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Self-Regulation of Mouse p45/NF-E2 during Murine Erythroleukemia Cell Differentiation

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Tung-Liang Lee, Shau-Ching Wen, Wei-Yuan Hsiao, Che-Kun James Shen, and Yu-Chiau Shyu (2009) Self-regulation of mouse p45/NF-E2 during murine erythroleukemia cell differentiation. *Zoological Studies* **48**(3): 362-369. The complicated process of murine erythroleukemia (MEL) cell differentiation is precisely controlled by a group of transcription factors. One of those transcription factors, p45/NF-E2, is important for globin gene expression. We analyzed the structure of the mouse *p45* gene which contains a putative nuclear factor erythroid-derived 2 (NF-E2) which binds to the Maf recognition element (MARE) located upstream of the erythroid-specific *p45* promoter. Chromatin immunoprecipitation (ChIP) assays showed that p45/NF-E2 bound to this MARE-like region during the period of dimethyl sulfoxide (DMSO)-induced MEL differentiation. Moreover, the ChIP analysis also showed that p45/NF-E2 binding to the GATA-1 binding region of the erythroid-specific *p45* promoter was similar to the binding to the MARE-like region. Analysis of the *p45* expression profile corresponded to the *p45* promoter binding capacity of p45/NF-E2 during MEL cell differentiation. This evidence suggests that the MARE-like binding site might function as an enhancer that interacts with the GATA-1 binding motif within the *p45* promoter to mediate the upregulation of *p45* mRNA in erythroid differentiation. Furthermore, we also found that the MARE binding repressor, Bach1, did not bind to the *p45* promoter, thus excluding any involvement of Bach1 in *p45* gene regulation before MEL differentiation. Together these results suggest that p45/NF-E2 self-regulation is a positive enhancer regulatory mechanism, which differs from the MARE-dependent regulatory mechanism that contributes to the rapid upregulation of p45/NF-E2 required for erythroid differentiation. http://zoolstud.sinica.edu.tw/Journals/48.3/362.pdf

Key words: Bach1, MEL, MARE, ChIP, GATA-1.

The hematopoietic activator nuclear factor erythroid-derived 2 (NF-E2) is a functional heterodimer, composed of an erythroid-specific p45 subunit, and ubiquitously expresses small Maf family subunits, which play critical roles in erythroid differentiation and megakaryocyte maturation (Andrews et al. 1993, Andrews 1998). NF-E2 is a positive regulatory transcription factor that recognizes the specific Maf recognition element (MARE) sequence, GCTGA(G/C)TCAGCA, present in the β globin gene and several genes essential to the heme biosynthesis pathway (Mignotte et al. 1989b, Ney et al. 1990, Cox et al. 1991, Tugores et al. 1994). The importance of NF-E2 is evident in the *p45*-deficient mouse erythroleukemia cell line (CB3) that shows deficient erythroid differentiation. *p45*-null CB3 cells fail to express large amounts of globin messenger (m)RNA, although this can be rescued by reintroducing the expression of p45/NF-E2 (Lu et al. 1994, Kotkow and Orkin 1995). The abnormal expression of p45/NF-E2 has been shown to have different effects on cell proliferation and differentiation (Labbaye et al. 1995, Sayer et al. 2000, Li et al. 2001). Overexpression of NF-E2 promotes erythropoietin (EPO)-independent erythroid maturation and also reprograms precursor cells differentiating into erythroid and megakaryocytic lineages (Sayer

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