INTRODUCTION

The Conclusion from the previous chapter on the transcription of native chromatin templates in vitro is that in some systems direct evidence for differential gene transcription can be demonstrated provided that appropriate controls are carried out.

For selective gene expression to occur there must be recognition of specific DNA sequences which directly or indirectly facilitate limitation of transcription. This could be mediated through the RNA polymerase itself or through regulatory elements built into the chromatin. In the following discussion the various possibilities including some prokaryotic situations are considered. Finally, the function of chromosomal non-histone proteins (NHP) as judged by chromatin reconstitution is described and the value of this technique for future studies is discussed.

RNA polymerase directed specificity

The multiplicity of animal RNA polymerases and their different intra-nuclear localisation might suggest that gene expression in animal cells could be regulated in part by distinct RNA polymerases with different template specificities (Widnell and Tata, 1964; Fogo et al., 1970; Roeder and Rutter, 1969; Kedinger et al., 1971; Chesterton and Butterworth, 1971). Whereas in bacteria a single RNA polymerase species transcribes r-RNA, 5s, t-RNA and mRNA, in animals separate polymerase species have been identified for the transcription of r-RNA (polymerase I) mRNA (II) and 5s and t-RNA (III). In some cases one or two sub-species have been identified within these groups however
the biological significance of these are unknown. There have been no reports of multiple forms of polymerase II which can transcribe individual genes or groups of genes specifically.

It is clear from bacterial studies that specificity can be conferred on a constant subunit structure by the participation of sigma factor. The factor acts as an allosteric effector and allows the holoenzyme to initiate transcription specifically from promoter regions. After initiation sigma is jettisoned. In the absence of sigma the core enzyme binds indiscriminately to the DNA phosphate backbone.

The activity of eukaryotic RNA polymerases can also be modulated by protein factors. Rat liver polymerases I and II can be stimulated with pi factor isolated from yeast cells (Di Mauro et al., 1972). Factors that stimulate the activity of polymerase II have been isolated from Novikoff ascites cells (Lee and Dahmus, 1973) calf thymus (Stein and Hausen, 1970) and rat liver (Seifart, 1970). These factors could affect either the initiation or elongation processes and as yet no information exists as to their effect on the specificity of transcription.

Although in bacteria sigma confers specificity for promoter regions in the DNA there is no evidence that they are responsible for selecting which genes are to be transcribed. Analagous situations to eukaryotic differentiation have been found in prokaryotes where there is an irreversible commitment independent of environment. For example in the case of T4 phage development (Goff and Weber, 1970; Travers, 1970; Schachner and Zillig, 1971; Synder, 1973) or the induction of sporulation in B. subtilis (Losick et al., 1970; Losick, 1972; Greenleaf et al., 1973) the existing polymerase is altered by modification of its subunits and loss of the original sigma factor.

The question of selective transcription by multiple specific RNA polymerase complexes is still contentious. Parker and Roeder (1977) examined the transcription of 5s genes in Xenopus oocyte chromatin by exogenous polymerases I and III from X. laevis ovaries, polymerase II from mouse phasmacytoma cells and E. Coli polymerase. In this system only polymerase III is capable of stimulating specifically the asymmetric synthesis of 5s RNA; the other enzymes stimulate total RNA synthesis. However, when the comparison is made on naked DNA the specificity shown by polymerase III is lost. This would suggest that chromatin associated proteins are required for the selective and asymmetric synthesis of 5s genes and that a specific polymerase is required to do this. At present there is no evidence that polymerase II can behave in this way; in fact the difficulties associated with in vitro transcription by exogenously added polymerase II suggest that factors as yet unknown may still be missing. It is apparent