Chapter 19
A Comparative Study of RNA Polymerase II Transcription Machinery in Yeasts

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Abstract The control of gene expression, predominantly at the level of transcription, plays a fundamental role in biological processes determining the phenotypic changes in cells and organisms. The eukaryotes have evolved a complex and sophisticated transcription machinery to transcribe DNA into RNA. RNA polymerase II enzyme lies at the centre of the transcription apparatus that comprises nearly 60 polypeptides and is responsible for the expression and regulation of protein-encoding genes. Much of our present understanding and knowledge of the RNA polymerase II transcription apparatus in eukaryotes has been derived from studies in *Saccharomyces cerevisiae*. More recently, *Schizosaccharomyces pombe* has emerged as a better model system to study transcription because the transcription mechanism in this yeast is closer to that in higher eukaryotes. Also, studies on components of the basal transcription machinery have revealed a number of properties that are

T. Satyanarayana, G. Kunze (eds.), *Yeast Biotechnology: Diversity and Applications*,
DOI: 10.1007/978-1-4020-8292-4_19,

common with other eukaryotes, but have also highlighted some features unique to *S. pombe*. In fact, the fungal transcription associated protein families show greater species specificity and only 15% of these proteins contain homologues shared between both *S. cerevisiae* and *S. pombe*. In this chapter, we compare the RNA polymerase II transcription apparatus in different yeasts.

Keywords Transcription, transcription machinery, RNA polymerase II, *S. cerevisiae*, *S. pombe*

19.1 Introduction

Regulated expression of protein-coding genes underlies fundamental biological processes, including development, differentiation, morphogenesis and oncogenesis. Most of this regulation occurs predominantly at the level of transcription initiation. More than 60 different proteins coordinate with each other to fine tune the spatial and temporal pattern of gene expression. These proteins can be grouped into three different classes (Fig. 19.1).

- General or basal transcription factors (GTFs) that are ubiquitous and bind to core promoter DNA sequences. These proteins enable the recruitment of RNA polymerase II (pol II) to the specific promoter sequences of protein-coding genes;

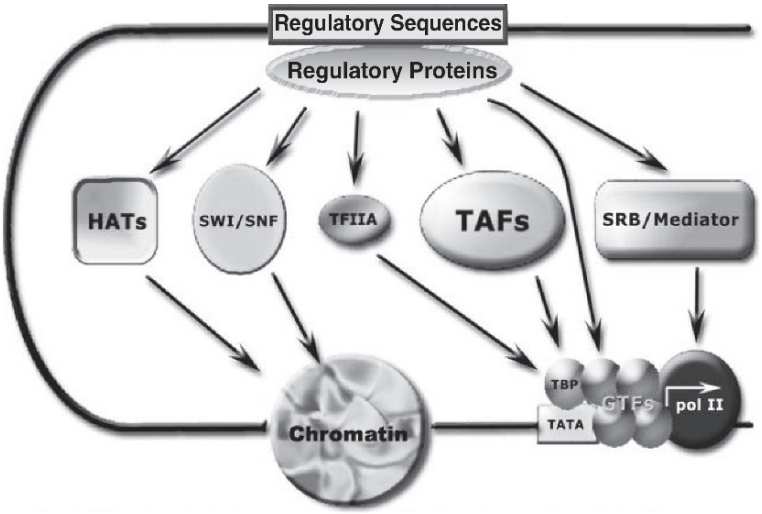

Fig. 19.1 Schematic representation of the key players involved in expression and regulation of protein encoding genes (see text for details)

- Regulatory proteins that bind to proximal promoter elements, enhancers or silencers in a sequence-dependent manner. They activate or repress transcription of target gene(s) either in a cell-type/developmental stage-specific manner or in response to external stimuli;
- Co-activators and co-repressors - proteins which interact with the regulatory proteins, and mediate their effects on the basal transcription machinery. These can be further sub-divided into two groups-those which affect chromatin structure, such as Swi/Snf complex and HATs (Histone Acetyl Transferases) and those which serve as an interface, integrating information from the different transcription regulatory proteins and transmitting it to the general transcription machinery. The latter category of protein is collectively referred to as 'mediators'.

Much of our current knowledge of the mechanism of transcription and the transcription machinery has come from biochemical and genetic studies carried out in the budding yeast, *Saccharomyces cerevisiae*. However advances in structural biology over the past few years, have helped in unveiling the structures of the *S. cerevisiae* pol II enzyme alone and in complex with several transcription factors. Specifically, structures of the 10 subunit pol II enzyme alone, i.e. lacking the Rpb4 and Rpb7 subunits (Cramer et al., 2000, 2001), the 12 subunit enzyme alone (Armache et al., 2003, 2005; Bushnell and Kornberg, 2003), the 10 subunit enzyme in the form of a transcribing complex with the general transcription factor TFIIB (Chen and Hahn, 2004; Bushnell et al., 2004), and also in complex with the transcription elongation factor TFIIS (Kettenberger et al., 2003, 2004) have been resolved. Lower resolution EM structures have also been determined for the pol II–mediator complex (Davis et al., 2002) and for pol II–TFIIF complex (Chung et al., 2003). Determination of structures of these large multiprotein complexes, have added a new dimension to our analysis of the different steps of the transcription reaction (reviewed by Hahn, 2004; Woychik and Hampsey, 2002).

Recently, fission yeast, *Schizosaccharomyces pombe*, has emerged as a complementary model system to study many of the biological processes. Cross-species comparisons between *S. cerevisiae* and *S. pombe* have proven a valuable tool in analyzing cell division cycle control, DNA repair and recombination (reviewed by Sunnerhagen, 2002). Several lines of evidence indicate that the mechanism of transcription initiation by *S. pombe* RNA polymerase II is more similar to higher eukaryotes than that of *S. cerevisiae*. Initiation of transcription by pol II occurs 25–30 bp downstream from the TATA box of the core promoter in both *S. pombe* and mammalian cells; but this distance may vary between 40 bp and 120 bp downstream of the TATA box in *S. cerevisiae* (Li et al., 1994). These observations raise the possibility that both *S. cerevisiae* and *S. pombe* may use different mechanisms to identify transcription start sites. It was also observed that transcriptional initiation from mammalian promoters introduced into *S. pombe* occurred at the same site as in mammalian cells (Toyoma and Okayama, 1990). Furthermore, the AP2 and CTF transcriptional activators did not stimulate transcription in *S. cerevisiae*, whereas they activated transcription in *S. pombe* and humans. This implies that the

transcription activation mechanism in *S. pombe* is closer to that of humans than *S. cerevisiae* (Remacle et al., 1997).

The scope of this chapter is to provide an overview of the structural features and functions of the various proteins that function in RNA pol II-mediated transcription initiation in yeast, with more emphasis on the evolutionary distant *S. cerevisiae* and *S. pombe*. To grasp the relationship between these proteins, it is imperative to briefly describe the steps involved in the transcription of protein-coding genes.

19.2 Overview of Pol II Transcription

Transcription of RNA polymerase II-dependent genes begins with the assembly of a pre-initiation complex at the promoter. The pre-initiation complex or PIC is a conglomerate of six different basal transcription factors-TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH and the RNA polymerase II enzyme (Fig. 19.2).

Formation of the PIC is followed by promoter melting or separation of the two strands of the template DNA to expose the transcription initiation site and the synthesis of the first phosphodiester bond in the nascent RNA. Subsequently many short RNA transcripts, 3 to 10 bases long, are transcribed by pol II and released. This phenomenon is referred to as 'abortive initiation'. Finally, longer transcripts of approximately 30 bases are synthesized and transcription switches from being abortive to productive, i.e. RNA polymerase II is released from the proteins

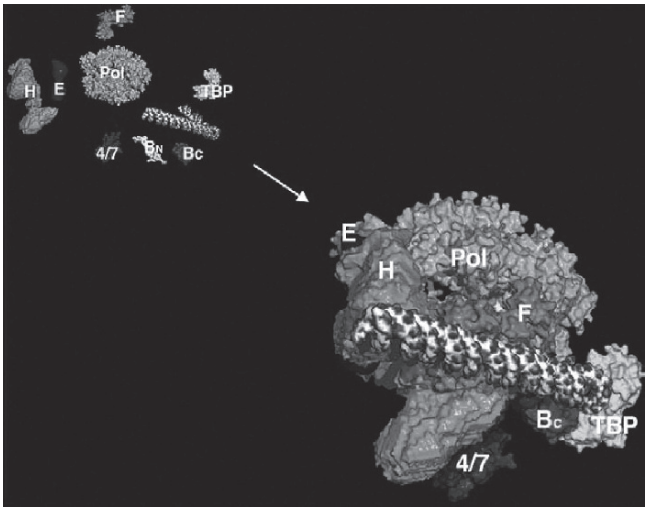

Fig. 19.2 RNA polymerase II transcription initiation complex. X-ray and electron microscopic structures (upper left) were assembled in a complete transcription initiation complex (lower right). Reprinted by permission of Federation of the European Biochemical Societies from Structural basis of eukaryotic gene transcription, by Boeger et al., 2005. *FEBS Lett.* 579: 899–903

assembled at the promoter (known as 'promoter clearance') and enters the stage of transcription elongation. Once the enzyme reaches the termination site, it dissociates from the DNA template and the nascent RNA transcript is released (reviewed by Orphanides et al., 1996).

The key players involved in transcription initiation of RNA pol II genes are described below:

19.3 RNA Polymerase II Core Enzyme: The 'CPU' of mRNA Synthesizing Machinery

RNA polymerase II core enzyme lies at the centre of the transcription apparatus responsible for decoding the information stored in DNA into its usable form, the mRNA. It resembles the CPU or the central processing unit of a computer receiving inputs from various internal and external stimuli, processing these inputs and then giving an output in the form of expression of specific genes or specific classes of genes (Fig. 19.3).

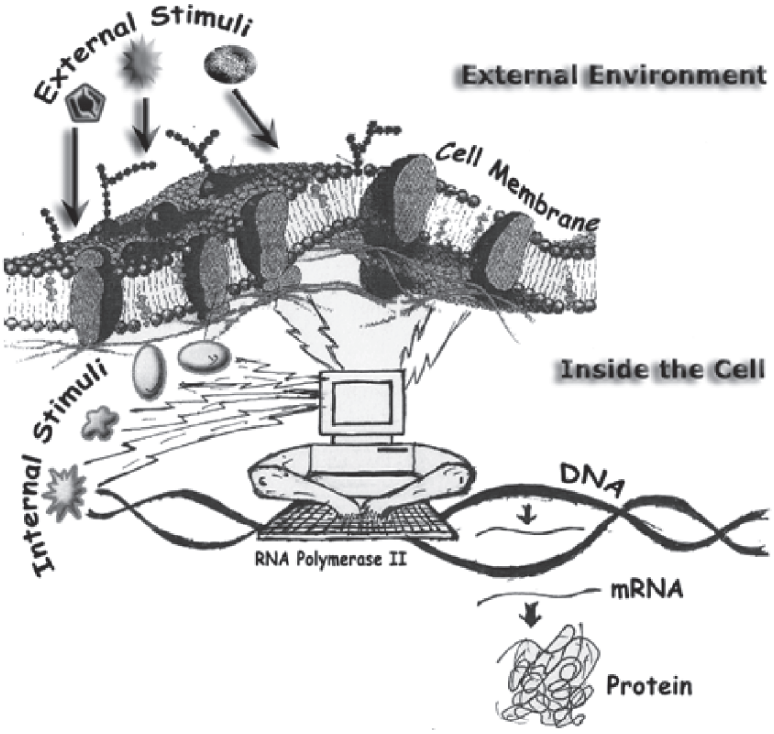

Fig. 19.3 A cartoon depicting the RNA polymerase II enzyme as the Central Processing Unit of the computer

Pol II from both *S. cerevisiae* and *S. pombe* consist of twelve subunits (reviewed by Kolodziez et al., 1990; Young, 1991; Mitsuzawa and Ishihama, 2004). These subunits, designated as Rpb1 to Rpb12, can be divided into three overlapping categories: (i) core subunits-Rpb1, Rpb2, Rpb3 and Rpb11; (ii) shared or common subunits-Rpb5, Rpb6, Rpb8, Rpb10 and Rpb12; and (iii) pol II unique subunits-Rpb4, Rpb7 and Rpb9. Pol II has also been isolated from other yeasts, including *Candida albicans* and *Candida utilis*. Interestingly, *Candida albicans* pol II contains only nine subunits, with apparent molecular weights of 170, 145, 120, 80, 62, 58, 45, 40 and 20 kDa. The mobility of these subunits on SDS-PAGE is different from the corresponding subunits from *S. cerevisiae* or *C. utilis* (Patturajan et al., 1999). In comparison to the *C. albicans* pol II, the enzyme from *C. utilis* comprises ten subunits, with molecular weights ranging from 205 kDa to 14 kDa (Patturajan, 1995). Interestingly, pol II from *C. utilis* could initiate transcription accurately upon addition of cell extracts from both *C. utilis* and *S. cerevisiae.* Moreover, the GTFs were also functionally interchangeable between these two yeasts (Patturajan et al.,1994). Both prokaryotic and eukaryotic RNA polymerases are zinc containing metalloproteins (reviewed by Archambault and Friesen, 1993). The *S. cerevisiae* enzyme contains two molecules of zinc, whose removal damages the enzyme conformation irreversibly (Mayalagu et al., 1997). In comparison, both *C. albicans* and *C. utilis* pol II contain five molecules of zinc bound to them. It was also shown that the three largest subunits of *C. albicans* pol II had the ability to bind zinc, whereas only the largest subunit of *C. utilis* pol II could bind zinc (Patturajan et al., 1999). In case of the *S. cerevisiae* enzyme, five subunits have been demonstrated to possess zinc binding ability (reviewed by Archambault and Friesen, 1993).

Following sections describe our current status of understanding of the different subunits of *S. cerevisiae* and *S. pombe* RNA polymerase II. Table 19.1 shows a comparison of some of the features of these subunits in *S. cerevisiae* and *S. pombe*.

Table 19.1 Comparison between orthologous subunits in *S. cerevisiae* and *S. pombe*

Subunit	Molecular weight (kDa) in *S. cerevisiae*	Molecular weight (kDa) in *S. pombe*	Deletion viability in *S. cerevisiae*	Deletion viability in *S. pombe*	Identity in *S. pombe*
Rpb1	192	194	Essential	Essential	59
Rpb2	139	138	Essential	Essential	67
Rpb3	35	34	Essential	Essential	47
Rpb4	25	15.4	Nonessential	Essential	20
Rpb5	25	24	Essential	Essential	56
Rpb6	18	16	Essential	Essential	54
Rpb7	19	19	Essential	Essential	52
Rpb8	17	14	Essential	Essential	34
Rpb9	14	13	Nonessential	Essential	47
Rpb10	8	8.3	Essential	Essential	72
Rpb11	14	14	Essential	Essential	44
Rpb12	8	7.2	Essential	Essential	39

19.3.1 Core Subunits: Rpb1, Rpb2, Rpb3 and Rpb11

The subunits- Rpb1, 2, 3 and 11, form the core catalytic domain of RNA polymerase II. Rpb1 and Rpb2 are homologous to the largest and second largest subunits of RNA polymerases I and III, and also share sequence homology with the β′ and β subunits respectively of the eubacterial RNA polymerase. Not surprisingly, this sequence similarity also extends to functional similarity: the Rpb1 and β′ subunits bind DNA, whereas the Rpb2 and β subunits are involved in binding nucleotide substrates. Specific mutations have been isolated in *S. cerevisiae* Rpb1 and Rpb2 subunits that affect accuracy of transcription initiation, thereby implying a role of these subunits in start site selection. Other mutations in these subunits confer sensitivity to 6-azauracil (6-AU), a phenotype linked to defects in transcription elongation, suggesting that these subunits help in overcoming transcriptional arrest (reviewed by Archambault and Friesen, 1993).

A unique feature of the pol II Rpb1 subunit is the presence of the carboxyl terminal repeat domain (CTD) containing tandem repeats of a heptad motif with the consensus sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser. Although the presence of CTD is a ubiquitous feature of all eukaryotic RNA polymerase II enzymes, the number of heptapeptide repeat varies between different organisms. The budding yeast pol II CTD contains 26 repeats (Allison et al., 1985), whereas fission yeast pol II CTD has 29 repeats (Azuma et al., 1991). Complete removal of the CTD from budding yeast pol II is lethal, but truncation of the number of repeats present in the CTD to 10–12, results in conditional phenotypes like cold sensitivity and the inability to grow on a variety of carbon sources (Nonet et al., 1987; Nonet and Young, 1989). A genetic screen carried out to isolate suppressors of the cold-sensitive phenotype identified mutations in nine different genes and these suppressors were called SRBs or Suppressors of RNA Polymerase B (Nonet and Young, 1989). The CTD plays myriad roles in coordination and regulation of transcription initiation, elongation and termination, DNA repair, mRNA processing and mRNA export, by interacting with proteins directly involved in these processes (Shilatifard et al., 2003). It is largely unstructured in the absence of interacting proteins (Cramer et al., 2001) and the binding of proteins depends on the phosphorylation status of the CTD. It undergoes extensive phosphorylation and dephosphorylation during the transcription cycle at serine residues present at positions 2 and 5 in each heptapeptide repeat. In both the budding yeast and the fission yeast, cyclin-dependent kinases are involved in CTD phosphorylation. RNA polymerase II containing an extensively phosphorylated CTD (designated form IIo) is found in the elongating complex, while the dephosphorylated form (designated as form IIa) preferentially enters the PIC. A phosphatase called Fcp1 (TFIIF-associating CTD phosphatase) has been identified in *S. cerevisiae* (Chambers and Kane, 1996) and *S. pombe* (Kimura et al., 2002), which predominantly dephosphorylates serine 2 in the CTD. Fcp1 interacts with TFIIB, TFIIF and the Rpb4 subunit of the *S. pombe* polymerase (Kimura et al., 2002). Another CTD phosphatase has also been isolated in *S. cerevisiae* called Ssu72, which can dephosphorylate serine 5 in vitro (Krishnamurthy et al., 2004).

Sakurai and Ishihama (2002) reported that the intracellular concentration of the pol II in fission yeast remains constant but phosphorylation of the serine, threonine and tyrosine residues in its carboxy terminal domain varies depending on the phase and rate of growth.

The Rpb2 subunit of the budding yeast features a strong negative charge cluster in the third quartile of the protein, comprising residues 665 to 724: there are 23 acidic residues with no basic residues (Brendel and Karlin, 1994). Recently, Kato et al. (2005) isolated a mutation in the Rpb2 subunit of fission yeast that resulted in loss of heterochromatic histone modifications, accumulation of pericentromeric transcripts and loss of siRNAs, indicating that RNA polymerase II may couple pericentromeric transcription with siRNA processing and heterochromatin assembly.

Rpb3 and Rpb11 subunits form a heterodimer. They also share sequence similarity with the bacterial RNA polymerase α subunit. Mutational analysis of Rpb3 revealed its role in the assembly of *S. cerevisiae* pol II (Kolodziez and Young, 1991) and it is likely that the pol II assembly is initiated by the formation of the Rpb3/Rpb11 heterodimer. More recently, Benga et al. (2005) have shown that the formation of *S. cerevisiae* Rpb3/Rpb11 heterodimer critically depends on the presence of the C-terminal region of Rpb11. Rpb3 is also important in activator-dependent transcription in *S. cerevisiae* (Tan et al., 2000). In case of *S. pombe*, exposure to 6M urea results in the dissociation of the pol II into Rpb2-Rpb3-Rpb11 subcomplex. This ternary complex is considered to be an intermediate in the assembly of *S. pombe* pol II (Kimura et al., 1997). Far western blot and GST-pull down assays with different Rpb3 deletion mutants demonstrated that amino acid residues 105 to 263 of Rpb3 were involved in binding to the Rpb5 subunit and amino acid residues 105 to 297 were required for binding the Rpb11 subunit of *S. pombe* pol II. In fact binding of Rpb5 stabilized the Rpb3-Rpb11 heterodimer (Yasui et al., 1998). In another study, analysis of temperature -sensitive mutants of *S. pombe* Rpb3 provided further evidence for a role of this subunit in assembly of pol II in *S. pombe* and also in transcription activation (Mitobe et al., 1999, 2001).

To examine if the *S. pombe* core subunits could functionally complement their respective *S. cerevisiae* counterparts, Shpakovski et al. (2000) replaced the core subunits of *S. cerevisiae* pol II with their respective *S. pombe* homologs. Interestingly, no heterospecific complementation was observed for the two largest subunits, Rpb1 and Rpb2. In contrast, the Rpb3 and Rpb11 subunits partially complemented the defect. They supported growth at 30°C but not at either high (37°C) or low (16°C, 25°C) temperatures. However, growth at these temperatures was restored by increasing the gene dosage of the *S. cerevisiae* Rpb11 or Rpb10 subunits.

19.3.2 Shared Subunits: Rpb5, Rpb6, Rpb8, Rpb10 and Rpb12

The five small subunits, Rpb5, -6, -8, -10 and -12 are common to all the three eukaryotic RNA polymerases. Despite accumulation of data from genetic, biochemical and structural experiments, the precise functions of each of these subunits

are still largely unclear! In *S. pombe*, it has been estimated that on an average 10–20% of these subunits are assembled in pol II and the remaining are either assembled in RNA polymerase I and III or exist as unassembled subunits (Sakurai and Ishihama, 2002). The Rpb5 subunit of *S. pombe* pol II complements the absence of the Rpb5 subunit in *S. cerevisiae* (Shpakovski et al., 2000). It has also been reported to form a binary complex with the Rpb3 subunit. These two subunits also bind to the Rpb1 and Rpb2 subunits independently (Miyao et al., 1996). Chemical crosslinking and far western blotting experiments carried out to determine subunit-subunit interactions within the *S. pombe* pol II have shown that the Rpb5 subunit associates with Rpb3 and Rpb6 subunits and stimulates the formation of the Rpb3-Rpb11 heterodimer (Kimura and Ishihama, 2002). The *S. cerevisiae* Rpb5 subunit interacts with the CTD of Rpb1 subunit and both have been proposed to have overlapping functions in transcription activation (Miyao and Woychik, 1998). Furthermore, *S. cerevisiae* Rpb5 has been shown to directly interact with the RAP30 subunit of the basal transcription factor, TFIIF (Wei et al., 2001).

The Rpb6 subunit is considered to be a homolog of the bacterial w subunit and both Rpb6 and the w subunits have been implicated in subunit assembly (Nouraini et al., 1996; Minakhin et al., 2001). However, Rpb6 is essential for pol II activity and cell viability, whereas the w subunit is neither required for the survival of bacterial cells nor for in vivo or in vitro transcription. The budding yeast Rpb6 subunit contains a highly acidic N-terminus (14 acidic residues among the 3–31 amino acid residues with no basic residue). It also displays a C-terminal alternating charge pattern with mostly hydrophobics intervening the charges (Brendel and Karlin, 1994). *S. pombe* Rpb6 gene can rescue the growth defect associated with the absence of the corresponding gene in *S. cerevisiae* (Shpakovski, 1994). Mutational and overexpression studies have implicated Rpb6 in transcription elongation in *S. pombe* (Ishiguro et al., 2000).

Alignment of the budding yeast and fission yeast Rpb8 amino acid sequence shows that a central segment spanning amino acid residues 65 and 88 in the budding yeast Rpb8 is absent in the *S. pombe* homolog. This segment is not essential for growth (Voutsina et al., 1999). *S. pombe* Rpb8 also did not rescue the growth defect associated with the lack of Rpb8 subunit in *S. cerevisiae* (Shpakovski et al., 2000). However, growth was recovered in the presence of a high genetic dosage of the largest subunit of RNA polymerase III (Voutsina et al., 1999). Yeast two-hybrid screen using *S. cerevisiae* Rpb8 as the 'bait' showed that it interacted with the 516–639 amino acids of the Rpb1 subunit of RNA polymerase I, II and III, which is in agreement with the pol II crystal structure (Cramer et al., 2001). A nucleoprotein, Nup82, was also identified as one of the Rpb8-interaction partners in this screen and extragenic suppression analysis identified Rpb6 as one of the physiological partners of Rpb8 (Briand et al., 2001). Kimura and Ishihama (2000) demonstrated a direct interaction between the Rpb8 and the Rpb3 subunits of *S. pombe* RNA polymerase II. They further showed that Rpb8 causes an enhancement of the Rpb1-Rpb3 interaction, albeit at a low level.

The genes encoding the other two common subunits, Rpb10 and Rpb12, have been cloned from both the budding and fission yeasts. Heterologous complementation

studies have shown that the *S. pombe* gene encoding the Rpb10 subunit is proficient in supporting the growth of an *rpb10* mutant of *S. cerevisiae* (Shpakovski et al., 2000). But very little information is available about their in vivo function(s). Apparently, both Rpb10 and Rpb12 play an indispensable role in assembly and maintenance of pol II, forming sub-complexes with the Rpb3-Rpb11 heterodimer during the early stages of the assembly of RNA pol II (Lalo et al., 1993).

19.3.3 Unique Subunits: Rpb4, Rpb7 and Rpb9

Rpb4, -7 and -9 subunits are unique to RNA polymerase II and Rpb4 and Rpb9 are the only two subunits that are dispensable for growth of budding yeast cells under optimal growth conditions (Woychik and Young, 1989; Woychik et al., 1991). In contrast, both these subunits are essential for viability in the fission yeast (reviewed by Mitsuzawa and Ishihama, 2004). The Rpb4 subunit also differs markedly in these two yeasts in several other aspects (Sakurai et al., 1999)

- The *S. pombe* subunit is smaller in size (135 amino acids), lacking several regions present in the *S. cerevisiae* subunit (221 amino acids);
- Stoichiometric amount of Rpb4 is present in the *S. pombe* pol II, whereas only 20% of the *S. cerevisiae* pol II prepared from log-phase cells contain Rpb4;
- Rpb4 is more tightly associated with the pol II in *S. pombe* as compared to its *S. cerevisiae* counterpart.

Despite all these differences, *S. pombe* Rpb4 could rescue the growth defect associated with the lack of Rpb4 in *S. cerevisiae* (Shpakovski et al., 1994). The Rpb4 subunit also forms a heterodimer with the Rpb7 subunit in archaebacteria, yeast, plants and humans (reviewed by Choder, 2004). Using deletion analysis, the regions involved in interaction between the *S. cerevisiae* Rpb4 and Rpb7 subunits have been delineated (Sareen et al., 2005). The in vivo functions of the Rpb4 subunit alone and in a complex with the Rpb7 subunit have been quite well characterized in *S. cerevisiae* (reviewed in Choder, 2004). Convergence of data from several studies suggests that this complex is essential for stress response and stress survival. More recently it has been shown that while Rpb4 promotes sporulation, Rpb7 enhances psudohyphae formation in budding yeast (Singh et al., 2007). Earlier studies provided evidence that Rpb4 is not required for constitutive transcription, but is important for activated transcription from a subset of promoters (Pillai et al., 2001). It plays a significant role in carbon and energy metabolism at moderate temperatures and sporulation (Pillai et al., 2003). It also has a dual role in controlling sub-pathways of transcription-coupled DNA repair-repressing the Rpb9-mediated sub-pathway and facilitating the Rad26-mediated subpathway (Li and Smerdon, 2002). Recently, the involvement of Rpb4 in the decay of specific class of mRNAs has been reported in *S. cerevisiae* (Lotan et al., 2005). In contrast, we are only beginning to decipher the functions of the Rpb4 subunit in *S. pombe*. Kimura et al. (2002) provided evidence that it interacts with the CTD phosphatase, Fcp1 and may play a role in the assembly of the Fcp1-pol II complex, thus stimulating

CTD dephosphorylation for the recruitment of pol II in a new cycle of transcription in *S. pombe*. Whole genome expression analysis in *S. pombe* has uncovered a new function for Rpb4 in cell separation (Sharma et al., 2006).

The Rpb7 subunit is one of the most highly conserved subunits of RNA polymerase II. The *S. cerevisiae*, *S. pombe* and *C. albicans* Rpb7 orthologs display a high sequence similarity in the central stretch of 20 amino acids and also in both the N-terminal and C-terminal regions of the protein (Sadhale and Woychik, 1994). The similarity between the *S. cerevisiae* and *S. pombe* protein is 75%, while the extent of similarity between the *S. cerevisiae* and *C. albicans* orthologs is 79% (Singh et al., 2004). Overexpression of *S. cerevisiae* Rpb7 in an *rpb4* deletion mutant rescues some of the phenotypes linked with the lack of Rpb4 in *S. cerevisiae*, thus suggesting that Rpb4 may also play a role in stabilizing the association of Rpb7 with the remaining pol II (Sharma and Sadhale, 1999; Sheffer et al., 1999; Pillai et al., 2003). These *rpb4*-deletion phenotypes could also be partially rescued by overexpression of either *C. albicans* Rpb7 or *S. pombe* Rpb7 (Singh et al., 2004). The *C. albicans* and *S. pombe* orthologs could also complement for the absence of *S. cerevisiae* Rpb7. In summary, these observations imply that the high sequence similarity seen in the Rpb7 orthologs from different yeasts also extends to a functional conservation. The budding yeast Rpb4/7 complex also binds single-stranded nucleic acids and mediates a post-recruitment step in transcription initiation (Orlicky et al., 2001). Pull down assays carried out in *S. pombe* identified glyceraldehyde-3-phosphate dehydrogenase and actin as proteins interacting with *S. pombe* Rpb7 subunit (Mitsuzawa et al., 2005). Another report showed that the Rpb7 protein associates with the Seb1 protein in fission yeast and with the Nrd1 protein in budding yeast, thus linking the Rpb7 protein to transcription termination of small nuclear and small nucleolar RNAs (Mitsuzawa et al., 2003). A recent study discovered a novel role for *S. pombe* Rpb7 in RNAi- directed chromatin silencing pathway (Djupedal et al., 2005).

The functions of the Rpb9 subunit have been investigated in detail in *S. cerevisiae*. Hull et al. (1995) demonstrated that *S. cerevisiae* cells lacking the Rpb9 subunit exhibited an upstream shift in the position of the start site. It was later shown that this alteration is associated with an impaired interaction between Rpb9 and TFIIF (Ziegler et al., 2003). Several studies provided evidence for the role of Rpb9 in transcription elongation in vitro and in vivo (Awrey et al., 1997; Hemming and Edwards, 2000; Hemming et al., 2000). Moreover, Mullem et al. (2002) observed that *rpb9* null mutants failed to grow when they also lacked the histone acetyl transferase activity of either the elongator or the SAGA complex, adding another facet to the role of Rpb9 in transcription elongation. They also showed a direct physical interaction between Rpb9 and the large subunit of TFIIE, and proposed that this may be the mechanism by which Rpb9 may contribute to the recruitment of TFIIE to pol II. Recent observations have illuminated the role of Rpb9 in maintaining transcriptional fidelity (Nesser et al., 2006). The Rpb9-encoding cDNA from *S. pombe* has also been cloned. From the cDNA sequence, the *S. pombe* Rpb9 subunit was found to consist of 113 amino acids with a molecular mass of approximately 13 kDa. It also possesses 47% identity in amino acid sequence with *S. cerevisiae* Rpb9 (Sakurai et al., 1998). Interestingly although both *S. cerevisiae* and *S. pombe*

differ in the position of the transcription initiation site, Rpb9 is readily exchangeable between these two yeasts. This suggests that the Rpb9 subunit does not directly determine the differences in start site selection between these yeasts.

19.3.4 Structure of pol II

As mentioned in the introduction, structure of the *S. cerevisiae* 10-subunit RNA polymerase II (lacking the Rpb4/7 subcomplex) in the absence of DNA has been determined at different resolutions. It is formed of four mobile elements, known as core, clamp, shelf and jaw lobe, which move relative to each other. The core element comprising the Rpb3, -10, -11, -12 and those parts of Rpb1 and Rpb2 that form the active centre, accounts for approximately half the mass of pol II and is composed predominantly of subunits shared among the three polymerases. A deep cleft is located at the centre of the enzyme, where incoming DNA enters from one side and the active site is buried at the base. This cleft is formed by all the four mobile elements and is present in both the open and closed conformations in the 10-subunit enzyme. The shelf and jaw lobe move relatively less and can rotate parallel to the active site cleft. In comparison, the clamp which is connected to the core through a set of flexible switches can move with a large swinging motion of upto 30A° to open and close the cleft.

Subsequently, structure of the complete 12-subunit enzyme from budding yeast was derived independently by two different groups (Armache et al., 2003; Bushnell and Kornberg, 2003). The polymerase models presented by both these groups were essentially identical, revealing the location of Rpb4/7 heterodimer in a pocket formed by the subunits, Rpb1, Rpb2 and Rpb6, at the base of the clamp. The position of Rpb7 in this pocket functions not only as a wedge to lock the clamp in a closed conformation, but both Rpb4 and Rpb7 provide a surface for binding of other transcription factors and also for RNA exiting the elongating Pol II.

19.4 General Transcription Factors: Pol II Helper Proteins

Unlike the *E. coli* RNA polymerase, eukaryotic RNA polymerase II does not have the ability to recognize the promoter. Thus, it requires the help of accessory proteins called the basal or general transcription factors (GTFs). Six GTFs have been purified from different systems, though the human and budding yeast proteins remain the most well characterized proteins. These GTFs assemble at the promoter along with pol II to form the PIC that initiates transcription. Two different pathways have been proposed to explain the assembly of the PIC in vivo. According to the ‘sequential’ or the step-wise’ assembly pathway, each GTF enters the assembling transcription apparatus individually and sequentially. The assembly is nucleated by the binding of TFIID, through the direct interaction of its TATA-box

binding protein (TBP) with the promoter. This interaction also depends on other factors, referred to as TAFs or the TBP-associated factors. Binding of the TFIID is followed by the sequential binding of TFIIA, TFIIB, TFIIF, RNA pol II, TFIIE and TFIIH, thus completing the assembly of the PIC.

An alternative pathway was proposed when several groups discovered that pol II could be isolated as a preassembled 'holoenzyme'. Although the exact composition of the holoenzyme complex varied according to the method of purification, it was isolated with or without a subset of the GTFs, but with mediator proteins and other proteins involved in chromatin-remodelling, mRNA processing, DNA repair and DNA replication. This holoenzyme is then recruited to the promoter as a single, large complex (reviewed by Lee and Young, 2000; Myers and Kornberg, 2000). We still do not know which of the two pathways operates in a cell, but it is possible that either both pathways exist or a mechanism in between these two extremes exists in vivo.

The properties and functions of the GTFs have been discussed below.

19.4.1 TFIID

TFIID is the first GTF that recognizes and associates with both TATA-containing and TATA-less promoters, to begin the assembly of the PIC. It is a multi-protein complex containing the TATA-binding protein (TBP) and TBP-associated Factors (TAFs). Besides its role in promoter binding, TFIID functions as a co-activator in mediating interaction between activators and the basal transcription machinery; it interacts with other basal transcription factors to enhance PIC assembly; and also acts as an enzyme to post-translationally modify chromatin and protein factors involved in transcriptional control.

Specifically, it is the TBP subunit of TFIID that has the ability to recognize the TATA box sequence in promoters. Crystal structures of budding yeast TBP in complex with the TATA box showed that TBP binding caused a severe bend in the DNA (Kim et al., 1993). In the bound and the unbound states, TBP resembles a molecular 'saddle' with a pair of 'stirrups' flanking the DNA-binding surface which help in bending the DNA. TBP associates with 14 different TAFs in the *S. cerevisiae* TFIID complex (Sanders and Weil, 2000). The elucidation of the functions of TAFs has been an area of intense research (reviewed by Albright and Tjian, 2000; Thomas and Chiang, 2006). Four TAFs in the *S. cerevisiae* TFIID complex-TAF17, TAF60, TAF48 AND TAF61, have domains similar to histones H3, H4, H2A and H2B respectively and these TAFS can form an octameric structure in vitro (Selleck et al., 2001). Studies in *S. cerevisiae* revealed that depletion or inactivation of individual TAFs does not have a global effect on transcription activation of many genes (Moqtaderi et al., 1996; Walker et al., 1996). Additional evidence supporting the view that TAFs may not be universally required for gene expression in vivo came from whole genome expression analysis with *S. cerevisiae* TAF mutants (Holstege et al., 1998; Lee et al., 2000). In vitro studies have also confirmed

that TAFs may not be essential for transcription of every gene. TBP, not bound to TAFs, has been found in *S. cerevisiae* (Kuras et al., 2000; Li et al., 2000) and can be biochemically separated from TAFs during in vitro fractionation of budding yeast TFIID (Sanders et al., 2002). Interestingly, some TAFs have also been found in other complexes, like TBP-free TAF_{11}–containing complex (TFTC), TFTC-related GCN5 complexes, SAGA complex and SAGA-like complexes. These TBP-lacking TAF-containing complexes are involved in diverse aspects of pol II-mediated transcription. In fact yeast genes have been categorized into two distinct classes-TAF-dependent and TAF-independent, based on the requirement of TAFs for their expression (Kuras et al., 2000; Li et al., 2000).

The gene-encoding *S. pombe* TFIID homolog has also been cloned (Hoffman et al., 1990). *S. pombe* TFIID contains 231 amino acids and shares a 93% identity with the *S. cerevisiae* ortholog. Not surprisingly, the *S. pombe* TFIID can complement a disruption of the *S. cerevisiae* TFIID (Fikes et al., 1990). The carboxy-terminal three quarters of the *S. pombe* TFIID protein exhibits an extraordinary degree of amino acid sequence homology with a corresponding region of *S. cerevisiae* TFIID. This region is necessary and sufficient for TATA-box-binding and basal transcription activation. In contrast, the amino-terminal region of *S. pombe* TFIID differs markedly in amino acid sequence and composition from its *S. cerevisiae* counterpart (Hoffman et al., 1990). *S. pombe* TFIID consists of TBP and 14 TAFs (reviewed by Thomas and Chiang, 2006). Out of these 14 TAFs, only five TAFs have been identified biochemically-TAF111, TAF72, TAF73, TAF50 and Ptr6. The TAF72-encoding gene was cloned using sequence homology by Yamamoto et al., 1997. Later, the genes encoding the TAF72 and TAF73 were isolated as high-copy number suppressors of cell cycle mutations (Mitsuzawa et al., 2001). Both these TAFs contain WD repeat motif and are components of the TFIID complex. TAF72 is also a component of the SAGA complex. *S. cerevisiae* TFIID contain only one TAF (TAF90) with a WD repeat motif and it has a stoichiometry of two (Sanders et al., 2002). This raises the possibility that the single species of the WD repeat TAF in *S. cerevisiae* is present in two copies in *S. pombe* TFIID (Mitsuzawa et al., 2001). TAF 50 was identified as a protein that interacts with TAF72 (Mitsuzawa and Ishihama, 2002). TAF50 also possesses limited homology to histone H4. The gene encoding Ptr6 (poly A+ RNA transport) was identified in a screen for mutants defective in mRNA export (Shibuya et al., 1999) and it is considered to be a homolog of the budding yeast TAF67. The *S. pombe* counterparts of the remaining TAFs were identified by a search of the *S. pombe* genome (Mitsuzawa and Ishihama, 2004). Tamayo et al. (2004) purified a TAF-containing complex from *S. pombe* and demonstrated that TAFs are not required for basal or activated transcription in vitro.

19.4.2 TFIIA

S. cerevisiae TFIIA is composed of two subunits, with apparent molecular masses of 32 and 13.5 kDa (Ranish and Hahn, 1991). The genes encoding these two subunits, TOA1 and TOA2, are essential for cell viability (Ranish et al., 1992). The role of

TFIIA as a general transcription factor is controversial. Early in vitro experiments revealed that TFIIA was required for transcription, while later studies showed that it was dispensable for basal transcription (reviewed by Orphanides et al., 1996). Subsequent studies suggested that TFIIA is more likely to function as an anti-repressor, rather than a basal transcription factor. It stabilizes the TBP-DNA binding (Weideman et al., 1997) by competing with the N-terminal domain of TAF145 that occludes the DNA binding surface of TBP when TFIID is not bound to DNA (Kokubo et al., 1998; Sanders et al., 2002). TFIIA can also compete with the negative regulatory factors, Mot1 and NC2, to stimulate TBP binding in vitro (Xie et al., 2000). In addition to its role as an anti-repressor, TFIIA also functions as a co-activator to stimulate overall transcription by interacting with various activators, and other components of the transcriptional machinery. It has also been reported to interact directly with TAF40 both in vivo and in vitro (Kraemer et al., 2001). Mutational studies abolishing the interaction of TFIIA with TBP have shown that TFIIA is essential for transcription of only a subset of genes (Stargell et al., 2000). Recently, Kraemer et al. (2006) examined the transcriptional profiles of different *S. cerevisiae* TFIIA mutants in order to further characterize the functions of TFIIA in the regulation of gene expression by pol II. It was observed that approximately 11–27% of the expressed genes exhibited altered expression levels depending on the particular TFIIA mutant. Surprisingly, all these affected genes contained the binding site for the Yap1, a transcription factor involved in oxidative stress. The dependence of Yap1 on TFIIA was also demonstrated in genetic and biochemical experiments, thus highlighting a novel role for TFIIA in response to oxidative stress.

19.4.3 TFIIB

S. cerevisiae TFIIB (also called factor e) is a monomer of approximately 41 kDa, encoded by the *SUA7* gene (Tschochner et al., 1992). Pinto et al. (1992) observed that mutations in the *SUA7* gene shifted the transcription start site downstream of the normal site, indicating a role for TFIIB in transcription start site selection in vivo. Berroteran et al. (1994) provided evidence that mutations in the Rpb1 encoding gene also resulted in alterations in the start site of transcription. Moreover, a functional interaction was demonstrated between TFIIB and the Rpb2 subunit of RNA polymerase II (Chen and Hampsey, 2004). Thus, the selection of the start site may depend on both TFIIB and pol II. By superposition of the structures of TFIIB-RNA pol II and DNA-TBP-TFIIB, it was observed that TFIIB acts as a bridge between TBP and RNA polymerase II such that the DNA template need only follow a straight path from the TATA box to position the start site in the active centre of pol II (Leuther et al., 1996). TFIIB interacts directly with TBP and enters the PIC after TBP. It is also a prerequisite for recruitment of pol II (Buratowski et al., 1989). TFIIB is also a direct target of many transcription activators and recruitment of TFIIB is the mechanism by which many activators stimulate transcription (Lin et al., 1991). In addition, a post-assembly function for TFIIB was also revealed by

the isolation of TFIIB mutants competent for assembly of the transcription complex, but defective for in vitro transcription (Cho and Buratowski, 1999).

The cloning, expression and functional characterization of *S. pombe* TFIIB was reported in 2002 by Tamayo and Maldonado. It is a 340 amino acid long protein with a calculated molecular mass of 37.4 kDa. It displays 38.8% identity to its *S. cerevisiae* homolog and 40.1% identity to its human counterpart. Earlier fission yeast TFIIB was also purified from cell extracts as a 35 kDa protein. It has been shown that pairwise replacement of TFIIB and pol II from *S. cerevisiae* by their respective *S. pombe* counterparts was sufficient to shift the start sites from the pattern characteristic of *S. cerevisiae* to the pattern characteristic of *S. pombe* (Li et al., 1994).

19.4.4 TFIIE

TFIIE (known as factor a), is a two subunit protein in *S. cerevisiae*. The apparent molecular weights of these subunits are 66 and 43 kDa. The genes encoding these subunits, TFA1 and TFA2, are present in a single copy and are essential for cell survival. Two functionally distinct domains have been identified by mutational analysis of the TFA1-encoded subunit: mutations in the N-terminal half confer growth defects at high temperatures, whereas mutations in the C-terminal half confer growth defects at low temperatures. TFIIE is able to bind single-stranded DNA, thus explaining the dispensability of TFIIE for transcription initiation from pre-melted template DNA (Holstege et al., 1995). TFIIE influences recruitment of TFIIH and subsequent control of TFIIH activities. Both TFIIE and TFIIH are required for ATP-dependent formation of the open complex before formation of the first phosphodiester bond. TFIIE and TFIIH, in cooperation with TFIIF, suppress promoter-proximal stalling, thereby facilitating early events in the transition of RNA pol II to productive elongation (Dvir et al., 1997). The functional link between TFIIE and TFIIH was elegantly demonstrated by the inability of the *S. cerevisiae* TFIIE to functionally substitute the *S. pombe* TFIIE ortholog in a constituted transcription system, unless they were exchanged as a TFIIE-TFIIH pair (Li et al., 1994). *S. pombe* TFIIE is made up of two subunits, α and β. The α subunit contains 434 amino acids, with a calculated molecular weight of 49.1 kDa. It shares a 26% amino acid sequence identity and a 50% similarity with its *S. cerevisiae* ortholog. The smaller β subunit contains 285 amino acids with a calculated molecular weight of 32.2 kDa, sharing a 38% amino acid sequence identity and 49% similarity with the *S. cerevisiae* ortholog (Hayashi et al., 2005). The genes encoding both these subunits have been cloned and are essential for cell viability as seen in *S. cerevisiae*. The functions of TFIIE have been investigated by biochemical and genetic approaches in *S. pombe* (Hayashi et al., 2005). Chromatin immunoprecipitation assays revealed that TFIIE was localized to the promoter and promoter-proximal regions. It was also observed that mutation of the C-terminal residues of fission yeast TFIIE β subunit conferred cold sensitivity. These mutations had earlier been shown to be linked to transcription defects, either at initiation or at the transition from initiation to elongation phase (Watanabe et al., 2003). These findings

confirm the role of TFIIE in transcription initiation and transition from the initiation to the elongation phase in fission yeast. Far western studies have shown that *S. pombe* TFIIE formed an α_2 β_2 heterotetramer with a molecular weight of 180 kDa in vitro. Further characterization of the binding specificities revealed that the β subunit of *S. pombe* TFIIE interacts predominantly with the Rpb2 and Rpb12 subunits of pol II and also weakly with the Rpb1 subunit. In comparison, the α subunit mainly binds to the Rpb5 subunit of pol II.

19.4.5 TFIIF

Three different subunits, designated as Tfg1, Tfg2 and Tfg3, constitute the *S. cerevisiae* TFIIF (known as factor g). The Tfg1 (105 kDa) and Tfg2 (54 kDa) are considered to be homologous to the RAP74 and RAP30 subunits of human TFIIF respectively. Tfg1 and Tfg2-encoding genes are essential for cell viability, whereas the gene encoding the Tfg3 subunit (30 kDa) is dispensable. Archambault et al. (1997) showed that the CTD phosphatase, Fcp1 binds to Tfg1. Cryo-electron microscopy resolved the structure of *S. cerevisiae* pol II in complex with the TFIIF (Chung et al., 2003). TFIIF interacts with a highly extended surface of pol II along the edge of the clamp element and also with the Rpb4/7 subcomplex. Tfg1 also interacts with the Rpb9 subunit of pol II in budding yeast (Ziegler et al., 2003; Ghazy et al., 2004). This stable Tfg1-pol II complex accounts for almost 50% of pol II isolated from *S. cerevisiae* nuclear extracts and is active in supporting multiple rounds of transcription (Rani et al., 2004).

Two subunits of TFIIF have been identified in *S. pombe* (Tamayo et al., 2004). The α subunit has 490 amino acids and shows a 33% amino acid identity with its *S. cerevisiae* counterpart, while the β subunit contains 301 amino acids and shares 37% identity with the *S. cerevisiae* ortholog. *S. pombe* TFIIF had earlier been isolated as part of a complex containing Fcp1 and pol II (Kimura et al., 2002) and further characterization of the complex showed that the *S. pombe* homolog of Tfg3 was indeed a constituent of the Fcp1/pol II/TFIIF complex. Deletion of Tfg3 in *S. pombe* is associated with temperature-sensitive phenotype and other stress-related phenotypes. Interaction of Tfg3 with TFIIB and TBP has also demonstrated (Kimura and Ishihama, 2004). Infact, the Tfg3 subunit is not only present in TFIID, but is also a component of the Swi/Snf and NueA complexes (Cairns et al., 1996). Therefore, it can be speculated that the Tfg3 subunit of TFIIF may act as an intermediary protein, facilitating interactions between Swi/Snf complex and the general transcription apparatus.

19.4.6 TFIIH

TFIIH, also called factor b, has a host of enzymatic activities. These include DNA dependent ATPase, two ATP-dependent DNA helicases with opposite polarity

(called Rad3 and Rad25 in humans) and CTD kinase (Cdk7-cyclin H). TFIIH can be separated into two sub-complexes- core TFIIH and the cyclin-kinase complex. In addition to its role in transcription, core TFIIH plays an important role in nucleotide excision repair. Till recently, the *S. cerevisiae* TFIIH was considered to contain nine different subunits. Ranish et al. (2004), discovered a tenth subunit called Tfb5 involved in DNA repair function of TFIIH. Mutations in genes encoding the different components of the core TFIIH, i.e. Tfb1, Tfb2, Ssl1 and Tfb4, caused defects in responding to UV irradiation, thus implying a role of these subunits in DNA damage. A ubiquitin-ligase activity associated with Ssl1 has been discovered recently because of the presence of a RING finger domain at its C-terminal region encompassing amino acid residues 403 to 454 (Takagi et al., 2005) and this activity can be enhanced by addition of another TFIIH RING-finger containing subunit Tfb4. The electron-crystal structure of core TFIIH has been solved at 13A° resolution (Chang and Kornberg, 2000). Many activators, including Gal4-VP16, have been demonstrated to bind to TFIIH (reviewed by Zurita and Merino, 2003).

The gene encoding the fission yeast homolog of the budding yeast TFIIH subunit, Ssl1, was cloned by Adachi et al. (1999) and the gene product was called p47. Although deletion of the p47 gene was not lethal, but it was required for normal growth. In contrast, an *ssl1* null mutation in budding yeast was lethal. A comparison of the primary amino acid sequence of Ssl1 and p47 revealed that p47 has a 45% identity to Ssl1. Both these yeast homologs contain a charged cluster in the most N-terminal region. The amounts of charged residues are 55% in p47 and 57% in Ssl1. However, the polarity as a whole within the charged cluster is acidic in Ssl1 and basic in p47. The *S. pombe* Tfb1 subunit of TFIIH contains 457 amino acids and shares a 29% amino acid sequence identity with its *S. cerevisiae* ortholog (Tamayo et al., 2004). Also, the Ssl2 subunit of *S. cerevisiae* is encoded by the *ptr8* gene in *S. pombe*, which is involved in mRNA transport (Mitsuzawa and Ishihama, 2004).

19.5 Mediator: The Link Between Transcriptional Regulators and pol II Machinery

Mediator is a multi-protein complex that provides the interface between gene-specific regulatory proteins and the general RNA polymerase transcription machinery. The first evidence for its existence came from squelching experiments in *S. cerevisiae* (Gill and Ptashne, 1988). In these experiments, the ability of one activator to inhibit transcription by another activator could not be rescued by addition of excess GTFs, but was rescued by addition of a partially purified yeast fraction. The factor(s) present in the partially purified fraction with the ability to support activated transcription was termed as the ‘mediator’. Our current understanding of the mediator complex in terms of its subunit composition, its structure and its role in regulation of gene expression can be summarized as follows.

19.5.1 Subunit Composition

The *S. cerevisiae* core mediator complex comprises 21 proteins as its 'bona fide' members (reviewed by Biddick and Young, 2005; Björklund and Gustaffsson, 2005). In addition to these bonafide members, four Srb proteins, Srb8-11, form a distinct sub-complex which may sometimes found to be associated with the mediator (Liao et al., 1995; Borggrefe et al., 2002). Many of these mediator components have been identified in genetic screens for mutations that influence transcription. The mediator subunits-Srb2, Srb4, Srb5 and Srb6 were identified as dominant suppressors of the cold sensitive phenotype associated with the truncation of the CTD of the Rpb1 subunit of pol II (reviewed by Myers and Kornberg, 2000), while the Srb8-Srb11 were identified as recessive suppressors of this phenotype (Hengartner et al., 1995; Liao et al., 1995). Genetic analysis demonstrated that the complex comprising the Srb8-11 subunits is involved in the negative regulation of transcription of a subset of genes (Holstege et al., 1995). When budding yeast cells were grown under conditions of nutrient limitation, the Srb8-11 module was degraded. This suggests that the conversion of an Srb8-11 containing mediator into a smaller active mediator may be a regulated event. Srb 10 and Srb11 form a cyclin-kinase pair. Srb10 has the unique ability to phosphorylate the CTD prior to formation of the initiation complex on promoter DNA, consequently inhibiting transcription (Hengartner et al., 1998). Eight other subunits of the mediator, i.e. Gal11, Nut2, Rgr1, Rox3, Sin4, Med3, Med9 and Med10, were identified in different genetic screens for positive as well as negative transcriptional regulators (reviewed by Carlson, 1997). The remaining seven subunits were identified as novel proteins present as part of the biochemically isolated mediator complex from the budding yeast (Kim et al., 1994). These subunits were called Med1, Med2, Med4, Med6, Med7, Med8 and Med11. Since different mediator subunits were identified in different screens by different groups of investigators, there is a lot of variation with respect to their nomenclature. Bourbon et al. (2004) have proposed a unified nomenclature in which all mediator subunits are designated MED followed by a number.

Purification and characterization of the RNA polymerase II holoenzyme from *S. pombe* led to the identification of the proteins constituting the fission yeast mediator complex (Spahr et al., 2000, 2001). 13 individual subunits were identified as mediator components. Ten of these subunits were homologs of the *S. cerevisiae* Rgr1, Nut2, Med4, Med6, Med7, Med8, Rox3, Srb4, Srb6 and Srb7 proteins. Three subunits, Pmc2, Pmc3 and Pmc6, lacked homologs in the *S. cerevisiae* mediator. Gene disruption experiments showed that the genes encoding the Pmc3 and Pmc6 subunits are non-essential for the viability of *S. pombe* cells. In comparison, deletion of genes encoding the *S. pombe* homologs of Srb4, Med4, Med7 and Med8 resulted in lethality (Spahr et al., 2001). The mediator present in the *S. pombe* RNA pol II holoenzyme stimulated phosphorylation of the CTD by TFIIH isolated from *S. pombe*. But if TFIIH was isolated from *S. cerevisiae*, this stimulation was not seen, demonstrating that the stimulation of CTD by TFIIH was species specific

(Spahr et al., 2000). It has been speculated that the essential subunits conserved between *S. cerevisiae* and *S. pombe* may constitute a core mediator, interacting with RNA polymerase II and TFIIH. *S. pombe* homologues of the Srb8-11 module of *S. cerevisiae* were identified later by Samuelsen et al. (2003). It was observed that the mediator containing these proteins was present in a free form, without RNA polymerase II. On the other hand, mediator lacking these proteins could associate with the polymerase. The Srb10 homolog has also been identified from *Kluyveromyces lactis* (Nunez et al., 2004) and it contains 593 amino acids and can complement the phenotypes of a *S. cerevisiae* haploid *srb10* null mutant. Orthologs of various mediator subunits across different species have been identified using a genome-wide search (Boube et al., 2002). A comparison of the primary sequence of mediator subunits present in the yeasts- *S. cerevisiae*, *S. pombe* and *C. albicans*, with that of their metazoan counterparts suggest that the overall subunit composition and therefore, the structural organization of mediator exhibits a remarkable degree of conservation.

19.5.2 Structure

The *S. cerevisiae* mediator complex is organized into three functionally and physically distinct sub-complexes or modules- the head module, the middle domain and the tail domain (Asturias et al., 1999; Dotson et al., 2000). Seven mediator subunits, i.e. Med6, Med8, Med11, Med17, Med18, Med20 and Med22, constitute the head domain, while Med1, Med4, Med5, Med7, Med9, Med10 and Med21 comprise the middle domain, and the tail domain contains Med2, Med3, Med15 and Med16. The Med 14 subunit connects the middle domain to the tail domain. The structure of the mediator alone and in complex with RNA polymerase II have been determined. Figure 19.4 shows a schematic representation of the pol II–mediator complex.

The mediator structure reveals that it can exist in two different conformational states- an elongated structure seen in the presence of pol II and a compact form observed in the absence of pol II. It remains in the elongated state even if the CTD is truncated, providing evidence that the mediator makes multiple contacts with the polymerase, in addition to the CTD. Recent findings show that over-expression of the proteins comprising the head module of the budding yeast mediator could complement the absence of a ‘headless’ mediator in transcription initiation in vitro. Interestingly, the head module interacted with the RNA pol II-TFIIF complex, but not with either of the two components separately and this interaction was lost in the presence of DNA template and associated RNA transcript. Also, disruption of the head module in vivo resulted in the release of the middle and the tail domains from a transcriptionally active promoter. In summary all these observations suggest that the head module regulates the interaction of the mediator with pol II and also with the promoter (Takagi et al., 2006).

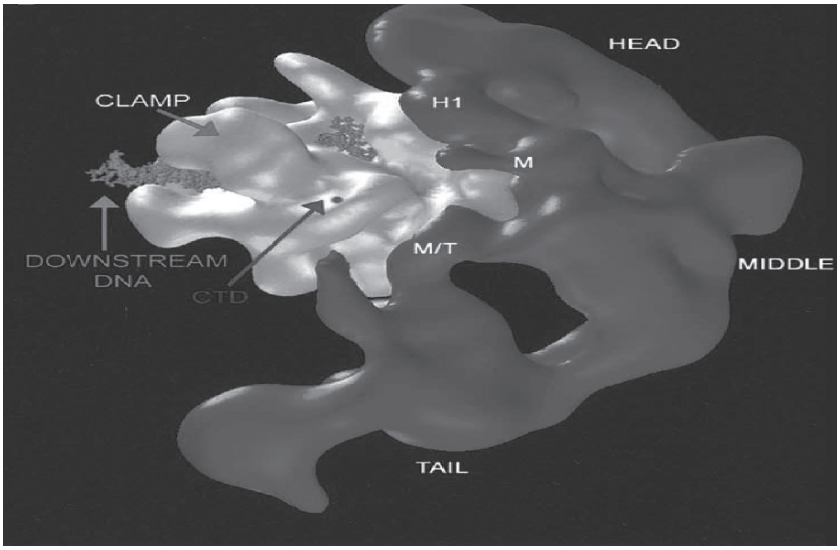

Fig. 19.4 RNA polymerase II-mediator complex. Reprinted by permission of Federation of the European Biochemical Societies from Structure of the yeast RNA polymerase II holoenzyme: Mediator conformation and polymerase Interaction by Davis et al. 2002. *Mol. Cell* 10: 409–415

19.5.3 Functions

Different approaches used to understand the functions of the mediator complex have led to the proposal that it acts as a global regulator of transcription. Although most evidence suggests that it functions as a co-activator, but several observations also point towards its negative role in transcription (reviewed by Biddick and Young, 2005).

Regulatory proteins directly interact with the mediator complex, but the specific subunit of the mediator that is contacted depends upon the regulatory protein. In *S. cerevisiae*, three acidic-rich transcriptional activators (Gal4, Gcn4 and VP16) interact with the mediator and this interaction requires the proteins constituting the tail module. In case of *S. pombe*, these mediator subunits are absent. Therefore, to determine if the *S. pombe* mediator had the ability to interact with these acidic activators, Spahr et al. (2001) carried out GST pull down assays to test the interaction between the mediator and VP16. These assays demonstrated that the mediator could still interact with VP16, suggesting that other subunits of the mediator could substitute for the missing subunits in *S. pombe*. In addition to its role in activator-dependent transcription, mediator is also known to stimulate basal pol II transcription in *S. cerevisiae* and it functions like any other general transcription factor involved in initiation of transcription (Takagi and Kornberg, 2006). Earlier work

had demonstrated that mediator was critical for the formation of a stable PIC (Koleske et al., 1992; Ranish et al., 1999). Subsequently, results of Nair et al. (2005) suggested that mediator may perform this function by incorporating and stabilizing TFIIH in PIC and re-initiation scaffold. Several studies have also demonstrated that mediator is recruited to the promoter separately from RNA polymerase II and general transcription factors (Kuras et al., 2003) and it continues to remain at the promoter even after transcription is initiated (Yudkovsky et al., 2000). Thus, it may serve as a scaffold for the assembly and re-assembly of the transcription complex during each cycle of transcription. This proposition was further supported by the existence of pol II-free mediator in *S. cerevisiae*, which was also the most abundant form of the mediator (Takagi et al., 2005). This view was challenged by the findings of Fan et al. (2006), which suggest that the mediator is not a stable component of the basic pol II transcription apparatus that binds to promoters in vivo and the intact mediator complex may not be required for transcription of many genes in wild type cells.

The role of mediator in stimulation of basal transcription has also been shown in *S. pombe*. Spahr et al. (2003) showed that the mediator lacking the Srb8-11 module had a stimulatory effect on basal transcription, while the mediator containing this module repressed basal transcription. An RNA polymerase II holoenzyme containing both the mediator and the RNA pol II enzyme was isolated from *S. pombe* that was more active in basal transcription in vitro than pol II alone and supported activated transcription in the absence of TAFs with proline-rich (AP2 and CTF) and acidic (VP16) activators, but not with Sp1 (Tamayo et al., 2004). Zhu et al. (2006) used chromatin immuno-precipitation assays and DNA microarrays to study genome-wide localization of mediator complex lacking the Srb8-11 module and the Srb8-11 sub-complex in *S. pombe*. Both of these complexes showed similar binding patterns and their interactions with promoters and UAS correlated with increased transcription activity. The mediator was also seen to interact with the downstream coding region of many genes.

Finally as mentioned before, the mediator also acts as a co-repressor of gene transcription, as exemplified by the Srb8-11 module of the mediator, but no universal mechanism has been proposed to explain this role of the mediator.

19.6 Elongation and Termination

Over the last 35 years, considerable effort has been invested in characterizing the early events of transcription, involving the GTFs- their interactions with each other, with RNA pol II and other regulatory proteins. But relatively little is known about the other events in the transcription cycle, like promoter clearance, elongation and termination. Several transcription elongation factors have been identified which enhance productive RNA synthesis, RNA processing, RNA export and chromatin modelling. Otero et al. (1999) isolated a novel protein complex, called elongator, as the major component of the elongating RNA polymerase II holoenzyme.

They proposed an interesting hypothesis, according to which the elongator is the counterpart of the mediator complex and it may be exchanged for the mediator, as transcription moves from the initiation to the elongation phase. However, to gain a complete understanding of the mechanism of transcription elongation, we still need to identify the entire repertoire of elongation factors, and define their precise functions. Also, in the light of growing evidence that some activator proteins function by enhancing the elongation efficiency and also that the elongating pol II interacts with proteins involved in mRNA processing, future studies will elucidate the role of pol II elongation complex not only as a target of regulatory proteins but also as a regulator of downstream steps in transcription of genes.

19.7 Conclusions and Future Perspective

Several groups identified components of the transcription machinery by fractionation of cell extracts, guided by transcription assays with naked DNA in vitro. The RNA pol II transcription machinery defined in this way consists of three different components- the 12-subunit RNA polymerase II, a set of GTFs and the mediator complex. Owing to the growing wealth of information, it is almost impossible to describe all the aspects of all the components of pol II transcription machinery. Hence, in this chapter we have attempted to review the information about the above mentioned components in different yeasts. RNA polymerase II shows a remarkable degree of conservation in terms of its structure and subunit composition. But the functions of the five common subunits in transcription still need to be dissected in detail. The observation that some of the subunits in *S. pombe* are essential for cell survival in contrast to their *S. cerevisiae* orthologs suggest that they may have more important roles to play in gene expression and regulation. Thus, future studies may entail a more detailed functional characterization of the *S. pombe* pol II subunits. The GTFs are obviously the most highly conserved components of the transcriptional machinery. Analysis of the structure of a PIC containing the 12-subunit *S. cerevisiae* pol II and GTFs bound to promoter DNA has revealed the specific roles of the GTFs: TBP configures DNA to pol II surface; TFIIB directs the DNA to the active site of pol II and stabilizes the transcription complex; TFIIE recognizes the closed complex of pol II and helps recruit TFIIH; TFIIF captures the template strand DNA when the double-stranded DNA melts to form the transcription bubble. Finally, the TFIIH helicase introduces negative supercoils into the promoter DNA, helping the pol II enzyme to move away from the promoter (Boeger et al., 2005). The most unique and intriguing component of the RNA polymerase II transcriptional machinery is the mediator. The subunits of the mediator complex exhibit low levels of primary sequence conservation, which may reflect a functional flexibility required to interact with specific transcriptional regulators in different systems. It is interesting to see how the combination of genetics, molecular genetics, biochemistry and structural biology have resulted in a nearly complete picture of the transcription initiation complex. However, in the future it will be important to

address the specific roles of mediator subunits and whether and how the mediator subunits are themselves regulated. Additional challenges will be to understand how the mediator receives and transduces signals to pol II transcriptional machinery, and finally to integrate all the information to build a step-by-step picture of one of the most fascinating processes of life!

Acknowledgements The authors would like to thank Suman Kumar Das for his help with the illustrations. We would also like to thank Sukriti Dewan and Dr. Jyoti Kumar Jaiswal for critical reading of the manuscript.

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