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# ISOLATION OF GENES HIGHLY EXPRESSED IN EARLY AND LATE STAGES OF FRIEND VIRUS-INDUCED ERYTHROLEUKEMIA IN MICE

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We isolated two genes that are highly expressed in Friend erythroleukemia. The first one, expressed at an extremely high level in a late-stage tumorigenic cell line F5-5, was found to be a species of intracisternal A-particle. Most of those genomes in F5-5 DNA were not amplified nor rearranged compared with those in the normal cell DNA, suggesting transcriptional activation. Another late-stage cell line T3K showed similar results. The second gene, highly expressed in early-stage cells but weaker in late-stage cells, was identified as one of the heme-synthesizing enzymes, uroporphyrinogen decarboxylase. Usefulness of these two genes for the analysis of erythroid transformation and differentiation will be discussed.


Murine Friend leukemia virus complex, Friend spleen focus forming virus (F-SFFV) and Friend leukemia virus rapidly induce early-stage erythroleukemia (polyclonal erythroblastosis) in the mice spleen within a week. Some of these polyclonal cells could change to monoclonal tumorigenic murine erythroleukemia (MEL) cells at a later stage, after a multi-step transforming process. These MEL cells still retain, to some extent, the capacity to differentiate, to produce hemoglobin and to become mature erythroblasts by several physiological and nonphysiological factors. Thus, the Friend leukemia system has been widely used as a model system for cell growth and differentiation of the erythroid lineage.

To isolate genes involved in erythroid cell growth, transformation and differentiation, we screened a cDNA library of Friend cells by differential hybridization method, which is a powerful technique for the identification of genes overexpressed in certain type of cells compared with control cells. In this

Abbreviations used: IAP, intracisternal A-particle; UPD, uroporphyrinogen decarboxylase; MEL, murine erythroleukemia cell line.

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study we found that some species of murine IAP is expressed at a very high level in a few MEL cell lines. Further, we isolated a cellular gene for heme synthesis whose expression appears to be stage-specific in the erythroid lineage.

## Materials and Methods

Preparation of cDNA libraries

Polycythemic strain of Friend SFFV and Friend helper virus complex were injected peritoneally into the DDD strain of mice, and the spleens containing early-stages of Friend erythroleukemia cells were isolated 10 days after virus infection. The mRNAs were prepared from these spleens, and a cDNA library was prepared with these mRNA samples using λgt10 vector DNA (1).

Differentiation hybridization screening

To isolate genes highly expressed in early-stage erythroleukemia cells or late-stage MEL cells, cDNA library was screened with several [$^{32}$P]-labeled cDNA probes prepared from normal spleen mRNA, early-stage cell mRNA and MEL F5-5 cell mRNA. cDNA clones which hybridized more extensively with early- or late-stage Friend cell probes rather than with normal spleen cell probe, were further screened and purified. cDNA clones carrying SFFV or Friend helper virus proviral DNA were eliminated in the course of screening.

Nucleotide sequencing

Nucleotide sequences of cDNAs were determined by the dideoxynucleotide sequencing method (2) with double strand plasmid DNA as templates described by Hattori and Sakaki (3).

## Results and Discussion

Overexpression of murine IAP at very high levels in a few MEL cell lines

Among several cDNA clones which expressed at a high level in Friend's disease, clone #24 probe detected about 5 kilobase (kb) RNA in early-stage Friend cells. This is a 3 to 5-fold increase compared to normal spleen cells (Fig. 1). Furthermore, #24 probe hybridized very strongly with RNAs of 7 to 8 kb long in MEL F5-5 cells (Fig. 1, lane 4 and 5). The expression of these 7 to 8 kb RNAs in F5-5 was 40 to 60-fold higher than those of the 7 to 8 kb RNAs in the early-stage Friend cells.

In addition to clone #24, a few other clones which show essentially the same pattern as #24 did in Northern blot analysis were isolated: these cDNA clones including #24 were subcloned into plasmid vectors, and the 5'- and 3'-regions were sequenced by dideoxynucleotide method. Computer search of the data base revealed that these cDNA clones encode murine intracisternal A particle (Fig. 2) (4 - 8).

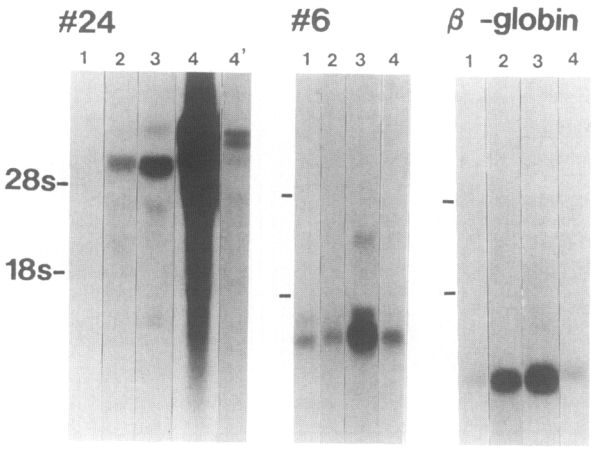


Fig. 1. Northern blot analysis using cDNA clone #24 and #6 as probes. 10 μg of total RNA from normal mouse liver (lane 1), normal mouse spleen (lane 2), Friend virus-induced early-stage erythroleukemia spleen (lane 3), and MEL F5-5 (lane 4) were electrophoresed, transferred to a nylon filter, and hybridized with the various probes indicated in the figure. Lane 4' was a short exposure of the sample corresponding to lane 4.

## IAP genomes are not amplified in MEL F5-5

To examine the mechanism of overexpression in some species of IAP genomes in MEL F5-5, cellular DNAs of normal spleen, early-stage Friend cells and MEL F5-5 cells were analyzed by Southern blot method using clone #24 DNA as a probe. It is well known that multi-copies of IAP genomes (500 to 1,000 copies per haploid genome) are present in mouse DNA. As expected, probe #24 detected multiple IAP bands in the genomic DNAs (Fig. 3). However, the intensity of each band was essentially identical among normal, early-stage Friend cells and MEL F5-5 cells after digestion with various restriction enzymes, except for HindIII digestion: a faint band of 6.3 kb long was detected in MEL F5-5 cells. This might indicate that a single or a few IAP genomes

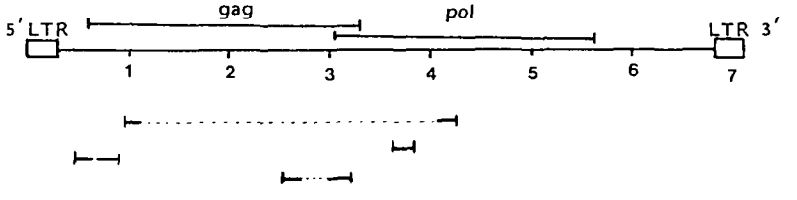


Fig. 2. Localization of IAP-derived cDNA clones on mouse IAP genome. Several cDNA clones including #24 were sequenced at the 5'- and 3'-termini, and compared with the sequence of mouse IAP reported by Mietz et al. (4).

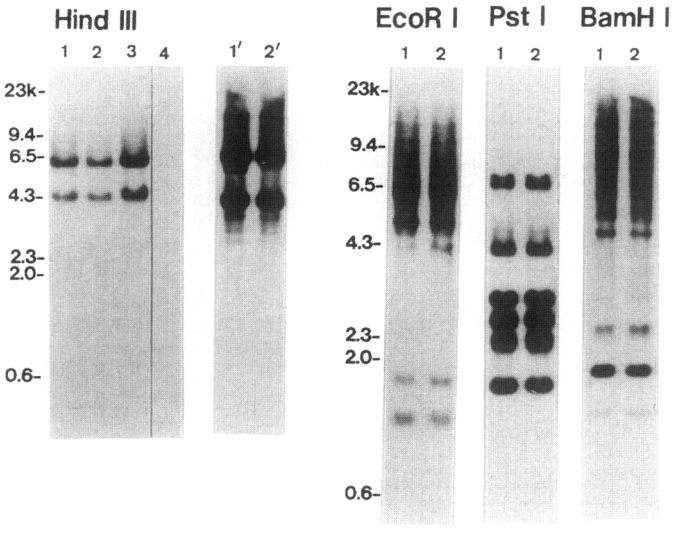


Fig. 3. Southern blot analysis of cellular DNA using clone #24 as a probe. 10 μg of genome DNAs were digested with the indicated restriction enzymes. DNA samples are: lane 1, MEL F5-5 ; 2, early-stage Friend erythroleukemia ; 3, normal mouse spleen; 4, human placenta. Lanes 1' and 2' were long exposure of lanes 1 and 2 in HindIII digestion.

are rearranged in F5-5 cells. Since the major bands of IAP in F5-5 were not amplified, the extremely high expression of IAP appears due to activation at the transcriptional level.

Fig. 4 shows the level of IAP RNAs in other murine hematopoietic cell lines including other MEL cell lines. MEL T3K cells and WEHI-3B cells also expressed IAP at very high levels. WEHI-3B cell line has been shown to carry an IL-3 gene

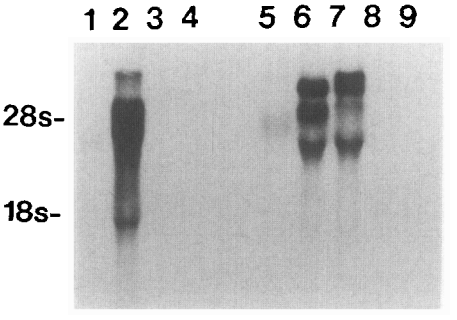


Fig. 4. Northern blot analysis of IAP gene expression in various hematopoietic cell lines using IAP probe. 10 μg of total RNA was probed with a DNA fragment derived from murine 7 kb-IAP. RNA samples are as follows: lane 1, EL-4; 2, WEHI-3B; 3, IC-2; 4, NFS-60; 5, MEL TSA8; 6, MEL T3K; 7, MEL F5-5; 8, early-stage Friend erythroleukemia; 9, normal mouse spleen.

transcriptionally activated by insertional mutation with IAP genome at the 5'-region (5, 9).

Up to now several tumor cell lines of mice were reported to overexpress IAP genomes (10, 11). Possible transcriptional activation of IAP in tumor cells including MEL F5-5 and T3K may indicate two interesting points: (i) The activation of the IAP promoter sequence may be useful to study trans-acting transcriptional factors functioning in these tumor cells. Such transcriptional factors might be crucial for cell transformation. (ii) Since the enhancer/promoter sequence in at least some population of IAP is very active in the IAP-overexpressing cells (12), these enhancer/promoter sequences could activate the adjacent cellular genes whose abnormal expression might play important roles in the maintenance or progression of the transformed state (Fig. 4) (5, 9). For example, in a plasmacytoma cell line XRPC24 the c-mos gene was reported to be activated by IAP promoter insertion (6, 7). Furthermore, in a GM-CSF or multi-CSF dependent cell line FDC-P-1, activation of these growth factor genes by IAP insertion resulted in factor-independent leukemic cells (13).

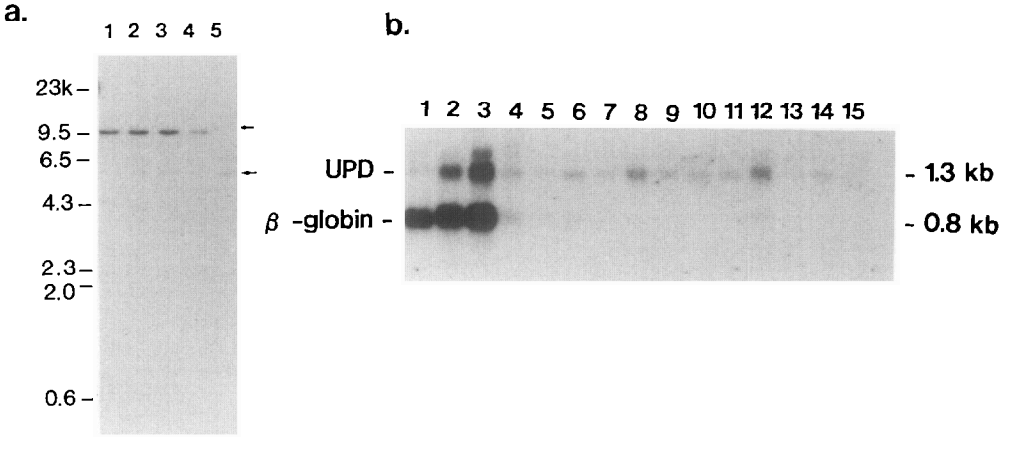


Fig. 5. DNA and RNA analyses of UPD gene. a). 10 μg of HindIII-digested DNA was hybridized with cDNA clone #6. Samples are: lane 1, MEL F5-5; 2, early-stage Friend erythroleukemia (spleen); 3, normal DDD mouse; 4, normal DR mouse; 5, human placenta. b). Expression of UPD gene in various erythroid cells and other hematopoietic cells. 10 μg of total RNA was analyzed by Northern blot method with clone #6 and β-globin cDNA as probes. Samples are as follows: lane 1, normal mouse spleen; 2, mouse anemic spleen 4 days after treatment with phenylhydrazine; 3, early-stage Friend erythroleukemia induced mouse spleen; 4, MEL F5-5; 5, MEL T3C12O; 6, MEL T3K; 7, MEL B8-3; 8, MEL F4-6; 9, MEL 754A; 10, MEL DR-2; 11, MEL DR-3; 12, MEL TSA8; 13, WEHI-3B; 14, IC-2; 15, FDC-P2.

High expression of UPD, a heme-synthesizing enzyme, in early-stage erythroleukemia cells

Another overexpressing clone #6 was isolated from early-stage Friend cell cDNA library by differential hybridization method (Fig. 1). #6 probe detected about 1.3 kb RNA which was highly expressed in early-stage Friend cells, but at a very low level in MEL F5-5, normal spleen or normal liver. Nucleotide sequence analysis revealed that this clone encodes a sequence closely related to rat and human UPD: the sequence of #6 clone was 93 % and 86 % identical to a region corresponding to nucleotide residue 480 to 700 in rat and human UPD, respectively (14, 15). To distinguish two possibilities, i.e. whether #6 clone is the mouse UPD gene itself or another gene closely related to UPD, rat and mouse cellular DNAs were examined by Southern blotting using #6 clone as a probe. #6 probe detected a HindIII fragment of 9.6 kb long in mouse DNA, whereas it hybridized a HindIII fragment of 5.5 kb long in human DNA whose size is identical to that of human UPD gene reported by Roméo et al. (15) (Fig. 5a). Thus, we conclude that #6 clone encodes the mouse UPD gene itself.

Since UPD is involved in heme synthesis, and since its RNA level is high in early-stage Friend erythroleukemia cells, but low in tumorigenic late-stage MEL cells, we assume that overexpression of UPD may not have a direct role in transformation of early-stage Friend cells to tumorigenic MEL cells, but rather reflect a certain stage of erythroid differentiation. The expression of $\beta$-globin gene is almost negligible in MEL cells but is relatively high in normal spleen (Fig. 1 and 5b). On the other hand, the expression of UPD gene is detectable in most of the MEL cells, but negligible in normal spleen (Fig. 5b). These results suggest that the expression of UPD is slightly earlier than that of the globin gene during erythroid differentiation. Therefore, UPD might be one of the useful markers for the detection of certain stage of erythropoiesis.

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