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).

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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


