SUMMARY AND CONCLUSIONS
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The evolution of a normal cell into a malignant one is a stepwise process that proceeds through a sequential selection of variant subpopulations originating from a common progenitor. Histological, cytogenetic and other analyses of a large number of tumours of both human and animal origin has led to this conclusion. Multiple genetic changes have been found to be associated with this transformation process. In order to investigate such genetic changes associated with a chemically induced tumour, we have used a convenient model system, the Zajdela Ascitic Hepatoma - a rat hepatoma induced by dimethyl-aminoaazobenzene. The major conclusions of the studies described in the thesis are summarised below.

1) Our analysis of ZAH for gross chromosomal changes has revealed that there is a drastic alteration in its karyotype. There is a wide variation in the chromosome number in the tumour cells. The modal chromosome number is 59-62. Associated with these alterations is the change in DNA content. The average DNA content is close to triploid. In addition, 18 tumour-specific marker chromosomes have been identified. Two of the marker chromosomes, M1 and M2, have been shown to have originated by pericentric inversion. In view of these chromosomal abnormalities we have investigated the amplification of large regions of DNA using the in-gel DNA-renaturation technique. There was no specific amplification of any of the sequences in the DNA samples from ZAH C and ZAH D.

2) The examination of cell free ascitic fluid of ZAH C and ZAH D for retroviruses and for reverse transcriptase activity indicated that there is no involvement of retroviruses either in the genesis of the tumour or during the adaptation of the tumour to the ascitic form.

3) Investigations were carried out to study specific alterations in oncogenes and their expression. We have analysed sis, fms, neu, trk, ras\(^{Ha}\), ras\(^{Ki}\), ras\(^{N}\), yes, abl, fes,
raf, fos, B-myc, c-myc and myb oncogenes for the alterations in the copy number using DNA-DNA dot hybridization. The results indicate that the copy number of these oncogenes in the tumour cells is similar to that in normal cells. Southern hybridization of ras\textsuperscript{Ha}, ras\textsuperscript{Ki}, ras\textsuperscript{N}, B-myc, abl, raf, myb, trk and c-myc oncogenes upon EcoRI and HindIII digestion showed that the organization of these genes is similar in normal and tumour cells.

4) The expression of sis, fms, neu, ras\textsuperscript{Ha}, ras\textsuperscript{Ki}, ras\textsuperscript{N}, abl, raf, fes, myb, B-myc, c-myc and fos oncogenes was studied using DNA-RNA slot blot hybridization. Among the oncogenes studied abl, fes, fos, fms, myb, neu and raf showed higher expression in both ZAH C and ZAH D compared to adult liver. There was a difference between ZAH C and ZAH D in the expression of fms, fos and neu. Notable is the two-fold increase in the expression of fos and neu in ZAH C over ZAH D. Interestingly, ZAH C is faster growing, with a cell cycle duration of 17.7 h, compared to ZAH D which shows a cell cycle duration of 24.4 h. We have also studied the expression of oncogenes in developing liver. There is a variation in the expression of different oncogenes in adult liver and fetal liver. The expression levels of c-myc are 3-4 fold higher in fetal liver. However the expression of fms oncogene is 1.5 fold higher in adult liver compared to fetal liver.

5) Differences were observed between the normal adult liver and ZAH C in the methylation pattern of the c-myc oncogene. The methylation pattern was studied by digesting the DNA with enzymes which discriminate significantly between methylated and unmethylated CG sequences, such as MspI, HpaII and HhaI. Our results indicate the presence of C\textsuperscript{mCGG}, GGC\textsuperscript{mCGG} or C\textsuperscript{mCGGCC} and G\textsuperscript{mCGC} in the adult liver DNA which are unmethylated in the tumour DNA and fetal liver DNA. These changes in the methylation pattern correlate with the expression pattern of the gene 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.

6) DNA from ZAH C and ZAH D was analysed for mutations in $\text{ras}^{\text{Ha}}$ and $\text{ras}^{\text{Ki}}$ genes at codons 12, 13, 61 or 63. These sites are mutational hot spots that lead to the activation of $\text{ras}$ genes in a large number of tumours. The regions around these codons were amplified by PCR and subsequently cloned and sequenced. Sequence analysis shows that codons 12, 13, 61 and 63 in ZAH C and ZAH D are identical to those in normal DNA, suggesting that there are no mutations that have occurred at these codons in $\text{ras}$ genes of the tumour DNA.

7) In order to check for the involvement of any of the dominantly activated oncogenes, we have looked at the transforming ability of ZAH DNA using both the focus forming assay and the co-transfection tumourigenicity assay. Results of the focus forming assay indicate that ZAH DNA has a strong transforming ability, with 0.9 and 0.8 foci being induced per $\mu$g of DNA in ZAH C and ZAH D respectively. In the co-transfection-tumourigenicity assay, injection of transfected NIH3T3 cells led to the development of tumours in the case of ZAH C transfections. Taken together, these results suggest that ZAH DNA harbours activated oncogenes, whose identity is yet to be established.

8) In view of the involvement of loss of function, either due to mutational inactivation or deletion of antioncogenes, in many human tumours, we have studied the involvement of p53 and RB antioncogenes in ZAH. Our analysis suggests that the copy number and organization of both these genes are similar to those in normal tissues. However, we observe that the expression of RB and p53 in ZAH C and ZAH D was markedly reduced as compared to adult liver, which could be a contributory factor in tumourigenesis.
9) The study was extended to understand the regulation of the RB antioncogene in specific adult rat tissues. We have studied the expression of RB gene in different adult tissues (brain, kidney, liver, spleen, lung, mammary, muscle and retina) and developing tissues (brain, kidney, liver and spleen) from rat. We observe a wide variation in the expression of the gene. Muscle and lung show high expression while kidney and liver show low levels of mRNAs for RB. Among the developing tissues there is an increase in the expression of the gene from embryonic to adult stages, except in the liver. These results suggest that the RB gene is developmentally regulated and differentially expressed in adult rat tissues.

10) In order to understand the regulation of RB further, we have carried out nuclear run-on transcription assays using nuclei prepared from brain and liver, the tissues in which we observe a considerable difference in RB expression. Our results indicate that the rate of transcription of RB genes is similar in both the tissues. RB expression was also studied upon cycloheximide treatment. There was an increase in the expression of the gene in all tissues except spleen. Further, the analysis of the 3′untranslated region of the RB genes revealed that the 3′UTR of RB genes is AU-rich (70%). There are 44 stretches of at least 10 bases each which are 90% AU-rich. In addition, there are 12 stretches of AUUUA consensus motifs which are found in highly unstable messages. The similarity of rates of transcription in brain and liver, the increase in expression of the gene in different tissues upon cycloheximide treatment and, the high AU content and the occurrence of the consensus AUUUA motifs in the 3′UTR together suggest that the RB gene is post-transcriptionally regulated and that the RB message is highly unstable.