DISCUSSION

The mechanism of gene expression is linked with genetic structure of organisms. Thus, the study of gene expression in different organisms will unravel the principles involved in the structure-function relation of chromatin. Yeast offers a unique opportunity as it is probably the first intermediate in the evolution of higher organisms. Yeast is potentially an excellent system for the study of the control of protein synthesis in eukaryotic cells. Not only can one study the way in which the synthesis of specific protein is switched on but also the events which occur at the cessation of growth by changing the environment.

In this report, I will consider several of the aspects of transcription and translation which are unique to eukaryotic cells. As our knowledge of the macromolecular biochemistry of higher organisms grows, it is apparent that the models of regulatory mechanisms derived from prokaryotes are increasingly unsatisfactory. The synthesis of proteins from the messenger RNA in eukaryotic cells is different from that of prokaryotes in two important ways. In the mammalian cells, the translation of the mRNA in the cytoplasm is remote from the transcription in the nucleus, whereas in bacteria these two processes occur almost simultaneously\(^3\). Bacterial protein synthesis ceases within several minutes after inhibition of RNA transcription with actinomycin D; hence the mRNA is short-lived. In metazoan eukaryotic cells, a similar experiment indicates that
the m⁴RNA is much more stable of the order of several hours\(^{18}\). Thus, protein synthesis is not immediately affected by transcription. In this report, I will summarize some of the findings in our laboratory which deal with the unique properties of the systems which transcribe DNA and regulate translation in eukaryotic cells.

Synthesis of inducible enzymes offers a useful tool in the study of gene expression. Most of the results on prokaryotic gene expression have been elucidated due to characterization of simple inducible catabolic enzymes. On the other hand, absence of a suitable inducible enzyme system is partly responsible for the slow progress in understanding the complexity of eukaryotic gene expression. In yeast, the synthesis of N-acetylglucosamine kinase, the first enzyme in the pathway of N-AcGm metabolism can be induced by its substrate\(^{43}\). It has also been observed that for continuous synthesis of the enzyme, the presence of the inducer is required. This enzyme is absent in the cells grown in other carbon sources. The appearance and also the synthesis of the enzyme activity can be blocked with inhibitors of DNA transcription and of RNA translation and the process therefore, appears to be due to de novo enzyme synthesis resulting from the initiation of gene transcription, but other explanations are also tenable. Mechanism of induction of enzyme synthesis in yeast may be similar to that in prokaryotes as at least in few cases
"Operon" like genetic units have been identified\(^\text{37}\). Since N-AcGm kinase is the first enzyme in the pathway for amino-sugar metabolism, studies which are being carried out now on the induction of other enzymes in the pathway and their functional interrelationship will be helpful in determining the genetic unit of yeast.

In higher organisms the genetic material is present in form of the nucleoprotein complex, chromatin, composed of DNA, RNA and the various chromosomal proteins. The arrangement of these components in chromatin as well as the role of the various components in transcription are still unsolved problems. In prokaryotes, the structural genes are in the form of clusters\(^\text{44}\). The process of information transfer takes place by synthesis of a single mRNA chain (polycistronic) which carries information of all structural genes of the operon\(^2\). During translation different polypeptides are sequentially synthesized. Main evidence for such a process comes from the isolation of different polar mutants in one operon. Polarity effect has been also observed in case of yeast\(^\text{41,42}\), but polycistronic message of the bacterial type has not yet been detected\(^\text{45}\). We have characterized a class of high molecular weight proteins in yeast with a very high turnover rate. Kinetics of labelling of different molecular weight classes of proteins reveal that these proteins rapidly decay to smaller polypeptides. It is very tempting to conclude that information transfer in yeast takes place via synthesis of polycistronic messages.
Active proteins are generated by post-translational cleavage of a larger poly peptide chain, a process similar to that observed in certain animal viruses\(^46\),\(^47\). In many other ways, yeast behaves like a prokaryote. For example, repression of RNA synthesis on glucose-starvation and subsequent relaxation by cycloheximide observed in different strains of yeast by us and other workers\(^48\). This type of effect has been reported in bacteria with chloroamphenicol\(^49\).

Cyclic AMP, a mediator of gene expression in both prokaryote and eukaryote, affects RNA and protein synthesis in yeast in a sequential manner. This type of effect has not yet been observed in other systems. Earlier studies show only a stimulation of RNA synthesis in glucose starved cells\(^48\). Cyclic AMP has been implicated in glucose-repression of catabolic enzymes in prokaryotes\(^50\). A direct correlation has been observed of intracellular level of c-AMP with concentration of glucose in the medium\(^51\). Surprisingly, in this system, glucose could not repress the synthesis of the enzyme N-acetylglucosamine kinase which suggests the heterogeneity in the genetic structure of inducible catabolic enzymes. However, more information is necessary to understand the mechanism of glucose repression in yeast.

It has been suggested that c-AMP modulates gene expression in higher eukaryotes by activating a series of specific protein kinases\(^52\). These enzymes phosphorylate
different proteins involved in the expression of a gene. However, in bacteria the effect of cAMP is not mediated through protein kinases instead by a direct interaction with the DNA along with binding protein 53. Our results indicate that cAMP not only stimulates protein synthesis in a sequential manner, but also enhances the stability of total proteins. Moreover, no new protein has been detected on cAMP treatment. Though there is no evidence for activation of protein kinases in yeast, it will not be out of place if phosphorylation of proteins is evoked as the biochemical process which is brought out by cAMP. Phosphorylated proteins will show different biochemical properties.

A number of steps are involved in the expression of an eukaryotic gene. In terms of regulatory processes, these steps can be classified into two broad categories: synthesis of cytoplasmic mRNA (that is, mRNA biogenesis) and translation of mRNA into active proteins. Though processes involved in mRNA biogenesis are fairly known (see fig. 1.), it is not yet clear what regulates the appearance of specific sequences in the cytoplasm. In case of lower eukaryotes like yeast, it has not yet been possible to detect and characterize the mRNA precursors in the nucleus. As a first step in the understanding of mRNA biogenesis in yeast, we have made an attempt to detect nuclear pre-mRNA by its decay constant. Due to very
rapid decay of this RNA, a simple kinetic method was devised. The first order decay constant is 1 min which is much smaller than the value reported for higher organisms (23 min). Such rapid processing time, along with the fact the high molecular weight hnRNA has not yet been detected suggests very few intermediates in the mRNA biogenesis which agrees with the absence of repetitive sequences in yeast genome.

The present study of RNA metabolism in yeast cells were made possible by the discovery that most of the mRNA of eukaryotic cells including yeast contained a poly(A) segment at the 3'-end. This homopolymer confers upon the mRNA an affinity to immobilized poly-U, poly-dT or in the present case, poly-U attached to a sepharose substrate. By making use of this affinity, mRNA can be separated from the overwhelming amount of rRNA and labelling and decay kinetics can be studied without resort to the selective inhibition of rRNA synthesis. Poly-adenylation is an important event in mRNA biogenesis. So far no suitable role could be assigned to poly(A) sequences attached to 3'-end of cytoplasmic mRNAs. Our experiments with cordycepin and c-AMP show a direct correlation between poly(A) containing RNA level and capacity for protein synthesis which suggests a possible role of poly(A) in the synthesis of proteins. In the case of cordycepin, accumulation of inducible enzyme N-acetylglucosamine kinase shows much more inhibition than
total protein synthesis. Similar results have also been observed by other workers in higher organisms\textsuperscript{55,56}. Modified mRNA containing shorter poly(A) segment, is released from nuclei on cordycepin treatment\textsuperscript{57}. It is proposed that modified mRNAs, though capable of directing incorporation of \(^3\)H-amino acids into acid precipitable fraction, code for proteins which are biologically inactive. Support for this hypothesis comes from the result on altered protein synthesized in presence of cordycepin. These results suggests a subtle role of poly-(A) in the function of mRNA.

Experiments with cycloheximide and certain amino acid requiring auxotrophs suggest a relation between transcription and translation in yeast\textsuperscript{58}. Recently, similar experiments in Chlamydomonas reinhardi show a different picture\textsuperscript{59}. It is apparent that cycloheximide is not a specific inhibitor of protein synthesis. Modification of transcription by cycloheximide has also been observed in \textit{in vitro} system derived from yeast\textsuperscript{60}. By studying inhibition of RNA synthesis by cycloheximide at different growth conditions we have observed two components in RNA synthesis inhibition. The major component is independent of temperature and growth conditions. The former might be due to direct interaction of cycloheximide with transcriptional machinery. In that case absence of new proteins required for transcription contributes for the second component. We have also observed a rapid suppression of stimulation of RNA synthesis by c-AMP under conditions...
when cycloheximide does not effect RNA synthesis. Recent results from glucose-starved and cycloheximide treated cells along with our observation suggest a model, based on closely linked transcription, post-transcriptional processing and translation \(^{43,61}\). At present of course, there are not enough information to distinguish between these two models.