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# MODULATION OF B2 CONTAINING SMALL RNAs DURING INDUCED DIFFERENTIATION OF MURINE ERYTHROLEUKEMIA CELLS

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**Summary :** We identified a B2 repetitive element approximately 1.9kb down stream from mouse p53 coding gene. This element was then used as a probe to investigate the expression of B2 containing RNA during the induced differentiation of murine erythroleukemia (MEL) cells. This probe revealed two nuclear and one cytoplasmic RNA species. Nuclear small RNAs had a biphasic variation: a decrease followed by a reaccumulation. The cytoplasmic species was essentially non polysomal, and disappeared after the induced differentiation. The presented results suggest that the regulation of these RNAs is associated to cell proliferation and differentiation respectively. 

MEL cells are murine proerythroblasts transformed by the Friend's viral complex. These cells are established as cell lines and can undergo an induced differentiation under the action of several chemical inducers (1). Early after the induced differentiation there is a change in the expression of several genes, namely those encoding nuclear proteins such as oncogenes and suppressor gene products (2, 3), chromatin components like histone $H1^0$ (4). Such modifications are followed by a transient accumulation of cells in the G1 phase of the cell cycle (5, 6).

The aim of this study was to investigate and characterize the expression of B2 repetitive elements during the early period of the induced differentiation of MEL cells in order to evaluate the putative association of such sequences with cell proliferation (7, 8), transformation (9,10, 11) and differentiation (12, 13, 14).

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## Materials and Methods

*Cell lines and culture:* MEL cells, clone F4NW0, were maintained in culture and induced to differentiate as described (2).

*Cell fractionation:* All cell fractionation procedures were performed at 0-4°C, using sterile glassware and diethylpyrocarbonate-treated solutions. MEL cells were seeded at $10^4$ cells/ml, and induced for various period of time with 4mM Hexamethylene bis acetamide (HMBA) (final concentration), as indicated in the figure legends. $10^8$ cells were harvested and rinsed with phosphate-buffered solution (PBS), and cytoplasmic and nuclear extracts were prepared as described (15). Cytoplasmic extracts were fractionated as described (15), in two main fractions: non polysomal ($\leqslant$80S) and polysomal (>80S).

*RNA preparation and analysis:* RNA were prepared from nuclei, cytoplasm and cytoplasmic subfractions as described (15).

*RNA determination:* RNA samples were analysed by Northern blots(16). GAPDH (Glyceraldehyde-3-phosphate deshydrogenase) mRNA was determined using a rat cDNA probe (gift from Dr. J.M. Blanchard). Radioactive labeling was performed by random priming (17), or *in vitro* transcription using an appropriate Boehringer-Mannheim kit.

*DNA sequencing:* DNA sequences were determined by the dideoxy sequencing method, using a Sequenase sequencing kit (United States Biochemical Corporation).

## Results

We first report the identification of a B2 repetitive element located approximately 1.9 kb downstream the mouse p53 gene, (Fig 1A). This B2 element is more than 90% homologous to the consensus B2 sequence (18), has an internal RNA polymerase III promoter and a double polyadenylation site ; it is oriented in the same direction as the p53 gene (Fig. 1B).

This B2 element has been subcloned and used as a probe in order to study the expression of B2 homologous sequences in MEL cells. We found that B2 like sequences are highly expressed in uninduced cells and are present in both high and low molecular weight RNA's. In the former group they are present in both orientation (sense and antisense with respect to RNA polymerase III promoter), but the sense orientation only, is found in the small RNA fraction (Fig. 2). A similar observation has been made by Kramerov et al. for B2 sequences in Erlich carcinoma cells (19, 20). It appears, at least in these two cell lines (MEL cells and Erlich carcinoma cells), that the antisense oriented B2 sequences cannot survive RNA processing and are no longer present in the low molecular weight RNA's.

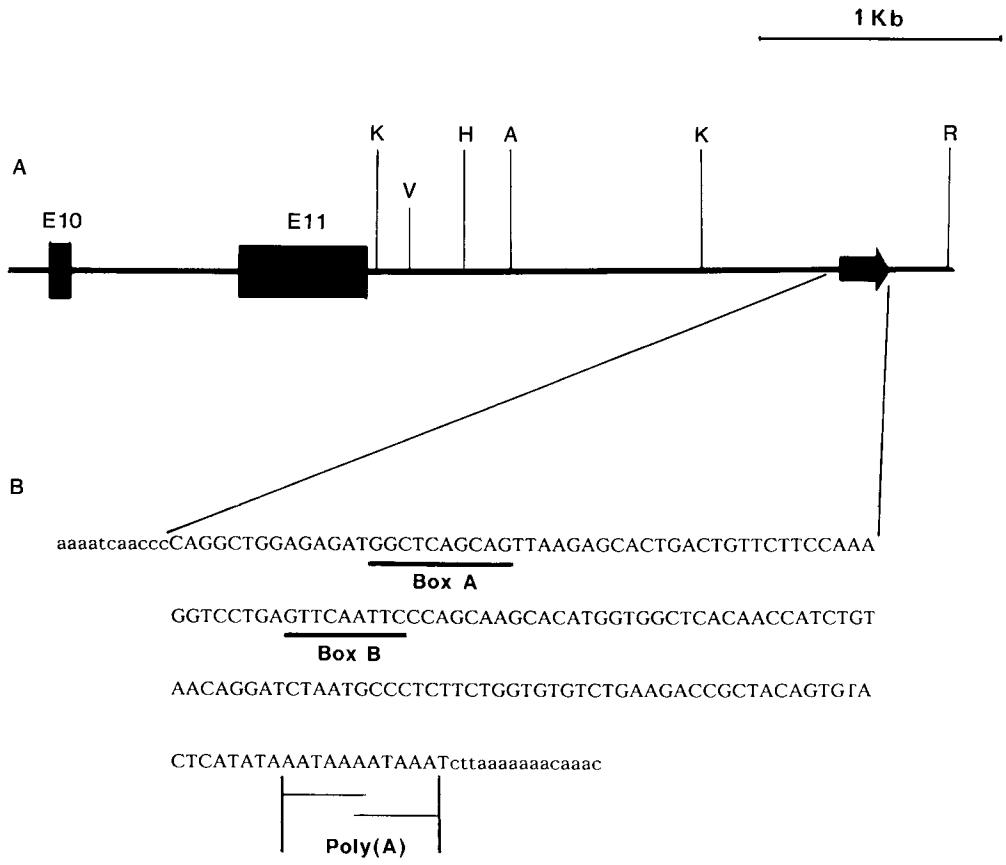



**Figure 1. Identification of a B2 repetitive element in the 3' flanking region of the mouse p53 coding gene.** A) Partial restriction map of 3' flanking region of the mouse p53 gene (21). A : Ava I ; H : Hind III ; K : Kpn I ; R : EcoR I ; V : Pvu II . Black boxes represent p53 exon 10 and 11, the arrow indicates the localization and the orientation of the B2 element. B) Sequence analysis of the B2 element. Internal RNA polymerase III promoter, box A and B are underlined. Two nested polyadenylation sites are gated.

This probe actually revealed two main bands in the low molecular weight nuclear RNA which are approximately 0.5 and 0.2 kb, and only one in the cytoplasmic fraction, with a length spanning between those of the two nuclear species (Fig. 2 and Fig. 4).

We have investigated the variation of the level of these small B2 containing RNA's during the induced differentiation of MEL cells. When total RNA extracted from cells induced for various times was analyzed, the broad band present in uninduced cells disappeared to give rise to two distinct bands located at the position of the two nuclear species identified above (Fig. 3B). This result suggests that the nuclear and cytoplasmic fractions are regulated differently. In order to confirm this hypothesis, we analyzed the variation of the level of the nuclear and the cytoplasmic species separately. The nuclear

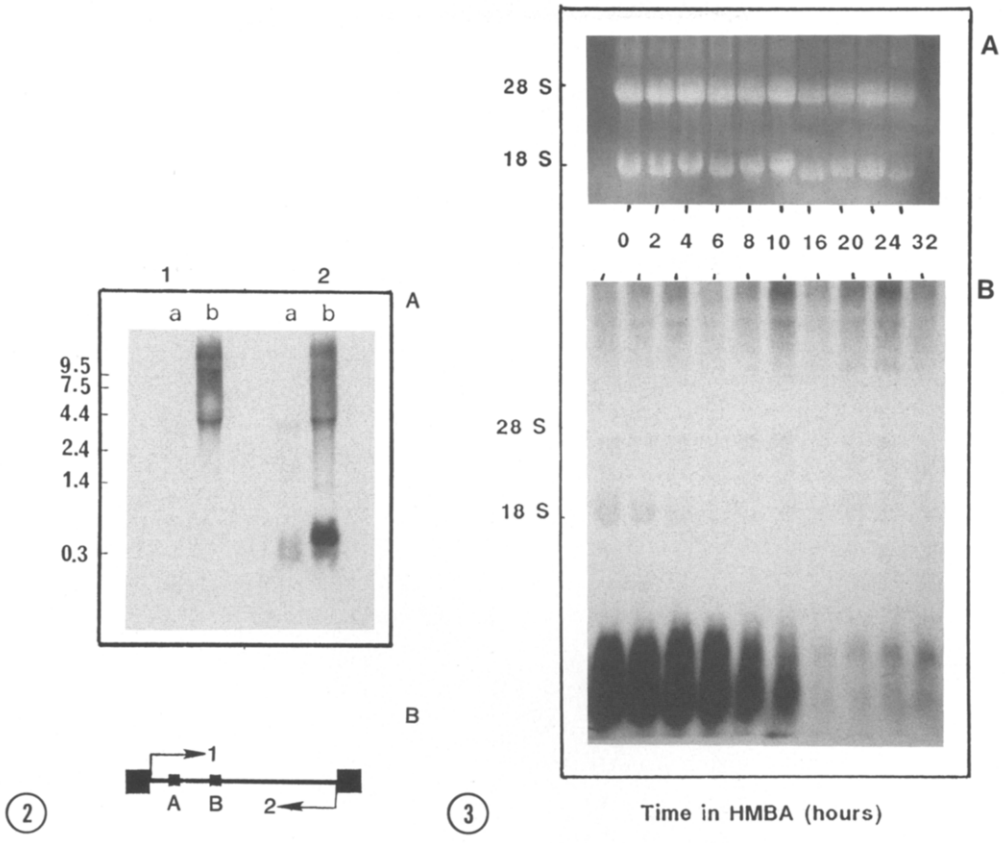
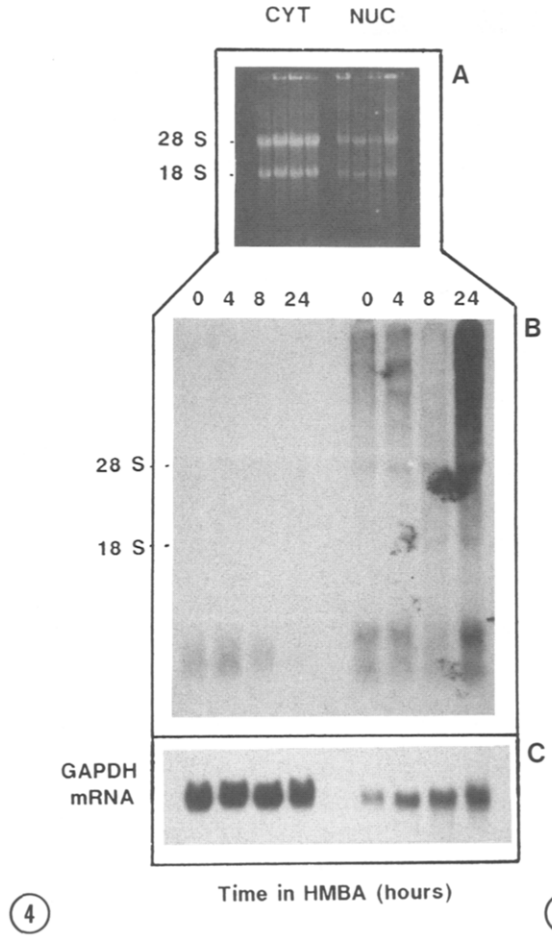



**Figure 2 . Expression of B2 containing RNA in uninduced MEL cells.** A) Cytoplasmic (a) and nuclear (b) RNA has been purified from uninduced MEL cells as described (15), and 50 $\mu$g of RNA from each fraction have been subjected to Northern blot analysis. Blots were probed with sense [1] or antisense [2] $^{32}$P *in vitro* labelled B2 containing riboprobe obtained as described below. The position of the BRL RNA ladder is indicated on the left side of the figure. B) Sense [1] and antisense [2] riboprobes were obtained from the appropriate viral promoters displayed in the figure. The position of A and B boxes in the B2 element is indicated.

**Figure 3 . Variation of small B2 containing RNA during the induced differentiation of MEL cells.** MEL cells were treated with the inducer (HMBA, 4 mM) for the indicated time before RNA purification. A) Ethidium bromide stained gel obtained after electrophoresis analysis of 50 $\mu$g RNA from each sample. 28S and 18S rRNA bands are shown. B) The corresponding Northern blot was probed with the double stranded $^{32}$P labelled genomic B2 element. The position of 28S and 18S rRNA bands is indicated on the left side of the figure.

RNA displayed a biphasic variation, starting by a decrease and followed by a reaccumulation (Fig. 4B). The cytoplasmic RNA decreased continuously.

The localization of the cytoplasmic B2 containing RNA with respect to the translation apparatus was further investigated. For this purpose cytoplasmic extract from cells induced for different times was fractionated on a sucrose gradient and RNA was



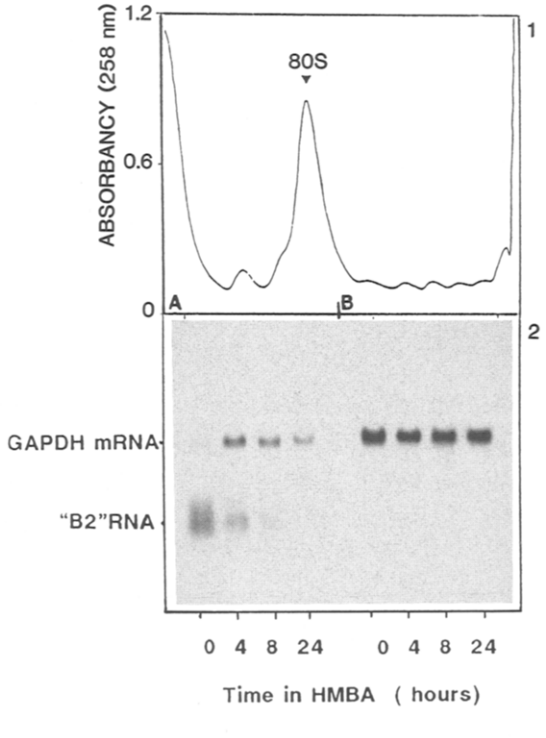




**Figure 4 . Variation of the nuclear and cytoplasmic B2 containing RNA's during the induced differentiation of MEL cells.** A) RNA was purified from the cytoplasmic (CYT) or the nuclear (NUC) fractions of cells treated for the indicated time ; 50 $\mu$g of RNA from each sample were analyzed by gel electrophoresis. The position of 28S and 18S rRNA bands is indicated. B) The corresponding Northern blot was probed with a $^{32}P$ labelled B2 element as in figure 3. C) The same blot was probed with a $^{32}P$ labelled GAPDH specific cDNA as a control.

**Figure 5 . Localization of the cytoplasmic B2 RNA with respect to the translation apparatus.** 1) Cytoplasm from cells induced to differentiate for the indicated time was fractionated on a 14-44% linear sucrose gradient as described (15) into non polysomal (A) ($\leqslant$80S) and polysomal (B),(>80S) fractions. 2) 25 $\mu$g of RNA were purified from each fraction and a Northern blot was obtained. The blot was then probed with a $^{32}P$ labelled B2 element and GAPDH cDNA respectively.

purified from non polysomal and polysomal fractions (Fig. 5). Northern blot analysis showed that the cytoplasmic RNA species was essentially associated with the non polysomal ribonucleoprotein material. GAPDH mRNA taken as a control, was present in both fractions, although the relative amount of this RNA in the two fractions was subjected to some kind of regulation during the induced differentiation.

## Discussion

After 10 hours of induction there is a drastic decrease of B2 containing nuclear RNA species. This time has a particular importance in the course of the induced differentiation : it corresponds to the transition of the induced cells to the irreversible recovery of the genetic program toward erythropoïesis. During the same period of time a minimal level of RNA coding for several oncogene products such as C-MYC and C-MYB and also p53 mRNA is observed (2, 3). After 24 hours of induction nuclear small B2 containing RNA's reaccumulate as well as the mRNA's described above. The minimum level of these nuclear RNA's corresponds to the maximum arrest of MEL cells in the G1 phase of the cell cycle and their reaccumulations parallel the escape of cells from this transient arrest. A detailed analysis of the cell cycle modifications has been described previously (5, 6). Thus the expression of the nuclear species seems to be tightly linked to the state of proliferation of the cells.

The cytoplasmic B2 containing RNA's decreased continuously after the induction of cell differentiation. This result suggests that their presence in the uninduced cells could reflect the fact that such sequences are associated to the transformed state, and that their decrease would be a necessary event for cells to become committed to differentiation. A decrease of B2 like small RNA has also been reported by Bladon et al. (14) and White et al. (12) during the induced differentiation of mouse embryonic carcinoma cells. Such correlations suggest that the B2 sequences could be involved in the regulation of genes whose expression is linked to the cell cycle modifications observed during the early times of induced differentiation. Interestingly, analogs of such repetitive sequences have been found expressed in several transformed cell lines but not in their normal counterparts (9, 10, 11). Our observation add to the growing body of evidences suggesting an association of small repetitive transcripts with the transformed phenotype and cell proliferation.

The biological function of B2 containing transcripts is not clear. B2 like sequences as shown here and by others (19, 20), are present in both orientations in hnRNA. Only the sense orientation is observed in low molecular weight RNA's and we found in the present study that two species of these RNA's are mainly located in the nuclear compartment. This observation provides an attractive model for pre-messenger RNA's maturation, which would involve sense-antisense recognition. The antisense oriented B2 sequences in the hnRNA, either alone or associated with other regulatory sequences,

could be privileged targets for the sense B2 sequences, and the hybrids between the two species is likely to interfere with the pre-messenger RNA processing.

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