SYNOPSIS

The operon hypothesis of Jacob and Monod has opened new vistas of enquiries into the molecular events underlying expression of information coded in DNA. Most of the knowledge has undoubtedly emanated from studies on bacteria and bacteriophages. It is believed that control mechanisms for gene action in higher organisms may be similar but, in addition, should be endowed with other regulatory devices by virtue of greater complexity of their cells and genetic apparatus as well as multicellularity. In the eukaryotic cells, the DNA is mainly confined to discrete nucleus and is associated with acidic and basic proteins and some amount of RNA. Practically very little is known regarding the exact roles played by the non-DNA components but several recent findings implicate them in specific regulation of gene expression. Additional control mechanisms are expected to become operative in the multicellular animal as compared to the unicellular eukaryotic organism.

Not all the approaches used in studies with prokaryotic systems could be employed for understanding control of gene expression in eukaryotes. One of the useful approaches that has been made in this direction is to subject organisms to stress conditions which may evoke specific changes in genetic information flow, for example, hormonal imbalance and nutritional deficiency. The studies outlined in this thesis are aimed at understanding various regulatory aspects of proteins and nucleic acid syntheses in higher organisms using the two stress conditions, (i) ionizing radiations and (ii) partial hepatectomy.

Part I

A brief survey of current information on regulation of gene expression and biosyntheses of macromolecules in eukaryotes with special reference to the topics covered in the subsequent parts of the thesis is presented in the introductory part (Part I).

Part II

In a number of studies, it has been observed that exposure of animals to ionizing radiations leads to enhanced rate of protein synthesis in the liver during 4-24 hr post-irradiation. This elevation in protein synthesis is found to be associated with increase in total ribosomal population and also with enrichment of polysomes at the expense of monosomes. The unusually high rate of protein synthesis observed at 18 hr after exposure of rats to lethal doses of ionizing radiations (1000 r, x-rays) seems to be mainly the consequence of the enhanced rate of RNA synthesis. The whole-body irradiation-provoked alterations in the liver transcription machinery hence provide an ideal system to investigate the control of eukaryotic transcription. Studies pertaining to whole-body x-irradiation effects on RNA biosynthesis in rat liver are investigated with this in view and these are covered in Section 1. RNA synthesis was increased as early as 4 hr following whole-body x-irradiation and a graded enhancement in this synthesis was observable up to 18 hr post-irradiation. Initial probes on the mechanisms involved revealed that the capacity of liver nuclei to polymerise RNA from ribonucleoside triphosphates in vitro was

increased following whole-body radiation exposure. In further experiments, activity of RNA polymerase (isolated free of DNA) and template activity of chromatin in the liver were separately analysed for identifying radiation-induced changes. There was no change in RNA polymerase activity in the liver following whole-body x-irradiation. On the other hand, it was found that template activity of the chromatin was raised considerably during 4 to 18 hr following whole-body radiation exposure. The number of RNA chain initiation sites on chromatin was also found to be significantly increased in response to whole-body radiation exposure. This would suggest that the increased RNA synthesis in the liver following whole-body radiation-exposure arises mainly from the activation of the chromatin transcription functions. The template activity of the liver DNA at corresponding periods after irradiation remained unchanged indicating that the increased template efficiency of the liver chromatin may be related to either changes in distribution of non-DNA chromatin components or modifications in their structures.

Studies on physico-chemical changes brought about in the liver chromatin by whole-body radiation-exposure are incorporated in Section 2. The gross chemical composition of the chromatin was found to remain unaltered after whole-body x-irradiation. Similarly, no alterations in the thermal melting profile or the UV-absorption spectrum of the chromatin isolated from normal and irradiated animals could be discerned.

Differences in physico-chemical characteristics in the chromatin as a result of whole-body x-irradiation are apparently too small to be detected by known analytical methods. Hence studies were directed to

see if any changes in the metabolism of chromosomal constituents are brought about in response to irradiation. The major constituents of the chromatin, other than DNA are proteins, which mask the DNA and make it unavailable as template for transcription. The exact role of these proteins in gene transcription is not well-understood. Evidence from several model systems suggests that nonhistone chromosomal proteins may regulate gene expression in eukaryotic cells. In the studies aimed at ascertaining whether turnovers of nonhistone chromosomal proteins play a role in radiation-induced modification in chromatin function, it was found that there was selective stimulation in syntheses of two nonhistone chromosomal proteins (mol.wt. 53000 and 31000 daltons) following whole-body irradiation. Further studies have revealed that there was also significant stimulation in phosphorylation of histones and phosphorylation, acetylation and methylation of nonhistone chromosomal proteins following whole-body radiation-exposure. The observed increases in phosphorylation of chromosomal proteins was the result of activation of phosphokinase activity associated with chromosomal nonhistone proteins following whole-body radiation exposure. These results would imply that modulations in turnovers of chromosomal proteins and also in their structures could have significant roles in regulatory mechanisms of gene expression.

The changes observed in turnovers and structures of chromosomal components could have resulted from direct action of radiations on chromosomal constituents or on other cell components. Studies carried out to elucidate primary events resulting from x-irradiation revealed that these changes are brought about by indirect mechanisms. These

studies form the subject-matter of Section 3. It was found that protection of the portion of the body containing the liver by shielding with lead sheets during total-body irradiation failed to suppress the radiation-induced changes in RNA synthesis. Further it was revealed that if the adrenals were removed prior to radiation exposure there was no increase in the liver chromatin template activity. The stimularoty effect of whole-body x-irradiation was also found to be totally prevented by protection of only the head portion during irradiation. These results indicate that the changes at transcriptional level may have been elicited by increased secretion of adrenal steroids via the hypothalamus-pituitary-adrenal axes in response to the radiation-exposure. These findings corroborate well with the reports that administration of glucocorticoids to adrenalectomised rats results in increased transcription efficiency of the liver chromatin. The findings are discussed in relation to current information on initial events responsible for changes in transcription efficiency of the chromatin in higher organisms.

Part III

This part of the thesis pertains to investigations on RNA metabolism in the rat liver following partial hepatectomy. Quiescent cells of the mammalian liver can be triggered to proliferate by chemical damage or by partial extirpation of the organ. This is known to be associated with a great upheaval in the RNA synthetic pattern in the liver. Unlike alterations in RNA synthesis caused by other stimuli, the modulations in transcription induced by partial hepatectomy are intimately

linked to subsequent processes which lead to cell division. Liver regeneration therefore offers a unique opportunity to study modulations in transcription associated with the cell cycle.

Sucrose density gradient centrifugation of RNAs in the regenerating liver clearly showed that the synthesis of all species of RNAs are elevated to the same extent. The capacity of the liver nuclei to polymerise RNA from ribonucleoside triphosphates in vitro was also elevated in partially hepatectomized rats. The activity of RNA polymerase(s) isolated free of DNA was increased as early as 4 hr and reached the maximum level around 18 hr post-operation. Further examination showed that the activity of RNA polymerase I (nucleolar enzyme) was stimulated to a much greater extent as compared to that of RNA polymerase II (nucleoplasmic enzyme). These polymerases probably have different transcriptive functions and the present results seem to suggest that relative proportions of these polymerases may fluctuate during the cell cycle. Other studies indicate that the increase in nuclear RNA polymerization could also due to increased template activity of liver chromatin during 4 to 18 hr following partial hepatectomy. The increased template efficiency of chromatin was correlatable with the increase in the number of RNA chain initiation sites on chromatin. In investigations aimed at ascertaining whether changes in chromosomal proteins could be responsible for such

changes in chromatin function, it was found that the turnover of a nonhistone chromosomal protein of the molecular weight of about 55000 daltons was selectively stimulated in the regenerating liver. It is pertinent to mention here that this protein is different from the two nonhistone chromosomal proteins the turnovers of which are stimulated in the liver following whole-body radiation exposure (see Part II - Section 2). These observations indicate that gene activation caused by different stimuli may be associated with stimulated syntheses (or turnovers) of different nonhistone chromosomal proteins.

The studies described in the thesis have thus endeavoured to unravel some facets of control mechanisms of RNA biosynthesis in mammals by the use of two stress conditions which evoke subtle perturbances in RNA synthetic patterns.