

Supplementary data

Details on Figure 1. Expression of key transcription factors during erythropoiesis.

GATA-2 is not expressed in ES cells (RT-PCR, Elefanty *et al*, 1997, and immunofluorescence (IF) data not shown), but is necessary for hemopoietic stem cells (HSC) to develop (Tsai *et al*, 1994) and is expressed in common myeloid progenitors including FDCP-mix cells (Northern blot, Nb, Cross *et al*, 1994; flow cytometry, Fc, and RT-PCR, Suzuki *et al*, 2003; IF, this work, see Fig 3). Later on, GATA-2 decreases in CFU-E (Fc, and RT-PCR, Suzuki *et al*, 2003) and is very low in uninduced MEL cells (IF and nuclease protection assay (npa) Figure 3 and data not shown), disappearing in proerythroblasts (Fc, Suzuki *et al*, 2003; IF, data not shown). GATA-1 is also negative in ES cells (RT-PCR, Elefanty *et al*, 1997, and IF, data not shown), but is detectable at low levels in FDCP-mix cells (Nb, Cross *et al*, 1994; RT-PCR, Hu *et al*, 1997; IF, Figure 3), increasing in CFU-E (Fc, and RT-PCR, Suzuki *et al*, 2003) and MEL cells (IF, Elefanty *et al*, 1996, and Figure 3). After MEL cell induction GATA-1 initially increases then decreases (IF, Elefanty *et al*, 1996, and Figure 3). Consistently, GATA-1 is present at very low levels in mature erythroid cells (Fc, Suzuki *et al*, 2003; Western blot, Wb, Dolznig *et al*, 2001). This step seems to be important for terminal maturation of erythrocytes (Mouthon *et al*, 1993 and data not shown).

GATA-1's cofactor FOG-1 has been reported at high levels in common myeloid progenitors and to follow GATA-1 expression in erythroid cells (Nb, Tsang *et al*, 1997). We have observed an increase in the expression of FOG-1 following induction of MEL cells (IF, data not shown).

SCL is not present in ES cells (RT-PCR, Elefanty *et al*, 1997; IF, data not shown), but is required for HSC to develop and is present in FDCP-mix cells (Nb, Cross *et al*, 1994; IF, data

not shown). In MEL cells, SCL increases after induction (Wb, Xu *et al*, 2003; npa, Figure 3; IF, data not shown), but the level decreases in late erythroid cells after GATA-1 (Wb, Xu *et al*, 2003; Wb, Dolznig *et al*, 2001). The p45 subunit of NF-E2 is absent from ES cells (Nb, Andrews *et al*, 1993a; RT-PCR, Elefanty *et al*, 1997). It can be detected in common myeloid progenitors including FDCP-mix cells (Nb, Andrews *et al*, 1993a; RT-PCR, Hu *et al*, 1997), but when these cells differentiate into erythroid colonies *in vitro*, the levels drop but increase again in the late stages of differentiation (cDNA Microarray, Bruno *et al*, 2004). In MEL cells, p45 is expressed (Nb, Andrews *et al*, 1993a; Andrews *et al*, 1993b; IF, Francastel *et al*, 2001) and increases with differentiation (nuclease protection assay Figure 3), as in erythroblasts (RT-PCR on TER119+ cells, Igarashi *et al*, 1995), until the very late stages when it starts to decrease after SCL (Wb, Dolznig *et al*, 2001). The p18 subunit of NF-E2 is expressed at similar levels in ES cells, uninduced and induced MEL cells (IF, data not shown; IF, Francastel *et al*, 2001), whilst, FDCP-Mix have a higher level of p18 (IF, data not shown). It is also weakly expressed in HSC (RT-PCR on Kit+/,Sca+/Lin- bone marrow cells, Igarashi *et al*, 1995), compared with TER119+ erythroblasts (RT-PCR, Igarashi *et al*, 1995).

Andrews NC, Erdjument-Bromage H, Davidson MB, Tempst P and Orkin SH (1993a)
Erythroid transcription factor NF-E2 is a haematopoietic-specific basic-leucine zipper protein. *Nature* **362**: 722-728

Andrews NC, Kotkow KJ, Ney PA, Erdjument-Bromage H, Tempst P and Orkin SH (1993b)
The ubiquitous subunit of erythroid transcription factor NF-E2 is a small basic-leucine zipper protein related to the v-maf oncogene. *Proc Natl Acad Sci U S A* **90**: 11488-11492

Bruno L, Hoffmann R, McBlane F, Brown J, Gupta R, Joshi C, Pearson S, Seidl T, Heyworth C and Enver T (2004) Molecular signatures of self-renewal, differentiation, and lineage choice in multipotential hemopoietic progenitor cells *in vitro*. *Mol Cell Biol* **24**: 741-756

- Cross MA, Heyworth CM, Murrell AM, Bockamp EO, Dexter TM and Green AR (1994) Expression of lineage restricted transcription factors precedes lineage specific differentiation in a multipotent haemopoietic progenitor cell line. *Oncogene* **9**: 3013-3016
- Dolznic H, Boulme F, Stangl K, Deiner EM, Mikulits W, Beug H and Mullner EW (2001) Establishment of normal, terminally differentiating mouse erythroid progenitors: molecular characterization by cDNA arrays. *Faseb J* **15**: 1442-1444
- Elefanti AG, Antoniou M, Custodio N, Carmo-Fonseca M and Grosveld FG (1996) GATA transcription factors associate with a novel class of nuclear bodies in erythroblasts and megakaryocytes. *Embo J* **15**: 319-333
- Elefanti AG, Robb L, Birner R and Begley CG (1997) Hematopoietic-specific genes are not induced during in vitro differentiation of scl-null embryonic stem cells. *Blood* **90**: 1435-1447
- Francastel C, Magis W and Groudine M (2001) Nuclear relocation of a transactivator subunit precedes target gene activation. *Proc Natl Acad Sci U S A* **98**: 12120-12125
- Hu M, Krause D, Greaves M, Sharkis S, Dexter M, Heyworth C and Enver T (1997) Multilineage gene expression precedes commitment in the hemopoietic system. *Genes Dev* **11**: 774-785
- Igarashi K, Itoh K, Motohashi H, Hayashi N, Matuzaki Y, Nakauchi H, Nishizawa M and Yamamoto M (1995) Activity and expression of murine small Maf family protein MafK. *J Biol Chem* **270**: 7615-7624
- Mouthon MA, Bernard O, Mitjavila MT, Romeo PH, Vainchenker W and Mathieu-Mahul D (1993) Expression of tal-1 and GATA-binding proteins during human hematopoiesis. *Blood* **81**: 647-655
- Suzuki N, Suwabe N, Ohneda O, Obara N, Imagawa S, Pan X, Motohashi H and Yamamoto M (2003) Identification and characterization of 2 types of erythroid progenitors that express GATA-1 at distinct levels. *Blood* **102**: 3575-3583

- Tsai FY, Keller G, Kuo FC, Weiss M, Chen J, Rosenblatt M, Alt FW and Orkin SH (1994)
An early haematopoietic defect in mice lacking the transcription factor GATA-2.
Nature **371**: 221-226
- Tsang AP, Visvader JE, Turner CA, Fujiwara Y, Yu C, Weiss MJ, Crossley M and Orkin SH
(1997) FOG, a multitype zinc finger protein, acts as a cofactor for transcription factor
GATA-1 in erythroid and megakaryocytic differentiation. *Cell* **90**: 109-119
- Xu Z, Huang S, Chang LS, Agulnick AD and Brandt SJ (2003) Identification of a TAL1
target gene reveals a positive role for the LIM domain-binding protein Ldb1 in
erythroid gene expression and differentiation. *Mol Cell Biol* **23**: 7585-7599