CHAPTER 4

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4. DISCUSSION

A study of genetic changes associated with tumour cells is crucial in understanding molecular events governing malignant transformation. In order to gain insights into the understanding of genetic changes, many model systems have been employed. These include skin tumours, colorectal cancers, mammary tumours and liver tumours. A convenient model system has been chemically induced liver tumours in experimental animals, as it provides distinct advantages. Among other things, it is possible, in liver tumours, to identify the earlier stages of development of the tumour and follow its progression.

In our studies, we have chosen the Zajdela Ascitic Hepatoma, a rat hepatoma induced by a chemical carcinogen, dimethylaminoazobenzene (DAB). We have carried out studies to understand some of the genetic changes associated with the tumour, such as, gross chromosomal changes and alterations in the organization and expression of oncogenes and antioncogenes. Any possible involvement of retroviruses in the genesis of the tumour has also been examined. Experiments to test the transforming ability of the tumour DNA were also carried out.

It is apparent from other studies that products of oncogenes and antioncogenes interact at different levels, both in the process of transformation and during normal development. There is considerable experimental evidence available, suggestive of the role played by oncogenes during normal development. However, information on the role of antioncogenes is lacking. Understanding the expression and regulation of antioncogenes will have important consequences on understanding their interaction with oncogene products. In view of this, apart from studying oncogenes and antioncogenes in ZAH, we have extended our studies to investigate the expression and regulation of RB antioncogene in different rat tissues.
4.1 Involvement of retroviruses

Retroviruses have been found to be involved in the origin of many kinds of animal tumours (Varmus, 1983). Apart from the involvement of exogenous viruses, endogenous retroviruses can also get activated under specific conditions (Kaplan, 1967). In order to investigate the possibility of activation of any of the endogenous retroviruses during chemical carcinogenesis or the involvement of exogenous retroviruses, cell-free ascitic fluids from ZAH C and D were subjected to equilibrium sucrose density gradient centrifugation. The gradients were fractionated and tested for absorbance at 260 nm. We did not observe any absorption peak in the density range of 1.16-1.22 g/cm³, characteristic of retroviruses. No reverse transcriptase activity was found in pellets obtained from pooled fractions between densities of 1.16 and 1.22 g/cm³. An examination of the cytoplasmic fraction of ZAH cells also did not show any evidence for the presence of retroviruses, ruling out the possibility of involvement of this class of viruses in the origin of ZAH.

4.2 Alterations in the DNA

During its natural history the tumour cell genome undergoes several gross changes. Such changes have been observed both in human and animal tumours. Ulah and Weber (1979) studied the karyotypes of slow and rapidly growing transplantable Morris solid hepatoma cells. In the rapidly growing solid hepatoma 3924A, the mitotic cells exhibited a range of 54 to 120 chromosomes. In this tumour, extensive chromosomal rearrangements were observed. As noticed in the case of solid hepatoma 3924A, ZAH also displays some interesting genomic alterations. The modal chromosome number varies from 59 to 63 and the DNA content is close to triploidy.

Some of these chromosomal changes might involve several-fold amplification of large regions of the DNA. Such amplifications can be characterized by the in-gel renaturation technique (Roninson, 1987). Using this technique it is possible to identify
and isolate genomic sequences which are amplified at least 30-40 times per haploid genome and Fairchild et al (1987) have used this technique and isolated DNA sequences spanning a region of 140 kb. These sequences were amplified 50- to 100-fold in the human breast carcinoma cell line AdR MCF-7.

The possibility of the amplification of large stretches of DNA in ZAH was investigated using the in-gel renaturation technique. When DNA from ZAH and normal liver was digested with HindIII and subjected to in-gel renaturation, no difference was observed in the pattern of annealed DNA fragments. This indicates that, in spite of gross chromosomal changes and an increase in the DNA content, there are no DNA sequences which are specifically amplified in ZAH.

Apart from amplifications involving large DNA regions, specific alterations of oncogenes such as rearrangement, truncation and amplification have also been observed in a variety of tumours. Increasing the gene dosage (gene amplification) is a cellular mechanism for increasing the number of transcribable genes (Alitalo and Schwab, 1986; Wang et al., 1987). Konopka et al (1984) have reported a 4 to 8 fold amplification of c-abl in human leukaemic cell line K562. c-myb was found to be amplified 10-fold in two human colon adenocarcinoma cell lines, COLO 201 and COLO 205 (Ekstrand et al., 1991). c-myc oncogene was found to be amplified in many human tumours of diverse tissue origin (Bigner et al., 1990; Little et al., 1983; Dalla Favera et al., 1982).

We have studied 15 known cellular oncogenes, namely sis, fms, neu, trk, rasHa, rasKi, rasN, yes, abl, fes, raf, fos, B-myc, c-myc and myb, in ZAH in an attempt to check for such alterations. ZAH DNA was hybridized with the radioactive clones of these oncogenes in a DNA-DNA dot hybridization. In contrast to the reports which have been discussed above, our study shows that the copy number of these genes was the
same in both the tumour cell lines, C and D, as compared to those in fetal and adult rat liver DNA.

An analysis of the genomic organization of \( ras^{Ha}, ras^{Ki}, ras^{N}, B\text{-}myc, myb, abl, raf \) and \( trk \) oncogenes in ZAH C and ZAH D, as compared to their organization in normal tissues, was carried out. Southern blot hybridization showed no significant changes in the Southern blot profiles. The analyses were carried out using the restriction enzymes \( EcoRI \) and \( HindIII \). In each set, there were no changes among the different DNAs. Again this observation is in contrast to a number of observations for several other tumours. Rearrangements of several of the oncogenes such as \( erb \text{B} \) (Libermann \textit{et al.}, 1985), \( mos \) (Rechavi \textit{et al.}, 1982), \( myb \) (Mushinski \textit{et al.}, 1983) and \( myc \) (Erisman and Astrin, 1988) have been shown to exist in a number of investigations.

4.3 Expression of oncogenes at the RNA level

After having examined the organization of oncogenes in ZAH DNA we have studied also the expression of different oncogenes at RNA level in the ZAH cells. Alterations in oncogenes resulting in the quantitative and/or qualitative changes in their expression could also play an important role in transformation. The studies on expression of oncogenes can provide important clues about specific oncogenes that are involved either in triggering transformation or in the maintenance of transformed state. In this context, we have studied the expression of several oncogenes in the tumour cell lines ZAH C and ZAH D.

Among the various oncogenes examined, \( abl, fes, fos, fms, myb, neu \) and \( raf \) showed higher expression in both ZAH C and ZAH D compared to their expression in adult- and fetal- rat liver. But the increased expression of these genes was marginal, except in the case of \( neu \) which was overexpressed 6-fold in ZAH C and 3 fold in ZAH D, as compared to adult liver. Oncogenes such as \( ras^{Ha}, ras^{Ki}, ras^{N} \) and \( sis \) did not
show any increase in their expression at the RNA level in both ZAH C and ZAH D. These findings are in contrast with many earlier investigations. In Morris Hepatoma cell lines and in neoplasms induced by 3'-methyl-4-dimethyl-aminoazobenzene, a 2-6 fold increase in \textit{ras}^{Ha} gene and a 2 fold increase in \textit{c-myc} was observed (Cote \textit{et al.}, 1985; Makino \textit{et al.}, 1984). A 2 to 25 fold increase in the expression of \textit{ras}^{Ha}, \textit{ras}^{Ki} and \textit{ras}^{N} genes was observed in Diethylnitrosamine-induced rat hepatocellular carcinomas (Corcos \textit{et al.}, 1984).

4.4 Oncogene expression and cell cycle behaviour

Our experiments with ZAH C and ZAH D showed that the cell cycle duration of ZAH C and that of ZAH D were 17.7 h and 24.4 h respectively. The duration of the different stages of cell cycle, G1, S and G2+M, for ZAH C, were 2.8, 11.7 and 3.3 h and those for ZAH D were 2.2, 18.8, 3.1 h respectively. Interestingly, ZAH C and ZAH D showed differences between themselves in the expression of some oncogenes. In ZAH C, \textit{fms}, \textit{fos} and \textit{neu} were expressed at a higher level as compared to ZAH D. There was a two-fold increase in the expression of both \textit{fos}, which encodes for a sequence-specific transcription factor, and \textit{neu}, which encodes for a transmembrane receptor with intrinsic tyrosine kinase activity. It is possible that the p185 \textit{neu} might be increasing the phosphorylation of specific proteins, while Fos protein might be driving the transcription of genes promoting the mitotic cycle. Such a mechanism can, in principle, explain the enhanced growth rate of ZAH C. It may be noted that despite the distinct differences in the generation time of the two cell lines ZAH C and ZAH D, there was no difference in the virulence of tumour caused by these cell lines (Table 2).

4.5 Undermethylation of \textit{c-myc} gene correlates with the expression of the gene

In addition to the changes in the organization and expression of oncogenes, changes in DNA methylation are also associated with the malignant phenotype. In a large number of primary human malignancies, an approximately 6% overall
reduction in the 5-methylcytosine content as compared to normal tissues has been observed (Gama-sosa et al., 1983). In colon carcinomas and lung carcinomas a substantial reduction in the methylation of a number of genes has been reported. These include the human growth hormone gene, α- and γ-globin genes, \( \text{ras}^{\text{Ha}} \) and \( \text{ras}^{\text{Ki}} \) genes (Feinberg and Vogelstein 1983b; Feinberg et al., 1988). We have examined the possibility of a similar phenomenon in ZAH using the \textit{c-myc} oncogene.

In mammalian DNAs, variations in methylation of cytosine residues are found almost exclusively at the dinucleotide CG (Doerfler, 1983). The enzymes that discriminate significantly between methylated and unmethylated CG sequences are therefore sensitive indicators of methylation. The presence of methylation at CCGG sequences can be conveniently analysed using \textit{MspI} and \textit{HpaII}. \textit{MspI} does not cleave this sequence when the first cytosine is methylated but is blind to the methylation status of the second cytosine. \textit{HpaII} cleaves the sequence only if the second cytosine is unmethylated, regardless of the methylation status of the first cytosine. Another methylation-specific restriction enzyme, \textit{HhaI}, which cleaves the sequence GCGC only if the first cytosine is unmethylated is also useful for studying DNA methylation. So these methylation-specific restriction enzymes were used to digest the DNAs with a view to investigate possible differences in the methylation pattern of \textit{c-myc} gene in tumour DNA as compared to normal DNA.

An examination by Southern blot hybridization using the \textit{c-myc} probe after digesting the DNA with methylation specific restriction enzymes revealed some novel features. The presence of a number of fragments (<1.2 kb size) hybridizing with the \textit{myc} probe in \textit{HpaII} digests of the tumour DNA, in comparison with corresponding digests of normal adult liver DNA (Fig 25), suggests the presence of several \( \text{CmCGG} \) sites in normal DNA which are unmethylated in the tumour DNA. Likewise a comparison of the \textit{MspI} digests of ZAH C and normal adult liver DNA would argue for the presence of fragments containing sequences such as \( \text{GGCmCGG} \) or \( \text{CmCGGCC} \).
in the adult DNA, which are unmethylated in the tumour DNA (Busslinger et al., 1983; Keshet and Cedar, 1983). Another interesting feature that may be noted is that both the MspI and HpaI digests of fetal DNA show at least two almost identically sized fragments (1.2 and 0.74 kb) which are absent in the corresponding digests of adult DNA. If fragments of the type GGCCmCGG, which are not restricted either by MspI or HpaII remain unmethylated in the fetal DNA, such sites become available for restriction by both MspI and HpaII. This type of situation is also obtained for the tumour DNA, in comparison with adult liver DNA. The HhaI digests suggest that a GCGC site, which is methylated in a 7 kb fragment of adult and fetal liver, is undermethylated in ZAH C cells as the disappearance of this band from the ZAH lane coincides with appearance of a 2 kb fragment and several fragments of the size of about 0.5 kb (Fig 26). Since our v-myc probe is known to hybridize to exons 2 and 3 of the c-myc gene in several species (mouse, rat, human), the small size of the fragments obtained on digestion of ZAH C DNA, either with HpaII or HhaI, suggests that the sites of undermethylation are in exons 2 and 3 (Bernard et al., 1983). In the study on human tumour cell lines, a specific undermethylated site was shown to be present exclusively in exon 3 of the human myc gene (Cheah et al., 1984).

It is of interest to note that these changes in the methylation of the c-myc gene are correlated with its expression in these tissues. ZAH C and fetal liver, the tissues in which we observe a decrease in the methylation of the gene compared to adult liver, also show a concomitant increase in the level of myc specific RNA.

Hypomethylation might inhibit chromosome condensation, which in turn might lead to problems in chromosome pairing and dysjunction. In support of such a mechanism for tumourigenesis, it has been shown that 5-azacytidine induces transformation of Chinese hamster embryo fibroblasts at high frequencies (Harrison et al., 1983). If hypomethylation leads to expression of genes in neoplastic growth, then
cells exhibiting a defect in the control of methylation may obtain a selective advantage (Holliday, 1979).

4.6 Hotspots of ras gene in the ZAH DNA

Among the ras family of genes, ras$^\text{Ha}$, ras$^\text{Ki}$ and ras$^\text{N}$ have been implicated in the development of a variety of tumours. The ras genes acquire transformation-inducing properties by point mutations and these have been localized to codons 12, 13, 61 and 63. Such mutations were originally observed in human bladder carcinoma (Reddy et al., 1982; Tabin et al., 1982).

In chemically induced tumours too, similar mutations have been observed. The reproducible activation of ras oncogenes in carcinogen-induced tumours has made it possible to correlate their activating mutations with the known mutagenic effects of certain carcinogens. In NMU-induced rat mammary carcinomas, the ras$^\text{Ha}$ gene was activated by a G to A transition at position 2 in codon 12 (Zarbl et al., 1985). Activation of the ras$^\text{Ha}$ gene has also been observed due to C to A transversions in the first base of codon 61 in HO-AAF induced tumours in mice (Wiseman et al., 1986). In mouse skin carcinomas induced by DMBA and phorbol esters, and in mouse hepatocellular carcinomas induced by vinyl carbamate, the mutations were present in the 61st codon (an A to T transversion) (Quintanilla et al., 1986; Bizub et al., 1986). These findings indicate that ras oncogenes can be directly activated by the mutagenic properties of carcinogens.

In the light of these findings we have carefully studied the mutations in ras$^\text{Ha}$ and ras$^\text{Ki}$ oncogenes in the ZAH DNA. The regions of these genes encompassing the codons 12, 13, 61 and 63 in the two cell lines of the tumour were amplified using the polymerase chain reaction and subsequently cloned and sequenced. The results presented in the previous chapter clearly indicate the absence of any mutations at any of the hotspots, either in ras$^\text{Ha}$ or ras$^\text{Ki}$, both in the ZAH C and ZAH D.
4.7 Involvement of transforming gene(s)

From the earlier discussion it is clear that in ZAH C and ZAH D there is no evidence for any amplification or translocation of the *ras* genes. Nor is there any experimental evidence for the existence of common mutations found during the conversion of normal *ras* to an oncogenic *ras*. Under these circumstances, it was thought logical to look for the existence of possible transforming genes in the tumour DNA. The transforming ability of tumour DNA is generally tested using DNA transfection experiments.

For transfection experiments, two assay systems are generally employed: the focus forming assay and the cotransfection-tumourigenicity assay. Both of these systems utilize NIH 3T3 cells as recipients for transformation. In the focus forming assay, where transformants are selected on the basis of their ability to grow as foci, a preponderance of the genes detected belong to the *ras* gene family (Der *et al.*, 1982; Parada *et al.*, 1982; Pulciani *et al.*, 1982a, b; Yuasa *et al.*, 1983) Apart from this, the focus forming assay does not preclude the possibility of selecting spontaneous transformants. To overcome this drawback the cotransfection-tumourigenicity assay is used. This is a variant of the focus forming assay and is based on cotransfection of NIH 3T3 cells with the tumour DNA and a selectable marker gene, *neo* (which imparts resistance to the antibiotic G418), followed by a tumourigenicity assay in nude mice. Cotransfection with selection for G418 resistance effectively removes from the population spontaneously transformed NIH 3T3 cells that have not incorporated the exogenous DNA. Furthermore, selections of colonies based purely on G418 resistance rules out the bias for selection for *ras* genes. The oncogenes detected and identified as transforming genes in tumourigenicity assay include *hst* from stomach carcinoma (Yuasa *et al.*, 1990), *mas* from epidermoid carcinoma (Young *et al.*, 1986) and *ros* and *rasN* from the mammary carcinoma cell line MCF-7 (Birchmeier *et al.*, 1986).
We have performed DNA-mediated gene transfer to ascertain whether transforming genes are present in DNA derived from the two cell lines of ZAH. The transforming ability of ZAH DNA was tested using the NIH 3T3 focus-forming as well as tumourigenicity assays. In both these experiments, appropriate positive and negative controls were used. The positive control was transfection by pEJ 6.6 plasmid, while the negative control was transfection by NIH 3T3 DNA and rat liver DNA.

ZAH DNA shows a strong focus inducing activity (0.93 and 0.8 foci per µg of DNA from ZAH C and ZAH D respectively) in contrast to DNA from rat liver tumours induced by aflatoxin B1 (0.01-0.19 foci per µg of DNA). The appearance of foci following transformation involving DNA from ZAH C and ZAH D indicated that both the cell lines harbour activated oncogene(s) in their genomes. Although a number of investigations have been carried out to characterize transforming genes from liver tumours, there are only few instances where positive evidence is available. Ishikawa et al (1986) have demonstrated the activation of raf gene in IQ-induced rat hepatocellular carcinoma using NIH 3T3 focus forming assay. The other oncogenes identified by transfection assay include ras^Ha in N-HO-AAF, vinyl carbamate and HO-DHE-induced hepatomas of the B6C3 F1 mice (Wiseman et al., 1987) and ras^Ki and ras^N in aflatoxin B1 induced rat liver tumours (McMahon et al., 1986; Sinha et al., 1988). However, Farber et al (1984) have not been able to detect any activated oncogenes using the NIH 3T3 focus forming assay in 20 different chemically induced rat liver carcinomas.

The Zajdela Ascitic Hepatoma belongs to the small category of liver tumours capable of transforming NIH 3T3 cells in transfection assays. The fact that the ras family genes are normal suggests that the target of DAB in ZAH might be a novel gene whose identity is yet to be discovered. Sequence analysis of the transforming gene and its comparison with known oncogenes is of immediate significance for zeroing in on the transforming gene.
4.8 Organization and expression of p53 and RB antioncogenes in ZAH

The regulation of cell proliferation is brought about by two classes of genes - one class that promotes cell division and the other that inhibits it. Maintenance of this fine balance between the two opposing states of cell proliferation can be disrupted by two mechanisms, one of which is mutations in genes that deregulate cell proliferation by overriding existing controls. Such deregulation involving the gain of function of mutated genes is generally believed to be the mechanism by which oncogenes function. As has been indicated earlier, such gain-of-function mutations that lead to activation of oncogenes have been observed in a large number of tumours which are either of spontaneous origin or have been chemically induced.

In contrast to the way of functioning of oncogenes, there exists a group of genes whose expression is necessary to keep the cell from dividing. Deletions of such genes or mutational inactivation of these genes could lead to cell division. Such genes are known as antioncogenes. Mutational inactivation of antioncogenes have been observed in many human tumours such as retinoblastoma, colorectal cancer and Wilms' tumour. However, their involvement in chemically induced tumours is not known.

RB is one of the most commonly studied antioncogenes. The role of RB in cell proliferation has been confirmed in experiments with DNA tumour viruses (Whyte et al., 1988; Ludlow et al., 1989). While there are indications of RB involvement in the regulation of c-fos, AP-1, c-jun (Robbins et al., 1990) and c-myc (Pietenpol et al., 1990) genes, its own regulation is largely unknown. Increased levels of RB transcripts have been observed in tumours carrying both the mutant alleles of RB and in adenovirus-transformed human retina cells, suggesting a transcriptional regulation of the RB gene (Goddard et al., 1988; Dunn et al., 1989). On the other hand, based on the similarity of its promoter elements with those of other house keeping genes,
together with the ubiquitous expression of RB, post-transcriptional regulation of RB also has been suggested (Chen et al., 1989; Hong et al., 1989).

We have studied the role of two antioncogenes, RB and p53, in ZAH. Experiments were carried out to investigate alterations in the copy number, organization and expression of these antioncogenes. We did not observe any changes with regards to the copy number and organization of RB and p53. However, it was observed that the expression of RB and p53 in ZAH C and ZAH D was markedly reduced as compared to adult liver. The reduction in the expression of p53 and RB in the tumour cells could be a contributory factor in tumourigenesis.

4.9 Expression of RB gene in different rat tissues

Investigations on RB were extended to study the expression of the gene in different rat tissues. This was considered important in view of the fact that it is becoming increasingly clear from recent studies that the proteins encoded by oncogenes interact with those encoded by antioncogenes. This co-operation has important consequences in malignant transformation and is likely to be important in events controlling development (Hunter, 1991). Although there is considerable experimental evidence available on the role of oncogenes during normal development, a similar level of understanding has not come about with regard to antioncogenes.

The RB gene is ubiquitously expressed in all tissues (Lee et al., 1987b). It encodes a 105 kilodalton nuclear protein which can be phosphorylated to give species of Mr 110-115 kilodaltons (Lee et al., 1987a). The RB protein undergoes cyclical changes of phosphorylation and dephosphorylation during specific stages of the cell cycle. The unphosphorylated form is found predominantly in G0 and G1 stages, whereas the phosphorylated form predominates during the S and G2 stages (Decaprio et al., 1989; Buchkovich et al., 1989; Chen et al., 1989; Xu et al., 1989; Mihara et al., 1989). In HL-60
cells induced to terminal differentiation, as well as in senescent and quiescent human diploid fibroblasts, the RB protein is unphosphorylated (Mihara et al., 1989; Stein et al., 1990). Based on these observations, it has been hypothesized that the unphosphorylated RB is the active form which acts as the negative regulator of cell division by retaining the cell in the G0 or G1 stage. The control clamped on cell division is removed as a consequence of phosphorylation, leading to cell division (Stanbridge, 1990).

We have studied the pattern of expression of the RB gene in different adult tissues (brain, kidney, liver, spleen, lung, mammary, muscle and retina) and developing tissues (brain, kidney, liver and spleen) from the rat (Fig 43). Total RNA from these tissues was hybridized with a human RB cDNA probe. The relative levels of the RB transcript in these tissues were quantitatively evaluated.

Among the adult tissues studied, there was a wide variation in the expression of the gene (Fig 43 and 44). While muscle and lung showed high expression, kidney and liver showed low levels of mRNAs. Among the developing tissues, there was an increase in the expression of the gene from embryonic to adult stages (Fig 43 and 45) except in the case of liver. The extent of increase in expression varied in different tissues, with adult spleen showing a six-fold and adult kidney a two-fold increase over their respective embryonic counterparts. In the case of spleen there was an increase in the expression of RNA from the embryonic to the neonatal stage, but a slight decrease from the neonatal to adult stage. Lee et al (1987b) have reported a higher level of RB transcript in the adult kidney compared to adult brain and spleen tissues. However, our data shows higher levels of RB transcripts in spleen and brain tissues.

Our results would suggest that the RB gene is developmentally regulated and differentially expressed in adult rat tissues. RB regulation can occur at different stages, such as at the transcriptional level or at the stages of mRNA processing, protein
synthesis or as post-transcriptional modifications (Buchkovich et al., 1989). In order to probe this question further, we have carried out nuclear run-on transcription assays with brain and liver nuclei, tissues in which considerable difference in RB expression were observed. Nascent RNA from the nuclei, labeled with \( \alpha-^{32}\text{P} \)UTP, was extracted and hybridized to linearised single stranded RB cDNA. The results are shown in Fig 46. Comparison of signals from these tissues indicated that the level of RB transcription in the brain and in the liver was nearly the same. The differential accumulation of RB transcripts in these tissues thus seems to be a post-transcriptional event. If the regulation were to be at the transcriptional level, one would expect the differences in the level of RB transcripts in brain and liver to be reflected in nuclear run-on signals.

RB expression was also studied in tissues obtained from cycloheximide treated rats. Total RNA isolated from the different tissues was hybridized with RB cDNA probe (Fig 47). Increase in the level of RB transcript upon cycloheximide treatment was observed in all the tissues studied except in spleen and lung. In fact, different tissues showed a wide variation in the level of RB transcript upon cycloheximide treatment, suggestive of differences in post-transcriptional regulation which are tissue specific. The increases observed could result from the accumulation of the message consequent to translational block by cycloheximide (Graves, 1987). Stability of the mRNA due to changes in the susceptibility to endonucleolytic processing enzymes would also affect the level of mRNAs. Post transcriptional regulation of mRNAs has also been observed for c-\text{myc} and p53 expression in an embryonal carcinoma cell line (Dony et al., 1985). Kindey and Sinshein(1986) have observed a transient stabilization of c-\text{myc} mRNA upon serum stimulation of cultured aortic smooth muscle cells. Similarly, a transient increase in the expression of c-\text{myc}, \text{jun B}, c-\text{jun} and \text{jun D} mRNA in mouse liver was observed upon cycloheximide treatment (Morello et al., 1990).
It has been shown that 3' UTR of mRNAs influence their stability (Atwater et al., 1990). The 3' UTRs of mRNAs with a short half-life show two important features, high AU content and consensus AUUUA motifs. In fact the stability of the mRNA conferred upon by the 3' UTR of mRNAs could play an important role in post transcriptional regulation of messages. In the case of c-fos, the deletion of a 67 base pair region in the 3' UTR stabilizes its mRNA and is sufficient to convert it into a transforming gene when assayed in cultured rat fibroblasts (Wilson and Treisman, 1988). Schuler and Cole (1988) have shown that linkage of the germ-line 3'-untranslated region of GM-CSF gene to a neo reporter gene in a monocytic tumour resulted in mRNA stabilization mediated by tumour-specific trans-acting factor(s). They also showed that similar fusion of c-myc and c-fos untranslated region to neo yielded mRNAs that turned over rapidly. These results suggest that AU-rich mRNA turnover signals are recognized differentially in trans within the same cell.

We have analysed the 3' UTR region of RB using the RB cDNA sequence. The analyses was carried out using the PC Gene software package. The analyses reveal that the 3' UTR region has a high (70%) AU-content. There are 44 stretches of at least 10 bases each which are 90% AU-rich (Fig 48). The longest stretch among these consists of 45 bases. Apart from these, there are 12 stretches of AUUUA motifs (Table 9). Both these features in the 3' UTR of the RB mRNA might influence its stability and would suggest that RB mRNAs are likely to have a shorter half life. The cycloheximide experiments, where increased levels of transcripts were observed, are suggestive of higher turnover of the transcripts. The present study demonstrates that there is a differential expression of RB gene in different adult and developing rat tissues. The RB mRNA is likely to be short lived with a high turnover in many tissues. The observed differences in the level of mRNA expression is likely to result from post-transcriptional regulation. However, the relevance of the tissue-specific regulation of the RB mRNA is not clear at present. Further experiments are in progress to delineate the significance of these observations in relation to development.