]. The *GATA1*
45 mRNA has been described to have an unusual 5' UTR structure, in that it is short and
46 predicted to have a less complex secondary structure [72, 76]. These 5' UTR features
47 are generally shared by mRNAs that are associated with increased translational
48 efficiencies and are particularly susceptible to changes in ribosomal levels, for example,
49 in ribosomopathies [72]. Remarkably, the *GATA1* 5' UTR structure is unique among a
50 number of critical hematopoietic transcription factors which, on average, have longer 5'
51 UTRs with more complex predicted structures [72]. 5' UTR swap experiments between
52 *GATA1* and other hematopoietic transcription factors, such as *GATA2* or *RUNX1*, failed
53
54
55
56
57
58
59
60

1
2
3 to rescue erythroid differentiation in a functional complementation assay in the *Gata1*-
4 null G1E proerythroblastic cell line [72]. Furthermore, *GATA1* mRNA translation appears
5 to have a greater requirement for eIF-dependent translation initiation [76]. Lastly, recent
6 evidence showed that *GATA1* mRNA translation also requires the ribonuclease inhibitor
7 1 (RNH1) [79]. RNH1 associates with ribosomes and enhances *GATA1* mRNA
8 translation by enabling its recruitment to the ribosomal complex [79]. Thus, the overall
9 picture emerging is that *GATA1* mRNA translation is highly regulated in erythroid cells
10 and disruptions in these regulatory mechanisms lead to reduced *GATA1* protein levels,
11 defective erythropoiesis and, ultimately, to disease (see below). Additionally, it appears
12 that disruption of the complex translational regulation of the *GATA1* mRNA affects
13 translation of both full length *GATA1* and of *GATA1*s [76].
14
15
16
17
18
19

20
21 Targeted degradation of mRNA by miRNAs is acknowledged as being a crucial
22 checkpoint for cellular processes and tumorigenesis, however, not much is known about
23 the miRNA-mediated regulation of *GATA1* mRNA. In the teleost *C. hamatus*, the 3' UTR
24 of the *GATA1* mRNA has been reported to be a target for miR-152 [80]. This interaction
25 reduces the expression levels of *GATA1* protein and, consequently, represses
26 hematopoiesis when miR-152 is overexpressed [80].
27
28
29
30
31

32 **Posttranslational modifications and *GATA1* protein stability**

33
34 *GATA1* undergoes several posttranslational modifications (PTMs), including
35 phosphorylation, acetylation, sumoylation and ubiquitination. Phosphorylation occurs in
36 seven serine residues, of which six are constitutively phosphorylated, whereas S310
37 phosphorylation, located near the C-ZnF of *GATA1*, is erythroid specific [81]. *GATA1*
38 phosphorylation occurs downstream of signaling pathways in erythroid cells, the main
39 one being Epo activation, which leads to PI3/AKT or MAPK phosphorylation of specific
40 *GATA1* serine residues [67, 81, 82]. For example, S26 is phosphorylated by MAPK upon
41 stimulation by IL-3 and has been linked to improved cell survival caused by increased
42 expression of Bcl-XL anti-apoptotic gene [83]. Whereas phosphorylation has been
43 proposed to affect *GATA1* properties such as DNA binding and transcriptional activation
44 [84], DNA bending [85] or protein interactions [86], its relevance to *GATA1 in vivo*
45 functions remains unclear [87].
46
47
48
49
50
51
52
53

54 *GATA1* has also been shown to be acetylated by the histone acetyltransferase (HAT)
55 CBP/p300, at evolutionarily conserved lysine residues in the carboxyl ends of its zinc-
56 finger domains (a.a.245-252 and a.a.308-316) [56, 88]. *GATA1* protein acetylation is
57 required for terminal erythroid differentiation and for *GATA1* chromatin occupancy and
58
59
60

1
2
3 transcriptional activation [55, 56, 89, 90]. Acetylated GATA1 interacts with Bromodomain
4 Protein 3 (BRD3) which stabilizes GATA1 binding to chromatin [91]. Interestingly, the
5 combinatorial action of GATA1 phosphorylation and acetylation has been proposed to
6 signal GATA1 degradation by the ubiquitin/proteasome protein pathway [82]. Under this
7 scenario, the acetylated, chromatin bound, “active” fraction of GATA1 becomes
8 phosphorylated in response to specific external signals, or as part of the erythroid
9 differentiation program, which in turn lead to GATA1 becoming ubiquitinated and
10 targeted for degradation [82]. Ubiquitin mediated proteasomal degradation of acetylated
11 GATA1 in erythroid cells appears to involve the HSP27 protein chaperone, which itself
12 is subjected to regulation via posttranslational modification [92]. This complex regulatory
13 network has been proposed to serve as a mean of controlling GATA1 activity at the
14 protein level, in response to specific signaling cues in erythropoiesis [82, 92]. However,
15 the *in vivo* relevance of such a mechanism remains unclear since mutating the GATA1
16 phosphorylatable residues does not appear to have an effect in erythropoiesis *in vivo* [87,
17 93], as would have been predicted by this model. In addition, recent work identified the
18 USP7 deubiquitylase as interacting with and stabilizing GATA1 protein levels in human
19 erythroid cells, by preventing its proteasomal degradation through the deubiquitination
20 of lysine 48 [94]. However, it is not presently known how the USP7 mediated GATA1
21 deubiquitination relates to the acetylation and phosphorylation mediated ubiquitination
22 of GATA1 proposed by Hernandez-Hernandez *et al.* [82].

23
24
25
26
27
28
29
30
31
32
33
34
35
36 GATA1 has also been shown to undergo sumoylation in erythroid cells [95-97].
37 Sumoylation does not appear to affect GATA1 protein levels [97], however its functional
38 significance remains contradictory. One study reported that sumoylation inhibits GATA1
39 chromatin occupancy and transcriptional activation [97], whereas another study
40 suggested the opposite, in that sumoylation appeared to enhance GATA1 chromatin
41 occupancy, subnuclear localization and transcriptional activation of a subset of
42 GATA1/FOG-1 dependent target genes [96]. Either way, GATA1 sumoylation appears
43 to add yet another layer to the regulation of GATA1 activity. However, the precise
44 crosstalk between all of GATA1’s PTMs (acetylation, phosphorylation, sumoylation and
45 ubiquitination) in regulating GATA1 protein levels and activity *in vivo*, remains to be
46 elucidated.

37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 **Caspase mediated GATA1 protein cleavage and terminal erythroid differentiation**

55
56
57 GATA1 protein has also been shown to be a target for caspase-mediated cleavage in
58 terminal erythroid differentiation in human. In a seminal study, De Maria *et al.* showed
59 that immature erythroid cells expressed several death receptors which, when activated
60

1
2
3 by addition of ligand (e.g. Fas-L) or by incubation with mature erythroblasts, could inhibit
4 erythroid maturation through the specific cleavage of GATA1 protein by caspases [74].
5
6 These observations led to the suggestion of a homeostatic mechanism whereby
7
8 accumulation of mature erythroid cells in the erythroblastic island would lead to the
9
10 activation of death receptors in immature cells, leading to caspase-mediated GATA1
11
12 protein cleavage and differentiation arrest and apoptosis [74].

13
14 In a subsequent study by Ribeil *et al.*, it was shown that caspase 3 was the predominant
15
16 caspase activated in the nucleus of terminally differentiating erythroid cells which can
17
18 cleave GATA1, for example, when starving erythroid precursors for Epo, resulting in cell
19
20 death by apoptosis [75]. Furthermore, Ribeil *et al.* uncovered a mechanism that protects
21
22 GATA1 from cleavage by caspase 3 activation in the nucleus during human erythroid
23
24 cell maturation. Specifically, they showed that Epo mediated erythroblast survival and
25
26 terminal differentiation results in the nuclear translocation of the HSP70 protein
27
28 chaperone and its specific interaction with GATA1 protein, thus protecting it from
29
30 cleavage by activated caspase 3 [75]. Under conditions of Epo starvation, HSP70
31
32 translocates to the nucleus and no longer protects GATA1 which now becomes cleaved
33
34 by caspase 3, thus leading to differentiation arrest and apoptosis [75]. Thus, HSP70
35
36 protects GATA1 from programmed caspase 3 activation in the Epo-dependent, early
37
38 stages of erythroid cell maturation as a cell survival and differentiation protective
39
40 mechanism, whereas at later stages, Epo-independent mature erythroid cells can signal
41
42 death receptor and caspase activation in immature cells, leading to GATA1 cleavage
43
44 and differentiation arrest as a homeostatic control mechanism. It is unclear whether this
45
46 mechanism also operates in the murine system, as murine GATA1 protein does not
47
48 share the caspase cleavage site with human GATA1 [74]. Interestingly, a recent study
49
50 further added to the GATA1/HSP70 protective axis by showing that nuclear HSP70 and
51
52 GATA1 protein levels were modulated by the p19^{INK4d} cyclin-dependent kinase inhibitor
53
54 [98]. Specifically, it was shown that p19^{INK4d} downregulated PEBP1
55
56 (phosphatidylethanolamine-binding protein 1), resulting in stabilized nuclear HSP70
57
58 protein levels through increased ERK phosphorylation, known to be implicated in HSP70
59
60 nuclear localization [98]. Importantly, the GATA1/HSP70 protective mechanism breaks
down in hematological disorders presenting with ineffective erythropoiesis, as outlined
below.

A recent study uncovered an additional caspase-mediated mechanism for regulating
GATA1 protein levels in hematopoiesis [99]. Inflammasome activation in HSCs due to
infection, led to caspase 1 activation and GATA1 cleavage and degradation, thus biasing

1
2
3 cell fate decisions towards the myeloid lineage. Inhibition of caspase 1 in murine HSCs
4 resulted in the upregulation of GATA1 protein and an increase in megakaryocytic-
5 erythroid (MegE) colony output at the expense of granulocyte-monocyte (GM) colonies,
6 suggesting an evolutionarily conserved mechanism for the inflammasome regulating
7 hematopoietic cell fate decisions by fine-tuning GATA1. In addition, the same study
8 showed that the cleavage of GATA1 by caspase 1 was required for the final steps of
9 hemin-induced erythroid differentiation in K562 cells, thus pointing at an additional
10 mechanism whereby the inflammasome could regulate erythropoiesis through GATA1.
11 Importantly, just like for caspase 3, GATA1 was indeed shown to be a substrate for
12 cleavage by caspase 1 *in vitro* [99].
13
14
15
16
17
18
19

20 **The effects of artificially altering GATA1 protein levels**

21
22 As outlined above, GATA1 translation and protein levels are exquisitely regulated in
23 hematopoiesis and it is now well established that tinkering with GATA1 protein levels has
24 important functional implications and may also result in hematological disease. For
25 example, the extremes of either a complete loss of GATA1 expression or GATA1
26 overexpression, result in both cases in embryonic lethal anemia [22, 100]. Another study
27 using inducible GATA1 expression in an immortalized GATA1-null proerythroblastic cell
28 line, demonstrated differential sensitivity of target genes to the dosage of GATA1 protein
29 [101]. Studies using transgenic mice under- or over-expressing GATA1 have been
30 particularly useful in investigating the effects of altering GATA1 protein levels in
31 hematopoiesis. Specifically, two GATA1 knockdown mouse models have been
32 generated by the Yamamoto and Orkin groups expressing GATA1 at 5% or 20% of its
33 physiological levels, respectively [102, 103]. The first mouse model, called GATA1.05,
34 was generated by the insertion of a neomycin resistance cassette in the GATA1 promoter
35 region, which resulted in GATA1 expression at 5% its physiological level [102].
36 Hemizygous male GATA1.05 embryos die from severe anemia between E11.5 and
37 E12.5, suggesting that 5% GATA1 expression is not sufficient to support primitive
38 erythropoiesis. Interestingly, using *in vitro* differentiated ES cells, it was shown that both
39 GATA1.05 and GATA1-null cells resulted in reduced expression of p16^{INK4A} and
40 accelerated cell cycle progression, however, 5% of GATA1 expression was sufficient to
41 maintain expression of the anti-apoptotic Bcl-X_L gene, thus protecting the cells from
42 apoptotic cell death normally observed with GATA1-null cells [104]. These results
43 provide further evidence for subtle alterations in GATA1 expression levels having
44 differential effects on the expression of GATA1 target genes.
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59

60 In contrast to male mice, heterozygous GATA1.05 female mice were born at slightly

1
2
3 lower than expected Mendelian frequencies and often developed perinatally varying
4 degrees of transient anemia (due to X inactivation), from which they recovered [102,
5 104]. However, between 3 and 6 months of age, GATA1.05 female mice frequently
6 develop erythroleukemia, characterized by the proliferation of immature erythroid
7 progenitors [105]. By contrast, heterozygous GATA1-null female mice do not present
8 with erythroleukemia. The difference between the two conditions (which only differ by
9 5% in the expression of GATA1), may lie in the observation that 5% of GATA1 is sufficient
10 to protect cells from apoptosis, but not from uncontrollable cell proliferation [104]. By
11 contrast, GATA1-null cells in a heterozygous female background would be eliminated by
12 apoptosis. Thus, additional genetic events that may occur in the proliferating GATA1.05
13 progenitor population can lead to leukemic transformation [105-107]. Interestingly, older
14 GATA1.05 mice also develop CD19⁺ B-cell leukemia through a leukemogenic
15 mechanism that is not yet understood, but may be related to lineage commitment
16 skewing towards lymphoid fates by proliferating progenitors expressing low levels of
17 GATA1 [105].

18
19 The second GATA1 knockdown mouse strain was generated by replacing an upstream
20 enhancer region in the GATA1 gene locus with a neomycin resistance cassette [103]. In
21 these mice, known as GATA1^{low} mice, GATA1 expression levels were originally reported
22 to be knocked down to 20% [103], although subsequent analyses have shown GATA1
23 mRNA reduced by as much as 100-fold in orthochromatic erythroblasts ([108] and
24 references therein). The majority of GATA1^{low} male mice die of anemia between
25 embryonic stages E13.5 and E14.5. In contrast to the GATA1.05 mice, a few GATA1^{low}
26 male mice are born alive but are severely anemic, but for some of them anemia resolves
27 around 4-5 weeks of age [103]. These observations suggest that ~20% GATA1
28 expression is insufficient to support primitive erythropoiesis but sufficient for adult
29 erythropoiesis. Interestingly, GATA1 expression in the megakaryocytes of GATA1^{low}
30 mice is completely abolished and the mice are born with severe thrombocytopenia,
31 associated with the expansion of abnormal megakaryocytic progenitors that, at 10
32 months of age, develops into a myeloproliferative condition characterized by fibrosis of
33 the bone marrow, closely resembling primary myelofibrosis [108, 109]. GATA1^{low} mice
34 also have defects in mast cell differentiation, as evidenced by the amplification of mast
35 cell progenitors, increased apoptotic precursors and defective differentiation of mature
36 cells [110]. These observations provide yet more evidence for the necessity of
37 maintaining appropriate GATA1 levels to support balanced hematopoiesis.

1
2
3 GATA1 overexpression has also been shown to have dramatic effects in erythropoiesis
4 *in vivo*. In particular, a mouse model was generated bearing an X-linked *GATA1*
5 transgene driven by the Locus Control Region (LCR) of the human β -globin locus (the
6 *GATA1-OX* transgene), resulting in the overexpression of *GATA1* beyond the
7 proerythroblast stage, at the moment of maximum globin transcription [100]. Male
8 embryos carrying the *GATA1-OX* transgene die *in utero* in mid gestation due to anemia,
9 whereas heterozygous females survive to term and develop normally. From this study it
10 was shown that *GATA1* overexpression at the proerythroblast stage causes cell cycle
11 arrest and apoptosis of erythroid progenitors beyond the proerythroblast stage.
12 Intriguingly, in heterozygous females it was shown that *GATA1-OX* progenitors were able
13 to mature at an almost normal rate [100]. The explanation for this phenomenon lies in
14 the erythroblastic island, i.e. the histological unit where erythropoiesis takes place in the
15 bone marrow [111, 112]. The erythroblastic island is composed of a central macrophage
16 surrounded by erythroid progenitors in successive stages of differentiation, emanating
17 from the center towards the periphery of the island. It is in this periphery where mature
18 erythroblasts will enucleate and the expelled pyrenocyte will be phagocytosed by the
19 central macrophage [113]. In this hierarchical histological unit, erythroid cells are in
20 contact and communicate with each other, thus providing an internal feedback
21 mechanism measuring differentiation rate and assuring maturation and production of red
22 blood cells in response to physiological demands at any given moment [114, 115]. Due
23 to X-chromosome inactivation, the erythroblastic islands of heterozygous *GATA1-OX*
24 females are chimeric in composition, i.e. approximately 50% of erythroid progenitors
25 overexpress *GATA1* (the X chromosome carrying the *GATA1-OX* transgene is active),
26 and 50% of erythroid progenitors express *GATA1* at normal levels (the wildtype X
27 chromosome is active). Based on this, it was proposed that maturing erythroid cells in
28 later stages of differentiation induce the terminal differentiation of earlier progenitors
29 through cell-cell signaling mechanisms, which result in the downregulation of *GATA1*
30 protein levels as a necessary regulatory cadency for proper erythroid terminal maturation
31 [100]. This provides a mechanism to ensure a smooth production of red blood cells in
32 wild type mice. However, in the heterozygous *GATA1-OX* females, this results in the
33 “rescue” of the *GATA1* overexpressing cells, which are able to differentiate when in close
34 proximity to wildtype maturing erythroid cells, which provide a signal directed towards
35 the downregulation of *GATA1* protein levels. This signal was tentatively named “REDS”
36 (red cell differentiation signal). A follow-up study using mice with different combinations
37 of the *GATA1-OX* transgene and a *GATA1*-knockout allele, provided further evidence for
38 REDS signaling by wildtype maturing erythroid cells: when crossed with *GATA1*
39 knockout mice, heterozygous *GATA1-OX* females died in mid gestation due to anemia
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

(similar to GATA1-OX males) [116]. However, to-date, the identity of the REDS receptor/ligand is not known. Regulation of GATA1 transcription at the terminal differentiation stages and recent work suggesting that REDS might act to regulate GATA1 levels through the inhibition of translation (L. Gutiérrez, manuscript in preparation), are possibilities. Overall, the REDS signal serves as another example of homeostatic regulation of erythropoiesis through GATA1 protein level regulation.

Lastly, it should be noted that artificially altering GATA1 expression levels through its enforced expression in hematopoietic progenitors, can push them to cell fates towards lineages that are normally regulated by GATA1 [117, 118]. For example, ectopic expression of GATA1 in avian myeloid progenitors [119] or in human primary myeloid progenitors [120] reprogrammed them to erythroid and megakaryocytic or eosinophilic cell fates, respectively. These studies support the notion that different thresholds of GATA1 expression levels in hematopoietic progenitors, can govern cell fate decisions towards different lineages.

Downregulation of GATA1 protein levels and ineffective erythropoiesis

Ineffective erythropoiesis (IE) or dyserythropoiesis is a pathological feature shared by a number of hematological diseases such as β thalassemia, myelodysplastic syndromes (MDS), Diamond-Blackfan anemia (DBA), 5q-syndrome and myelofibrosis [121]. IE is characterized by the abnormal differentiation of erythroid progenitors resulting in an expanded progenitor compartment, increased destruction of abnormal erythroblasts and a paucity in the production of mature erythroid cells. Recent work has shown that the downregulation of GATA1 protein levels is a critical aspect of IE in hematological disease. Three mechanisms have been described to account for reduced GATA1 protein levels in IE: (i) inherited GATA1 mutations greatly reducing, or eliminating altogether, expression of the full length GATA1 protein [122, 123]; (ii) impaired translation of full length GATA1 [72, 76], and (iii) impaired HSP70-mediated protection of GATA1 protein from cleavage and degradation [124].

The best example of inherited GATA1 mutations in IE is that of Diamond-Blackfan Anemia (DBA). This is an inherited bone marrow failure syndrome characterized by anemia, macrocytosis, reticulopenia and selective reduction or absence of erythroid precursors (BFU-Es) in an otherwise normocellular bone marrow [125]. More than 200 heterozygous mutations in 19 ribosomal protein (RP) genes have been recorded to-date, accounting for most (approximately 70%), but not all, of the DBA cases [126, 127]. Sankaran and colleagues were the first to identify through exome sequencing a GATA1 mutation associated with DBA [128]. This mutation was a c.220G>C transversion that

1
2
3 caused the skipping of exon 2, resulting in the loss of full length GATA1 protein, with the
4 continued expression of the GATA1s isoform. Additional cases in DBA with similar
5 GATA1 mutations have been reported [76, 129, 130], including a mutation that resulted
6 in the loss of the first translation initiation codon responsible for the production of full
7 length GATA1 protein, again without affecting GATA1s expression [76, 130]. These
8 findings are significant in explaining dyserythropoiesis in these patients, as it has been
9 shown that the GATA1s isoform cannot fully support erythropoiesis [20, 21, 131].
10 Furthermore, these results show that the mutations in the *GATA1* gene that give rise to
11 reduced, or absent, full length GATA1 protein, in the absence of RP gene mutations, are
12 sufficient to cause disease. In addition, Hollanda *et al.* reported a family with a similar G
13 > C transition at the boundary of exon 2 in the *GATA1* gene in male members, which
14 also resulted in the exclusive expression of GATA1s giving rise to macrocytic anemia
15 and variable neutropenia, with no apparent thrombocytopenia [132]. The differences in
16 disease manifestation between the two reports of the G > C mutation in exon 2 of the
17 *GATA1* gene remain unclear, but it has been suggested that they may be due to
18 differences in the levels of total GATA1 protein expressed [128]. Lastly, Sankaran and
19 colleagues recently identified two patients with dyserythropoietic anemia and
20 thrombocytopenia, due to a mutation that affects canonical splicing of the *GATA1* mRNA
21 [133]. Specifically, a single nucleotide change was identified 24 nucleotides upstream of
22 the canonical splice site in intron 5 of the *GATA1* gene. This mutation results in an
23 increase in the use of the mutated site as an alternative splice acceptor site, with a
24 reduction in canonical splicing. This leads to a partial intron retention event that
25 introduces an extra 5 amino acids C-terminally to the C-terminal zinc finger of GATA1,
26 with a concomitant reduction in the expression of wild-type GATA1 to ~ 40% its normal
27 levels. Importantly, the mutant GATA1 protein was functionally inactive as it could not
28 rescue erythroid differentiation in functional complementation assays in the GATA1-null
29 G1E murine proerythroblastic cell line [133].
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

47 The analysis of the molecular basis of DBA, again provided important clues as to the
48 implication of reduced GATA1 protein levels in IE in hematological disease. As outlined
49 above, DBA is primarily associated with heterozygous mutations in a number of RP
50 genes. It is now known that deficiencies in RPs act upstream of GATA1 in
51 downregulating its protein translation levels, whilst mRNA levels remain unaffected [76].
52 In accordance with this, knocking down RPS19 expression, or that of other RP genes,
53 led to a significant reduction in GATA1 protein levels resulting in deficient erythropoiesis,
54 reminiscent of DBA [76]. As described above, it appears that the particular sensitivity of
55 the *GATA1* mRNA to RP levels is mediated by the particular sequence and structure of
56
57
58
59
60

1
2
3 its 5' UTR, as it was shown that the presence of the 5' UTR is critical for GATA1 erythroid
4 function [72]. Interestingly, mutations in the RPS14 gene were also found in patients with
5 primary myelofibrosis and were again associated with reduced GATA1 protein levels and
6 impaired megakaryopoiesis [134], thus linking RP gene mutations and reduced GATA1
7 protein levels in hematological disease affecting a hematopoietic lineage other than the
8 erythroid lineage. Furthermore, it was recently shown that heme levels are retroactively
9 involved in this spectrum of phenomena around ribosomal protein dependent GATA1
10 protein level regulation [135]. This study showed that in early erythroid progenitors, heme
11 induces the translation of ribosomal proteins, ensuring sustained globin synthesis.
12 However, at later stages of differentiation, heme accumulation associates with
13 downregulation of *Gata1* through an still undescribed mechanism [135].
14
15
16
17
18
19
20
21

22 As described above, GATA1 protein is normally protected from caspase-mediated
23 cleavage in the early stages of erythroid differentiation, through its interaction in the
24 nucleus with the HSP70 protein chaperone [75]. This GATA1 protective mechanism
25 appears to break down in hematological disorders manifesting with IE, such as DBA and
26 myelodysplastic syndromes (MDS). In DBA, it was recently shown that HSP70 protein is
27 downregulated due to its increased ubiquitination and proteasomal degradation in
28 RPL11- and RPL5-deficient human primary erythroid cells, but not in RPS19-deficient
29 cells, resulting in proliferation and differentiation defects and increased apoptosis in
30 erythroid cells, resembling DBA features [136]. A subsequent study showed a
31 disequilibrium of globin chain and heme synthesis in RPL11- and RPL5-deficient human
32 erythroid cells, resulting in excessive free heme which downregulated HSP70 protein
33 [137]. The study by Doty et al. also suggests that aberrant accumulation of heme,
34 contributes to the amplification of ribosomal protein imbalance, thus causing premature
35 downregulation of GATA1 levels, responsible for the IE that is characteristic of DBA and
36 MDS [135]. Hence, it is clear in that in DBA, HSP70 downregulation results in a significant
37 drop in GATA1 protein levels due to caspase mediated cleavage, which underlies the
38 observed erythroid maturation defects [136]. The HSP70 mediated protection of GATA1
39 is also disrupted in β -thalassemia, which also manifests with IE. Here, it was shown in
40 primary human β -thalassemic erythroblasts that free α -globin chains sequester HSP70
41 to the cytoplasm, resulting in significant downregulation of nuclear GATA1 protein and a
42 blockade in terminal erythroid differentiation [138]. Lastly, myelodysplastic syndromes
43 are clonal disorders also characterized by IE associated with defective erythroid
44 maturation and increased apoptosis of erythroid precursors [121]. Here too, it was shown
45 that nuclear GATA1 protein was significantly lower as a result of aberrant HSP70
46 localization in the cytoplasm, accounting for the blockade in erythroid differentiation in
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 MDS [124]. Thus, downregulation or absence of full length GATA1 protein in erythroid
4 precursors, either directly through mutations in the *GATA1* gene itself, or indirectly
5 through defects in *GATA1* mRNA translation or the breakdown of the HSP70/GATA1
6 protective axis, result in defective erythropoiesis and disease. This is an addition to
7 hematological deficiencies or malignancies affecting other hematopoietic lineages,
8 where downregulation or loss of the full length GATA1 protein play a causative role, for
9 example, in primary myelofibrosis affecting the megakaryocytic lineage [108] or in
10 trisomy 21 associated transient myeloproliferative disorder (TMD), acute
11 megakaryoblastic leukemia (AMKL) and clonal eosinophilia [139, 140].
12
13
14
15
16
17
18

19 **Conclusions**

20
21 The fine-tuning of GATA1 protein levels has emerged as a key mechanism for regulating
22 erythropoiesis. High GATA1 protein levels are required in the EPO-dependent early
23 stages of erythropoiesis in providing cell survival functions, in addition to activating the
24 erythroid transcription program. Adequate GATA1 protein levels in the early stages of
25 erythroid differentiation are ensured by the efficient translation of GATA1 mRNA and its
26 protection from cleavage by activated caspase 3 through GATA1's interaction with the
27 HSP70 protein chaperone. Subsequently, GATA1 needs to be drastically downregulated
28 for the terminal stage of erythroid maturation to take place, in a homeostatic mechanism
29 that balances the production of red blood cells depending on surplus or demand. GATA1
30 protein levels also affect cell fate decisions, as evidenced by the reprogramming of
31 myeloid progenitors towards MegE fates by GATA1 overexpression. Furthermore,
32 inflammasomes appear to exploit the caspase-mediated cleavage of GATA1 in HSCs in
33 order to tip the balance towards enhanced myeloid differentiation in response to
34 infection. Lastly, the combined action of GATA1 post translational modifications has been
35 proposed as a mechanism to control the active, chromatin-bound fraction of GATA1 by
36 targeting it for proteasomal degradation as a mechanism for regulating GATA1
37 transcriptional activity in response to signals. It has also become clear that, not only
38 genetic mutations affecting *GATA1*, but also defects in the different levels of regulatory
39 checkpoints for physiological GATA1 expression, play a key role in the physiopathology
40 of disease hematological diseases associated with inefficient erythropoiesis. With the
41 advent of novel, unbiased, single cell-based technologies, we stand to gain a much better
42 understanding of GATA1 regulatory mechanisms and the cascading effects of their
43 deregulation in vertebrate hematopoiesis and in human disease. Newly gained
44 knowledge will help the development of novel therapies targeting the restoration of
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 GATA1 regulation in treating inefficient erythropoiesis and anemia in hematological
4 disease in man.
5
6
7
8
9
10
11
12

13 **Acknowledgments**

14
15
16 We apologize to the many of our colleagues whose work we were not able to cite due to
17 space restrictions. L.G. is supported by a Ramón y Cajal Fellowship (RYC-2013-12587;
18 Ministerio de Economía y Competitividad, Spain). E.K. is supported by a Scholarship for
19 Strengthening Post-Doctoral Research from the Greek State Scholarships Foundation
20 (IKY), within the framework of the Operational Program “Human Resources
21 Development program, Education and Life-Long learning”.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

1. Adam, R. C., Yang, H., Rockowitz, S., Larsen, S. B., Nikolova, M., Oristian, D. S., Polak, L., Kadaja, M., Asare, A., Zheng, D. & Fuchs, E. (2015) Pioneer factors govern super-enhancer dynamics in stem cell plasticity and lineage choice, *Nature*. **521**, 366-70.
2. Brown, G., Ceredig, R. & Tsapogas, P. (2018) The Making of Hematopoiesis: Developmental Ancestry and Environmental Nurture, *Int J Mol Sci*. **19**.
3. Jacobsen, S. E. W. & Nerlov, C. (2019) Haematopoiesis in the era of advanced single-cell technologies, *Nat Cell Biol*. **21**, 2-8.
4. Hofer, T. & Rodewald, H. R. (2018) Differentiation-based model of hematopoietic stem cell functions and lineage pathways, *Blood*. **132**, 1106-1113.
5. Dzierzak, E. & Philipsen, S. (2013) Erythropoiesis: development and differentiation, *Cold Spring Harb Perspect Med*. **3**, a011601.
6. Liang, R. & Ghaffari, S. (2016) Advances in understanding the mechanisms of erythropoiesis in homeostasis and disease, *Br J Haematol*. **174**, 661-73.
7. Palis, J. (2014) Primitive and definitive erythropoiesis in mammals, *Front Physiol*. **5**, 3.
8. Kerenyi, M. A. & Orkin, S. H. (2010) Networking erythropoiesis, *J Exp Med*. **207**, 2537-41.
9. Ferreira, R., Ohneda, K., Yamamoto, M. & Philipsen, S. (2005) GATA1 function, a paradigm for transcription factors in hematopoiesis, *Mol Cell Biol*. **25**, 1215-27.
10. Tremblay, M., Sanchez-Ferras, O. & Bouchard, M. (2018) GATA transcription factors in development and disease, *Development*. **145**.
11. Lowry, J. A. & Atchley, W. R. (2000) Molecular evolution of the GATA family of transcription factors: conservation within the DNA-binding domain, *J Mol Evol*. **50**, 103-15.
12. Weiss, M. J. & Orkin, S. H. (1995) GATA transcription factors: key regulators of hematopoiesis, *Exp Hematol*. **23**, 99-107.
13. Katsumura, K. R., Bresnick, E. H. & Group, G. F. M. (2017) The GATA factor revolution in hematology, *Blood*. **129**, 2092-2102.
14. Ko, L. J. & Engel, J. D. (1993) DNA-binding specificities of the GATA transcription factor family, *Mol Cell Biol*. **13**, 4011-22.
15. Vyas, P., Ault, K., Jackson, C. W., Orkin, S. H. & Shivdasani, R. A. (1999) Consequences of GATA-1 deficiency in megakaryocytes and platelets, *Blood*. **93**, 2867-75.
16. Yu, C., Cantor, A. B., Yang, H., Browne, C., Wells, R. A., Fujiwara, Y. & Orkin, S. H. (2002) Targeted deletion of a high-affinity GATA-binding site in the GATA-1 promoter leads to selective loss of the eosinophil lineage in vivo, *J Exp Med*. **195**, 1387-95.
17. Nei, Y., Obata-Ninomiya, K., Tsutsui, H., Ishiwata, K., Miyasaka, M., Matsumoto, K., Nakae, S., Kanuka, H., Inase, N. & Karasuyama, H. (2013) GATA-1 regulates the generation and function of basophils, *Proc Natl Acad Sci U S A*. **110**, 18620-5.
18. Gutierrez, L., Nikolic, T., van Dijk, T. B., Hammad, H., Vos, N., Willart, M., Grosveld, F., Philipsen, S. & Lambrecht, B. N. (2007) Gata1 regulates dendritic-cell development and survival, *Blood*. **110**, 1933-41.
19. Sasaki, H., Kurotaki, D. & Tamura, T. (2016) Regulation of basophil and mast cell development by transcription factors, *Allergol Int*. **65**, 127-134.
20. Byrska-Bishop, M., VanDorn, D., Campbell, A. E., Betensky, M., Arca, P. R., Yao, Y., Gadue, P., Costa, F. F., Nemiroff, R. L., Blobel, G. A., French, D. L., Hardison, R. C., Weiss, M. J. & Chou, S. T. (2015) Pluripotent stem cells reveal erythroid-specific activities of the GATA1 N-terminus, *J Clin Invest*. **125**, 993-1005.
21. Ling, T., Birger, Y., Stankiewicz, M. J., Ben-Haim, N., Kalisky, T., Rein, A., Kugler, E., Chen, W., Fu, C., Zhang, K., Patel, H., Sikora, J. W., Goo, Y. A., Kelleher, N., Zou, L., Izraeli, S. & Crispino, J. D. (2019) Chromatin occupancy and epigenetic analysis reveal new insights into the function of GATA1 N-terminus in erythropoiesis, *Blood*.
22. Pevny, L., Simon, M. C., Robertson, E., Klein, W. H., Tsai, S. F., D'Agati, V., Orkin, S. H. & Costantini, F. (1991) Erythroid differentiation in chimaeric mice blocked by a targeted mutation in the gene for transcription factor GATA-1, *Nature*. **349**, 257-60.
23. Pevny, L., Lin, C. S., D'Agati, V., Simon, M. C., Orkin, S. H. & Costantini, F. (1995) Development of hematopoietic cells lacking transcription factor GATA-1, *Development*. **121**, 163-72.

- 1
2
3 24. Weiss, M. J. & Orkin, S. H. (1995) Transcription factor GATA-1 permits survival and
4 maturation of erythroid precursors by preventing apoptosis, *Proc Natl Acad Sci U S A.* **92**, 9623-
5 7.
- 6 25. Gutierrez, L., Tsukamoto, S., Suzuki, M., Yamamoto-Mukai, H., Yamamoto, M., Philipsen,
7 S. & Ohneda, K. (2008) Ablation of Gata1 in adult mice results in aplastic crisis, revealing its
8 essential role in steady-state and stress erythropoiesis, *Blood.* **111**, 4375-85.
- 9 26. Cheng, Y., Wu, W., Kumar, S. A., Yu, D., Deng, W., Tripic, T., King, D. C., Chen, K. B.,
10 Zhang, Y., Drautz, D., Giardine, B., Schuster, S. C., Miller, W., Chiaromonte, F., Zhang, Y.,
11 Blobel, G. A., Weiss, M. J. & Hardison, R. C. (2009) Erythroid GATA1 function revealed by
12 genome-wide analysis of transcription factor occupancy, histone modifications, and mRNA
13 expression, *Genome Res.* **19**, 2172-84.
- 14 27. Fujiwara, T., O'Geen, H., Keles, S., Blahnik, K., Linnemann, A. K., Kang, Y. A., Choi, K.,
15 Farnham, P. J. & Bresnick, E. H. (2009) Discovering hematopoietic mechanisms through
16 genome-wide analysis of GATA factor chromatin occupancy, *Mol Cell.* **36**, 667-81.
- 17 28. Yu, M., Riva, L., Xie, H., Schindler, Y., Moran, T. B., Cheng, Y., Yu, D., Hardison, R.,
18 Weiss, M. J., Orkin, S. H., Bernstein, B. E., Fraenkel, E. & Cantor, A. B. (2009) Insights into
19 GATA-1-mediated gene activation versus repression via genome-wide chromatin occupancy
20 analysis, *Mol Cell.* **36**, 682-95.
- 21 29. Papadopoulos, G. L., Karkoulia, E., Tsamardinos, I., Porcher, C., Ragoussis, J., Bungert, J.
22 & Strouboulis, J. (2013) GATA-1 genome-wide occupancy associates with distinct epigenetic
23 profiles in mouse fetal liver erythropoiesis, *Nucleic Acids Res.* **41**, 4938-48.
- 24 30. Huang, D. Y., Kuo, Y. Y., Lai, J. S., Suzuki, Y., Sugano, S. & Chang, Z. F. (2004) GATA-1
25 and NF-Y cooperate to mediate erythroid-specific transcription of Gfi-1B gene, *Nucleic Acids
26 Res.* **32**, 3935-46.
- 27 31. Vilaboa, N., Bermejo, R., Martinez, P., Bornstein, R. & Cales, C. (2004) A novel E2 box-
28 GATA element modulates Cdc6 transcription during human cells polyploidization, *Nucleic Acids
29 Res.* **32**, 6454-67.
- 30 32. Fernandez-Morales, B., Pavon, L. & Cales, C. (2012) CDC6 expression is regulated by
31 lineage-specific transcription factor GATA1, *Cell Cycle.* **11**, 3055-66.
- 32 33. Papetti, M., Wontakal, S. N., Stopka, T. & Skoultschi, A. I. (2010) GATA-1 directly regulates
33 p21 gene expression during erythroid differentiation, *Cell Cycle.* **9**, 1972-80.
- 34 34. Gregory, T., Yu, C., Ma, A., Orkin, S. H., Blobel, G. A. & Weiss, M. J. (1999) GATA-1 and
35 erythropoietin cooperate to promote erythroid cell survival by regulating bcl-xL expression,
36 *Blood.* **94**, 87-96.
- 37 35. Tanaka, H., Matsumura, I., Nakajima, K., Daino, H., Sonoyama, J., Yoshida, H., Oritani, K.,
38 Machii, T., Yamamoto, M., Hirano, T. & Kanakura, Y. (2000) GATA-1 blocks IL-6-induced
39 macrophage differentiation and apoptosis through the sustained expression of cyclin D1 and
40 bcl-2 in a murine myeloid cell line M1, *Blood.* **95**, 1264-73.
- 41 36. Chou, S. T., Khandros, E., Bailey, L. C., Nichols, K. E., Vakoc, C. R., Yao, Y., Huang, Z.,
42 Crispino, J. D., Hardison, R. C., Blobel, G. A. & Weiss, M. J. (2009) Graded repression of
43 PU.1/Sfpi1 gene transcription by GATA factors regulates hematopoietic cell fate, *Blood.* **114**,
44 983-94.
- 45 37. Kadauke, S., Udugama, M. I., Pawlicki, J. M., Achtman, J. C., Jain, D. P., Cheng, Y.,
46 Hardison, R. C. & Blobel, G. A. (2012) Tissue-specific mitotic bookmarking by hematopoietic
47 transcription factor GATA1, *Cell.* **150**, 725-37.
- 48 38. Tsang, A. P., Visvader, J. E., Turner, C. A., Fujiwara, Y., Yu, C., Weiss, M. J., Crossley, M.
49 & Orkin, S. H. (1997) FOG, a multitype zinc finger protein, acts as a cofactor for transcription
50 factor GATA-1 in erythroid and megakaryocytic differentiation, *Cell.* **90**, 109-19.
- 51 39. Crispino, J. D., Lodish, M. B., MacKay, J. P. & Orkin, S. H. (1999) Use of altered specificity
52 mutants to probe a specific protein-protein interaction in differentiation: the GATA-1:FOG
53 complex, *Mol Cell.* **3**, 219-28.
- 54 40. Chang, A. N., Cantor, A. B., Fujiwara, Y., Lodish, M. B., Droho, S., Crispino, J. D. & Orkin,
55 S. H. (2002) GATA-factor dependence of the multitype zinc-finger protein FOG-1 for its
56 essential role in megakaryopoiesis, *Proc Natl Acad Sci U S A.* **99**, 9237-42.
- 57 41. Nichols, K. E., Crispino, J. D., Poncz, M., White, J. G., Orkin, S. H., Maris, J. M. & Weiss,
58 M. J. (2000) Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited
59 mutation in GATA1, *Nat Genet.* **24**, 266-70.
- 60 42. Rodriguez, P., Bonte, E., Krijgsveld, J., Kolodziej, K. E., Guyot, B., Heck, A. J., Vyas, P., de
Boer, E., Grosveld, F. & Strouboulis, J. (2005) GATA-1 forms distinct activating and repressive
complexes in erythroid cells, *EMBO J.* **24**, 2354-66.

- 1
2
3 43. Hong, W., Nakazawa, M., Chen, Y. Y., Kori, R., Vakoc, C. R., Rakowski, C. & Blobel, G. A.
4 (2005) FOG-1 recruits the NuRD repressor complex to mediate transcriptional repression by
5 GATA-1, *EMBO J.* **24**, 2367-78.
- 6 44. Wadman, I. A., Osada, H., Grutz, G. G., Agulnick, A. D., Westphal, H., Forster, A. &
7 Rabbitts, T. H. (1997) The LIM-only protein Lmo2 is a bridging molecule assembling an
8 erythroid, DNA-binding complex which includes the TAL1, E47, GATA-1 and Ldb1/NLI proteins,
9 *EMBO J.* **16**, 3145-57.
- 10 45. Kassouf, M. T., Hughes, J. R., Taylor, S., McGowan, S. J., Soneji, S., Green, A. L., Vyas, P.
11 & Porcher, C. (2010) Genome-wide identification of TAL1's functional targets: insights into its
12 mechanisms of action in primary erythroid cells, *Genome Res.* **20**, 1064-83.
- 13 46. Soler, E., Andrieu-Soler, C., de Boer, E., Bryne, J. C., Thongjuea, S., Stadhouders, R.,
14 Palstra, R. J., Stevens, M., Kockx, C., van Ijcken, W., Hou, J., Steinhoff, C., Rijkers, E.,
15 Lenhard, B. & Grosveld, F. (2010) The genome-wide dynamics of the binding of Ldb1
16 complexes during erythroid differentiation, *Genes Dev.* **24**, 277-89.
- 17 47. Jurata, L. W., Pfaff, S. L. & Gill, G. N. (1998) The nuclear LIM domain interactor NLI
18 mediates homo- and heterodimerization of LIM domain transcription factors, *J Biol Chem.* **273**,
19 3152-7.
- 20 48. Love, P. E., Warzecha, C. & Li, L. (2014) Ldb1 complexes: the new master regulators of
21 erythroid gene transcription, *Trends Genet.* **30**, 1-9.
- 22 49. Liu, G. & Dean, A. (2019) Enhancer long-range contacts: The multi-adaptor protein LDB1 is
23 the tie that binds, *Biochim Biophys Acta Gene Regul Mech.* **1862**, 625-633.
- 24 50. Kang, Y., Kim, Y. W., Yun, J., Shin, J. & Kim, A. (2015) KLF1 stabilizes GATA-1 and TAL1
25 occupancy in the human beta-globin locus, *Biochim Biophys Acta.* **1849**, 282-9.
- 26 51. Merika, M. & Orkin, S. H. (1995) Functional synergy and physical interactions of the
27 erythroid transcription factor GATA-1 with the Kruppel family proteins Sp1 and EKLF, *Mol Cell*
28 *Biol.* **15**, 2437-47.
- 29 52. Kuo, Y. Y. & Chang, Z. F. (2007) GATA-1 and Gfi-1B interplay to regulate Bcl-xL
30 transcription, *Mol Cell Biol.* **27**, 4261-72.
- 31 53. Kadam, S. & Emerson, B. M. (2003) Transcriptional specificity of human SWI/SNF BRG1
32 and BRM chromatin remodeling complexes, *Mol Cell.* **11**, 377-89.
- 33 54. Kadri, Z., Shimizu, R., Ohneda, O., Maouche-Chretien, L., Gisselbrecht, S., Yamamoto, M.,
34 Romeo, P. H., Leboulch, P. & Chretien, S. (2009) Direct binding of pRb/E2F-2 to GATA-1
35 regulates maturation and terminal cell division during erythropoiesis, *PLoS Biol.* **7**, e1000123.
- 36 55. Blobel, G. A., Nakajima, T., Eckner, R., Montminy, M. & Orkin, S. H. (1998) CREB-binding
37 protein cooperates with transcription factor GATA-1 and is required for erythroid differentiation,
38 *Proc Natl Acad Sci U S A.* **95**, 2061-6.
- 39 56. Hung, H. L., Lau, J., Kim, A. Y., Weiss, M. J. & Blobel, G. A. (1999) CREB-Binding protein
40 acetylates hematopoietic transcription factor GATA-1 at functionally important sites, *Mol Cell*
41 *Biol.* **19**, 3496-505.
- 42 57. Zon, L. I., Tsai, S. F., Burgess, S., Matsudaira, P., Bruns, G. A. & Orkin, S. H. (1990) The
43 major human erythroid DNA-binding protein (GF-1): primary sequence and localization of the
44 gene to the X chromosome, *Proc Natl Acad Sci U S A.* **87**, 668-72.
- 45 58. Shimizu, R. & Yamamoto, M. (2005) Gene expression regulation and domain function of
46 hematopoietic GATA factors, *Semin Cell Dev Biol.* **16**, 129-36.
- 47 59. McDevitt, M. A., Fujiwara, Y., Shivdasani, R. A. & Orkin, S. H. (1997) An upstream, DNase I
48 hypersensitive region of the hematopoietic-expressed transcription factor GATA-1 gene confers
49 developmental specificity in transgenic mice, *Proc Natl Acad Sci U S A.* **94**, 7976-81.
- 50 60. Valverde-Garduno, V., Guyot, B., Anguita, E., Hamlett, I., Porcher, C. & Vyas, P. (2004)
51 Differences in the chromatin structure and cis-element organization of the human and mouse
52 GATA1 loci: implications for cis-element identification, *Blood.* **104**, 3106-16.
- 53 61. Nishimura, S., Takahashi, S., Kuroha, T., Suwabe, N., Nagasawa, T., Trainor, C. &
54 Yamamoto, M. (2000) A GATA box in the GATA-1 gene hematopoietic enhancer is a critical
55 element in the network of GATA factors and sites that regulate this gene, *Mol Cell Biol.* **20**, 713-
56 23.
- 57 62. Vyas, P., McDevitt, M. A., Cantor, A. B., Katz, S. G., Fujiwara, Y. & Orkin, S. H. (1999)
58 Different sequence requirements for expression in erythroid and megakaryocytic cells within a
59 regulatory element upstream of the GATA-1 gene, *Development.* **126**, 2799-811.
- 60 63. Guyot, B., Valverde-Garduno, V., Porcher, C. & Vyas, P. (2004) Deletion of the major
GATA1 enhancer HS 1 does not affect eosinophil GATA1 expression and eosinophil
differentiation, *Blood.* **104**, 89-91.

- 1
2
3 64. Yamamoto, M., Takahashi, S., Onodera, K., Muraosa, Y. & Engel, J. D. (1997) Upstream
4 and downstream of erythroid transcription factor GATA-1, *Genes Cells*. **2**, 107-15.
- 5 65. Kobayashi, M. & Yamamoto, M. (2007) Regulation of GATA1 gene expression, *J Biochem*.
6 **142**, 1-10.
- 7 66. Deindl, P., Klar, M., Drews, D., Cremer, M., Gammella, E., Gassmann, M. & Dame, C.
8 (2014) Mice over-expressing human erythropoietin indicate that erythropoietin enhances
9 expression of its receptor via up-regulated Gata1 and Tal1, *Haematologica*. **99**, e205-7.
- 10 67. Zhao, W., Kitidis, C., Fleming, M. D., Lodish, H. F. & Ghaffari, S. (2006) Erythropoietin
11 stimulates phosphorylation and activation of GATA-1 via the PI3-kinase/AKT signaling pathway,
12 *Blood*. **107**, 907-15.
- 13 68. Zon, L. I., Youssoufian, H., Mather, C., Lodish, H. F. & Orkin, S. H. (1991) Activation of the
14 erythropoietin receptor promoter by transcription factor GATA-1, *Proc Natl Acad Sci U S A*. **88**,
15 10638-41.
- 16 69. Leonard, M., Brice, M., Engel, J. D. & Papayannopoulou, T. (1993) Dynamics of GATA
17 transcription factor expression during erythroid differentiation, *Blood*. **82**, 1071-9.
- 18 70. Suzuki, N., Suwabe, N., Ohneda, O., Obara, N., Imagawa, S., Pan, X., Motohashi, H. &
19 Yamamoto, M. (2003) Identification and characterization of 2 types of erythroid progenitors that
20 express GATA-1 at distinct levels, *Blood*. **102**, 3575-83.
- 21 71. Gutierrez, L., Lindeboom, F., Ferreira, R., Drissen, R., Grosveld, F., Whyatt, D. & Philipsen,
22 S. (2005) A hanging drop culture method to study terminal erythroid differentiation, *Exp*
23 *Hematol*. **33**, 1083-91.
- 24 72. Khajuria, R. K., Munschauer, M., Ulirsch, J. C., Fiorini, C., Ludwig, L. S., McFarland, S. K.,
25 Abdulhay, N. J., Specht, H., Keshishian, H., Mani, D. R., Jovanovic, M., Ellis, S. R., Fulco, C. P.,
26 Engreitz, J. M., Schutz, S., Lian, J., Gripp, K. W., Weinberg, O. K., Pinkus, G. S., Gehrke, L.,
27 Regev, A., Lander, E. S., Gazda, H. T., Lee, W. Y., Panse, V. G., Carr, S. A. & Sankaran, V. G.
28 (2018) Ribosome Levels Selectively Regulate Translation and Lineage Commitment in Human
29 Hematopoiesis, *Cell*. **173**, 90-103 e19.
- 30 73. Pali, C. G., Cheng, Q., Gillespie, M. A., Shannon, P., Mazurczyk, M., Napolitani, G., Price,
31 N. D., Ranish, J. A., Morrissey, E., Higgs, D. R. & Brand, M. (2019) Single-Cell Proteomics
32 Reveal that Quantitative Changes in Co-expressed Lineage-Specific Transcription Factors
33 Determine Cell Fate, *Cell Stem Cell*. **24**, 812-820 e5.
- 34 74. De Maria, R., Zeuner, A., Eramo, A., Domenichelli, C., Bonci, D., Grignani, F., Srinivasula,
35 S. M., Alnemri, E. S., Testa, U. & Peschle, C. (1999) Negative regulation of erythropoiesis by
36 caspase-mediated cleavage of GATA-1, *Nature*. **401**, 489-93.
- 37 75. Ribeil, J. A., Zermati, Y., Vandekerckhove, J., Cathelin, S., Kersual, J., Dussiot, M., Coulon,
38 S., Moura, I. C., Zeuner, A., Kirkegaard-Sorensen, T., Varet, B., Solary, E., Garrido, C. &
39 Hermine, O. (2007) Hsp70 regulates erythropoiesis by preventing caspase-3-mediated
40 cleavage of GATA-1, *Nature*. **445**, 102-5.
- 41 76. Ludwig, L. S., Gazda, H. T., Eng, J. C., Eichhorn, S. W., Thiru, P., Ghazvinian, R., George,
42 T. I., Gotlib, J. R., Beggs, A. H., Sieff, C. A., Lodish, H. F., Lander, E. S. & Sankaran, V. G.
43 (2014) Altered translation of GATA1 in Diamond-Blackfan anemia, *Nat Med*. **20**, 748-53.
- 44 77. Crispino, J. D. (2005) GATA1 in normal and malignant hematopoiesis, *Semin Cell Dev Biol*.
45 **16**, 137-47.
- 46 78. Martin, D. I. & Orkin, S. H. (1990) Transcriptional activation and DNA binding by the
47 erythroid factor GF-1/NF-E1/Eryf 1, *Genes Dev*. **4**, 1886-98.
- 48 79. Chennupati, V., Veiga, D. F., Maslowski, K. M., Andina, N., Tardivel, A., Yu, E. C.,
49 Stilianovic, M., Simillion, C., Duchosal, M. A., Quadroni, M., Roberts, I., Sankaran, V. G.,
50 MacDonald, H. R., Fasel, N., Angelillo-Scherrer, A., Schneider, P., Hoang, T. & Allam, R. (2018)
51 Ribonuclease inhibitor 1 regulates erythropoiesis by controlling GATA1 translation, *J Clin Invest*.
52 **128**, 1597-1614.
- 53 80. Chan, J., Hu, X., Wang, C. & Xu, Q. (2018) miRNA-152 targets GATA1 to regulate
54 erythropoiesis in *Chionodraco hamatus*, *Biochem Biophys Res Commun*. **501**, 711-717.
- 55 81. Crossley, M. & Orkin, S. H. (1994) Phosphorylation of the erythroid transcription factor
56 GATA-1, *J Biol Chem*. **269**, 16589-96.
- 57 82. Hernandez-Hernandez, A., Ray, P., Litos, G., Ciro, M., Ottolenghi, S., Beug, H. & Boyes, J.
58 (2006) Acetylation and MAPK phosphorylation cooperate to regulate the degradation of active
59 GATA-1, *EMBO J*. **25**, 3264-74.
- 60 83. Yu, Y. L., Chiang, Y. J., Chen, Y. C., Papetti, M., Juo, C. G., Skoultchi, A. I. & Yen, J. J.
(2005) MAPK-mediated phosphorylation of GATA-1 promotes Bcl-XL expression and cell
survival, *J Biol Chem*. **280**, 29533-42.

84. Partington, G. A. & Patient, R. K. (1999) Phosphorylation of GATA-1 increases its DNA-binding affinity and is correlated with induction of human K562 erythroleukaemia cells, *Nucleic Acids Res.* **27**, 1168-75.
85. Omichinski, J. G., Trainor, C., Evans, T., Gronenborn, A. M., Clore, G. M. & Felsenfeld, G. (1993) A small single-"finger" peptide from the erythroid transcription factor GATA-1 binds specifically to DNA as a zinc or iron complex, *Proc Natl Acad Sci U S A.* **90**, 1676-80.
86. Towatari, M., Ciro, M., Ottolenghi, S., Tsuzuki, S. & Enver, T. (2004) Involvement of mitogen-activated protein kinase in the cytokine-regulated phosphorylation of transcription factor GATA-1, *Hematol J.* **5**, 262-72.
87. Rooke, H. M. & Orkin, S. H. (2006) Phosphorylation of Gata1 at serine residues 72, 142, and 310 is not essential for hematopoiesis in vivo, *Blood.* **107**, 3527-30.
88. Boyes, J., Byfield, P., Nakatani, Y. & Ogryzko, V. (1998) Regulation of activity of the transcription factor GATA-1 by acetylation, *Nature.* **396**, 594-8.
89. Boyes, J., Omichinski, J., Clark, D., Pikaart, M. & Felsenfeld, G. (1998) Perturbation of nucleosome structure by the erythroid transcription factor GATA-1, *J Mol Biol.* **279**, 529-44.
90. Lamonica, J. M., Vakoc, C. R. & Blobel, G. A. (2006) Acetylation of GATA-1 is required for chromatin occupancy, *Blood.* **108**, 3736-8.
91. Lamonica, J. M., Deng, W., Kadauke, S., Campbell, A. E., Gamsjaeger, R., Wang, H., Cheng, Y., Billin, A. N., Hardison, R. C., Mackay, J. P. & Blobel, G. A. (2011) Bromodomain protein Brd3 associates with acetylated GATA1 to promote its chromatin occupancy at erythroid target genes, *Proc Natl Acad Sci U S A.* **108**, E159-68.
92. de Thonel, A., Vandekerckhove, J., Lanneau, D., Selvakumar, S., Courtois, G., Hazoume, A., Brunet, M., Maurel, S., Hammann, A., Ribeil, J. A., Zermati, Y., Gabet, A. S., Boyes, J., Solary, E., Hermine, O. & Garrido, C. (2010) HSP27 controls GATA-1 protein level during erythroid cell differentiation, *Blood.* **116**, 85-96.
93. Lin, K. R., Li, C. L., Yen, J. J. & Yang-Yen, H. F. (2013) Constitutive phosphorylation of GATA-1 at serine(2)(6) attenuates the colony-forming activity of erythrocyte-committed progenitors, *PLoS One.* **8**, e64269.
94. Liang, L., Peng, Y., Zhang, J., Zhang, Y., Roy, M., Han, X., Xiao, X., Sun, S., Liu, H., Nie, L., Kuang, Y., Zhu, Z., Deng, J., Xia, Y., Sankaran, V. G., Hillyer, C. D., Mohandas, N., Ye, M., An, X. & Liu, J. (2019) Deubiquitylase USP7 regulates human terminal erythroid differentiation by stabilizing GATA1, *Haematologica.*
95. Collavin, L., Gostissa, M., Avolio, F., Secco, P., Ronchi, A., Santoro, C. & Del Sal, G. (2004) Modification of the erythroid transcription factor GATA-1 by SUMO-1, *Proc Natl Acad Sci U S A.* **101**, 8870-5.
96. Lee, H. Y., Johnson, K. D., Fujiwara, T., Boyer, M. E., Kim, S. I. & Bresnick, E. H. (2009) Controlling hematopoiesis through sumoylation-dependent regulation of a GATA factor, *Mol Cell.* **36**, 984-95.
97. Yu, L., Ji, W., Zhang, H., Renda, M. J., He, Y., Lin, S., Cheng, E. C., Chen, H., Krause, D. S. & Min, W. (2010) SENP1-mediated GATA1 deSUMOylation is critical for definitive erythropoiesis, *J Exp Med.* **207**, 1183-95.
98. Han, X., Zhang, J., Peng, Y., Peng, M., Chen, X., Chen, H., Song, J., Hu, X., Ye, M., Li, J., Sankaran, V. G., Hillyer, C. D., Mohandas, N., An, X. & Liu, J. (2017) Unexpected role for p19INK4d in posttranscriptional regulation of GATA1 and modulation of human terminal erythropoiesis, *Blood.* **129**, 226-237.
99. Tyrkalska, S. D., Perez-Oliva, A. B., Rodriguez-Ruiz, L., Martinez-Morcillo, F. J., Alcaraz-Perez, F., Martinez-Navarro, F. J., Lachaud, C., Ahmed, N., Schroeder, T., Pardo-Sanchez, I., Candel, S., Lopez-Munoz, A., Choudhuri, A., Rossmann, M. P., Zon, L. I., Cayuela, M. L., Garcia-Moreno, D. & Mulero, V. (2019) Inflammasome Regulates Hematopoiesis through Cleavage of the Master Erythroid Transcription Factor GATA1, *Immunity.* **51**, 50-63 e5.
100. Whyatt, D., Lindeboom, F., Karis, A., Ferreira, R., Milot, E., Hendriks, R., de Bruijn, M., Langeveld, A., Gribnau, J., Grosveld, F. & Philipsen, S. (2000) An intrinsic but cell-nonautonomous defect in GATA-1-overexpressing mouse erythroid cells, *Nature.* **406**, 519-24.
101. Johnson, K. D., Kim, S. I. & Bresnick, E. H. (2006) Differential sensitivities of transcription factor target genes underlie cell type-specific gene expression profiles, *Proc Natl Acad Sci U S A.* **103**, 15939-44.
102. Takahashi, S., Onodera, K., Motohashi, H., Suwabe, N., Hayashi, N., Yanai, N., Nabesima, Y. & Yamamoto, M. (1997) Arrest in primitive erythroid cell development caused by promoter-specific disruption of the GATA-1 gene, *J Biol Chem.* **272**, 12611-5.

- 1
2
3 103. McDevitt, M. A., Shivdasani, R. A., Fujiwara, Y., Yang, H. & Orkin, S. H. (1997) A
4 "knockdown" mutation created by cis-element gene targeting reveals the dependence of
5 erythroid cell maturation on the level of transcription factor GATA-1, *Proc Natl Acad Sci U S A.*
6 **94**, 6781-5.
- 7 104. Pan, X., Ohneda, O., Ohneda, K., Lindeboom, F., Iwata, F., Shimizu, R., Nagano, M.,
8 Suwabe, N., Philipsen, S., Lim, K. C., Engel, J. D. & Yamamoto, M. (2005) Graded levels of
9 GATA-1 expression modulate survival, proliferation, and differentiation of erythroid progenitors,
10 *J Biol Chem.* **280**, 22385-94.
- 11 105. Shimizu, R., Kuroha, T., Ohneda, O., Pan, X., Ohneda, K., Takahashi, S., Philipsen, S. &
12 Yamamoto, M. (2004) Leukemogenesis caused by incapacitated GATA-1 function, *Mol Cell*
13 *Biol.* **24**, 10814-25.
- 14 106. Shimizu, R. & Yamamoto, M. (2012) Contribution of GATA1 dysfunction to multi-step
15 leukemogenesis, *Cancer Sci.* **103**, 2039-44.
- 16 107. Shimizu, R., Engel, J. D. & Yamamoto, M. (2008) GATA1-related leukaemias, *Nat Rev*
17 *Cancer.* **8**, 279-87.
- 18 108. Ling, T., Crispino, J. D., Zingariello, M., Martelli, F. & Migliaccio, A. R. (2018) GATA1
19 insufficiencies in primary myelofibrosis and other hematopoietic disorders: consequences for
20 therapy, *Expert Rev Hematol.* **11**, 169-184.
- 21 109. Vannucchi, A. M., Bianchi, L., Cellai, C., Paoletti, F., Rana, R. A., Lorenzini, R., Migliaccio,
22 G. & Migliaccio, A. R. (2002) Development of myelofibrosis in mice genetically impaired for
23 GATA-1 expression (GATA-1(low) mice), *Blood.* **100**, 1123-32.
- 24 110. Migliaccio, A. R., Rana, R. A., Sanchez, M., Lorenzini, R., Centurione, L., Bianchi, L.,
25 Vannucchi, A. M., Migliaccio, G. & Orkin, S. H. (2003) GATA-1 as a regulator of mast cell
26 differentiation revealed by the phenotype of the GATA-1low mouse mutant, *J Exp Med.* **197**,
27 281-96.
- 28 111. Socolovsky, M. (2013) Exploring the erythroblastic island, *Nat Med.* **19**, 399-401.
- 29 112. Yeo, J. H., Lam, Y. W. & Fraser, S. T. (2019) Cellular dynamics of mammalian red blood
30 cell production in the erythroblastic island niche, *Biophys Rev.*
- 31 113. McGrath, K. E., Kingsley, P. D., Koniski, A. D., Porter, R. L., Bushnell, T. P. & Palis, J.
32 (2008) Eucleation of primitive erythroid cells generates a transient population of "pyrenocytes"
33 in the mammalian fetus, *Blood.* **111**, 2409-17.
- 34 114. Mohandas, N. & Chasis, J. A. (2010) The erythroid niche: molecular processes occurring
35 within erythroblastic islands, *Transfus Clin Biol.* **17**, 110-1.
- 36 115. Chasis, J. A. & Mohandas, N. (2008) Erythroblastic islands: niches for erythropoiesis,
37 *Blood.* **112**, 470-8.
- 38 116. Gutierrez, L., Lindeboom, F., Langeveld, A., Grosveld, F., Philipsen, S. & Whyatt, D.
39 (2004) Homotypic signalling regulates Gata1 activity in the erythroblastic island, *Development.*
40 **131**, 3183-93.
- 41 117. Graf, T. (2002) Differentiation plasticity of hematopoietic cells, *Blood.* **99**, 3089-101.
- 42 118. Iwasaki, H., Mizuno, S., Wells, R. A., Cantor, A. B., Watanabe, S. & Akashi, K. (2003)
43 GATA-1 converts lymphoid and myelomonocytic progenitors into the megakaryocyte/erythrocyte
44 lineages, *Immunity.* **19**, 451-62.
- 45 119. Kulesa, H., Frampton, J. & Graf, T. (1995) GATA-1 reprograms avian myelomonocytic
46 cell lines into eosinophils, thromboblats, and erythroblasts, *Genes Dev.* **9**, 1250-62.
- 47 120. Hirasawa, R., Shimizu, R., Takahashi, S., Osawa, M., Takayanagi, S., Kato, Y., Onodera,
48 M., Minegishi, N., Yamamoto, M., Fukao, K., Taniguchi, H., Nakauchi, H. & Iwama, A. (2002)
49 Essential and instructive roles of GATA factors in eosinophil development, *J Exp Med.* **195**,
50 1379-86.
- 51 121. Valent, P., Busche, G., Theurl, I., Uras, I. Z., Germing, U., Stauder, R., Sotlar, K., Fureder,
52 W., Bettelheim, P., Pfeilstocker, M., Oberbauer, R., Sperr, W. R., Geissler, K., Schwaller, J.,
53 Moriggl, R., Bene, M. C., Jager, U., Horny, H. P. & Hermine, O. (2018) Normal and pathological
54 erythropoiesis in adults: from gene regulation to targeted treatment concepts, *Haematologica.*
55 **103**, 1593-1603.
- 56 122. Barbarani, G., Fugazza, C., Strouboulis, J. & Ronchi, A. E. (2019) The Pleiotropic Effects
57 of GATA1 and KLF1 in Physiological Erythropoiesis and in Dyserythropoietic Disorders, *Front*
58 *Physiol.* **10**, 91.
- 59 123. Crispino, J. D. & Horwitz, M. S. (2017) GATA factor mutations in hematologic disease,
60 *Blood.* **129**, 2103-2110.
124. Frisan, E., Vandekerckhove, J., de Thonel, A., Pierre-Eugene, C., Sternberg, A., Arlet, J.
B., Floquet, C., Gyan, E., Kosmider, O., Dreyfus, F., Gabet, A. S., Courtois, G., Vyas, P., Ribeil,

- J. A., Zermati, Y., Lacombe, C., Mayeux, P., Solary, E., Garrido, C., Hermine, O. & Fontenay, M. (2012) Defective nuclear localization of Hsp70 is associated with dyserythropoiesis and GATA-1 cleavage in myelodysplastic syndromes, *Blood*. **119**, 1532-1542.
125. Ellis, S. R. & Lipton, J. M. (2008) Diamond Blackfan anemia: a disorder of red blood cell development, *Curr Top Dev Biol*. **82**, 217-41.
126. Ulirsch, J. C., Verboon, J. M., Kazerounian, S., Guo, M. H., Yuan, D., Ludwig, L. S., Handsaker, R. E., Abdulhay, N. J., Fiorini, C., Genovese, G., Lim, E. T., Cheng, A., Cummings, B. B., Chao, K. R., Beggs, A. H., Genetti, C. A., Sieff, C. A., Newburger, P. E., Niewiadomska, E., Matysiak, M., Vlachos, A., Lipton, J. M., Atsidaftos, E., Glader, B., Narla, A., Gleizes, P. E., O'Donohue, M. F., Montel-Lehry, N., Amor, D. J., McCarroll, S. A., O'Donnell-Luria, A. H., Gupta, N., Gabriel, S. B., MacArthur, D. G., Lander, E. S., Lek, M., Da Costa, L., Nathan, D. G., Korostelev, A. A., Do, R., Sankaran, V. G. & Gazda, H. T. (2018) The Genetic Landscape of Diamond-Blackfan Anemia, *Am J Hum Genet*. **103**, 930-947.
127. Boria, I., Garelli, E., Gazda, H. T., Aspesi, A., Quarello, P., Pavesi, E., Ferrante, D., Meerpohl, J. J., Kartal, M., Da Costa, L., Proust, A., Leblanc, T., Simansour, M., Dahl, N., Frojmark, A. S., Pospisilova, D., Cmejla, R., Beggs, A. H., Sheen, M. R., Landowski, M., Buros, C. M., Clinton, C. M., Dobson, L. J., Vlachos, A., Atsidaftos, E., Lipton, J. M., Ellis, S. R., Ramenghi, U. & Dianzani, I. (2010) The ribosomal basis of Diamond-Blackfan Anemia: mutation and database update, *Hum Mutat*. **31**, 1269-79.
128. Sankaran, V. G., Ghazvinian, R., Do, R., Thiru, P., Vergilio, J. A., Beggs, A. H., Sieff, C. A., Orkin, S. H., Nathan, D. G., Lander, E. S. & Gazda, H. T. (2012) Exome sequencing identifies GATA1 mutations resulting in Diamond-Blackfan anemia, *J Clin Invest*. **122**, 2439-43.
129. Klar, J., Khalfallah, A., Arzoo, P. S., Gazda, H. T. & Dahl, N. (2014) Recurrent GATA1 mutations in Diamond-Blackfan anaemia, *Br J Haematol*. **166**, 949-51.
130. Parrella, S., Aspesi, A., Quarello, P., Garelli, E., Pavesi, E., Carando, A., Nardi, M., Ellis, S. R., Ramenghi, U. & Dianzani, I. (2014) Loss of GATA-1 full length as a cause of Diamond-Blackfan anemia phenotype, *Pediatr Blood Cancer*. **61**, 1319-21.
131. Chlon, T. M., McNulty, M., Goldenson, B., Rosinski, A. & Crispino, J. D. (2015) Global transcriptome and chromatin occupancy analysis reveal the short isoform of GATA1 is deficient for erythroid specification and gene expression, *Haematologica*. **100**, 575-84.
132. Hollanda, L. M., Lima, C. S., Cunha, A. F., Albuquerque, D. M., Vassallo, J., Ozelo, M. C., Joazeiro, P. P., Saad, S. T. & Costa, F. F. (2006) An inherited mutation leading to production of only the short isoform of GATA-1 is associated with impaired erythropoiesis, *Nat Genet*. **38**, 807-12.
133. Abdulhay, N. J., Fiorini, C., Verboon, J. M., Ludwig, L. S., Ulirsch, J. C., Zieger, B., Lareau, C. A., Mi, X., Roy, A., Obeng, E. A., Erlacher, M., Gupta, N., Gabriel, S. B., Ebert, B. L., Niemeyer, C. M., Khoriaty, R. N., Ancliff, P., Gazda, H. T., Wlodarski, M. W. & Sankaran, V. G. (2019) Impaired human hematopoiesis due to a cryptic intronic GATA1 splicing mutation, *J Exp Med*. **216**, 1050-1060.
134. Gilles, L., Arslan, A. D., Marinaccio, C., Wen, Q. J., Arya, P., McNulty, M., Yang, Q., Zhao, J. C., Konstantinoff, K., Lasho, T., Pardananani, A., Stein, B., Plo, I., Sundaravel, S., Wickrema, A., Migliaccio, A., Gurbuxani, S., Vainchenker, W., Plataniias, L. C., Tefferi, A. & Crispino, J. D. (2017) Downregulation of GATA1 drives impaired hematopoiesis in primary myelofibrosis, *J Clin Invest*. **127**, 1316-1320.
135. Doty, R. T., Yan, X., Lausted, C., Munday, A. D., Yang, Z., Yi, D., Jabbari, N., Liu, L., Keel, S. B., Tian, Q. & Abkowitz, J. L. (2019) Single-cell analyses demonstrate that a heme-GATA1 feedback loop regulates red cell differentiation, *Blood*. **133**, 457-469.
136. Gastou, M., Rio, S., Dussiot, M., Karboul, N., Moniz, H., Leblanc, T., Sevin, M., Gonin, P., Larghero, J., Garrido, C., Narla, A., Mohandas, N., Vainchenker, W., Hermine, O., Solary, E., Da Costa, L., French Society of, H., French Society of, I. & Hematology (2017) The severe phenotype of Diamond-Blackfan anemia is modulated by heat shock protein 70, *Blood Adv*. **1**, 1959-1976.
137. Rio, S., Gastou, M., Karboul, N., Derman, R., Suriyun, T., Manceau, H., Leblanc, T., El Benna, J., Schmitt, C., Azouzi, S., Larghero, J., Karim, Z., Macias-Garcia, A., Chen, J. J., Hermine, O., Courtois, G., Puy, H., Gouya, L., Mohandas, N. & Da Costa, L. (2019) Regulation of globin-heme balance in Diamond-Blackfan anemia by HSP70/GATA1, *Blood*. **133**, 1358-1370.
138. Arlet, J. B., Ribeil, J. A., Guillem, F., Negre, O., Hazoume, A., Marcion, G., Beuzard, Y., Dussiot, M., Moura, I. C., Demarest, S., de Beauchene, I. C., Belaid-Choucair, Z., Sevin, M., Maciel, T. T., Auclair, C., Leboulch, P., Chretien, S., Tchertanov, L., Baudin-Creuz, V.,

- 1
2
3 Seigneuric, R., Fontenay, M., Garrido, C., Hermine, O. & Courtois, G. (2014) HSP70
4 sequestration by free alpha-globin promotes ineffective erythropoiesis in beta-thalassaemia,
5 *Nature*. **514**, 242-6.
- 6 139. Bhatnagar, N., Nizery, L., Tunstall, O., Vyas, P. & Roberts, I. (2016) Transient Abnormal
7 Myelopoiesis and AML in Down Syndrome: an Update, *Curr Hematol Malig Rep*. **11**, 333-41.
- 8 140. Maroz, A., Stachorski, L., Emmrich, S., Reinhardt, K., Xu, J., Shao, Z., Kabler, S.,
9 Dertmann, T., Hitzler, J., Roberts, I., Vyas, P., Juban, G., Hennig, C., Hansen, G., Li, Z., Orkin,
10 S., Reinhardt, D. & Klusmann, J. H. (2014) GATA1s induces hyperproliferation of eosinophil
11 precursors in Down syndrome transient leukemia, *Leukemia*. **28**, 1259-70.
- 12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

Figure Legends:

Figure 1: GATA1 protein levels during murine erythroid differentiation

Intracellular flow cytometry analysis of GATA1 protein expression in mouse fetal and adult tissues. In brief, one million cells were stained with Ter119-PE antibody (fetal tissues) or a cocktail of KIT-PECy7/CD71-APC/Ter119-PE (adult tissues), and subsequently fixed and permeabilized using BD Cytofix/Cytoperm kit (BD Biosciences), after which they were incubated with anti-GATA1-N6 antibody (1:100) followed by anti-rat-FITC antibody (1:100) with a wash step after each incubation.

A) GATA1 protein levels were assessed using intracellular flow cytometry in murine fetal liver and fetal blood at E13.5. Representative data shown. In the top row, dot plots are depicted of GATA1 levels (MFI) against the Forward Scatter in fetal liver and fetal blood samples of the same mouse. In the mid row, dotplots are depicted of GATA1 levels (MFI) against Ter119 surface expression, including the Forward Scatter variable as a Heatmap Statistic colorcode in fetal liver and fetal blood samples of the same mouse. The plots on the right depict the overlay of respective fetal liver (red) and fetal blood (blue) samples on both rows. The bottom row is a composite figure, where representative pictures from cytopins of erythroid differentiating cells *in vitro* in hanging drop assays [71] and fetal blood nucleated and enucleated erythroid cells [116], are overlaid on the mid-row plots, as an indication of how differentiation takes place, with the remark that these cells do not correspond to prospectively isolated cells from the same samples.

B) GATA1 protein levels were assessed using intracellular flow cytometry in murine bone marrow cells. The graph represents GATA1 expression levels (GeoMFI) in successive stages of erythroid maturation based on surface marker expression, in 5 mice. The populations are from immature to mature, as follows: KIT⁺CD71⁻Ter119⁻ (which includes other hematopoietic progenitors than erythroid), KIT⁺CD71⁺Ter119⁻, KIT⁻CD71⁺Ter119⁺, KIT⁻CD71⁻Ter119⁺. The picture of maturing erythroid cells, is a composite of differentiating cells in hanging drop assays *in vitro* [71], and is used to exemplify how

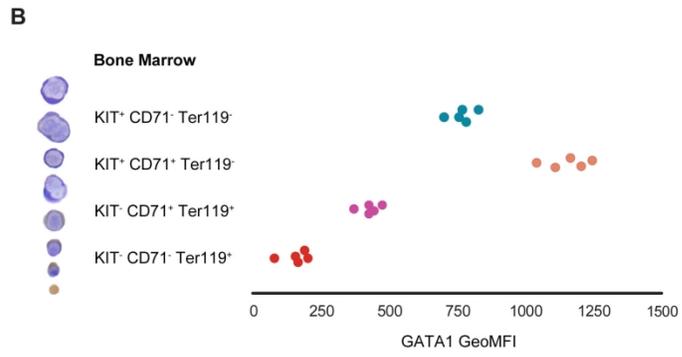
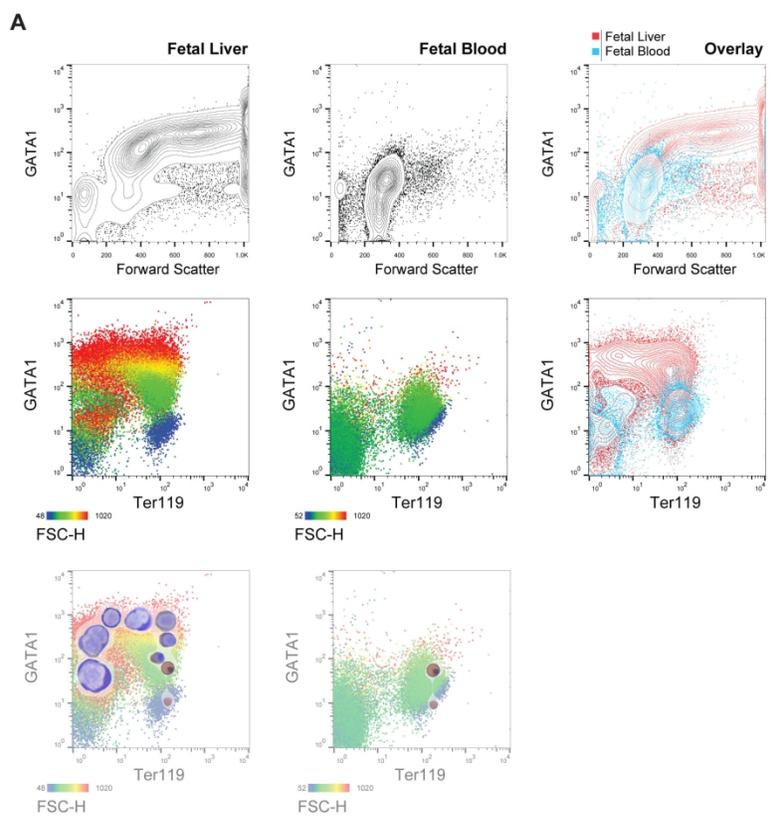
1
2
3 erythroid differentiation takes place, from top to bottom, following the order of gated
4
5 populations in the bone marrow.
6
7
8

9 **Figure 2: Model of GATA1 protein level regulation during erythroid maturation**

10
11 Summary of the different mechanisms regulating GATA1 protein abundance, which lead
12
13 to disease when deregulated.
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

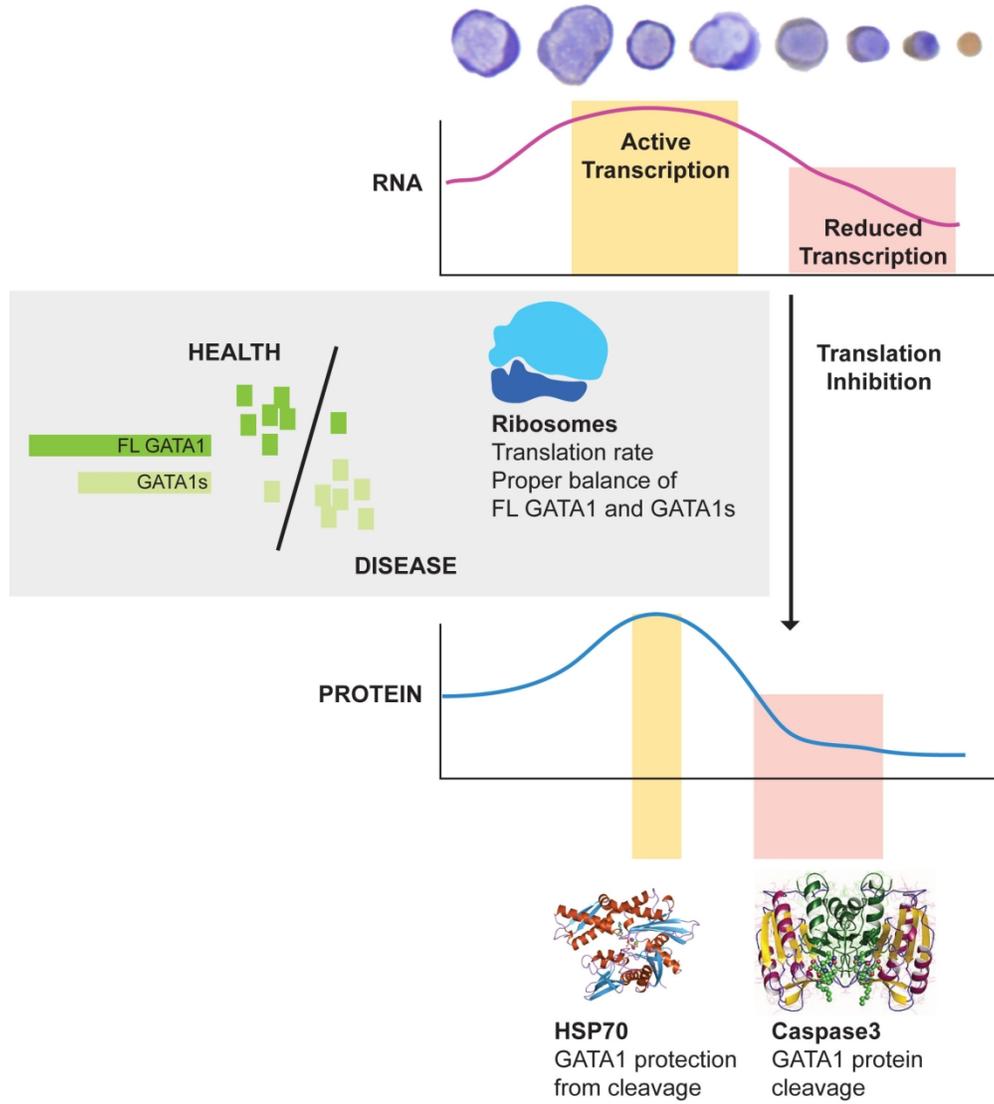
For Peer Review Only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



GATA1 protein levels during murine erythroid differentiation

176x261mm (300 x 300 DPI)



Model of GATA1 protein level regulation during erythroid maturation

179x199mm (300 x 300 DPI)

Regulation of GATA1 levels in erythropoiesis

Laura Gutiérrez^{1,2*}, Noemí Caballero¹, Luis Fernández-Calleja¹, Elena Karkoulia³, John Strouboulis^{4*}

1 Platelet Research Lab, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain

2 Dept. of Medicine, Universidad de Oviedo, Spain

3 Institute of Molecular Biology and Biotechnology, Foundation of Research & Technology Hellas, Heraklion, Crete, Greece

4 Cancer Comprehensive Center, School of Cancer and Pharmaceutical Sciences, King's College London, United Kingdom

** Corresponding authors: gutierrezglaura@uniovi.es, john.strouboulis@kcl.ac.uk*

Abstract

GATA1 is considered as the “master” transcription factor in erythropoiesis. It regulates at the transcriptional level all aspects of erythroid maturation and function, as revealed by gene knockout studies in mice and by genome-wide occupancies in erythroid cells. The GATA1 protein contains two zinc finger domains and an N-terminal activation domain. *GATA1* translation results in the production of the full-length protein and of a shorter variant (GATA1s) lacking the N-terminal activation domain, which is functionally deficient in supporting erythropoiesis. GATA1 protein abundance is highly regulated in erythroid cells at different levels, including transcription, mRNA translation, posttranslational modifications and protein degradation, in a differentiation-stage specific manner. Maintaining high GATA1 protein levels is essential in the early stages of erythroid maturation, whereas downregulating GATA1 protein levels is a necessary step in terminal erythroid differentiation. The importance of maintaining proper GATA1 protein homeostasis in erythropoiesis is demonstrated by the fact that both GATA1 loss and its overexpression, result in lethal anemia. Importantly, alterations in any of those GATA1 regulatory checkpoints has been recognized as an important cause of hematological disorders such as dyserythropoiesis (with or without thrombocytopenia), β -thalassemia, Diamond Blackfan anemia, myelodysplasia or leukemia. In this review, we provide an overview of the multi-level regulation of GATA1 protein homeostasis in erythropoiesis and of its deregulation in hematological disease.

Introduction

Cell lineage specification and differentiation is a complex process that must be tightly regulated through cell-type specific transcriptional programs. These programs are regulated by master transcription factors which play essential roles in any given cell lineage to successfully commit and differentiate with the appropriate temporal and spatial coordinates and at the exact demanded rate, thus assuring homeostatic balance in the organismal tissue composition [1]. Hematopoiesis has long served as an excellent model system for studying the molecular principles governing transcription factor functions in regulating cell lineage commitment and differentiation. The diversity of mature blood cells derives from a common progenitor, the hematopoietic stem cell (HSC), which undergoes a series of commitment and differentiation steps in response to specific stimuli in specialized tissue microenvironments (e.g. bone marrow, thymus etc.), to generate not only the circulating blood cells (white blood cells, red blood cells and platelets), but also the immune and non-immune organ resident immune cells [2-4]. Amongst the circulating blood cells, the highly specialized, oxygen-carrying, enucleated erythroid (red blood) cells must be produced daily at high rates, according to a very specialized and well characterized cell differentiation program, which includes progressive size reduction, cell cycle arrest, massive synthesis of globin chains and enucleation [5-7]. In this respect, GATA1 has been acknowledged as the master transcriptional regulator of erythropoiesis, controlling all aspects of differentiation, survival and cell cycle arrest [8, 9]. Maintaining GATA1 protein homeostasis has been shown to be a highly regulated process in erythropoiesis, with deregulation being a major factor in ineffective erythropoiesis or leukemic transformation in hematological disease. In this review we comprehensively discuss GATA1 functions in erythroid differentiation and, further, we focus on the regulation of GATA1 abundance in physiological and pathological erythropoiesis.

GATA Transcription Factors

GATA1 belongs to the GATA family of transcription factors which comprises six members: GATA1 to GATA6. The GATA family of transcription factors plays an important role in the regulation of several biological processes, such as embryonic development, cell growth, cell differentiation and tissue morphogenesis [10]. All members of the GATA family of transcription factors recognize and bind to a specific DNA motif, (A/T)GATA(A/G) (WGATAR), through two highly conserved Cys4-type zinc finger regions. This DNA consensus sequence is evolutionarily conserved in fungi, plants, invertebrates and vertebrates, such as zebrafish, *Xenopus*, chicken and mammals, further supporting the notion that GATA family members play important roles in the regulation of crucial cellular processes [11]. In mammals, GATA1, GATA2 and GATA3

1
2
3 have important roles primarily, but not exclusively, within the hematopoietic system [12,
4 13] and GATA4, GATA5, and GATA6 drive the differentiation of mesoderm and
5 endoderm derived tissues, thus regulating diverse developmental processes within the
6 cardiac, gastrointestinal, endocrine and gonadal systems [10]. The binding of GATA
7 transcription factors to the WGATAR sequence motif depends highly on the C-terminal
8 zinc finger (C-ZnF), whilst the N-terminal zinc finger (N-ZnF) domain may bind to sites
9 containing additional canonical, non-canonical (GATC), or palindromic GATA motifs, or
10 may strengthen binding through interaction with other surrounding DNA motifs, mainly in
11 partnership with other transcriptional co-factors [10, 14]. GATA transcription factor
12 tissue/lineage specificity is afforded by the transactivation domains surrounding the zinc
13 finger domains, the homology of which is weaker amongst the members of the GATA
14 transcription factor family [9]. The global sequence landscape at each specific locus will
15 determine the transcriptional activity and the higher order transcription factor complexes
16 involved in its regulation. GATA1, the founding member of the GATA family of
17 transcription factors, was initially shown to be essential for the erythroid/megakaryocytic
18 lineages and subsequently in eosinophils/basophils, mast cells and in dendritic cells [15-
19 19]. However, it is the study of GATA1 in erythropoiesis that has provided the most
20 valuable insight into GATA1 functions and regulation.
21
22
23
24
25
26
27
28
29
30
31
32

33 **GATA1 functions in erythropoiesis**

34 GATA1 is considered the “master” transcription factor of erythropoiesis as it
35 transcriptionally regulates all aspects of erythroid maturation and function [9]. The
36 GATA1 protein has three main functional domains: a N-terminal activation domain (N-
37 TAD) and two homologous ZnF domains in the C-terminal half of the protein. The C-ZnF
38 binds to DNA and the N-ZnF binds to the GATA1 main cofactor **Friend of GATA1 (FOG-1)**
39 and modulates the affinity of GATA1 for binding to complex and palindromic sites *in vitro*.
40 Translation of the *GATA1* mRNA produces two isoforms: the full-length protein and a
41 shorter variant (GATA1s), translated from codon 84 within the third exon. GATA1s lacks
42 the N-TAD and results in a protein with aberrant functional properties [20, 21]. The *Gata1*
43 gene knockout in the mouse results in embryonic lethality at around day E10.5-E11.5
44 due to severe anemia, with *Gata1*-null cells undergoing apoptosis at the proerythroblastic
45 stage [22-24]. The conditional erythroid knockout in adult mice causes aplastic anemia,
46 revealing its essential role in both steady-state and stress erythropoiesis [25].
47
48
49
50
51
52
53
54
55
56

57 Genome-wide occupancy studies (ChIPseq) have confirmed that all known erythroid
58 genes are regulated by GATA1 [26-29]. **These have confirmed previously known GATA1**
59 **functions in regulating globin expression, heme biosynthesis and red cell membrane**
60

1
2
3 genes and further revealed new GATA1 functions in transcriptionally regulating
4 processes essential for erythroid maturation such as autophagy and exosome
5 suppression. For example, it was shown by Bresnick and colleagues that GATA1
6 activates genes involved in autophagy and in lysosomal functions, the former being an
7 essential process for eliminating organelles through lysosomal clearance during
8 erythroid cell maturation [30]. GATA1 was also shown to repress the exosome complex,
9 an RNA-degrading and processing machine that promotes early erythroblast
10 proliferation, thus allowing maturation of erythroid cells to proceed [31, 32]. In addition,
11 GATA1 is critical for the positive regulation of genes involved in cell cycle regulation,
12 such as *GFI1B* [33], *CDC6* [34, 35] and p21 (*Cdkn1a*) [36]. GATA1 also activates genes
13 important for cell survival, such as Bcl-2 (*Bcl2*) and Bcl-xL (*Bcl2l1*) [37, 38]. Furthermore,
14 GATA1 has also been described as a regulator of restrictive lineage specification through
15 the repression of myeloid genes in the erythroid lineage. Repressed targets of GATA1
16 include *Gata2*, the *Myb* and *Myc* oncogenes and PU.1 (*Spi1*) [39]. Beyond gene
17 expression regulation, GATA1 regulates the transcription of a number of miRNAs, which
18 serve to fine-tune the cellular gene/proteome footprint. Specifically, GATA1 regulates the
19 expression of clusters miR-27a/24 and miR144/451, which have been shown to
20 modulate erythropoiesis, as their deletion causes erythroid-specific defects and
21 deregulation of a plethora of erythroid-relevant lineage specification/differentiation genes
22 [40-42]. Another important feature of GATA1 in the erythroid lineage differentiation, is its
23 role as a chromatin bookmark as it has been shown that GATA1 binding to certain gene
24 loci is preserved through mitosis, assuring the immediate expression of a given set of
25 genes after resumption of cell cycling [43].

26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41 GATA1 transcription functions in erythropoiesis are mediated by the formation of
42 activating and repressive transcription complexes, through GATA1 interactions with a
43 number of other transcription factors, co-factors and chromatin remodeling and
44 modification proteins and complexes [8]. The best studied GATA1 interactions are with
45 FOG-1, the SCL/TAL1/E2A/LDB1/LMO2 complex and with PU.1 (SPI1). FOG-1 interacts
46 with GATA1 through the latter's N-ZnF in the erythroid and megakaryocytic lineages,
47 with their interaction being essential for differentiation [44, 45]. Genetic studies in mice
48 and the identification of disease-causing mutations in humans, confirmed the importance
49 *in vivo* of the GATA1/FOG-1 interaction in erythropoiesis [46, 47]. GATA1/FOG-1
50 mediated repression involves interactions with the NuRD (Nucleosome Remodeling and
51 Deacetylating) complex, which is contacted directly by FOG-1 [48, 49]. Importantly,
52 GATA1/FOG-1 interactions have a dual function in the activation of the erythroid-specific
53 program, as well as the repression of alternative hematopoietic transcription programs,
54
55
56
57
58
59
60

1
2
3 often supported in both cases by NuRD [48-50].
4
5

6 Another critical GATA1 transcription complex in erythropoiesis is the so-called
7 pentameric complex with the SCL/TAL1/E2A/LDB1/LMO2 factors which is associated
8 with transcriptional activation [51]. The pentameric complex binds GATA-E-box motifs
9 comprised of a GATA consensus sequence located proximally (7-9 bp) to an E-box
10 CANNTG consensus motif bound by SCL/TAL1 [51]. These motifs occur primarily in
11 erythroid specific genes that become activated by the pentameric complex [52, 53].
12 LMO2 serves as the bridge between SCL/TAL1 and GATA1 [51], whereas LDB1
13 associates with LMO2 and is capable of self-association (dimerization) through its N-
14 terminus domain [54]. This dimerization allows LDB1-mediated long-range DNA looping
15 to activate transcription, as occurs, for example, in the β -globin locus [55, 56]. GATA1
16 and the myeloid transcription factor PU.1 have been reported to physically interact in
17 antagonizing each other's functions by displacing other protein co-factors and/or
18 preventing DNA binding, thus influencing cell lineage commitment decisions and
19 activation of associated transcription programs ([57] and references therein). GATA1
20 also interacts in erythroid cells with other transcription factors such as KLF1, SP1 and
21 GFI1B [58-60], as well as with chromatin remodeling factors such as ACF/WCRF [48],
22 BRG1 [61] and pRB/E2F [62]. Lastly, GATA1 interacts with the CBP/p300 histone
23 acetyltransferase which mediates acetylation of the GATA1 ZnFs, modulating its DNA
24 binding properties and/or protein stability (see below) [63, 64].
25
26
27
28
29
30
31
32
33
34
35
36
37

38 ***Cis* regulation of GATA1 gene expression**

39 *GATA1* is located on the X chromosome in mouse and human and is comprised of 6
40 exons distributed over 8 Kb [65]. Exon I is noncoding, exon II contains the starting codon
41 for the full length GATA1 protein and exons IV and V code for the two ZnF domains. The
42 *Gata1* gene has been largely studied in mice. It comprises two tissue-specific promoters:
43 the distal (IT) promoter drives expression of the *Gata1* gene in Sertoli cells in the testis,
44 whereas the proximal (IE) promoter located 8kb downstream of the IT promoter and 5'
45 of exon II, directs *Gata1* expression in hematopoietic cells [66]. The transcriptional
46 regulation of *Gata1* in hematopoietic cells is coordinated by *cis*-regulatory regions
47 clustered within three DNase I hypersensitive (HS) sites [67, 68]. HS1 is located between
48 3.9 and 2.6kb upstream of IE; HS2 corresponds to the putative region of the *Gata1* IE
49 promoter and contains a double palindromic GATA site and CACCC boxes and HS3 is
50 located in intron I. The regulated expression of *Gata1* throughout hematopoiesis involves
51 the differential contribution of these *cis*-regulatory elements. Thus, in the megakaryocytic
52 lineage, *Gata1* expression requires the extended (317 bp) HS1 region, whereas for
53
54
55
56
57
58
59
60

1
2
3 erythroid expression only the first 62bp of HS1 are necessary [69, 70]. In eosinophils,
4 *Gata1* expression is independent of the HS1 region [71], although it does require the
5 double GATA motif that is present in the HS2 region [16]. Whereas utilization of the
6 isolated HS sites in gene expression constructs confers normal *Gata1* expression in
7 committed hematopoietic cells, physiological repression of *Gata1* in the HSC
8 compartment requires the interspaced sequences between the HS sites [72, 73].
9
10
11
12
13

14 **GATA1 expression in hematopoiesis**

15 From earlier progenitors towards the terminally mature erythroid cell, *GATA1* expression
16 levels fluctuate. Most of the studies have been performed in murine model systems, while
17 more recent studies tackling this aspect using single-cell approaches have been
18 performed both in mouse and human models. HSCs and common myeloid progenitors
19 (CMP) express very low levels of *Gata1* mRNA ([73] and references therein). *Gata1*
20 expression increases moderately in early erythroid progenitors and burst-forming units-
21 erythroid (BFU-E). High *GATA1* expression levels have been shown in colony-forming
22 units-erythroid (CFU-E): at this stage *Gata1* activates the expression of the erythropoietin
23 receptor (EpoR) and, in turn, EpoR mediated signals enhance the *GATA1* transcriptional
24 program in erythroid progenitor cells, as has been shown *in vitro* [37, 74-76]. After this
25 point, expression levels of *Gata1* gradually decrease from the proerythroblast stage as
26 differentiation proceeds through the basophilic, polychromatic and orthochromatic
27 erythroblast stages [73]. In summary, *GATA1* mRNA is expressed at low levels in early
28 progenitors, its expression levels reach a maximum prior to the proerythroblast stage
29 and gradually declines towards the final maturation of erythroid cells, as has been shown
30 in mice and human model systems [73, 77, 78]. *GATA1* protein levels follow this pattern,
31 and decrease towards terminal maturation, as was shown in mouse erythroid progenitors
32 using a hanging-drop culture method, and comparing the levels of *GATA1* protein in the
33 same number of cells amongst successive stages of differentiation [79]. Studies using
34 intracellular flow cytometry or flow cytometry analysis of bone marrow progenitors of
35 *GATA1*-reporter transgenic mice, clearly supported this notion (Figure 1 and [78]).
36
37
38
39
40
41
42
43
44
45
46
47
48
49

50 Recent studies at the single cell level have also provided renewed insight as to *GATA1*
51 protein expression in human erythropoiesis, from hematopoietic stem and progenitor
52 cells (HSPCs) to terminally differentiated erythroid cells. In one study, *GATA1* protein
53 could be detected at low levels in a subset of HSPCs, gradually increasing as cells
54 differentiate, reaching high levels in lineage committed erythroid cells [80]. Similarly, in
55 another study, *GATA1* protein was clearly detectable at the CMP stage and gradually
56 increased with cell commitment and differentiation through the CFU-E, proerythroblast
57
58
59
60

1
2
3 and basophilic erythroblast and orthochromatic erythroblast stages [81]. Previously, it
4 has been shown that GATA1 protein levels decline at the very last stages of terminal
5 erythroid differentiation [82, 83], in what appears to be a regulated event necessary for
6 terminal erythroid maturation (see below).
7
8
9

12 Regulation of GATA1 mRNA translation

14 The translation of the *GATA1* mRNA in erythroid cells gives rise to the production of two
15 protein isoforms as a result of the utilization of two ATG start codons: one in exon II which
16 gives rise to full length *GATA1* encoding a 413 amino acid protein and a second ATG in
17 exon III which gives rise to a shorter isoform (*GATA1s* [84, 85]) lacking the N terminal-
18 most 83 amino acids, which span a so-called transactivation domain [86]. The *GATA1*
19 mRNA has been described to have an unusual 5' UTR structure, in that it is short and
20 predicted to have a less complex secondary structure [80, 84]. These 5' UTR features
21 are generally shared by mRNAs that are associated with increased translational
22 efficiencies and are particularly susceptible to changes in ribosomal levels, for example,
23 in ribosomopathies [80]. Remarkably, the *GATA1* 5' UTR structure is unique among a
24 number of critical hematopoietic transcription factors which, on average, have longer 5'
25 UTRs with more complex predicted structures [80]. 5' UTR swap experiments between
26 *GATA1* and other hematopoietic transcription factors, such as *GATA2* or *RUNX1*, failed
27 to rescue erythroid differentiation in a functional complementation assay in the *Gata1*-
28 null G1E proerythroblastic cell line [80]. Furthermore, *GATA1* mRNA translation appears
29 to have a greater requirement for eIF-dependent translation initiation [84]. Lastly, recent
30 evidence showed that *GATA1* mRNA translation also requires the ribonuclease inhibitor
31 1 (RNH1) [87]. RNH1 associates with ribosomes and enhances *GATA1* mRNA
32 translation by enabling its recruitment to the ribosomal complex [87]. Thus, the overall
33 picture emerging is that *GATA1* mRNA translation is highly regulated in erythroid cells
34 and disruptions in these regulatory mechanisms lead to reduced *GATA1* protein levels,
35 defective erythropoiesis and, ultimately, to disease (see below). Additionally, it appears
36 that disruption of the complex translational regulation of the *GATA1* mRNA affects
37 translation of both full length *GATA1* and of *GATA1s* [84].
38
39
40
41
42
43
44
45
46
47
48
49
50
51

52 Targeted degradation of mRNA by miRNAs is acknowledged as being a crucial
53 checkpoint for cellular processes and tumorigenesis, however, not much is known about
54 the miRNA-mediated regulation of *GATA1* mRNA. In the teleost *C. hamatus*, the 3' UTR
55 of the *GATA1* mRNA has been reported to be a target for miR-152 [88]. This interaction
56 reduces the expression levels of *GATA1* protein and, consequently, represses
57
58
59
60

1
2
3 hematopoiesis when miR-152 is overexpressed [88]. **In mammals, regulation of GATA-**
4 **1 levels by microRNAs has not been validated yet, including miRNAs that have been**
5 **described to modulate erythropoiesis [89-91].**
6
7

8 **Posttranslational modifications and GATA1 protein stability**

9
10
11 GATA1 undergoes several posttranslational modifications (PTMs), including
12 phosphorylation, acetylation, sumoylation and ubiquitination. Phosphorylation occurs in
13 seven serine residues, of which six are constitutively phosphorylated, whereas S310
14 phosphorylation, located near the C-ZnF of GATA1, is erythroid specific [92]. GATA1
15 phosphorylation occurs downstream of signaling pathways in erythroid cells, the main
16 one being Epo activation, which leads to PI3/AKT or MAPK phosphorylation of specific
17 GATA1 serine residues [75, 92, 93]. For example, S26 is phosphorylated by MAPK upon
18 stimulation by IL-3 and has been linked to improved cell survival caused by increased
19 expression of Bcl-XL anti-apoptotic gene [94]. Whereas phosphorylation has been
20 proposed to affect GATA1 properties such as DNA binding and transcriptional activation
21 [95], DNA bending [96] or protein interactions [97], its relevance to GATA1 *in vivo*
22 functions remains unclear [98].
23
24
25
26
27
28
29

30
31 GATA1 has also been shown to be acetylated by the histone acetyltransferase (HAT)
32 CBP/p300, at evolutionarily conserved lysine residues in the carboxyl ends of its zinc-
33 finger domains (a.a.245-252 and a.a.308-316) [64, 99]. GATA1 protein acetylation is
34 required for terminal erythroid differentiation and for GATA1 chromatin occupancy and
35 transcriptional activation [63, 64, 100, 101]. Acetylated GATA1 interacts with
36 Bromodomain Protein 3 (BRD3) which stabilizes GATA1 binding to chromatin [102].
37 Interestingly, the combinatorial action of GATA1 phosphorylation and acetylation has
38 been proposed to signal GATA1 degradation by the ubiquitin/proteasome protein
39 pathway [93]. Under this scenario, the acetylated, chromatin bound, "active" fraction of
40 GATA1 becomes phosphorylated in response to specific external signals, or as part of
41 the erythroid differentiation program, which in turn lead to GATA1 becoming ubiquitinated
42 and targeted for degradation [93]. Ubiquitin mediated proteasomal degradation of
43 acetylated GATA1 in erythroid cells appears to involve the HSP27 protein chaperone,
44 which itself is subjected to regulation via posttranslational modification [103]. This
45 complex regulatory network has been proposed to serve as a mean of controlling GATA1
46 activity at the protein level, in response to specific signaling cues in erythropoiesis [93,
47 103]. However, the *in vivo* relevance of such a mechanism remains unclear since
48 mutating the GATA1 phosphorylatable residues does not appear to have an effect in
49 erythropoiesis *in vivo* [98, 104], as would have been predicted by this model. In addition,
50
51
52
53
54
55
56
57
58
59
60

1
2
3 recent work identified the USP7 deubiquitylase as interacting with and stabilizing GATA1
4 protein levels in human erythroid cells, by preventing its proteasomal degradation
5 through the deubiquitination of lysine 48 [105]. However, it is not presently known how
6 the USP7 mediated GATA1 deubiquitination relates to the acetylation and
7 phosphorylation mediated ubiquitination of GATA1 proposed by Hernandez-Hernandez
8 *et al.* [93].
9
10
11
12

13 GATA1 has also been shown to undergo sumoylation in erythroid cells [106-108].
14 Sumoylation does not appear to affect GATA1 protein levels [108], however its functional
15 significance remains contradictory. One study reported that sumoylation inhibits GATA1
16 chromatin occupancy and transcriptional activation [108], whereas another study
17 suggested the opposite, in that sumoylation appeared to enhance GATA1 chromatin
18 occupancy, subnuclear localization and transcriptional activation of a subset of
19 GATA1/FOG-1 dependent target genes [107]. Either way, GATA1 sumoylation appears
20 to add yet another layer to the regulation of GATA1 activity. However, the precise
21 crosstalk between all of GATA1's PTMs (acetylation, phosphorylation, **sumoylation** and
22 ubiquitination) in regulating GATA1 protein levels and activity *in vivo*, remains to be
23 elucidated.
24
25
26
27
28
29
30
31
32
33

34 **Caspase mediated GATA1 protein cleavage and terminal erythroid differentiation**

35
36
37 GATA1 protein has also been shown to be a target for caspase-mediated cleavage in
38 terminal erythroid differentiation in human. In a seminal study, De Maria *et al.* showed
39 that immature erythroid cells expressed several death receptors which, when activated
40 by addition of ligand (e.g. Fas-L) or by incubation with mature erythroblasts, could inhibit
41 erythroid maturation through the specific cleavage of GATA1 protein by caspases [82].
42 These observations led to the suggestion of a homeostatic mechanism whereby
43 accumulation of mature erythroid cells in the erythroblastic island would lead to the
44 activation of death receptors in immature cells, leading to caspase-mediated GATA1
45 protein cleavage and differentiation arrest and apoptosis [82].
46
47
48
49
50

51
52 In a subsequent study by Ribeil *et al.*, it was shown that caspase 3 was the predominant
53 caspase activated in the nucleus of terminally differentiating erythroid cells which can
54 cleave GATA1, for example, when starving erythroid precursors for Epo, resulting in cell
55 death by apoptosis [83]. Furthermore, Ribeil *et al.* uncovered a mechanism that protects
56 GATA1 from cleavage by caspase 3 activation in the nucleus during human erythroid
57 cell maturation. Specifically, they showed that Epo mediated erythroblast survival and
58
59
60

1
2
3 terminal differentiation results in the nuclear translocation of the HSP70 protein
4 chaperone and its specific interaction with GATA1 protein, thus protecting it from
5 cleavage by activated caspase 3 [83]. Under conditions of Epo starvation, HSP70
6 translocates **out of** the nucleus and no longer protects GATA1 which now becomes
7 cleaved by caspase 3, thus leading to differentiation arrest and apoptosis [83]. Thus,
8 HSP70 protects GATA1 from programmed caspase 3 activation in the Epo-dependent,
9 early stages of erythroid cell maturation as a cell survival and differentiation protective
10 mechanism, whereas at later stages, Epo-independent mature erythroid cells can signal
11 death receptor and caspase activation in immature cells, leading to GATA1 cleavage
12 and differentiation arrest as a homeostatic control mechanism. It is unclear whether this
13 mechanism also operates in the murine system, as murine GATA1 protein does not
14 share the caspase cleavage site with human GATA1 [82]. Interestingly, a recent study
15 further added to the GATA1/HSP70 protective axis by showing that nuclear HSP70 and
16 GATA1 protein levels were modulated by the p19^{INK4d} cyclin-dependent kinase inhibitor
17 [109]. Specifically, it was shown that p19^{INK4d} downregulated PEBP1
18 (phosphatidylethanolamine-binding protein 1), resulting in stabilized nuclear HSP70
19 protein levels through increased ERK phosphorylation, known to be implicated in HSP70
20 nuclear localization [109]. Importantly, the GATA1/HSP70 protective mechanism breaks
21 down in hematological disorders presenting with ineffective erythropoiesis, as outlined
22 below.

23
24
25 A recent study uncovered an additional caspase-mediated mechanism for regulating
26 GATA1 protein levels in hematopoiesis [110]. Inflammasome activation in HSCs due to
27 infection, led to caspase 1 activation and GATA1 cleavage and degradation, thus biasing
28 cell fate decisions towards the myeloid lineage. Inhibition of caspase 1 in murine HSCs
29 resulted in the upregulation of GATA1 protein and an increase in megakaryocytic-
30 erythroid (MegE) colony output at the expense of granulocyte-monocyte (GM) colonies,
31 suggesting an evolutionarily conserved mechanism for the inflammasome regulating
32 hematopoietic cell fate decisions by fine-tuning GATA1. In addition, the same study
33 showed that the cleavage of GATA1 by caspase 1 was required for the final steps of
34 hemin-induced erythroid differentiation in K562 cells, thus pointing at an additional
35 mechanism whereby the inflammasome could regulate erythropoiesis through GATA1.
36 Importantly, just like for caspase 3, GATA1 was indeed shown to be a substrate for
37 cleavage by caspase 1 *in vitro* [110].

38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57 **The role of the proteasome in erythropoiesis has been highlighted through the years,**
58 **and it is well known that proteasome-inhibitor drugs used as anti-tumoral agents,**
59
60

1
2
3 especially in multiple myeloma, may cause anemia as an adverse event [111-113].
4 However, perhaps counterintuitively, chemical inhibition of the proteasome in murine
5 erythroid progenitors, or loss of the proteasome shuttle protein Rad23b, results in
6 reduced levels of GATA1 leading to early apoptosis *in vitro* and to anemia *in vivo*,
7 respectively [114]. A better understanding of the action of the proteasome in
8 erythropoiesis and how it may regulate directly or indirectly GATA1 levels, is currently
9 the research focus of several groups [112, 113, 115, 116].
10
11
12
13
14

15 **The effects of artificially altering GATA1 protein levels**

16
17 As outlined above, GATA1 translation and protein levels are exquisitely regulated in
18 hematopoiesis and it is now well established that tinkering with GATA1 protein levels has
19 important functional implications and may also result in hematological disease. For
20 example, the extremes of either a complete loss of GATA1 expression or GATA1
21 overexpression, result in both cases in embryonic lethal anemia [22, 117]. Another study
22 using inducible GATA1 expression in an immortalized GATA1-null proerythroblastic cell
23 line, demonstrated differential sensitivity of target genes to the dosage of GATA1 protein
24 [118]. Studies using transgenic mice under- or over-expressing GATA1 have been
25 particularly useful in investigating the effects of altering GATA1 protein levels in
26 hematopoiesis. Specifically, two GATA1 knockdown mouse models have been
27 generated by the Yamamoto and Orkin groups expressing GATA1 at 5% or 20% of its
28 physiological levels, respectively [119, 120]. The first mouse model, called GATA1.05,
29 was generated by the insertion of a neomycin resistance cassette in the GATA1 promoter
30 region, which resulted in GATA1 expression at 5% its physiological level [119].
31 Hemizygous male GATA1.05 embryos die from severe anemia between E11.5 and
32 E12.5, suggesting that 5% GATA1 expression is not sufficient to support primitive
33 erythropoiesis. Interestingly, using *in vitro* differentiated ES cells, it was shown that both
34 GATA1.05 and GATA1-null cells resulted in reduced expression of p16^{INK4A} and
35 accelerated cell cycle progression, however, 5% of GATA1 expression was sufficient to
36 maintain expression of the anti-apoptotic Bcl-X_L gene, thus protecting the cells from
37 apoptotic cell death normally observed with GATA1-null cells [121]. These results
38 provide further evidence for subtle alterations in GATA1 expression levels having
39 differential effects on the expression of GATA1 target genes.
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54

55 In contrast to male mice, heterozygous GATA1.05 female mice were born at slightly
56 lower than expected Mendelian frequencies and often developed perinatally varying
57 degrees of transient anemia (due to X inactivation), from which they recovered [119,
58 121]. However, between 3 and 6 months of age, GATA1.05 female mice frequently
59
60

1
2
3 develop erythroleukemia, characterized by the proliferation of immature erythroid
4 progenitors [122]. By contrast, heterozygous GATA1-null female mice do not present
5 with erythroleukemia. The difference between the two conditions (which only differ by
6 5% in the expression of GATA1), may lie in the observation that 5% of GATA1 is sufficient
7 to protect cells from apoptosis, but not from uncontrollable cell proliferation [121]. By
8 contrast, GATA1-null cells in a heterozygous female background would be eliminated by
9 apoptosis. Thus, additional genetic events that **may** occur in the proliferating GATA1.05
10 progenitor population can lead to leukemic transformation [122-124]. Interestingly, older
11 GATA1.05 mice also develop CD19⁺ B-cell leukemia through a leukemogenic
12 mechanism that is not yet understood, but may be related to lineage commitment
13 skewing towards lymphoid fates by proliferating progenitors expressing low levels of
14 GATA1 [122].

15
16 The second GATA1 knockdown mouse strain was generated by replacing an upstream
17 enhancer region in the GATA1 gene locus with a neomycin resistance cassette [120]. In
18 these mice, known as GATA1^{low} mice, GATA1 expression levels were originally reported
19 to be knocked down to 20% [120], although subsequent analyses have shown GATA1
20 mRNA reduced by as much as 100-fold in orthochromatic erythroblasts ([125] and
21 references therein). The majority of GATA1^{low} male mice die of anemia between
22 embryonic stages E13.5 and E14.5. In contrast to the GATA1.05 mice, a few GATA1^{low}
23 male mice are born alive but are severely anemic, but for some of them anemia resolves
24 around 4-5 weeks of age [120]. These observations suggest that ~20% GATA1
25 expression is insufficient to support primitive erythropoiesis but sufficient for adult
26 erythropoiesis. Interestingly, GATA1 expression in the megakaryocytes of GATA1^{low}
27 mice is completely abolished and the mice are born with severe thrombocytopenia,
28 associated with the expansion of abnormal megakaryocytic progenitors that, at 10
29 months of age, develops into a myeloproliferative condition characterized by fibrosis of
30 the bone marrow, closely resembling primary myelofibrosis [125, 126]. GATA1^{low} mice
31 also have defects in mast cell differentiation, as evidenced by the amplification of mast
32 cell progenitors, increased apoptotic precursors and defective differentiation of mature
33 cells [127]. These observations provide yet more evidence for the necessity of
34 maintaining appropriate GATA1 levels to support balanced hematopoiesis.

35
36 GATA1 overexpression has also been shown to have dramatic effects in erythropoiesis
37 *in vivo*. In particular, a mouse model was generated bearing an X-linked *GATA1*
38 transgene driven by the Locus Control Region (LCR) of the human β -globin locus (the
39 GATA1-OX transgene), resulting in the overexpression of GATA1 beyond the
40

1
2
3 proerythroblast stage, at the moment of maximum globin transcription [117]. Male
4 embryos carrying the GATA1-OX transgene die *in utero* in mid gestation due to anemia,
5 whereas heterozygous females survive to term and develop normally. From this study it
6 was shown that GATA1 overexpression at the proerythroblast stage causes cell cycle
7 arrest and apoptosis of erythroid progenitors beyond the proerythroblast stage.
8 Intriguingly, in heterozygous females it was shown that GATA1-OX progenitors were able
9 to mature at an almost normal rate [117]. The explanation for this phenomenon lies in
10 the erythroblastic island, i.e. the histological unit where erythropoiesis takes place in the
11 bone marrow [128, 129]. The erythroblastic island is composed of a central macrophage
12 surrounded by erythroid progenitors in successive stages of differentiation, emanating
13 from the center towards the periphery of the island. It is in this periphery where mature
14 erythroblasts will enucleate and the expelled pyrenocyte will be phagocytosed by the
15 central macrophage [130]. In this hierarchical histological unit, erythroid cells are in
16 contact and communicate with each other, thus providing an internal feedback
17 mechanism measuring differentiation rate and assuring maturation and production of red
18 blood cells in response to physiological demands at any given moment [131, 132]. Due
19 to X-chromosome inactivation, the erythroblastic islands of heterozygous GATA1-OX
20 females are chimeric in composition, i.e. approximately 50% of erythroid progenitors
21 overexpress GATA1 (the X chromosome carrying the GATA1-OX transgene is active),
22 and 50% of erythroid progenitors express GATA1 at normal levels (the wildtype X
23 chromosome is active). Based on this, it was proposed that maturing erythroid cells in
24 later stages of differentiation induce the terminal differentiation of earlier progenitors
25 through cell-cell signaling mechanisms, which result in the downregulation of GATA1
26 protein levels as a necessary regulatory cadency for proper erythroid terminal maturation
27 [117]. This provides a mechanism to ensure a smooth production of red blood cells in
28 wild type mice. However, in the heterozygous GATA1-OX females, this results in the
29 “rescue” of the GATA1 overexpressing cells, which are able to differentiate when in close
30 proximity to wildtype maturing erythroid cells, which provide a signal directed towards
31 the downregulation of GATA1 protein levels. This signal was tentatively named “REDS”
32 (red cell differentiation signal). A follow-up study using mice with different combinations
33 of the GATA1-OX transgene and a GATA1-knockout allele, provided further evidence for
34 REDS signaling by wildtype maturing erythroid cells: when crossed with GATA1
35 knockout mice, heterozygous GATA1-OX females died in mid gestation due to anemia
36 (similar to GATA1-OX males) [133]. However, to-date, the identity of the REDS
37 receptor/ligand is not known. Regulation of GATA1 transcription at the terminal
38 differentiation stages and recent work suggesting that REDS might act to regulate
39 GATA1 levels through the inhibition of translation (L. Gutiérrez, manuscript in
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

preparation), are possibilities. Overall, the REDS signal serves as another example of homeostatic regulation of erythropoiesis through GATA1 protein level regulation.

Lastly, it should be noted that artificially altering GATA1 expression levels through its enforced expression in hematopoietic progenitors, can push them to cell fates towards lineages that are normally regulated by GATA1 [134, 135]. For example, ectopic expression of GATA1 in avian myeloid progenitors [136] or in human primary myeloid progenitors [137] reprogrammed them to erythroid and megakaryocytic or eosinophilic cell fates, respectively. These studies support the notion that different thresholds of GATA1 expression levels in hematopoietic progenitors, can govern cell fate decisions towards different lineages.

Downregulation of GATA1 protein levels and ineffective erythropoiesis

Ineffective erythropoiesis (IE) or dyserythropoiesis is a pathological feature shared by a number of hematological diseases such as β thalassemia, myelodysplastic syndromes (MDS), Diamond-Blackfan anemia (DBA), 5q-syndrome and myelofibrosis [138]. IE is characterized by the abnormal differentiation of erythroid progenitors resulting in an expanded progenitor compartment, increased destruction of abnormal erythroblasts and a paucity in the production of mature erythroid cells. Recent work has shown that the downregulation of GATA1 protein levels is a critical aspect of IE in hematological disease. Three mechanisms have been described to account for reduced GATA1 protein levels in IE: (i) inherited GATA1 mutations greatly reducing, or eliminating altogether, expression of the full length GATA1 protein [139, 140]; (ii) impaired translation of full length GATA1 [80, 84], and (iii) impaired HSP70-mediated protection of GATA1 protein from cleavage and degradation [141].

The best example of inherited GATA1 mutations in IE is that of Diamond-Blackfan Anemia (DBA). This is an inherited bone marrow failure syndrome characterized by anemia, macrocytosis, reticulopenia and selective reduction or absence of erythroid precursors (BFU-Es) in an otherwise normocellular bone marrow [142]. More than 200 heterozygous mutations in 19 ribosomal protein (RP) genes have been recorded to-date, accounting for most (approximately 70%), but not all, of the DBA cases [143, 144]. Sankaran and colleagues were the first to identify through exome sequencing a GATA1 mutation associated with DBA [145]. This mutation was a c.220G>C transversion that caused the skipping of exon 2, resulting in the loss of full length GATA1 protein, with the continued expression of the GATA1s isoform. Additional cases in DBA with similar GATA1 mutations have been reported [84, 146, 147], including a mutation that resulted

1
2
3 in the loss of the first translation initiation codon responsible for the production of full
4 length GATA1 protein, again without affecting GATA1s expression [84, 147]. These
5 findings are significant in explaining dyserythropoiesis in these patients, as it has been
6 shown that the GATA1s isoform cannot fully support erythropoiesis [20, 21, 148].
7 Furthermore, these results show that the mutations in the *GATA1* gene that give rise to
8 reduced, or absent, full length GATA1 protein, in the absence of RP gene mutations, are
9 sufficient to cause disease. In addition, Hollanda *et al.* reported a family with a similar G
10 > C transition at the boundary of exon 2 in the *GATA1* gene in male members, which
11 also resulted in the exclusive expression of GATA1s giving rise to macrocytic anemia
12 and variable neutropenia, with no apparent thrombocytopenia [149]. The differences in
13 disease manifestation between the two reports of the G > C mutation in exon 2 of the
14 *GATA1* gene remain unclear, but it has been suggested that they may be due to
15 differences in the levels of total GATA1 protein expressed [145]. Lastly, Sankaran and
16 colleagues recently identified two patients with dyserythropoietic anemia and
17 thrombocytopenia, due to a mutation that affects canonical splicing of the *GATA1* mRNA
18 [150]. Specifically, a single nucleotide change was identified 24 nucleotides upstream of
19 the canonical splice site in intron 5 of the *GATA1* gene. This mutation results in an
20 increase in the use of the mutated site as an alternative splice acceptor site, with a
21 reduction in canonical splicing. This leads to a partial intron retention event that
22 introduces an extra 5 amino acids C-terminally to the C-terminal zinc finger of GATA1,
23 with a concomitant reduction in the expression of wild-type GATA1 to ~ 40% its normal
24 levels. Importantly, the mutant GATA1 protein was functionally inactive as it could not
25 rescue erythroid differentiation in functional complementation assays in the GATA1-null
26 G1E murine proerythroblastic cell line [150].
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41

42 The analysis of the molecular basis of DBA, again provided important clues as to the
43 implication of reduced GATA1 protein levels in IE in hematological disease. As outlined
44 above, DBA is primarily associated with heterozygous mutations in a number of RP
45 genes. It is now known that deficiencies in RPs act upstream of GATA1 in
46 downregulating its protein translation levels, whilst mRNA levels remain unaffected [84].
47 In accordance with this, knocking down RPS19 expression, or that of other RP genes,
48 led to a significant reduction in GATA1 protein levels resulting in deficient erythropoiesis,
49 reminiscent of DBA [84]. As described above, it appears that the particular sensitivity of
50 the *GATA1* mRNA to RP levels is mediated by the particular sequence and structure of
51 its 5' UTR, as it was shown that the presence of the 5' UTR is critical for GATA1 erythroid
52 function [80]. Interestingly, mutations in the RPS14 gene were also found in patients with
53 primary myelofibrosis and were again associated with reduced GATA1 protein levels and
54
55
56
57
58
59
60

1
2
3 impaired megakaryopoiesis [151], thus linking RP gene mutations and reduced GATA1
4 protein levels in hematological disease affecting a hematopoietic lineage other than the
5 erythroid lineage. Furthermore, it was recently shown that heme levels are retroactively
6 involved in this spectrum of phenomena around ribosomal protein dependent GATA1
7 protein level regulation [152]. This study showed that in early erythroid progenitors, heme
8 induces the translation of ribosomal proteins, ensuring sustained globin synthesis.
9 However, at later stages of differentiation, heme accumulation associates with
10 downregulation of *Gata1* through an still undescribed mechanism [152].
11
12
13
14
15
16

17 As described above, GATA1 protein is normally protected from caspase-mediated
18 cleavage in the early stages of erythroid differentiation, through its interaction in the
19 nucleus with the HSP70 protein chaperone [83]. This GATA1 protective mechanism
20 appears to break down in hematological disorders manifesting with IE, such as DBA and
21 myelodysplastic syndromes (MDS). In DBA, it was recently shown that HSP70 protein is
22 downregulated due to its increased ubiquitination and proteasomal degradation in
23 RPL11- and RPL5-deficient human primary erythroid cells, but not in RPS19-deficient
24 cells, resulting in proliferation and differentiation defects and increased apoptosis in
25 erythroid cells, resembling DBA features [153]. A subsequent study showed a
26 disequilibrium of globin chain and heme synthesis in RPL11- and RPL5-deficient human
27 erythroid cells, resulting in excessive free heme which downregulated HSP70 protein
28 [154]. The study by Doty et al. also suggests that aberrant accumulation of heme,
29 contributes to the amplification of ribosomal protein imbalance, thus causing premature
30 downregulation of GATA1 levels, responsible for the IE that is characteristic of DBA and
31 MDS [152]. Hence, it is clear in that in DBA, HSP70 downregulation results in a significant
32 drop in GATA1 protein levels due to caspase mediated cleavage, which underlies the
33 observed erythroid maturation defects [153]. The HSP70 mediated protection of GATA1
34 is also disrupted in β -thalassemia, which also manifests with IE. Here, it was shown in
35 primary human β -thalassemic erythroblasts that free α -globin chains sequester HSP70
36 to the cytoplasm, resulting in significant downregulation of nuclear GATA1 protein and a
37 blockade in terminal erythroid differentiation [155]. Lastly, myelodysplastic syndromes
38 are clonal disorders also characterized by IE associated with defective erythroid
39 maturation and increased apoptosis of erythroid precursors [138]. Here too, it was shown
40 that nuclear GATA1 protein was significantly lower as a result of aberrant HSP70
41 localization in the cytoplasm, accounting for the blockade in erythroid differentiation in
42 MDS [141]. Thus, downregulation or absence of full length GATA1 protein in erythroid
43 precursors, either directly through mutations in the *GATA1* gene itself, or indirectly
44 through defects in *GATA1* mRNA translation or the breakdown of the HSP70/GATA1
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 protective axis, result in defective erythropoiesis and disease. This is an addition to
4 hematological deficiencies or malignancies affecting other hematopoietic lineages,
5 where downregulation or loss of the full length GATA1 protein play a causative role, for
6 example, in primary myelofibrosis affecting the megakaryocytic lineage [125] or in
7 trisomy 21 associated transient myeloproliferative disorder (TMD), acute
8 megakaryoblastic leukemia (AMKL) and clonal eosinophilia [156, 157].
9
10
11
12
13

14 Interestingly enough, a balanced erythropoietic production is disturbed in the presence
15 of subjacent inflammation, a phenomenon that has been named as anemia of
16 inflammation or anemia of chronic disease. The most frequent scenario occurs when the
17 body feels a need to increase white blood cell (WBC) production in response to infection,
18 at the expense of red blood cell (RBC) production; in this case, the anemia is caused by
19 a rise in IL-6 and hepcidin levels, with subsequent inhibition of iron absorption, ultimately
20 resulting in reduced erythropoiesis [158]. It has been shown that inflammatory molecules
21 themselves (*i.e.* TNF α , IL6, IFN γ) are able to promote lymphoid production at the
22 expense of the erythroid compartment, either skewing lineage specification, or even
23 targeting directly GATA1 levels in erythroid progenitors [159-161]. It has been shown
24 that the proinflammatory cytokine TNF α , induces an erythroid defect in K562 cells *in*
25 *vitro*. The molecular mechanism implies downregulation of both GATA1 mRNA and
26 protein levels and decreased GATA1 DNA binding capacity. The study by Buck *et al*
27 identifies GATA2-dependent transcriptional deregulation of GATA1, proteasome-
28 dependent degradation of GATA1, and altered post-translational modification of GATA1
29 (acetylation) as a consequence of TNF α treatment [161]. Curiously, another study
30 showed that, *in vitro*, hematopoietic precursors bearing mutations in *RPS19* (accounting
31 for 25% of mutations associated with DBA), present with deficient erythropoiesis which
32 is accompanied by overproduction of TNF α by co-cultured non-erythroid cells. Addition
33 of the TNF α inhibitor Etarnecept overcomes partially the erythroid defect [158]. Bibikova
34 *et al* apply this therapeutic strategy on an experimental zebrafish morphant model
35 deficient for *rps19*, which phenocopies the dyserythropoiesis and TNF α increase of DBA,
36 and are able to rescue partially the erythropoietic defect by inhibiting TNF α [159]. In a
37 transgenic mouse model of anemia of chronic disease, characterized by enhanced
38 CD27-mediated costimulation and a strong increase in the formation of IFN γ -producing
39 effector T cells, progressive anemia is due to inhibition of erythropoiesis through the IRF-
40 1-dependent activation of PU.1 transcription factor in hematopoietic precursors [159].
41 This exemplifies how the hematopoietic production balance is self-regulated through
42 transcriptional reprogramming of hematopoietic precursors, GATA1 being a main
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 physiological target the levels of which fine-tune erythroid production in health and in
4 disease through natural mechanisms that could be used to develop novel and efficient
5 therapeutic strategies in well-known erythroid-related disorders.
6
7
8

9 **Conclusions**

10
11 The fine-tuning of GATA1 protein levels has emerged as a key mechanism for regulating
12 erythropoiesis. High GATA1 protein levels are required in the EPO-dependent early
13 stages of erythropoiesis in providing cell survival functions, in addition to activating the
14 erythroid transcription program. Adequate GATA1 protein levels in the early stages of
15 erythroid differentiation are ensured by the efficient translation of GATA1 mRNA and its
16 protection from cleavage by activated caspase 3 through GATA1's interaction with the
17 HSP70 protein chaperone. Subsequently, GATA1 needs to be drastically downregulated
18 for the terminal stage of erythroid maturation to take place, in a homeostatic mechanism
19 that balances the production of red blood cells depending on surplus or demand. GATA1
20 protein levels also affect cell fate decisions, as evidenced by the reprogramming of
21 myeloid progenitors towards MegE fates by GATA1 overexpression. Furthermore,
22 inflammasomes appear to exploit the caspase-mediated cleavage of GATA1 in HSCs in
23 order to tip the balance towards enhanced myeloid differentiation in response to
24 infection. Lastly, the combined action of GATA1 post-translational modifications has
25 been proposed as a mechanism to control the active, chromatin-bound fraction of GATA1
26 by targeting it for proteasomal degradation as a mechanism for regulating GATA1
27 transcriptional activity in response to signals (Figure 2). It has also become clear that, in
28 addition to genetic mutations affecting *GATA1*, defects in the different levels of regulatory
29 checkpoints for physiological GATA1 expression also play a key role in the
30 pathophysiology of disease hematological diseases associated with inefficient
31 erythropoiesis. With the advent of novel, unbiased, single cell-based technologies, we
32 stand to gain a much better understanding of GATA1 regulatory mechanisms and the
33 cascading effects of their deregulation in vertebrate hematopoiesis and in human
34 disease. Newly gained knowledge will help the development of novel therapies targeting
35 the restoration of GATA1 regulation in treating inefficient erythropoiesis and anemia in
36 hematological disease in man.
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Acknowledgments

1
2
3 We apologize to the many of our colleagues whose work we were not able to cite due to
4 space restrictions. L.G. is supported by a Ramón y Cajal Fellowship (RYC-2013-12587;
5 Ministerio de Economía y Competitividad, Spain). E.K. is supported by a Scholarship for
6 Strengthening Post-Doctoral Research from the Greek State Scholarships Foundation
7 (IKY), within the framework of the Operational Program “Human Resources
8 Development program, Education and Life-Long learning”. We are thankful to Dr. Martijn
9 Nolte and Dr. Sten Libregts (Sanquin Research, Amsterdam, the Netherlands) for
10 performing the experiments and providing the data for the intracellular GATA1 staining
11 in murine bone marrow, performed as a previous joint collaboration with L.G.
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

References

1. Adam, R. C., Yang, H., Rockowitz, S., Larsen, S. B., Nikolova, M., Oristian, D. S., Polak, L., Kadaja, M., Asare, A., Zheng, D. & Fuchs, E. (2015) Pioneer factors govern super-enhancer dynamics in stem cell plasticity and lineage choice, *Nature*. **521**, 366-70.
2. Brown, G., Ceredig, R. & Tsapogas, P. (2018) The Making of Hematopoiesis: Developmental Ancestry and Environmental Nurture, *Int J Mol Sci*. **19**.
3. Jacobsen, S. E. W. & Nerlov, C. (2019) Haematopoiesis in the era of advanced single-cell technologies, *Nat Cell Biol*. **21**, 2-8.
4. Hofer, T. & Rodewald, H. R. (2018) Differentiation-based model of hematopoietic stem cell functions and lineage pathways, *Blood*. **132**, 1106-1113.
5. Dzierzak, E. & Philipsen, S. (2013) Erythropoiesis: development and differentiation, *Cold Spring Harb Perspect Med*. **3**, a011601.
6. Liang, R. & Ghaffari, S. (2016) Advances in understanding the mechanisms of erythropoiesis in homeostasis and disease, *Br J Haematol*. **174**, 661-73.
7. Palis, J. (2014) Primitive and definitive erythropoiesis in mammals, *Front Physiol*. **5**, 3.
8. Kerenyi, M. A. & Orkin, S. H. (2010) Networking erythropoiesis, *J Exp Med*. **207**, 2537-41.
9. Ferreira, R., Ohneda, K., Yamamoto, M. & Philipsen, S. (2005) GATA1 function, a paradigm for transcription factors in hematopoiesis, *Mol Cell Biol*. **25**, 1215-27.
10. Tremblay, M., Sanchez-Ferras, O. & Bouchard, M. (2018) GATA transcription factors in development and disease, *Development*. **145**.
11. Lowry, J. A. & Atchley, W. R. (2000) Molecular evolution of the GATA family of transcription factors: conservation within the DNA-binding domain, *J Mol Evol*. **50**, 103-15.
12. Weiss, M. J. & Orkin, S. H. (1995) GATA transcription factors: key regulators of hematopoiesis, *Exp Hematol*. **23**, 99-107.
13. Katsumura, K. R., Bresnick, E. H. & Group, G. F. M. (2017) The GATA factor revolution in hematology, *Blood*. **129**, 2092-2102.
14. Ko, L. J. & Engel, J. D. (1993) DNA-binding specificities of the GATA transcription factor family, *Mol Cell Biol*. **13**, 4011-22.
15. Vyas, P., Ault, K., Jackson, C. W., Orkin, S. H. & Shivdasani, R. A. (1999) Consequences of GATA-1 deficiency in megakaryocytes and platelets, *Blood*. **93**, 2867-75.
16. Yu, C., Cantor, A. B., Yang, H., Browne, C., Wells, R. A., Fujiwara, Y. & Orkin, S. H. (2002) Targeted deletion of a high-affinity GATA-binding site in the GATA-1 promoter leads to selective loss of the eosinophil lineage in vivo, *J Exp Med*. **195**, 1387-95.
17. Nei, Y., Obata-Ninomiya, K., Tsutsui, H., Ishiwata, K., Miyasaka, M., Matsumoto, K., Nakae, S., Kanuka, H., Inase, N. & Karasuyama, H. (2013) GATA-1 regulates the generation and function of basophils, *Proc Natl Acad Sci U S A*. **110**, 18620-5.
18. Gutierrez, L., Nikolic, T., van Dijk, T. B., Hammad, H., Vos, N., Willart, M., Grosveld, F., Philipsen, S. & Lambrecht, B. N. (2007) Gata1 regulates dendritic-cell development and survival, *Blood*. **110**, 1933-41.
19. Sasaki, H., Kurotaki, D. & Tamura, T. (2016) Regulation of basophil and mast cell development by transcription factors, *Allergol Int*. **65**, 127-134.
20. Byrska-Bishop, M., VanDorn, D., Campbell, A. E., Betensky, M., Arca, P. R., Yao, Y., Gadue, P., Costa, F. F., Nemiroff, R. L., Blobel, G. A., French, D. L., Hardison, R. C., Weiss, M. J. & Chou, S. T. (2015) Pluripotent stem cells reveal erythroid-specific activities of the GATA1 N-terminus, *J Clin Invest*. **125**, 993-1005.
21. Ling, T., Birger, Y., Stankiewicz, M. J., Ben-Haim, N., Kalisky, T., Rein, A., Kugler, E., Chen, W., Fu, C., Zhang, K., Patel, H., Sikora, J. W., Goo, Y. A., Kelleher, N., Zou, L., Izraeli, S. & Crispino, J. D. (2019) Chromatin occupancy and epigenetic analysis reveal new insights into the function of GATA1 N-terminus in erythropoiesis, *Blood*.
22. Pevny, L., Simon, M. C., Robertson, E., Klein, W. H., Tsai, S. F., D'Agati, V., Orkin, S. H. & Costantini, F. (1991) Erythroid differentiation in chimaeric mice blocked by a targeted mutation in the gene for transcription factor GATA-1, *Nature*. **349**, 257-60.
23. Pevny, L., Lin, C. S., D'Agati, V., Simon, M. C., Orkin, S. H. & Costantini, F. (1995) Development of hematopoietic cells lacking transcription factor GATA-1, *Development*. **121**, 163-72.
24. Weiss, M. J. & Orkin, S. H. (1995) Transcription factor GATA-1 permits survival and maturation of erythroid precursors by preventing apoptosis, *Proc Natl Acad Sci U S A*. **92**, 9623-7.

25. Gutierrez, L., Tsukamoto, S., Suzuki, M., Yamamoto-Mukai, H., Yamamoto, M., Philipsen, S. & Ohneda, K. (2008) Ablation of Gata1 in adult mice results in aplastic crisis, revealing its essential role in steady-state and stress erythropoiesis, *Blood*. **111**, 4375-85.
26. Cheng, Y., Wu, W., Kumar, S. A., Yu, D., Deng, W., Tripic, T., King, D. C., Chen, K. B., Zhang, Y., Drautz, D., Giardine, B., Schuster, S. C., Miller, W., Chiaromonte, F., Zhang, Y., Blobel, G. A., Weiss, M. J. & Hardison, R. C. (2009) Erythroid GATA1 function revealed by genome-wide analysis of transcription factor occupancy, histone modifications, and mRNA expression, *Genome Res*. **19**, 2172-84.
27. Fujiwara, T., O'Geen, H., Keles, S., Blahnik, K., Linnemann, A. K., Kang, Y. A., Choi, K., Farnham, P. J. & Bresnick, E. H. (2009) Discovering hematopoietic mechanisms through genome-wide analysis of GATA factor chromatin occupancy, *Mol Cell*. **36**, 667-81.
28. Yu, M., Riva, L., Xie, H., Schindler, Y., Moran, T. B., Cheng, Y., Yu, D., Hardison, R., Weiss, M. J., Orkin, S. H., Bernstein, B. E., Fraenkel, E. & Cantor, A. B. (2009) Insights into GATA-1-mediated gene activation versus repression via genome-wide chromatin occupancy analysis, *Mol Cell*. **36**, 682-95.
29. Papadopoulos, G. L., Karkoulia, E., Tsamardinos, I., Porcher, C., Ragoussis, J., Bungert, J. & Strouboulis, J. (2013) GATA-1 genome-wide occupancy associates with distinct epigenetic profiles in mouse fetal liver erythropoiesis, *Nucleic Acids Res*. **41**, 4938-48.
30. Kang, Y. A., Sanalkumar, R., O'Geen, H., Linnemann, A. K., Chang, C. J., Bouhassira, E. E., Farnham, P. J., Keles, S. & Bresnick, E. H. (2012) Autophagy driven by a master regulator of hematopoiesis, *Mol Cell Biol*. **32**, 226-39.
31. Mclver, S. C., Kang, Y. A., DeVilbiss, A. W., O'Driscoll, C. A., Ouellette, J. N., Pope, N. J., Camprecios, G., Chang, C. J., Yang, D., Bouhassira, E. E., Ghaffari, S. & Bresnick, E. H. (2014) The exosome complex establishes a barricade to erythroid maturation, *Blood*. **124**, 2285-97.
32. Mclver, S. C., Katsumura, K. R., Davids, E., Liu, P., Kang, Y. A., Yang, D. & Bresnick, E. H. (2016) Exosome complex orchestrates developmental signaling to balance proliferation and differentiation during erythropoiesis, *Elife*. **5**.
33. Huang, D. Y., Kuo, Y. Y., Lai, J. S., Suzuki, Y., Sugano, S. & Chang, Z. F. (2004) GATA-1 and NF-Y cooperate to mediate erythroid-specific transcription of Gfi-1B gene, *Nucleic Acids Res*. **32**, 3935-46.
34. Vilaboa, N., Bermejo, R., Martinez, P., Bornstein, R. & Cales, C. (2004) A novel E2 box-GATA element modulates Cdc6 transcription during human cells polyploidization, *Nucleic Acids Res*. **32**, 6454-67.
35. Fernandez-Morales, B., Pavon, L. & Cales, C. (2012) CDC6 expression is regulated by lineage-specific transcription factor GATA1, *Cell Cycle*. **11**, 3055-66.
36. Papetti, M., Wontakal, S. N., Stopka, T. & Skoultschi, A. I. (2010) GATA-1 directly regulates p21 gene expression during erythroid differentiation, *Cell Cycle*. **9**, 1972-80.
37. Gregory, T., Yu, C., Ma, A., Orkin, S. H., Blobel, G. A. & Weiss, M. J. (1999) GATA-1 and erythropoietin cooperate to promote erythroid cell survival by regulating bcl-xL expression, *Blood*. **94**, 87-96.
38. Tanaka, H., Matsumura, I., Nakajima, K., Daino, H., Sonoyama, J., Yoshida, H., Oritani, K., Machii, T., Yamamoto, M., Hirano, T. & Kanakura, Y. (2000) GATA-1 blocks IL-6-induced macrophage differentiation and apoptosis through the sustained expression of cyclin D1 and bcl-2 in a murine myeloid cell line M1, *Blood*. **95**, 1264-73.
39. Chou, S. T., Khandros, E., Bailey, L. C., Nichols, K. E., Vakoc, C. R., Yao, Y., Huang, Z., Crispino, J. D., Hardison, R. C., Blobel, G. A. & Weiss, M. J. (2009) Graded repression of PU.1/Sfp1 gene transcription by GATA factors regulates hematopoietic cell fate, *Blood*. **114**, 983-94.
40. Dore, L. C., Amigo, J. D., Dos Santos, C. O., Zhang, Z., Gai, X., Tobias, J. W., Yu, D., Klein, A. M., Dorman, C., Wu, W., Hardison, R. C., Paw, B. H. & Weiss, M. J. (2008) A GATA-1-regulated microRNA locus essential for erythropoiesis, *Proc Natl Acad Sci U S A*. **105**, 3333-8.
41. Rasmussen, K. D., Simmini, S., Abreu-Goodger, C., Bartonicek, N., Di Giacomo, M., Bilbao-Cortes, D., Horos, R., Von Lindern, M., Enright, A. J. & O'Carroll, D. (2010) The miR-144/451 locus is required for erythroid homeostasis, *J Exp Med*. **207**, 1351-8.
42. Wang, F., Zhu, Y., Guo, L., Dong, L., Liu, H., Yin, H., Zhang, Z., Li, Y., Liu, C., Ma, Y., Song, W., He, A., Wang, Q., Wang, L., Zhang, J., Li, J. & Yu, J. (2014) A regulatory circuit comprising GATA1/2 switch and microRNA-27a/24 promotes erythropoiesis, *Nucleic Acids Res*. **42**, 442-57.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
43. Kadauke, S., Udugama, M. I., Pawlicki, J. M., Achtman, J. C., Jain, D. P., Cheng, Y., Hardison, R. C. & Blobel, G. A. (2012) Tissue-specific mitotic bookmarking by hematopoietic transcription factor GATA1, *Cell*. **150**, 725-37.
 44. Tsang, A. P., Visvader, J. E., Turner, C. A., Fujiwara, Y., Yu, C., Weiss, M. J., Crossley, M. & Orkin, S. H. (1997) FOG, a multitype zinc finger protein, acts as a cofactor for transcription factor GATA-1 in erythroid and megakaryocytic differentiation, *Cell*. **90**, 109-19.
 45. Crispino, J. D., Lodish, M. B., MacKay, J. P. & Orkin, S. H. (1999) Use of altered specificity mutants to probe a specific protein-protein interaction in differentiation: the GATA-1:FOG complex, *Mol Cell*. **3**, 219-28.
 46. Chang, A. N., Cantor, A. B., Fujiwara, Y., Lodish, M. B., Droho, S., Crispino, J. D. & Orkin, S. H. (2002) GATA-factor dependence of the multitype zinc-finger protein FOG-1 for its essential role in megakaryopoiesis, *Proc Natl Acad Sci U S A*. **99**, 9237-42.
 47. Nichols, K. E., Crispino, J. D., Poncz, M., White, J. G., Orkin, S. H., Maris, J. M. & Weiss, M. J. (2000) Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited mutation in GATA1, *Nat Genet*. **24**, 266-70.
 48. Rodriguez, P., Bonte, E., Krijgsveld, J., Kolodziej, K. E., Guyot, B., Heck, A. J., Vyas, P., de Boer, E., Grosveld, F. & Strouboulis, J. (2005) GATA-1 forms distinct activating and repressive complexes in erythroid cells, *EMBO J*. **24**, 2354-66.
 49. Hong, W., Nakazawa, M., Chen, Y. Y., Kori, R., Vakoc, C. R., Rakowski, C. & Blobel, G. A. (2005) FOG-1 recruits the NuRD repressor complex to mediate transcriptional repression by GATA-1, *EMBO J*. **24**, 2367-78.
 50. Miccio, A., Wang, Y., Hong, W., Gregory, G. D., Wang, H., Yu, X., Choi, J. K., Shelat, S., Tong, W., Poncz, M. & Blobel, G. A. (2010) NuRD mediates activating and repressive functions of GATA-1 and FOG-1 during blood development, *EMBO J*. **29**, 442-56.
 51. Wadman, I. A., Osada, H., Grutz, G. G., Agulnick, A. D., Westphal, H., Forster, A. & Rabbitts, T. H. (1997) The LIM-only protein Lmo2 is a bridging molecule assembling an erythroid, DNA-binding complex which includes the TAL1, E47, GATA-1 and Ldb1/NLI proteins, *EMBO J*. **16**, 3145-57.
 52. Kassouf, M. T., Hughes, J. R., Taylor, S., McGowan, S. J., Soneji, S., Green, A. L., Vyas, P. & Porcher, C. (2010) Genome-wide identification of TAL1's functional targets: insights into its mechanisms of action in primary erythroid cells, *Genome Res*. **20**, 1064-83.
 53. Soler, E., Andrieu-Soler, C., de Boer, E., Bryne, J. C., Thongjuea, S., Stadhouders, R., Palstra, R. J., Stevens, M., Kockx, C., van Ijcken, W., Hou, J., Steinhoff, C., Rijkers, E., Lenhard, B. & Grosveld, F. (2010) The genome-wide dynamics of the binding of Ldb1 complexes during erythroid differentiation, *Genes Dev*. **24**, 277-89.
 54. Jurata, L. W., Pfaff, S. L. & Gill, G. N. (1998) The nuclear LIM domain interactor NLI mediates homo- and heterodimerization of LIM domain transcription factors, *J Biol Chem*. **273**, 3152-7.
 55. Love, P. E., Warzecha, C. & Li, L. (2014) Ldb1 complexes: the new master regulators of erythroid gene transcription, *Trends Genet*. **30**, 1-9.
 56. Liu, G. & Dean, A. (2019) Enhancer long-range contacts: The multi-adaptor protein LDB1 is the tie that binds, *Biochim Biophys Acta Gene Regul Mech*. **1862**, 625-633.
 57. Morceau, F., Schnekenburger, M., Dicato, M. & Diederich, M. (2004) GATA-1: friends, brothers, and coworkers, *Ann N Y Acad Sci*. **1030**, 537-54.
 58. Kang, Y., Kim, Y. W., Yun, J., Shin, J. & Kim, A. (2015) KLF1 stabilizes GATA-1 and TAL1 occupancy in the human beta-globin locus, *Biochim Biophys Acta*. **1849**, 282-9.
 59. Merika, M. & Orkin, S. H. (1995) Functional synergy and physical interactions of the erythroid transcription factor GATA-1 with the Kruppel family proteins Sp1 and EKLF, *Mol Cell Biol*. **15**, 2437-47.
 60. Kuo, Y. Y. & Chang, Z. F. (2007) GATA-1 and Gfi-1B interplay to regulate Bcl-xL transcription, *Mol Cell Biol*. **27**, 4261-72.
 61. Kadam, S. & Emerson, B. M. (2003) Transcriptional specificity of human SWI/SNF BRG1 and BRM chromatin remodeling complexes, *Mol Cell*. **11**, 377-89.
 62. Kadri, Z., Shimizu, R., Ohneda, O., Maouche-Chretien, L., Gisselbrecht, S., Yamamoto, M., Romeo, P. H., Leboulch, P. & Chretien, S. (2009) Direct binding of pRb/E2F-2 to GATA-1 regulates maturation and terminal cell division during erythropoiesis, *PLoS Biol*. **7**, e1000123.
 63. Blobel, G. A., Nakajima, T., Eckner, R., Montminy, M. & Orkin, S. H. (1998) CREB-binding protein cooperates with transcription factor GATA-1 and is required for erythroid differentiation, *Proc Natl Acad Sci U S A*. **95**, 2061-6.

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
64. Hung, H. L., Lau, J., Kim, A. Y., Weiss, M. J. & Blobel, G. A. (1999) CREB-Binding protein acetylates hematopoietic transcription factor GATA-1 at functionally important sites, *Mol Cell Biol.* **19**, 3496-505.
 65. Zon, L. I., Tsai, S. F., Burgess, S., Matsudaira, P., Bruns, G. A. & Orkin, S. H. (1990) The major human erythroid DNA-binding protein (GF-1): primary sequence and localization of the gene to the X chromosome, *Proc Natl Acad Sci U S A.* **87**, 668-72.
 66. Shimizu, R. & Yamamoto, M. (2005) Gene expression regulation and domain function of hematopoietic GATA factors, *Semin Cell Dev Biol.* **16**, 129-36.
 67. McDevitt, M. A., Fujiwara, Y., Shivdasani, R. A. & Orkin, S. H. (1997) An upstream, DNase I hypersensitive region of the hematopoietic-expressed transcription factor GATA-1 gene confers developmental specificity in transgenic mice, *Proc Natl Acad Sci U S A.* **94**, 7976-81.
 68. Valverde-Garduno, V., Guyot, B., Anguita, E., Hamlett, I., Porcher, C. & Vyas, P. (2004) Differences in the chromatin structure and cis-element organization of the human and mouse GATA1 loci: implications for cis-element identification, *Blood.* **104**, 3106-16.
 69. Nishimura, S., Takahashi, S., Kuroha, T., Suwabe, N., Nagasawa, T., Trainor, C. & Yamamoto, M. (2000) A GATA box in the GATA-1 gene hematopoietic enhancer is a critical element in the network of GATA factors and sites that regulate this gene, *Mol Cell Biol.* **20**, 713-23.
 70. Vyas, P., McDevitt, M. A., Cantor, A. B., Katz, S. G., Fujiwara, Y. & Orkin, S. H. (1999) Different sequence requirements for expression in erythroid and megakaryocytic cells within a regulatory element upstream of the GATA-1 gene, *Development.* **126**, 2799-811.
 71. Guyot, B., Valverde-Garduno, V., Porcher, C. & Vyas, P. (2004) Deletion of the major GATA1 enhancer HS 1 does not affect eosinophil GATA1 expression and eosinophil differentiation, *Blood.* **104**, 89-91.
 72. Yamamoto, M., Takahashi, S., Onodera, K., Muraosa, Y. & Engel, J. D. (1997) Upstream and downstream of erythroid transcription factor GATA-1, *Genes Cells.* **2**, 107-15.
 73. Kobayashi, M. & Yamamoto, M. (2007) Regulation of GATA1 gene expression, *J Biochem.* **142**, 1-10.
 74. Deindl, P., Klar, M., Drews, D., Cremer, M., Gammella, E., Gassmann, M. & Dame, C. (2014) Mice over-expressing human erythropoietin indicate that erythropoietin enhances expression of its receptor via up-regulated Gata1 and Tal1, *Haematologica.* **99**, e205-7.
 75. Zhao, W., Kitidis, C., Fleming, M. D., Lodish, H. F. & Ghaffari, S. (2006) Erythropoietin stimulates phosphorylation and activation of GATA-1 via the PI3-kinase/AKT signaling pathway, *Blood.* **107**, 907-15.
 76. Zon, L. I., Youssoufian, H., Mather, C., Lodish, H. F. & Orkin, S. H. (1991) Activation of the erythropoietin receptor promoter by transcription factor GATA-1, *Proc Natl Acad Sci U S A.* **88**, 10638-41.
 77. Leonard, M., Brice, M., Engel, J. D. & Papayannopoulou, T. (1993) Dynamics of GATA transcription factor expression during erythroid differentiation, *Blood.* **82**, 1071-9.
 78. Suzuki, N., Suwabe, N., Ohneda, O., Obara, N., Imagawa, S., Pan, X., Motohashi, H. & Yamamoto, M. (2003) Identification and characterization of 2 types of erythroid progenitors that express GATA-1 at distinct levels, *Blood.* **102**, 3575-83.
 79. Gutierrez, L., Lindeboom, F., Ferreira, R., Drissen, R., Grosveld, F., Whyatt, D. & Philipsen, S. (2005) A hanging drop culture method to study terminal erythroid differentiation, *Exp Hematol.* **33**, 1083-91.
 80. Khajuria, R. K., Munschauer, M., Ulirsch, J. C., Fiorini, C., Ludwig, L. S., McFarland, S. K., Abdulhay, N. J., Specht, H., Keshishian, H., Mani, D. R., Jovanovic, M., Ellis, S. R., Fulco, C. P., Engreitz, J. M., Schutz, S., Lian, J., Gripp, K. W., Weinberg, O. K., Pinkus, G. S., Gehrke, L., Regev, A., Lander, E. S., Gazda, H. T., Lee, W. Y., Panse, V. G., Carr, S. A. & Sankaran, V. G. (2018) Ribosome Levels Selectively Regulate Translation and Lineage Commitment in Human Hematopoiesis, *Cell.* **173**, 90-103 e19.
 81. Pali, C. G., Cheng, Q., Gillespie, M. A., Shannon, P., Mazurczyk, M., Napolitani, G., Price, N. D., Ranish, J. A., Morrissey, E., Higgs, D. R. & Brand, M. (2019) Single-Cell Proteomics Reveal that Quantitative Changes in Co-expressed Lineage-Specific Transcription Factors Determine Cell Fate, *Cell Stem Cell.* **24**, 812-820 e5.
 82. De Maria, R., Zeuner, A., Eramo, A., Domenichelli, C., Bonci, D., Grignani, F., Srinivasula, S. M., Alnemri, E. S., Testa, U. & Peschle, C. (1999) Negative regulation of erythropoiesis by caspase-mediated cleavage of GATA-1, *Nature.* **401**, 489-93.
 83. Ribeil, J. A., Zermati, Y., Vandekerckhove, J., Cathelin, S., Kersual, J., Dussiot, M., Coulon, S., Moura, I. C., Zeuner, A., Kirkegaard-Sorensen, T., Varet, B., Solary, E., Garrido, C. &

- Hermine, O. (2007) Hsp70 regulates erythropoiesis by preventing caspase-3-mediated cleavage of GATA-1, *Nature*. **445**, 102-5.
84. Ludwig, L. S., Gazda, H. T., Eng, J. C., Eichhorn, S. W., Thiru, P., Ghazvinian, R., George, T. I., Gotlib, J. R., Beggs, A. H., Sieff, C. A., Lodish, H. F., Lander, E. S. & Sankaran, V. G. (2014) Altered translation of GATA1 in Diamond-Blackfan anemia, *Nat Med*. **20**, 748-53.
85. Crispino, J. D. (2005) GATA1 in normal and malignant hematopoiesis, *Semin Cell Dev Biol*. **16**, 137-47.
86. Martin, D. I. & Orkin, S. H. (1990) Transcriptional activation and DNA binding by the erythroid factor GF-1/NF-E1/Eryf 1, *Genes Dev*. **4**, 1886-98.
87. Chennupati, V., Veiga, D. F., Maslowski, K. M., Andina, N., Tardivel, A., Yu, E. C., Stilianovic, M., Simillion, C., Duchosal, M. A., Quadroni, M., Roberts, I., Sankaran, V. G., MacDonald, H. R., Fasel, N., Angelillo-Scherrer, A., Schneider, P., Hoang, T. & Allam, R. (2018) Ribonuclease inhibitor 1 regulates erythropoiesis by controlling GATA1 translation, *J Clin Invest*. **128**, 1597-1614.
88. Chan, J., Hu, X., Wang, C. & Xu, Q. (2018) miRNA-152 targets GATA1 to regulate erythropoiesis in *Chionodraco hamatus*, *Biochem Biophys Res Commun*. **501**, 711-717.
89. Kim, M., Civin, C. I. & Kingsbury, T. J. (2019) MicroRNAs as regulators and effectors of hematopoietic transcription factors, *Wiley Interdiscip Rev RNA*. **10**, e1537.
90. Lessard, S., Beaudoin, M., Orkin, S. H., Bauer, D. E. & Lettre, G. (2018) 14q32 and let-7 microRNAs regulate transcriptional networks in fetal and adult human erythroblasts, *Hum Mol Genet*. **27**, 1411-1420.
91. Wang, F., Yu, J., Yang, G. H., Wang, X. S. & Zhang, J. W. (2011) Regulation of erythroid differentiation by miR-376a and its targets, *Cell Res*. **21**, 1196-209.
92. Crossley, M. & Orkin, S. H. (1994) Phosphorylation of the erythroid transcription factor GATA-1, *J Biol Chem*. **269**, 16589-96.
93. Hernandez-Hernandez, A., Ray, P., Litos, G., Ciro, M., Ottolenghi, S., Beug, H. & Boyes, J. (2006) Acetylation and MAPK phosphorylation cooperate to regulate the degradation of active GATA-1, *EMBO J*. **25**, 3264-74.
94. Yu, Y. L., Chiang, Y. J., Chen, Y. C., Papetti, M., Juo, C. G., Skoultchi, A. I. & Yen, J. J. (2005) MAPK-mediated phosphorylation of GATA-1 promotes Bcl-XL expression and cell survival, *J Biol Chem*. **280**, 29533-42.
95. Partington, G. A. & Patient, R. K. (1999) Phosphorylation of GATA-1 increases its DNA-binding affinity and is correlated with induction of human K562 erythroleukaemia cells, *Nucleic Acids Res*. **27**, 1168-75.
96. Omichinski, J. G., Trainor, C., Evans, T., Gronenborn, A. M., Clore, G. M. & Felsenfeld, G. (1993) A small single-"finger" peptide from the erythroid transcription factor GATA-1 binds specifically to DNA as a zinc or iron complex, *Proc Natl Acad Sci U S A*. **90**, 1676-80.
97. Towatari, M., Ciro, M., Ottolenghi, S., Tsuzuki, S. & Enver, T. (2004) Involvement of mitogen-activated protein kinase in the cytokine-regulated phosphorylation of transcription factor GATA-1, *Hematol J*. **5**, 262-72.
98. Rooke, H. M. & Orkin, S. H. (2006) Phosphorylation of Gata1 at serine residues 72, 142, and 310 is not essential for hematopoiesis in vivo, *Blood*. **107**, 3527-30.
99. Boyes, J., Byfield, P., Nakatani, Y. & Ogryzko, V. (1998) Regulation of activity of the transcription factor GATA-1 by acetylation, *Nature*. **396**, 594-8.
100. Boyes, J., Omichinski, J., Clark, D., Pikaart, M. & Felsenfeld, G. (1998) Perturbation of nucleosome structure by the erythroid transcription factor GATA-1, *J Mol Biol*. **279**, 529-44.
101. Lamonica, J. M., Vakoc, C. R. & Blobel, G. A. (2006) Acetylation of GATA-1 is required for chromatin occupancy, *Blood*. **108**, 3736-8.
102. Lamonica, J. M., Deng, W., Kadauke, S., Campbell, A. E., Gamsjaeger, R., Wang, H., Cheng, Y., Billin, A. N., Hardison, R. C., Mackay, J. P. & Blobel, G. A. (2011) Bromodomain protein Brd3 associates with acetylated GATA1 to promote its chromatin occupancy at erythroid target genes, *Proc Natl Acad Sci U S A*. **108**, E159-68.
103. de Thonel, A., Vandekerckhove, J., Lanneau, D., Selvakumar, S., Courtois, G., Hazoume, A., Brunet, M., Maurel, S., Hammann, A., Ribeil, J. A., Zermati, Y., Gabet, A. S., Boyes, J., Solary, E., Hermine, O. & Garrido, C. (2010) HSP27 controls GATA-1 protein level during erythroid cell differentiation, *Blood*. **116**, 85-96.
104. Lin, K. R., Li, C. L., Yen, J. J. & Yang-Yen, H. F. (2013) Constitutive phosphorylation of GATA-1 at serine(2)(6) attenuates the colony-forming activity of erythrocyte-committed progenitors, *PLoS One*. **8**, e64269.

- 1
2
3 105. Liang, L., Peng, Y., Zhang, J., Zhang, Y., Roy, M., Han, X., Xiao, X., Sun, S., Liu, H., Nie,
4 L., Kuang, Y., Zhu, Z., Deng, J., Xia, Y., Sankaran, V. G., Hillyer, C. D., Mohandas, N., Ye,
5 M., An, X. & Liu, J. (2019) Deubiquitylase USP7 regulates human terminal erythroid
6 differentiation by stabilizing GATA1, *Haematologica*.
- 7 106. Collavin, L., Gostissa, M., Avolio, F., Secco, P., Ronchi, A., Santoro, C. & Del Sal, G. (2004)
8 Modification of the erythroid transcription factor GATA-1 by SUMO-1, *Proc Natl Acad Sci U S*
9 *A.* **101**, 8870-5.
- 10 107. Lee, H. Y., Johnson, K. D., Fujiwara, T., Boyer, M. E., Kim, S. I. & Bresnick, E. H. (2009)
11 Controlling hematopoiesis through sumoylation-dependent regulation of a GATA factor, *Mol*
12 *Cell.* **36**, 984-95.
- 13 108. Yu, L., Ji, W., Zhang, H., Renda, M. J., He, Y., Lin, S., Cheng, E. C., Chen, H., Krause, D.
14 S. & Min, W. (2010) SENP1-mediated GATA1 deSUMOylation is critical for definitive
15 erythropoiesis, *J Exp Med.* **207**, 1183-95.
- 16 109. Han, X., Zhang, J., Peng, Y., Peng, M., Chen, X., Chen, H., Song, J., Hu, X., Ye, M., Li, J.,
17 Sankaran, V. G., Hillyer, C. D., Mohandas, N., An, X. & Liu, J. (2017) Unexpected role for
18 p19INK4d in posttranscriptional regulation of GATA1 and modulation of human terminal
19 erythropoiesis, *Blood.* **129**, 226-237.
- 20 110. Tyrkalska, S. D., Perez-Oliva, A. B., Rodriguez-Ruiz, L., Martinez-Morcillo, F. J., Alcaraz-
21 Perez, F., Martinez-Navarro, F. J., Lachaud, C., Ahmed, N., Schroeder, T., Pardo-Sanchez,
22 I., Candel, S., Lopez-Munoz, A., Choudhuri, A., Rossmann, M. P., Zon, L. I., Cayuela, M. L.,
23 Garcia-Moreno, D. & Mulero, V. (2019) Inflammasome Regulates Hematopoiesis through
24 Cleavage of the Master Erythroid Transcription Factor GATA1, *Immunity.* **51**, 50-63 e5.
- 25 111. Richardson, P. G., Sonneveld, P., Schuster, M. W., Irwin, D., Stadtmauer, E. A., Facon, T.,
26 Harousseau, J. L., Ben-Yehuda, D., Lonial, S., Goldschmidt, H., Reece, D., San-Miguel, J.
27 F., Blade, J., Boccadoro, M., Cavenagh, J., Dalton, W. S., Boral, A. L., Esseltine, D. L., Porter,
28 J. B., Schenkein, D., Anderson, K. C. & Assessment of Proteasome Inhibition for Extending
29 Remissions, I. (2005) Bortezomib or high-dose dexamethasone for relapsed multiple
30 myeloma, *N Engl J Med.* **352**, 2487-98.
- 31 112. Khandros, E., Thom, C. S., D'Souza, J. & Weiss, M. J. (2012) Integrated protein quality-
32 control pathways regulate free alpha-globin in murine beta-thalassemia, *Blood.* **119**, 5265-
33 75.
- 34 113. Nguyen, A. T., Prado, M. A., Schmidt, P. J., Sendamarai, A. K., Wilson-Grady, J. T., Min,
35 M., Campagna, D. R., Tian, G., Shi, Y., Dederer, V., Kawan, M., Kuehnle, N., Paulo, J. A.,
36 Yao, Y., Weiss, M. J., Justice, M. J., Gygi, S. P., Fleming, M. D. & Finley, D. (2017) UBE2O
37 remodels the proteome during terminal erythroid differentiation, *Science.* **357**.
- 38 114. Bergink, S., Theil, A. F., Toussaint, W., De Cuyper, I. M., Kulu, D. I., Clapes, T., van der
39 Linden, R., Demmers, J. A., Mul, E. P., van Alphen, F. P., Marteiijn, J. A., van Gent, T., Maas,
40 A., Robin, C., Philipsen, S., Vermeulen, W., Mitchell, J. R. & Gutierrez, L. (2013)
41 Erythropoietic defect associated with reduced cell proliferation in mice lacking the 26S
42 proteasome shuttling factor Rad23b, *Mol Cell Biol.* **33**, 3879-92.
- 43 115. Khandros, E. & Weiss, M. J. (2010) Protein quality control during erythropoiesis and
44 hemoglobin synthesis, *Hematol Oncol Clin North Am.* **24**, 1071-88.
- 45 116. Thom, C. S., Traxler, E. A., Khandros, E., Nickas, J. M., Zhou, O. Y., Lazarus, J. E., Silva,
46 A. P., Prabhu, D., Yao, Y., Aribéana, C., Fuchs, S. Y., Mackay, J. P., Holzbaur, E. L. & Weiss,
47 M. J. (2014) Trim58 degrades Dynein and regulates terminal erythropoiesis, *Dev Cell.* **30**,
48 688-700.
- 49 117. Whyatt, D., Lindeboom, F., Karis, A., Ferreira, R., Milot, E., Hendriks, R., de Bruijn, M.,
50 Langeveld, A., Gribnau, J., Grosveld, F. & Philipsen, S. (2000) An intrinsic but cell-
51 nonautonomous defect in GATA-1-overexpressing mouse erythroid cells, *Nature.* **406**, 519-
52 24.
- 53 118. Johnson, K. D., Kim, S. I. & Bresnick, E. H. (2006) Differential sensitivities of transcription
54 factor target genes underlie cell type-specific gene expression profiles, *Proc Natl Acad Sci U*
55 *S A.* **103**, 15939-44.
- 56 119. Takahashi, S., Onodera, K., Motohashi, H., Suwabe, N., Hayashi, N., Yanai, N., Nabesima,
57 Y. & Yamamoto, M. (1997) Arrest in primitive erythroid cell development caused by promoter-
58 specific disruption of the GATA-1 gene, *J Biol Chem.* **272**, 12611-5.
- 59 120. McDevitt, M. A., Shivdasani, R. A., Fujiwara, Y., Yang, H. & Orkin, S. H. (1997) A
60 "knockdown" mutation created by cis-element gene targeting reveals the dependence of
erythroid cell maturation on the level of transcription factor GATA-1, *Proc Natl Acad Sci U S*
A. **94**, 6781-5.

- 1
2
3 121. Pan, X., Ohneda, O., Ohneda, K., Lindeboom, F., Iwata, F., Shimizu, R., Nagano, M.,
4 Suwabe, N., Philipsen, S., Lim, K. C., Engel, J. D. & Yamamoto, M. (2005) Graded levels of
5 GATA-1 expression modulate survival, proliferation, and differentiation of erythroid
6 progenitors, *J Biol Chem.* **280**, 22385-94.
- 7 122. Shimizu, R., Kuroha, T., Ohneda, O., Pan, X., Ohneda, K., Takahashi, S., Philipsen, S. &
8 Yamamoto, M. (2004) Leukemogenesis caused by incapacitated GATA-1 function, *Mol Cell*
9 *Biol.* **24**, 10814-25.
- 10 123. Shimizu, R. & Yamamoto, M. (2012) Contribution of GATA1 dysfunction to multi-step
11 leukemogenesis, *Cancer Sci.* **103**, 2039-44.
- 12 124. Shimizu, R., Engel, J. D. & Yamamoto, M. (2008) GATA1-related leukaemias, *Nat Rev*
13 *Cancer.* **8**, 279-87.
- 14 125. Ling, T., Crispino, J. D., Zingariello, M., Martelli, F. & Migliaccio, A. R. (2018) GATA1
15 insufficiencies in primary myelofibrosis and other hematopoietic disorders: consequences for
16 therapy, *Expert Rev Hematol.* **11**, 169-184.
- 17 126. Vannucchi, A. M., Bianchi, L., Cellai, C., Paoletti, F., Rana, R. A., Lorenzini, R., Migliaccio,
18 G. & Migliaccio, A. R. (2002) Development of myelofibrosis in mice genetically impaired for
19 GATA-1 expression (GATA-1(low) mice), *Blood.* **100**, 1123-32.
- 20 127. Migliaccio, A. R., Rana, R. A., Sanchez, M., Lorenzini, R., Centurione, L., Bianchi, L.,
21 Vannucchi, A. M., Migliaccio, G. & Orkin, S. H. (2003) GATA-1 as a regulator of mast cell
22 differentiation revealed by the phenotype of the GATA-1low mouse mutant, *J Exp Med.* **197**,
23 281-96.
- 24 128. Socolovsky, M. (2013) Exploring the erythroblastic island, *Nat Med.* **19**, 399-401.
- 25 129. Yeo, J. H., Lam, Y. W. & Fraser, S. T. (2019) Cellular dynamics of mammalian red blood
26 cell production in the erythroblastic island niche, *Biophys Rev.*
- 27 130. McGrath, K. E., Kingsley, P. D., Koniski, A. D., Porter, R. L., Bushnell, T. P. & Palis, J.
28 (2008) Eucleation of primitive erythroid cells generates a transient population of
29 "pyrenocytes" in the mammalian fetus, *Blood.* **111**, 2409-17.
- 30 131. Mohandas, N. & Chasis, J. A. (2010) The erythroid niche: molecular processes occurring
31 within erythroblastic islands, *Transfus Clin Biol.* **17**, 110-1.
- 32 132. Chasis, J. A. & Mohandas, N. (2008) Erythroblastic islands: niches for erythropoiesis, *Blood.*
33 **112**, 470-8.
- 34 133. Gutierrez, L., Lindeboom, F., Langeveld, A., Grosveld, F., Philipsen, S. & Whyatt, D. (2004)
35 Homotypic signalling regulates Gata1 activity in the erythroblastic island, *Development.* **131**,
36 3183-93.
- 37 134. Graf, T. (2002) Differentiation plasticity of hematopoietic cells, *Blood.* **99**, 3089-101.
- 38 135. Iwasaki, H., Mizuno, S., Wells, R. A., Cantor, A. B., Watanabe, S. & Akashi, K. (2003) GATA-
39 1 converts lymphoid and myelomonocytic progenitors into the megakaryocyte/erythrocyte
40 lineages, *Immunity.* **19**, 451-62.
- 41 136. Kulesa, H., Frampton, J. & Graf, T. (1995) GATA-1 reprograms avian myelomonocytic cell
42 lines into eosinophils, thromboblats, and erythroblasts, *Genes Dev.* **9**, 1250-62.
- 43 137. Hirasawa, R., Shimizu, R., Takahashi, S., Osawa, M., Takayanagi, S., Kato, Y., Onodera,
44 M., Minegishi, N., Yamamoto, M., Fukao, K., Taniguchi, H., Nakauchi, H. & Iwama, A. (2002)
45 Essential and instructive roles of GATA factors in eosinophil development, *J Exp Med.* **195**,
46 1379-86.
- 47 138. Valent, P., Busche, G., Theurl, I., Uras, I. Z., Germing, U., Stauder, R., Sotlar, K., Fureder,
48 W., Bettelheim, P., Pfeilstocker, M., Oberbauer, R., Sperr, W. R., Geissler, K., Schwaller, J.,
49 Moriggl, R., Bene, M. C., Jager, U., Horny, H. P. & Hermine, O. (2018) Normal and
50 pathological erythropoiesis in adults: from gene regulation to targeted treatment concepts,
51 *Haematologica.* **103**, 1593-1603.
- 52 139. Barbarani, G., Fugazza, C., Strouboulis, J. & Ronchi, A. E. (2019) The Pleiotropic Effects
53 of GATA1 and KLF1 in Physiological Erythropoiesis and in Dyserythropoietic Disorders, *Front*
54 *Physiol.* **10**, 91.
- 55 140. Crispino, J. D. & Horwitz, M. S. (2017) GATA factor mutations in hematologic disease,
56 *Blood.* **129**, 2103-2110.
- 57 141. Frisan, E., Vandekerckhove, J., de Thonel, A., Pierre-Eugene, C., Sternberg, A., Arlet, J.
58 B., Floquet, C., Gyan, E., Kosmider, O., Dreyfus, F., Gabet, A. S., Courtois, G., Vyas, P.,
59 Ribeil, J. A., Zermati, Y., Lacombe, C., Mayeux, P., Solary, E., Garrido, C., Hermine, O. &
60 Fontenay, M. (2012) Defective nuclear localization of Hsp70 is associated with
dyserythropoiesis and GATA-1 cleavage in myelodysplastic syndromes, *Blood.* **119**, 1532-
1542.

- 1
2
3 142. Ellis, S. R. & Lipton, J. M. (2008) Diamond Blackfan anemia: a disorder of red blood cell
4 development, *Curr Top Dev Biol.* **82**, 217-41.
- 5 143. Ulirsch, J. C., Verboon, J. M., Kazerounian, S., Guo, M. H., Yuan, D., Ludwig, L. S.,
6 Handsaker, R. E., Abdulhay, N. J., Fiorini, C., Genovese, G., Lim, E. T., Cheng, A.,
7 Cummings, B. B., Chao, K. R., Beggs, A. H., Genetti, C. A., Sieff, C. A., Newburger, P. E.,
8 Niewiadomska, E., Matysiak, M., Vlachos, A., Lipton, J. M., Atsidaftos, E., Glader, B., Narla,
9 A., Gleizes, P. E., O'Donohue, M. F., Montel-Lehry, N., Amor, D. J., McCarroll, S. A.,
10 O'Donnell-Luria, A. H., Gupta, N., Gabriel, S. B., MacArthur, D. G., Lander, E. S., Lek, M., Da
11 Costa, L., Nathan, D. G., Korostelev, A. A., Do, R., Sankaran, V. G. & Gazda, H. T. (2018)
12 The Genetic Landscape of Diamond-Blackfan Anemia, *Am J Hum Genet.* **103**, 930-947.
- 13 144. Boria, I., Garelli, E., Gazda, H. T., Aspesi, A., Quarello, P., Pavesi, E., Ferrante, D.,
14 Meerpohl, J. J., Kartal, M., Da Costa, L., Proust, A., Leblanc, T., Simansour, M., Dahl, N.,
15 Frojmark, A. S., Pospisilova, D., Cmejla, R., Beggs, A. H., Sheen, M. R., Landowski, M.,
16 Buros, C. M., Clinton, C. M., Dobson, L. J., Vlachos, A., Atsidaftos, E., Lipton, J. M., Ellis, S.
17 R., Ramenghi, U. & Dianzani, I. (2010) The ribosomal basis of Diamond-Blackfan Anemia:
18 mutation and database update, *Hum Mutat.* **31**, 1269-79.
- 19 145. Sankaran, V. G., Ghazvinian, R., Do, R., Thiru, P., Vergilio, J. A., Beggs, A. H., Sieff, C. A.,
20 Orkin, S. H., Nathan, D. G., Lander, E. S. & Gazda, H. T. (2012) Exome sequencing identifies
21 GATA1 mutations resulting in Diamond-Blackfan anemia, *J Clin Invest.* **122**, 2439-43.
- 22 146. Klar, J., Khalfallah, A., Arzoo, P. S., Gazda, H. T. & Dahl, N. (2014) Recurrent GATA1
23 mutations in Diamond-Blackfan anaemia, *Br J Haematol.* **166**, 949-51.
- 24 147. Parrella, S., Aspesi, A., Quarello, P., Garelli, E., Pavesi, E., Carando, A., Nardi, M., Ellis, S.
25 R., Ramenghi, U. & Dianzani, I. (2014) Loss of GATA-1 full length as a cause of Diamond-
26 Blackfan anemia phenotype, *Pediatr Blood Cancer.* **61**, 1319-21.
- 27 148. Chlon, T. M., McNulty, M., Goldenson, B., Rosinski, A. & Crispino, J. D. (2015) Global
28 transcriptome and chromatin occupancy analysis reveal the short isoform of GATA1 is
29 deficient for erythroid specification and gene expression, *Haematologica.* **100**, 575-84.
- 30 149. Hollanda, L. M., Lima, C. S., Cunha, A. F., Albuquerque, D. M., Vassallo, J., Ozelo, M. C.,
31 Joazeiro, P. P., Saad, S. T. & Costa, F. F. (2006) An inherited mutation leading to production
32 of only the short isoform of GATA-1 is associated with impaired erythropoiesis, *Nat Genet.*
33 **38**, 807-12.
- 34 150. Abdulhay, N. J., Fiorini, C., Verboon, J. M., Ludwig, L. S., Ulirsch, J. C., Zieger, B., Lareau,
35 C. A., Mi, X., Roy, A., Obeng, E. A., Erlacher, M., Gupta, N., Gabriel, S. B., Ebert, B. L.,
36 Niemeyer, C. M., Khoriaty, R. N., Ancliff, P., Gazda, H. T., Wlodarski, M. W. & Sankaran, V.
37 G. (2019) Impaired human hematopoiesis due to a cryptic intronic GATA1 splicing mutation,
38 *J Exp Med.* **216**, 1050-1060.
- 39 151. Gilles, L., Arslan, A. D., Marinaccio, C., Wen, Q. J., Arya, P., McNulty, M., Yang, Q., Zhao,
40 J. C., Konstantinoff, K., Lasho, T., Pardanani, A., Stein, B., Plo, I., Sundaravel, S., Wickrema,
41 A., Migliaccio, A., Gurbuxani, S., Vainchenker, W., Plataniias, L. C., Tefferi, A. & Crispino, J.
42 D. (2017) Downregulation of GATA1 drives impaired hematopoiesis in primary myelofibrosis,
43 *J Clin Invest.* **127**, 1316-1320.
- 44 152. Doty, R. T., Yan, X., Lausted, C., Munday, A. D., Yang, Z., Yi, D., Jabbari, N., Liu, L., Keel,
45 S. B., Tian, Q. & Abkowitz, J. L. (2019) Single-cell analyses demonstrate that a heme-GATA1
46 feedback loop regulates red cell differentiation, *Blood.* **133**, 457-469.
- 47 153. Gastou, M., Rio, S., Dussiot, M., Karboul, N., Moniz, H., Leblanc, T., Sevin, M., Gonin, P.,
48 Larghero, J., Garrido, C., Narla, A., Mohandas, N., Vainchenker, W., Hermine, O., Solary, E.,
49 Da Costa, L., French Society of, H., French Society of, I. & Hematology (2017) The severe
50 phenotype of Diamond-Blackfan anemia is modulated by heat shock protein 70, *Blood Adv.*
51 **1**, 1959-1976.
- 52 154. Rio, S., Gastou, M., Karboul, N., Derman, R., Suriyun, T., Manceau, H., Leblanc, T., El
53 Benna, J., Schmitt, C., Azouzi, S., Larghero, J., Karim, Z., Macias-Garcia, A., Chen, J. J.,
54 Hermine, O., Courtois, G., Puy, H., Gouya, L., Mohandas, N. & Da Costa, L. (2019)
55 Regulation of globin-heme balance in Diamond-Blackfan anemia by HSP70/GATA1, *Blood.*
56 **133**, 1358-1370.
- 57 155. Arlet, J. B., Ribeil, J. A., Guillem, F., Negre, O., Hazoume, A., Marcion, G., Beuzard, Y.,
58 Dussiot, M., Moura, I. C., Demarest, S., de Beauchene, I. C., Belaid-Choucair, Z., Sevin, M.,
59 Maciel, T. T., Auclair, C., Leboulch, P., Chretien, S., Tchertanov, L., Baudin-Creuz, V.,
60 Seigneure, R., Fontenay, M., Garrido, C., Hermine, O. & Courtois, G. (2014) HSP70
61 sequestration by free alpha-globin promotes ineffective erythropoiesis in beta-thalassaemia,
62 *Nature.* **514**, 242-6.

- 1
2
3 156. Bhatnagar, N., Nizery, L., Tunstall, O., Vyas, P. & Roberts, I. (2016) Transient Abnormal
4 Myelopoiesis and AML in Down Syndrome: an Update, *Curr Hematol Malig Rep.* **11**, 333-41.
5 157. Maroz, A., Stachorski, L., Emmrich, S., Reinhardt, K., Xu, J., Shao, Z., Kabler, S., Dertmann,
6 T., Hitzler, J., Roberts, I., Vyas, P., Juban, G., Hennig, C., Hansen, G., Li, Z., Orkin, S.,
7 Reinhardt, D. & Klusmann, J. H. (2014) GATA1s induces hyperproliferation of eosinophil
8 precursors in Down syndrome transient leukemia, *Leukemia.* **28**, 1259-70.
9 158. Bibikova, E., Youn, M. Y., Danilova, N., Ono-Uruga, Y., Konto-Ghiorghi, Y., Ochoa, R.,
10 Narla, A., Glader, B., Lin, S. & Sakamoto, K. M. (2014) TNF-mediated inflammation represses
11 GATA1 and activates p38 MAP kinase in RPS19-deficient hematopoietic progenitors, *Blood.*
12 **124**, 3791-8.
13 159. Libregts, S. F., Gutierrez, L., de Bruin, A. M., Wensveen, F. M., Papadopoulos, P., van
14 Ijcken, W., Ozgur, Z., Philipsen, S. & Nolte, M. A. (2011) Chronic IFN-gamma production in
15 mice induces anemia by reducing erythrocyte life span and inhibiting erythropoiesis through
16 an IRF-1/PU.1 axis, *Blood.* **118**, 2578-88.
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

Figure Legends:

Figure 1: GATA1 protein levels during murine erythroid differentiation

Intracellular flow cytometry analysis of GATA1 protein expression in mouse fetal and adult tissues. In brief, one million cells were stained with Ter119-PE antibody (fetal tissues) or a cocktail of KIT-PECy7/CD71-APC/Ter119-PE (adult tissues), and subsequently fixed and permeabilized using BD Cytofix/Cytoperm kit (BD Biosciences), after which they were incubated with anti-GATA1-N6 antibody (1:100) followed by anti-rat-FITC antibody (1:100) with a wash step after each incubation.

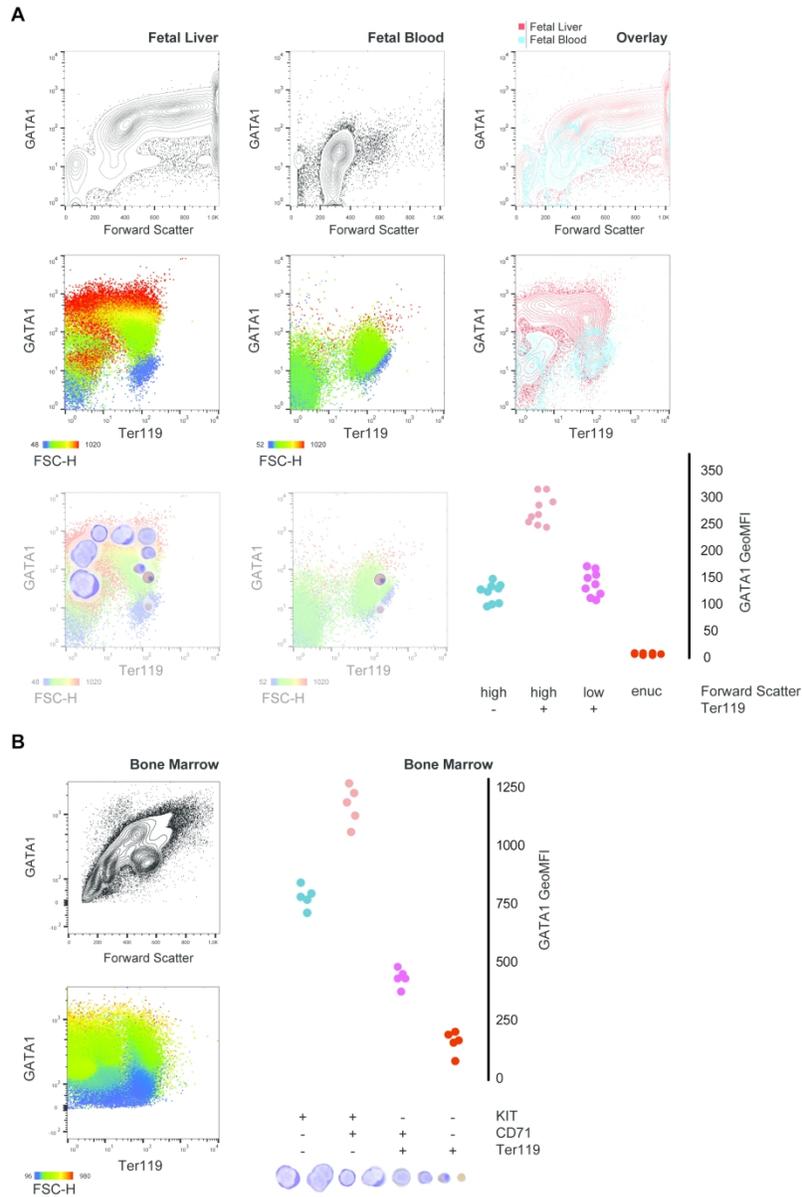
A) GATA1 protein levels were assessed using intracellular flow cytometry in murine fetal liver and fetal blood at E13.5. Representative data shown. In the top row, dot plots are depicted representing GATA1 expression level (MFI) against the Forward Scatter in fetal liver and fetal blood samples of the same mouse. In the mid row, dot plots are depicted of GATA1 levels (MFI) against Ter119 surface expression, including the Forward Scatter variable as a Heatmap Statistic colorcode in fetal liver and fetal blood samples of the same mouse. The plots on the right depict the overlay of respective fetal liver (red) and fetal blood (blue) samples on both rows. The bottom row is a composite figure, where representative pictures from cytopins of erythroid differentiating cells *in vitro* in hanging drop assays [79] and fetal blood nucleated and enucleated erythroid cells [133], are overlaid on the mid-row plots, as an indication of how differentiation takes place, with the remark that these cells do not correspond to prospectively isolated cells from the same samples. On the bottom right, a graph depicting GATA1 expression levels (GeoMFI) in successive stages of differentiation in fetal liver samples from 9 mice, based on Forward Scatter and Ter119 expression. "Enuc" means enucleated.

B) GATA1 protein levels were assessed using intracellular flow cytometry in murine bone marrow cells (data provided by Dr. Martijn Nolte). On the left, dot plots are depicted representing GATA1 expression level (MFI) against the Forward Scatter (top), and against Ter119 surface expression, including the Forward Scatter variable as a Heatmap

1
2
3 **Statistic colorcode (bottom)**. The graph represents GATA1 expression levels (GeoMFI)
4
5 in successive stages of erythroid maturation based on surface marker expression, in 5
6
7 mice. The populations are from immature to mature, as follows: KIT⁺CD71⁻Ter119⁻
8
9 (which includes other hematopoietic progenitors than erythroid), KIT⁺CD71⁺Ter119⁻, KIT⁻
10
11 CD71⁺Ter119⁺ **and** KIT⁻CD71⁻Ter119⁺. The picture of maturing erythroid cells, is a
12
13 composite of differentiating cells in hanging drop assays *in vitro* [79], and is used to
14
15 exemplify how erythroid differentiation takes place, **from left to right**, following the order
16
17 of gated populations in the bone marrow.
18
19
20
21

22 **Figure 2: Model of GATA1 protein level regulation during erythroid maturation**

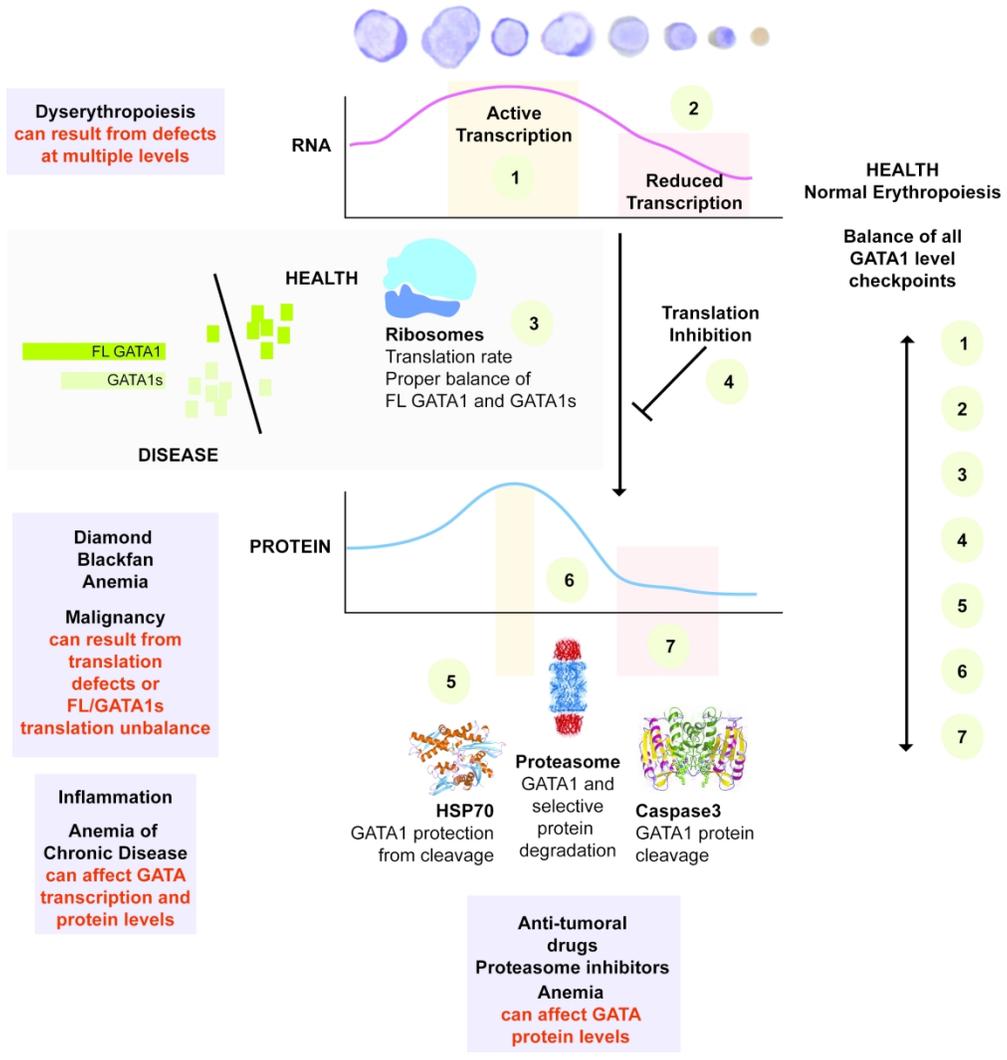
23
24 Summary of the different mechanisms regulating GATA1 protein abundance, which lead
25
26 to disease when deregulated.
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



GATA1 protein levels during murine erythroid differentiation

198x299mm (300 x 300 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



Model of GATA1 protein level regulation during erythroid maturation

228x241mm (300 x 300 DPI)