# Molecular and Cell Biology of Yeasts

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# Preface

Our knowledge of the molecular and cell biology of yeast has advanced rapidly in recent years. The accumulation of this knowledge has not only promoted industrial applications but has also aided our fundamental understanding of cellular processes in lower and higher eukaryotes. Advances in the subject have not been reviewed for some time, and so we hope that this book is timely. We have aimed to cover both the fundamentals and the complexities of molecular and cellular function, and have included yeasts other than *Saccharomyces cerevisiae* which have made a considerable impact on our understanding of cellular processes—in particular, *Schizosaccharomyces pombe* and the methylotrophic yeasts, *Pichia pastoris* and *Hansenula polymorpha*.

The study of yeasts has been greatly facilitated by their fast, simple growth, accessible biochemistry and ease of genetic manipulation. Lower eukaryotes, especially *S. cerevisiae*, have long been used as model organisms in studying the cellular biology of higher eukaryotes. This book documents the high degree of conservation in cellular function and process, and demonstrates how yeast facilitates our understanding of both higher and lower eukaryotic biology. We feel, therefore, that the book will be of interest to the non-yeast biologist as well as to those active in yeast research.

There have been extensive studies of gene expression in *Saccharomyces cerevisiae* since the advent of genetic engineering and the development of new techniques of molecular biology. Research into yeast promoter structure and function is important to our understanding of both gene regulation and the means by which heterologous genes can be expressed to high levels for the production of commercially important proteins in the food/drink and pharmaceutical/healthcare industries.

Since gene regulation is of importance to our understanding of cellular differentiation and development in all eukaryotes, we have included a chapter on the negative regulation of gene expression.

After transcription, the fate of mRNA can be quite varied: some mRNA molecules are long-lived, others short-lived. The stability of mRNA is of considerable interest, not only because it reflects a level of control on cellular processes but also because it influences the efficiency with which a particular gene can be expressed to the high levels required for commercial purposes. For this reason, we have included a chapter reviewing the current status of our knowledge of mRNA translation and stability, a topic not normally covered in detail.

Research on the expression and secretion of heterologus proteins has resulted in considerable advances in the techniques of producing proteins and

peptides in yeast. The problems and solutions to some of these problems have been included in the book.

A level of control of cellular functions and processes is found in the area of post-translational proteolytic processing and protein degradation. Significant advances have been made here, and so we have included a comprehensive review which will also be of interest to those studying such processes in other organisms, and to those concerned with the impact of these processes in the production of proteins and peptides in yeast.

For over a quarter of a century, the study of killer toxin of the type 1 killer system in *S. cerevisiae* has been pursued with vigour. This area of research has led to the discovery of *ds*RNA genomes, which encode both the killer toxin itself and the information required for the replication and maintenance of *ds*RNA in yeast. The study of this mycovirus has revealed how intracellular protein trafficking and post-translational modification confers immunity on the killer toxin. The recent extensive research is reviewed by the leading scientists in this field.

In higher multi-cellular eukaryotes, individual cells respond to external signals through ligand-receptor interaction. Signal transduction is a complex chain of interactions which is readily studied in yeast. The GTP-binding proteins (G-proteins), key components in this interaction, have been the target for extensive study over the past five years. These proteins have functions identical to those in higher eukaryotes and share homologous structural domains. Their function in yeast is the transmission of signals for the control of cell proliferation in response to external stimuli: thus the study of these proteins is important for understanding the mechanisms of oncogenesis in mammalian cells. Recently, it has been found that G-proteins are involved not only in the transfer of signals across membranes from the external environment to the internal components of the cell but also in the transfer of signals between intracellular membranes—in particular, the membranes of the secretory pathway. Thus the role of G-proteins in cellular functions may be broader than first envisaged.

The regulation of cell proliferation in both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* has been investigated for almost two decades, and work on cell division cycle mutants has resulted in the isolation of genes and the discovery of functions that regulate cell proliferation. The isolation of mutants which accelerate progression through the cell cycle as small or '*wee*' mutants in *S. pombe* has added considerably to our knowledge of cell cycle control. We have included a review of these advances, with an emphasis on *S. pombe*, where most of the recent discoveries have been made.

*Saccharomyces cerevisiae* has been exploited in the brewing and baking industries for centuries. With the aid of genetic engineering, brewing yeast can now be manipulated with greater ease, allowing improvements in beer quality and production costs. We felt it relevant to include a chapter covering this

rapid progress with brewing yeast, since it has played an important role in the foundation of one of the earliest biotechnological processes.

Several yeast organisms other than *Saccharomyces cerevisiae* have been investigated as possible host organisms for the expression and secretion of heterologous proteins. Work on the methylotrophic yeasts *Pichia pastoris* and *Hansenula polymorpha* has revealed that these organisms can be extremely efficient at both intracellular production and extracellular secretion of proteins. We have included a chapter reviewing both these organisms in detail.

The 2 $\mu$m circle plasmid of *Saccharomyces cerevisiae* is commonly used in part or in whole for the replication of heterologous genes to high copy number. Endogeneous 2 $\mu$m remains at steady-state copy number levels and the mechanism of this homeostasis is at present being elucidated. Our understanding of the mechanisms involved in plasmid copy number control and stability is important for the application of 2 $\mu$m to high copy number expression vectors in yeast. In the last few years this understanding has approached new levels, and a review of the current status of the subject is included in the book.

We would like to thank our contributors for the speed with which they completed their work to ensure that the volume was published rapidly, and we hope that our readers will find the book a valuable reference to the remarkable advances in yeast molecular and cell biology over the past five years.

E.F.W.
G.T.Y.

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# 1 The activation and initiation of transcription by the promoters of *Saccharomyces cerevisiae*

JANE MELLOR

## 1.1 Introduction to gene regulation

A coherent explanation for the mechanisms used by bacteria to control the expression of their genetic material in response to environmental conditions was formulated in the early 1960s (Jacob and Monod, 1961; Jacob *et al.*, 1964). A gene can be divided into three distinct functional parts. The structural unit encodes the gene product, the promoter allows the expression of the structural region and the regulatory sequences ensure that expression occurs only in response to the correct environmental stimuli. Gene regulation occurs primarily by controlling the rate of transcription initiation at the promoter which acts in *cis* and is the site recognized by RNA polymerase. The regulatory sequences control the rate of transcription initiation through interaction with specific *trans*-acting regulatory proteins that may act positively to enhance the rate of transcription or negatively to repress transcription. A general outline of the basic principles of gene regulation is shown in Figure 1.1

A prokaryotic promoter consists of two distinct elements located at $-10$ and $-35$ relative to the RNA initiation site at $+1$. Both are necessary for interaction with the RNA polymerase holoenzyme and are sufficient for directing basal level constitutive transcription from a discrete initiation site. Different forms of the holoenzyme exist that are distinguished by the sigma subunit. This subunit is essential for promoter recognition. Thus some form of regulation can be imposed by enzymes containing distinct sigma subunits interacting with specific DNA elements in different promoters. Positive control of transcription is achieved by a second protein–DNA interaction between an activator protein and the regulatory site which is usually located just upstream of the promoter elements. Enhancement of the rate of transcription is probably achieved by protein–protein interactions between the RNA polymerase and the activator protein. Negative control of transcription is achieved by preventing RNA polymerase–promoter interaction. The element to which the repressor protein binds is usually located within the region of DNA that normally interacts with RNA polymerase, and this bound repressor prevents functional interactions and transcription is impaired.

Recent developments in recombinant DNA technology have allowed the isolation and characterization of many eukaryotic genes. It is clear that the majority of eukaryotic genes have a far more complex organization than those

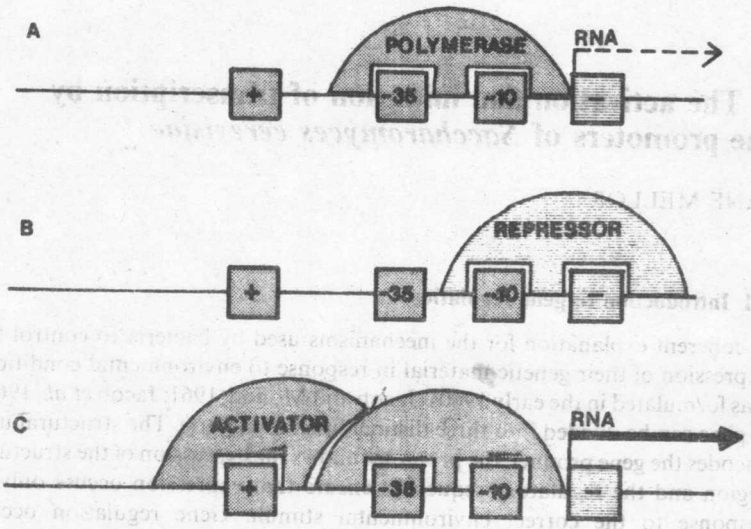


**Figure 1.1** Transcriptional regulation in prokaryotes. Promoter (− 35 and − 10) and regulatory (− and +) sequences for a generalized gene are shown. The position of the regulatory elements with respect to the promoter may vary. Panel *A* represents basal level transcription, with the RNA polymerase interacting with promoter elements. Panel *B* shows a specific DNA–protein interaction between the repressor protein and the operator site which prevents polymerase binding to the promoter. Panel *C* describes positive activation of transcription, with an activator protein interacting with both specific DNA sequences and the RNA polymerase.

found in prokaryotes. Structural regions of the gene may be interspersed with non-coding DNA, and the promoter/regulatory sequences are more complex and are often found far upstream or downstream from the structural gene. Despite the many differences that exist between eukaryotic and prokaryotic genes, a major event in the control of gene expression in eukaryotes occurs at the point of transcription initiation, and these events are regulated by positive or negative *trans*-acting proteins. Thus the basic principles of gene regulation may be applied to eukaryotic genes, but it is now clear that the properties of promoter/regulatory elements are quite distinct from prokaryotes and the molecular interactions are different. In this chapter, the organization of, and functional interactions that occur at, promoter and regulatory elements in genes of the baker's yeast, *Saccharomyces cerevisiae*, will be discussed.

## 1.2 Organization and expression of the yeast genome

Yeast is a simple single-cell eukaryotic organism that shares many characteristics with higher eukaryotic cells (Strathern *et al.*, 1981). The ease with which yeast can be manipulated genetically, coupled with its obvious industrial uses, has

made this a favoured system for studying gene regulatory events, and much progress has been made since the advent of recombinant DNA technology and the development of a transformation system for yeast in the late 1970s. The yeast genome contains 16 linear chromosomes. Each chromosome contains multiple origins of replication, two telomeres and a centromere, and is in the form of chromatin in a nucleus. The genome contains about $10^4$ kbp of DNA, of which about 50% is transcribed under normal growth conditions (Hereford and Rosbash, 1977; Kaback *et al.*, 1979), and thus contains about 5000 protein coding genes which must be packed quite closely together. The genetic map shows that genes of similar function are generally not grouped together, and so differ from prokaryotic genes which are often clustered into operons. Two well-studied exceptions are the genes involved in galactose utilization (*GAL7*, *GAL10*, *GAL1*) and the genes at the mating type locus (*MAT*$\alpha$*1* and *MAT*$\alpha$*2*).

In yeast, most genes are transcribed at similar low levels of approximately one to two molecules of mRNA per cell (St John and Davis, 1979; Struhl and Davis, 1981). Most of the few tens of yeast genes that have been analysed in detail to date were isolated by virtue of their high expression levels or tight *regulatory* response to given environmental conditions. Thus much of our detailed knowledge of yeast promoters has come from genes that are probably not representative of the majority of yeast genes. Most are likely to be expressed *constitutively* at the low basal level of one to two molecules per cell under all conditions. In any particular situation where regulation is likely to occur, such as change of carbon source, environmental stress, amino acid starvation, progression through the cell cycle and changes in cell type, transcription rates change for only a small number of genes.

Like all eukaryotes there are three functionally distinct RNA polymerase activities in yeast. RNA polymerase I transcribes only the clustered genes for ribosomal RNA which represents about 70% of the total cellular RNA at steady state; RNA polymerase III transcribes the tRNA genes and the 5S ribosomal RNA genes which together account for about 30% of the total cellular RNA; RNA polymerase II transcribes all the remaining 5000 or so protein coding genes which together represent only 1% of total cellular RNA at steady state. Much of our current knowledge of promoter structure and function is derived from studies of genes transcribed by RNA polymerase II, and thus this discussion will focus on the promoters of protein coding genes.

The term promoter will be used to describe the region of DNA found upstream of the structural part of a protein coding gene. Three elements within this region are necessary and sufficient for the regulation, efficiency and accuracy of transcription initiation; the upstream regulatory sequences, the TATA element and initiator (I) element. The general organization of the promoter of a typical gene transcribed by RNA polymerase II is shown in Figure 1.2. In any yeast promoter, one of each of these elements can be identified, which together are sufficient for properly regulated transcription, although most yeast promoters have a more complex organization with

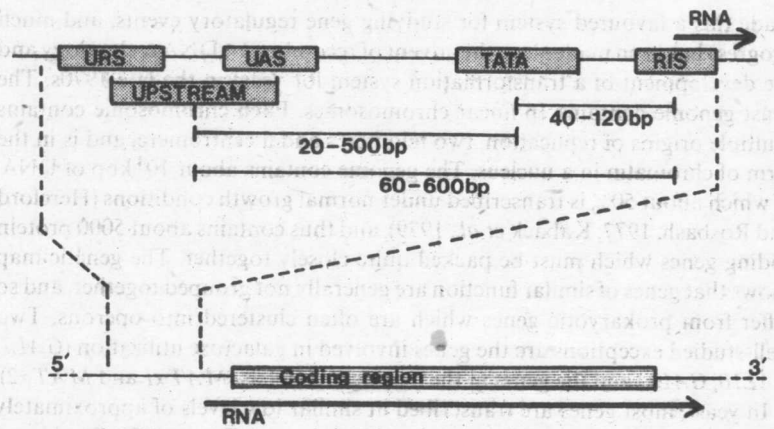


**Figure 1.2** A schematic representation of elements and their relative position in a yeast promoter. The position and number of the upstream sequences may vary from that shown.

multiple elements being present. Noticeable features of yeast promoters are their relatively large size and the variability in the distances separating individual elements when compared to their prokaryotic counterparts (Faye *et al.*, 1981; Struhl, 1981; Beier and Young, 1982; Guarente *et al.*, 1982*a*; Struhl, 1982*a*; Dobson *et al.*, 1982*a*, *b*; Siliciano and Tatchell, 1984; Sarokin and Carlson, 1984; Wright and Zitomer, 1984). The values for distances given in Figure 1.2 are averages, and in some genes essential regulatory functions can be located up to 1.5 kb distal to the structural gene (Miller and Nasmyth, 1984). In other genes, such as *MATα*, *PET56* and *TRP1*, promoter function lies within a region only 100 bp upstream from the RNA initiation site (Siliciano and Tatchell, 1984; Struhl, 1985*a*; Kim *et al.*, 1986). In the majority of promoters, essential sequences are found dispersed over a region extending about 500 bp upstream from the RNA start site.

## 1.3 Upstream promoter elements

### 1.3.1 *General properties of upstream elements*

Sequential deletion of sequences residing 5′ to the structural region of the gene of interest is the method used to determine the limits of the promoter necessary for wild-type levels and correct regulation of transcription. More extensive deletions will alter transcription or its regulation because essential promoter sequences, or upstream elements, are removed and thus their position on the promoter can be estimated. Three functionally distinct upstream elements have been defined in yeast promoters, upstream activator sequences (UASs),

upstream repressor sequences (URSs) and constitutive promoter elements. The upstream activator sequence is defined as the element in a yeast promoter at which positive activation and regulation of transcription occurs (for a brief review see Guarente, 1984, and Struhl, 1987). However, some positive regulatory sites do not correspond with the upstream activation sequence. Thus transcriptional activation and positive control can be functionally distinct properties of yeast promoters. Generally, deletion of the region that defines the UAS results in a significant drop in transcription which is no longer responsive to regulatory stimuli. The activity of the UASs may be modulated by upstream repressor sequences when they are located in the same promoter. The URSs usually have a negative influence on transcription and respond to specific regulatory signals to lower the efficiency of transcription to a greater or lesser extent. These sequences are also known as negative control elements, and when deleted may result in increased transcription or inappropriate regulation. Constitutive promoter elements activate transcription but are not regulatory sites. Deletion of these elements generally reduces transcription to undetectable levels.

Upstream elements generally share common features. They are found, and can function, at long and variable distances upstream from the RNA initiation site. They are functional when inverted with respect to their normal position, and thus in some situations may act bidirectionally. Upstream activating and regulating sequences, both positive and negative, have additional properties not shared with constitutive elements. They are defined by short sequences that confer on a promoter a particular regulatory response. These elements are exchangeable, and if inserted into other promoters they confer the regulatory response characteristic of the promoter in which they are normally found. In this section, the organization and characteristics of elements that mediate positive and negative control of transcription are considered.

### 1.3.2 *Upstream activator sequences*

(1) *The upstream activator sequence confers promoter specificity.* Upstream activator sequences (UASs) were first recognized in the early 1980s by 5′ deletion analyses of the *CYC1* and *HIS3* promoters (Guarente and Ptashne, 1981; Struhl, 1981, 1982*a*,*b*). Subsequently, similar elements have been found in a number of yeast promoters, most notably *GAL1–10*, *HIS4*, *SUC2* and *CYC7* (Guarente *et al.*, 1982*a*, Donahue *et al.*, 1982; West *et al.*, 1984; Sarokin and Carlson, 1984; Wright and Zitomer, 1984). A number of regulatory genes have been defined genetically as mediators of positive control of transcription. At the time that these loci were defined it was supposed that their products would interact *in trans* at some sequence within the gene they regulate. For example, transcription of the genes involved in galactose utilization at the *GAL1*, *GAL7* and *GAL10* loci are uninducible in yeast strains bearing recessive mutations in the *GAL4* gene