Chapter

Transcriptional Initiation in Ribosomal Protein Genes in the Fission Yeast *Schizosaccharomyces pombe*

Diego A. Rojas, Sandra Moreira-Ramos, Fabiola Urbina and Edio Maldonado

Abstract

Transcription of class II genes in eukaryotic organisms is carried out by the multi-subunit enzyme RNA polymerase II (RNA pol II) and includes the general transcription factors and the mediator. The region inside the promoters, which recruits and specifies the transcriptional machinery, is called "core promoter" and contains sub-regions called "core promoter elements," which are necessary for transcription initiation, where the most studied and classic element is the TATA-box. Ribosome protein gene (RPG) promoters do not possess a TATA-box (TATA-less promoters), and those, in particular, in the fission yeast *Schizosaccharomyces pombe* have a TATA-box analog called the HomolD-box. The transcription of RPG promoters is dependent on the RNA pol II transcription system and the HomolD-box is recognized by the transcription factor Rrn7. In this chapter, the authors will describe the general mechanisms associated to the transcription of TATA-less promoters in eukaryotic organisms and how the transcription initiation is carried out in the RPG promoters from those organisms, particularly in *Schizosaccharomyces pombe*. Finally, the authors will analyze the role of the HomolD-box and the transcription factor Rrn7 in the coordination of transcription initiation from RPG promoters and other ribosome-related genes and the presence of transcriptional modules in their promoters, which could be coordinated and regulated by a discrete number of transcription factors.

Keywords: transcription, ribosomal protein gene (RPG), RNA polymerase, TATA-less promoter, *Schizosaccharomyces pombe*

1. Introduction

Protein synthesis in eukaryotic organisms includes several steps and requires many regulatory events [1, 2]. One of these critical steps is ribosome biogenesis, which includes ribosomal protein gene (RPG) transcription and rRNA synthesis. As in many other central events in the cell, ribosome biosynthesis must be a regulated and coordinated process. A typical coordinated regulation of gene and protein expression is the presence of common DNA elements in the promoters of related genes, which are co-regulated by a discrete number of transcription factors. Those genes under the control of a common DNA element form a transcriptional

module (regulon). In this chapter, the authors will describe the state of the art of several topics associated to the transcription initiation from TATA-less promoters in eukaryotic organisms, such as the transcriptional regulation of RPGs in metazoan cells and the description of a novel mechanism of regulation present in the RPG of the fission yeast *Schizosaccharomyces pombe*.

2. General features of transcription initiation in the eukaryotic organisms and TATA-less CPEs

Transcription in eukaryotic organisms is carried out by RNA polymerases (RNA pols), which are enzymatic complexes composed by at least 12 subunits. In general, eukaryotic genes are classified as class I, II, and III, where class I genes codify rRNAs; class II codify pre-mRNAs; and class III codify 5S rRNA, tRNAs, and snRNAs, respectively. Transcription of each class of genes is carried out by a different RNA pol. Class I genes are transcribed by RNA pol I, class II genes are transcribed by RNA pol II, and class III genes are transcribed by RNA pol III, respectively. This specific transcription is based on the recognition of specific DNA sequences in the promoters of each class of genes by different transcription factors (TFs) that are able to recruit each specific RNA pol. These sequences are named "core promoter elements" (CPEs) and are located inside the region of the promoter named "core promoter" (CP) that is able to direct the formation of a pre-initiation complex (PIC) and initiate specific transcription of the gene. The CPEs are recognized by TFs specific to each RNA pol, which are called "general transcription factors" (GTFs). In summary, each RNA pol has a set of specific GTFs and these protein factors are able to recognize the CPEs associated to each class' gene promoters.

RNA pol II has been widely studied due to the enzyme that transcribes protein-coding genes. One of the first CPEs described in the promoters of class II genes was the so-called TATA-box [3–5]. This CPE is distributed in the promoters of most eukaryotic organisms and is located 25–40 bp upstream from the transcription initiation site. The formation of a PIC on the promoters containing a TATA-box has been extensively studied and characterized [6–8]. The formation of a PIC on the TATA-box starts with the recognition and binding of the transcription factor TATA-binding protein (TBP) to the TATA-box which in turn recruits the other GTFs and RNA pol II to form the PIC, which is able to initiate transcription upon the addition of the ribonucleotides [9, 10] (**Figure 1**). As it can be seen from the model, RNA pol II is integrated into the PIC in association with TFIIF when the promoter-TBP-TFIIB complex is formed. On the other hand, a fraction of RNA pol II can be purified from cell extracts in association with TFIIF and the mediator, and since those complexes are preformed inside the nucleus, a fast recruitment of the PIC to the promoter could be produced [11–13]. The multi-subunit complex named mediator (a general transcriptional coactivator) is also necessary for the transcription *in vivo*, in crude cell extracts, of class II genes [14]. In addition, recently, it has been demonstrated that another protein complex is recruited *in vivo* at most of the class II gene promotes in *S. cerevisiae*, where it plays a fundamental role in transcription. This multi-protein complex is named SAGA and is composed of several subunits including Gcn5, which have histone acetyltransferase (HAT) activity Spt gene products and TBP-associated factors (TAFs) that are shared with the complex TFIID [15].

In metazoan cells, the transcription factor TBP is tightly associated to TAFs and the TBP-TAF complex is named TFIID [16]. The role of TAFs seems to be the recognition of certain CPEs such as the Inr, motif ten element (MTE), and downstream promoter element (DPE) (see below). However, in yeast, this complex seems to be unstable, since it is possible to purify TBP free of TAFs from yeast cell extracts. Although TAFs are required for *in vivo* transcription of *S. cerevisiae* genes, the exact

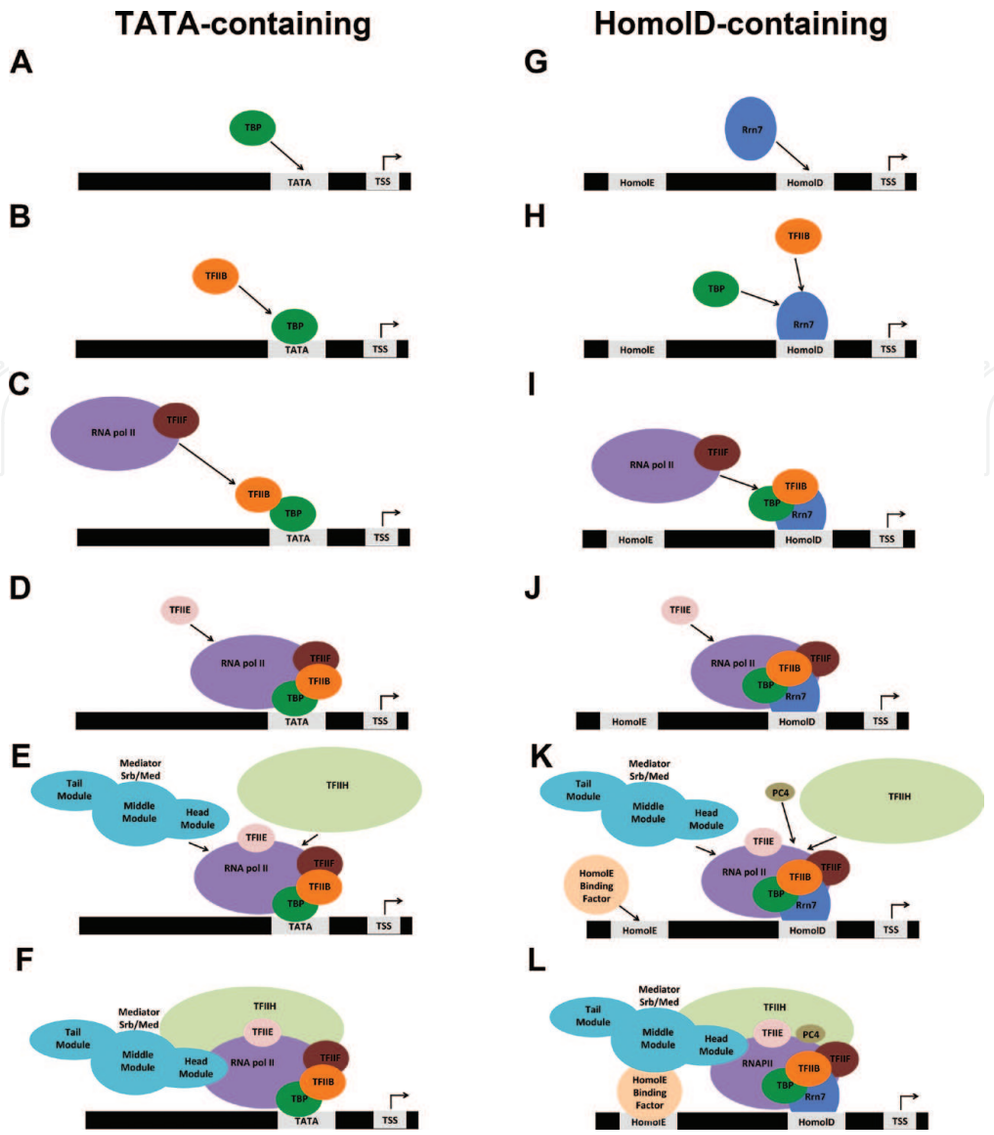

Figure 1.
PIC formation on TATA-containing and HomolD-containing promoters. *Classical PIC formation on a TATA-containing promoter is outlined in A–F. First, TBP binds to the TATA-box and then TFIIB is recruited to the promoter-TBP complex. This allows the RNA pol II-TFIIF complex to be incorporated into the promoter-TBP-TFIIB complex (C). Once that RNA pol II-TFIIF is loaded onto the complex, the transcription factor TFIIE is incorporated (D) followed by the binding of TFIIH (E). The mediator complex might be incorporated into the complex after the binding of RNA pol II-TFIIF and TFIIE (D). At step F, the complete PIC is formed and it is competent for transcription initiation. A competent PIC is formed on HomolD-box containing promoters, such as RPG promoters, to initiate RNA pol II-dependent transcription (G–L). The first step is the binding of the transcription factor Rrn7 to the HomolD-box sequence (G). Then, transcription factors TBP and TFIIB bind to Rrn7 (H). This DNA-protein complex is recognized by RNA pol II-TFIIF (I) and TFIIE (J). This complex is competent to initiate HomolD-box-dependent transcription. However, coactivators such as the mediator, PC4, and the HomolE-binding factor would be necessary to modulate transcription initiation (K and L). TSS: transcription start site. Note that steps C to F for PIC formation on TATA-box-containing promoters might be common with steps I to L on PIC formation on HomolD-box-containing promoters.*

mechanism of their function has not been identified yet. Interestingly, using an *in vitro* approach using TFIID-depleted yeast cell extracts, it was found that transcription from both TATA-containing and TATA-less promoters is dependent on TFIID, but isolated recombinant TBP can only rescue the transcription of TATA-containing promoters, indicating that additional interactions are necessary to efficiently transcribe TATA-less promoters [17].

However, our vision of transcription initiation on TATA-box-containing promoters cannot explain the mechanisms of transcription initiation on all the class II genes, because the analysis of several other class II gene promoter sequences showed that

in most of them the TATA-box is absent. Different studies have determined that only 10–15% of mammal core promoters contain a TATA-box element [18–20]. Those promoters that do not contain a TATA-box were named TATA-less promoters and they have also been studied and different CPEs have been characterized.

After the identification of TATA-box sequence, other conserved promoter elements were identified. One of them is the initiator element (Inr), identified as a conserved DNA element in the region near to the transcription start site [21]. This element can not only direct transcription initiation by itself if other CPEs are not present, but also act synergistically in the presence of a TATA-box [22]. The proteins $TAF_{II}150$ and $TAF_{II}250$ have been identified as the transcription factors that are able to recognize the Inr and allow the formation of the PIC in Inr-containing promoters [23, 24]. However, other Inr-containing promoters might be able to direct transcription initiation in a TAFs-independent manner. In those promoters, a few proteins have been identified as Inr-binding factors, such as TF_{II}-I and YY1 [25, 26]. Also, in other reports, transcription initiation from the human DNA beta polymerase promoter and from the human dihydrofolate reductase (DHFR) promoter, both TATA-less and Inr-containing promoters, has been achieved using solely TBP, IIB, IIE, IIF, IIH, and RNA pol II [26, 27]. This suggests that in some TATA-less promoters, the formation of a functional PIC might follow a common pathway with those TATA-containing promoters.

Another CPE that has been described in TATA-less promoters is the downstream promoter element (DPE), identified first in *Drosophila melanogaster* [28]. This element is widely distributed in metazoan organisms and is located 28–32 bp downstream from the transcription start site and can be contained in the context of a TATA-box and/or an Inr. Studies in *Drosophila* have shown that proteins $TAF_{II}40$ and $TAF_{II}60$ might bind to the DPE to improve transcription initiation [29, 30]. Similar elements have not been found in yeast yet.

Several other CPEs have been identified in TATA-less promoters but their contribution to transcription initiation is still poorly understood. Such is the case of motif ten element (MTE) [31]; TFIIB recognition element (BRE) [32]; X core promoter element 1 and 2 (XCPE1 and 2) [33, 34], both of which are able to direct transcription initiation; and the poly-pyrimidine initiator motif (TCT) motif [35]. The TCT motif element will be described in another section of this chapter.

However, using the information from the sequencing of the genomes of other organisms and the new bioinformatics technologies, it is expected that novel conserved CPEs will be identified and characterized and the transcription initiation mechanisms of TATA-less promoters will be revealed. Such is the case of the ribosomal protein genes (RPGs) in the fission yeast *Schizosaccharomyces pombe*, whose promoters do not contain a TATA-box; instead they possess a conserved sequence, acting as a TATA-analog to direct transcription initiation in those genes. In the next section, the RPG promoter of the fission yeast will be described and the transcription initiation mechanism will be discussed.

3. Characterization of ribosomal protein gene (RPG) promoters of *Schizosaccharomyces pombe* and their transcription initiation mechanism

3.1 The *Schizosaccharomyces pombe* RPG transcriptional module: the HomolD-box

The characterization of the promoter sequences of 14 RPGs from the fission yeast *Schizosaccharomyces pombe* showed discrete conserved modules, which were

named Homol A, B, C, D, and E (**Table 1**) [36–38]. These homology regions were completely different from those described in promoters of genes from other yeasts and mammals, such as TATA-box, Inr, or DPEs. The function of each Homol element was studied using a promoter-deletion mutant approach [37]. This work showed that the role of Homol A, B, C, and E is associated to the regulation of transcription initiation, and that they might have a upstream activation sequence (UAS)-like function. Only the HomolD sequence was able to function as an element that could direct transcription initiation in the same way as the TATA-box [36]. The conserved sequence of the HomolD-box is the octamer CAGTCACA/G; however, in several sequences, this element is found in the inverted form as TGTGACTG. The HomolD-box is located 39–52 bp upstream of the transcription start site in the same position as the TATA-box in the fission yeast promoters. In an *in vivo* approach, using reporter-gene assays in *S. pombe* cells, it was shown that the HomolD-box is necessary to direct and initiate transcription from the RPG and was postulated to act as a TATA-box analog; in the same work, using an electrophoretic mobility shift assay (EMSA), a novel protein complex that binds to the HomolD-box was identified [36]. In other studies using an *in vitro* approach, it was shown that point mutations in the HomolD-box sequence abolish completely the ability of this element to direct transcription initiation from the RPG [39].

Currently, we know that the genome of *Schizosaccharomyces pombe* contains 141 RPGs encoding the full set of 79 ribosomal proteins. Interestingly, the analysis of the promoter sequences showed that 140 RPGs contained a highly conserved HomolD-box in the region 49–104 bp upstream of the ATG start codon [40]. Additionally, other 59 non-RPGs also showed the presence of the HomolD-box in their promoters. In addition, using promoter databases, it was possible to find HomolD-box sequences in several promoters from other eukaryotic organisms, such as humans and plants, indicating the broad distribution of this novel CPE. Moreover, a functional HomolD-box was found in the human ATPV1H gene where RECQL/DDB1 complex binds to this sequence and is required for *in vitro* transcription [41].

Interestingly, HomolD-boxes in RPG promoters are broadly distributed in the *Ascomycota* fungus phylum [42]. However, in those organisms closely related to the yeast *Saccharomyces cerevisiae,* other CPEs, in the same position as the HomolD-box, are present in RPG promoters. These elements are named Rap1 and bind the transcription factor Rap1p [43]. It seems likely that Rap1 replaced the HomolD-box of *Schizosaccharomyces pombe* in *Saccharomyces cerevisiae* during evolution. Moreover, several other yeast species share both HomolD-box and Rap1 promoter elements [42]. Taking all those observations together, we suggest that RPGs from *S. pombe*, *S. cerevisiae*, *Drosophila,* and mammals form a transcriptional module that is under the control of the HomolD-box, Rap1-box, and TCT motif (*Drosophila* promoter element), respectively.

Homol	Consensus	Binding TF	Function	Reference
HomolA	TCAGTAACGAA	Unknown	UAS-like	[48]
HomolB	AAAAGCTATG	Unknown	UAS-like	[48]
HomolC	AAGAGTAAAATCT	Unknown	UAS-like	[48]
HomolD	CAGTCACA/G	Rrn7 (*S. pombe*) RECQL/DDB1 (Human, *S. pombe*)	Transcription initiation and regulation of RPG expression	[36, 39, 48]
HomolE	AGGGTAGGGT	Unknown	UAS-like	[37, 48]

Table 1.
Homol sequences identified in RPG promoters in S. pombe.

3.2 The role of Rrn7 and CK2 in RPG transcription initiation in *Schizosaccharomyces pombe*

The HomolD-box present in the RPG promoters of the fission yeast is the target of a DNA-binding protein with biochemical features different from TBP. The identification of the HomolD-box-binding protein was achieved using DNA affinity chromatography with double-stranded tandem HomolD-boxes covalently attached to a resin. Proteins bound to the resin were eluted and analyzed by mass spectrometry. The result was that the transcription factor Rrn7 was identified in the protein DNA-bound fraction [39]. This factor is a member of the RNA pol I transcriptional machinery and its function is to transcribe rDNA in the nucleolus. In the rDNA promoter, this factor is able to bind to a conserved box, which is similar to a HomolD-box. Rrn7 showed a specific HomolD-box-binding activity and it is required for the specific transcription of RPGs containing a HomolD-box [39]. Moreover, the GTFs and RNA pol II were required for accurate transcription initiation of a HomolD-box-containing promoter.

Rrn7 is part of the Zn-ribbon protein family related to TFIIB, including the mammalian ortholog TAF1B [44]. It possesses a Zn-ribbon domain in the N-terminal region and two cyclin-like domains in the carboxy-terminal region, displaying domain conservation with the TFIIB family members [44]. Recently, it has been demonstrated that *Schizosaccharomyces pombe* Rrn7 is able to interact with casein kinase 2 (CK2) both *in vitro* and *in vivo*, leading to a functional phosphorylation of threonine 67 in the N-terminal domain. This modification modulates negatively the transcriptional activity of Rrn7, affecting HomolD-directed transcription and DNA-binding activity [45]. Studies in *S. pombe* cell cultures using the specific CK2 inhibitor 4,5,6,7-tetrabromobenzotriazole (TBB) have shown the potentiation of RPG expression during CK2 inhibition. Moreover, using chromatin immunoprecipitation assays, it has been found that CK2 is associated with RPG promoters, suggesting that this kinase has a role in the modulation of ribosomal protein abundance [45].

3.3 Preinitiation complex (PIC) formation on the RPG promoters in *Schizosaccharomyces pombe*

As stated before, RPGs that contain a HomolD-box are transcribed by the RNA pol II transcription apparatus [39]. The formation of the PIC on a HomolD-box-containing promoter was recently described [45, 46]. The first step in the formation of a PIC on these promoters is the binding of Rrn7 to the HomolD-box. As mentioned previously, this step in the PIC establishment might be regulated by phosphorylation of Rrn7 via CK2 protein kinase [45]. Upon the binding of Rrn7 to the HomolD-box, the general transcription factors TBP and TFIIB are able to recognize this DNA-protein complex [46]. After the binding of TBP/TFIIB to the complex, the RNA pol II/TFIIF complex is recruited, which in turn allows the TFIIE factor to be incorporated into the complex [46]. Finally, the mediator and the coactivator PC4 may be incorporated into the PIC and might modulate basal transcription through a putative HomolE-binding factor in those promoters that contain this DNA element. All the steps describing the pathway of complex formation are summarized in **Figure 1**.

4. Regulation of RPG expression in *Schizosaccharomyces pombe*

The expression of genes containing the HomolD-box in their promoters is almost unknown. However, data from analysis of the RPG expression profiles during

several biological processes in *S. pombe*, for example, the switch from vegetative to meiotic growth and growth under stress conditions, have revealed a tightly coordinated expression for all 141 RPGs. For example, during the switch from vegetative to meiotic growth, transcription of RPG is down-regulated, but then, within a short time, strong reactivation of RPG expression is observed at the beginning of meiosis. The same co-regulation profile is observed in 32 of the 59 non-RPGs that contain a HomolD-box in their core promoter [40]. Many, but not all, of these non-RPGs encode components whose homologs in other organisms are involved in protein biosynthesis and signal transduction [40].

Several promoters of *Schizosaccharomyces pombe* RPGs have been isolated and characterized [47–50], showing that individual ribosomal proteins are encoded by two or three related genes whose promoters contain a HomolD-box. Interestingly, in each gene family, at least one promoter possesses a tandem repeat ACCCTACCCT or the inverted form (AGGGTAGGGT) upstream of the HomolD-box. This sequence corresponds to the HomolE-box, which is considered a proximal UAS-like sequence for HomolD-box-containing promoters, since the presence of this element strongly increases *in vivo* transcription directed by the HomolD-box. Both promoter elements HomolD- and HomolE-boxes must be in the same orientation to be functional. The distance between the boxes is critical in transcription modulation of RPGs, and it has been described that the smaller the distance between HomolD and HomolE, the higher the transcription activity. This distance ranges from 0 to 32 nucleotides.

Now that the complete genome of *Schizosaccharomyces pombe* is available and searchable, it is known that of the 141 RPG promoters, 140 promoters contain a HomolD-box and 62 contain a HomolE-box upstream of the HomolD-box. In contrast, only 5 of the 59 non-RPG promoters containing a HomolD-box contain a HomolE-box [40].

The activity of the HomolE-box must be related to a transcriptional activator in the same manner as the IFH1 element is recognized by a TF (Ifh1p) in *Saccharomyces cerevisiae* RPGs [51, 52]. This element has been identified upstream of the Rap1 sequence in RPG promoters. It is likely that a member of the same family of Ifh1p recognizes the HomolE-box in *S. pombe*. However, the gene encoding this protein has not been identified yet.

The modular architecture of the *Schizosaccharomyces pombe* RPG promoters where the HomolE-box is always found upstream of HomolD indicates that some of the promoters are under the control of the HomolE-box. This fact suggests that there must be a mechanism that regulates RPG transcription through the HomolE-box under specific growth conditions.

Further investigations must be performed to understand how RPG expression is regulated and which are the mechanisms involved in the coordination between HomolD- and HomolE-boxes during RPG transcription. Despite the fact that there are several factors and mechanisms studied in RNA pol II-directed transcription, most of the promoters studied possess a TATA-box, whereas RPG promoters are TATA-less. Moreover, transcriptional initiation and activation from TATA-less promoters are poorly understood both in metazoan and yeast cells. Thus, the RPG promoters and the arrangement of HomolE-HomolD could provide a model to study transcription in TATA-less promoters using a promoter element such as HomolD that is analogous to the TATA-box.

5. Coordinated regulation of the expression of ribosomal components in *Schizosaccharomyces pombe*

Ribosome biogenesis is one of the most complicated processes in eukaryotic cells, requiring coordinated expression of all ribosome components, which are

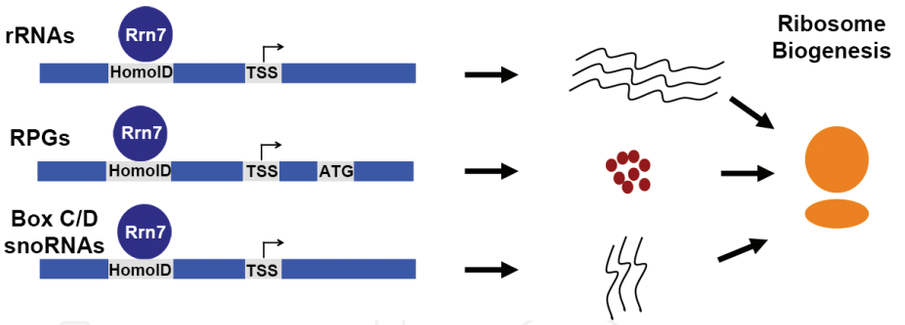

Figure 2.
Role of HomolD-box-containing promoters in ribosome biogenesis. *It is believed that the coordinated expression of genes that encode ribosome components (rRNA, RPG, box C/D snoRNA) is due to a common DNA element (HomolD-box) that is able to bind a protein factor (Rrn7). The presence of the HomolD-box in the promoters of several genes encoding ribosome components indicates a common regulation. Until now, experiments have demonstrated that Rrn7 binds to rRNA and RPG promoters in Schizosaccharomyces pombe. However, binding of Rrn7 to box C/D snoRNA promoters has not been demonstrated yet, although those promoters contain a HomolD-box, which is critical for in vivo transcription.*

essential for accurate translation activity. The coordinated regulation and expression of the RPG with other ribosomal components is still poorly understood. However, in the fission yeast *Schizosaccharomyces pombe*, it is known that rRNAs, RPGs, and box C/D snoRNAs contain in their promoters a HomolD-box [39, 53], which might be able to control the expression of those genes. Moreover, the Rrn7 transcription factor, which is the HomolD-box-binding protein in RPG, was found to be responsible for the control of the gene expression of box C/D snoRNAs and RPGs *in vivo* in *Schizosaccharomyces pombe* cells [53]. Interestingly, the yeast orthologs of the human RECQL/DDB1 complex may recognize the HomolD-box and down-regulate RPG expression [53]. Taking all these results together, we propose a model, in which the HomolD-box is bound by Rrn7 and co-regulates the transcription of RPG, box C/D snoRNA and rRNA genes in the fission yeast. This model is summarized in **Figure 2**.

Unlike *Schizosaccharomyces pombe* in the case of *Saccharomyces cerevisiae*, there is accumulated evidence that show a putative coordinated model to regulate biogenesis of ribosome components. In this model, CK2 protein kinase is part of protein complexes that regulate RPG expression and rRNA synthesis [54] and interact with the protein Fhl1p that is associated to Ifh1p, which binds to the IFH1 element near to the Rap1 sequence. CK2 and Ifh1p are part of the complex CURI associated to rRNA processing and RPG transcription [55]. Also, in *S. cerevisiae,* another protein has been identified and named protein HmoI, which is associated with the transcription regulation of RPGs and rRNAs [56].

In addition to the role of CK2 to modulate Rrn7 function in *Schizosaccharomyces pombe* during HomolD-box directed transcription, there might be another points of regulation related to protein complexes, such as those described in the yeast *Saccharomyces cerevisiae*.

6. The TCT-motif module in metazoan RPG

The analyses of insect and mammalian RPG promoters have shown the presence of a common core promoter element that is part of the poly-pyrimidine initiator (TCT)-motif family, which is a novel core promoter element necessary to initiate transcription in those genes [35, 57]. In these promoters, the transcription start site (TSS) involves the TCT motif and is positioned around −2 to +6 relative to TSS, competing with exactly the same position as the Inr. However, the features of

the TCT-containing promoters are dissimilar to those Inr-containing promoters. The function of a TCT motif cannot be replaced by an Inr, and the TFIID complex cannot bind to the TCT motif [57]. Recently, studies in *Drosophila* RPG promoters, which contain a TCT motif, have shown the dependence on a TBP-related factor 2 (TRF2) but not TBP. Using a TRF2-depleted *Drosophila* whole cell extract, it was shown that human TRF2 [58, 59] and *Drosophila* TRF2 [60] were able to support TCT-dependent transcription. The TATA-binding protein TBP was unable to support TCT-dependent transcription. However, whether or not the TBP factor is required for TCT-dependent transcription remains to be determined. The proteins able to recognize this element are still unknown, because TRF2 is unable to bind directly to the TCT motif. It is possible that TRF2 interacts with other members of the RNA pol II basal transcription machinery and forms a PIC associated with the TCT motif. In addition, TRF2 is able to bind to the vicinity of the TSS of other genes, since it can be crosslinked and immunoprecipitated from that region, but whether or not this factor binds directly to the Inr motif is still unknown [61].

7. Conclusions

The fission yeast *Schizosaccharomyces pombe* provides an excellent biological model to study the coordinated expression of ribosome components. The finding that rDNAs, RPGs, and box C/D snoRNAs genes contain a HomolD-box, which is most likely bound by Rrn7, provides the starting point to investigate this issue. The most important questions to answer are: (i) to determine whether or not box C/D snoRNA genes are transcribed by the same transcription apparatus that transcribes RPG; (ii) to identify the signal that activates transcription of HomolD-box containing genes, and (iii) to identify the HomolE-binding protein. The resolution of all these issues would contribute to understand the regulation of RPG transcription in the fission yeast and most likely could be extrapolated to metazoan organisms.

Acknowledgements

This work has been funded by the Corporación Nacional de Ciencia y Tecnología de Chile (CONICYT), Fondo de Desarrollo de Ciencia y Tecnología (FONDECYT), and FONDEF (ID16I10145). We are very grateful to Mrs. Teresa Donoso for her continued support to F. U. and E. M.

Conflict of interest

The authors do not have any conflict of interest.

Abbreviations

CP	core promoter
CPE	core promoter element
DPE	downstream promoter element
GTF	general transcription factor
Inr	initiator
PIC	pre-initiation complex
RNA pol	RNA polymerase

RPG	ribosomal protein genes
rRNA	ribosomal RNA
snoRNA	small nucleolar RNA
TAF	TBP-associated factor
TBP	TATA-binding protein
TCT	poly-pyrimidine initiator motif
TF	transcription factor
TRF	TBP-related factor
TSS	transcription start site

Author details

Diego A. Rojas[1], Sandra Moreira-Ramos[2], Fabiola Urbina[2] and Edio Maldonado[2*]

1 Programa de Microbiología y Micología, Instituto de Ciencias Biomedicas, Facultad de Medicina, Universidad de Chile, Independencia, Santiago, Chile

2 Programa de Biología Celular y Molecular, Instituto de Ciencias Biomedicas, Facultad de Medicina, Universidad de Chile, Independencia, Santiago, Chile

*Address all correspondence to: emaldona@med.uchile.cl

References

[1] Kandiah E, Trowitzsch S, Gupta K, Haffke M, Berger I. More pieces to the puzzle: Recent structural insights into class II transcription initiation. Current Opinion in Structural Biology. 2014;**24**:91-97. DOI: 10.1016/j.sbi.2013.12.005

[2] Shandilya J, Roberts SG. The transcription cycle in eukaryotes: From productive initiation to RNA polymerase II recycling. Biochimica et Biophysica Acta. 2012;**1819**:391-400. DOI: 10.1016/j.bbagrm.2012.01.010

[3] Wasylyk B, Derbyshire R, Guy A, Molko D, Roget A, Teoule R, et al. Specific in vitro transcription of conalbumin gene is drastically decreased by single-point mutation in T-A-T-A box homology sequence. Proceedings of the National Academy of Sciences of the United States of America. 1980;**77**:7024-7028

[4] Sassone-Corsi P, Corden J, Kedinger C, Chambon P. Promotion of specific in vitro transcription by excised "TATA" box sequences inserted in a foreign nucleotide environment. Nucleic Acids Research. 1981;**9**:3941-3958

[5] Concino MF, Lee RF, Merryweather JP, Weinmann R. The adenovirus major late promoter TATA box and initiation site are both necessary for transcription in vitro. Nucleic Acids Research. 1984;**12**:7423-7433

[6] Orphanides G, Reinberg D. A unified theory of gene expression. Cell. 2002;**108**:439-451. DOI: 10.1016/S0092-8674(02)00655-4

[7] Thomas M, Chiang C. The general transcription machinery and general cofactors. Critical Reviews in Biochemistry and Molecular Biology. 2006;**41**:105-178. DOI: 10.1080/10409230600648736

[8] Sikorski T, Buratowski S. The basal initiation machinery: Beyond the general transcription factors. Current Opinion in Cell Biology. 2009;**21**:344-351. DOI: 10.1016/j.ceb.2009.03.006

[9] Maldonado E, Ha I, Cortes P, Weis L, Reinberg D. Factors involved in specific transcription by mammalian RNA polymerase II: Role of transcription factors IIA, IID, and IIB during formation of a transcription-competent complex. Molecular and Cellular Biology. 1990;**10**:6335-6347. DOI: 10.1128/MCB.10.12.6335

[10] Murakami K, Elmlund H, Kalisman N, Bushnell DA, Adams CM, Azubel M, et al. Architecture of an RNA polymerase II transcription pre-initiation complex. Science. 2013;**342**:1238724. DOI: 10.1126/science.1238724

[11] Lee TI, Young RA. Transcription of eukaryotic protein-coding genes. Annual Review of Genetics. 2000;**34**:77-137. DOI: 10.1146/annurev.genet.34.1.77

[12] Hahn S. Structure and mechanism of the RNA polymerase II transcription machinery. Nature Structural & Molecular Biology. 2004;**11**:394-403. DOI: 10.1038/nsmb763

[13] Esnault C. Mediator-dependent recruitment of TFIIH modules in preinitiation complex. Molecular Cell. 2008;**31**:337-346. DOI: 10.1016/j.molcel.2008.06.021

[14] Jeronimo C, Robert F. The mediator complex: At the nexus of RNA polymerase II transcription. Trends in Cell Biology. 2017;**27**:765-783. DOI: 10.1016/j.tcb.2017.07.001

[15] Baptista T, Grünberg S, Minoungou N, Koster MJE, Timmers HTM, Hahn S, et al. SAGA Is a general cofactor for RNA polymerase II transcription.

Molecular Cell. 2017;**68**:130-143. DOI: 10.1016/j.molcel.2017.08.016

[16] Burley SK, Roeder RG. Biochemistry and structural biology of transcription factor IID (TFIID). Annual Review of Biochemistry. 1996;**65**:769-799. DOI: 10.1146/annurev.bi.65.070196.004005

[17] Donczew R, Hahn S. Mechanistic differences in transcription initiation at TATA-less and TATA-containing promoters. Molecular and Cellular Biology. 2017;**38**:e00448-e00417. DOI: 10.1128/MCB.00448-17

[18] Kim T, Barrera L, Zheng M, Qu C, Singer M, Richmond TA, et al. A high-resolution map of active promoters in the human genome. Nature. 2005;**436**: 876-880. DOI: 10.1038/nature03877

[19] Carninci P, Sandelin A, Lenhard B, Katayama S, Shimokawa K, Ponjavic J, et al. Genome-wide analysis of mammalian promoter architecture and evolution. Nature Genetics. 2006;**38**:626-635. DOI: 10.1038/ng1789

[20] Cooper S, Trinklein N, Anton E, Nguyen L, Myers R. Comprehensive analysis of transcriptional promoter structure and function in 1% of the human genome. Genome Research. 2006;**16**:1-10. DOI: 10.1101/gr.4222606

[21] Smale ST, Baltimore D. The "initiator" as a transcription control element. Cell. 1989;**57**:103-113

[22] O'Shea-Greenfield A, Smale ST. Roles of TATA and initiator elements in determining the start site location and direction of RNA polymerase II transcription. The Journal of Biological Chemistry. 1992;**267**:1391-1402

[23] Verrijzer CP, Chen JL, Yokomori K, Tjian R. Binding of TAFs to core elements directs promoter selectivity by RNA polymerase II. Cell. 1995;**81**:1115-1125. DOI: 10.1016/S0092-8674(05)80016-9

[24] Kauffmann J, Ahrens K, Koop R, Smale ST, Müller R. CIF150, a human cofactor for transcription factor IID-dependent initiator function. Molecular and Cellular Biology. 1998;**18**:233-239. DOI: 10.1128/MCB.18.1.233

[25] Cheriyath V, Novina CD, Roy AL. TFII-I regulates Vβ promoter activity through an initiator element. Molecular and Cellular Biology. 1998;**18**:4444-4454. DOI: 10.1128/MCB.18.8.4444

[26] Weis L, Reinberg D. Accurate positioning of RNA polymerase II on a natural TATA-less promoter is independent of TATA-binding-protein-associated factors and initiator-binding proteins. Molecular and Cellular Biology. 1997;**17**:2973-2984. DOI: 10.1128/MCB.17.6.2973

[27] Aso T, Conaway JW, Conaway RC. Role of core promoter structure in assembly of the RNA polymerase II preinitiation complex. A common pathway for formation of preinitiation intermediates at many TATA and TATA-less promoters. The Journal of Biological Chemistry. 1994;**269**:26575-26583

[28] Burke TW, Kadonaga JT. *Drosophila* TFIID binds to a conserved downstream basal promoter element that is present in many TATA-box-deficient promoters. Genes & Development. 1996;**10**: 711-724. DOI: 10.1101/gad.10.6.711

[29] Burke TW, Kadonaga JT. The downstream core promoter element, DPE, is conserved from *Drosophila* to humans and is recognized by TAFII60 of *Drosophila*. Genes & Development. 1997;**11**:3020-3031

[30] Kutach AK, Kadonaga JT. The downstream promoter element DPE appears to be as widely used as the TATA box in *Drosophila* core promoters. Molecular and Cellular Biology. 2000;**20**:4754-4764. DOI: 10.1128/MCB.20.13.4754-4764.
2000

[31] Lim CY, Santoso B, Boulay T, Dong E, Ohler U, Kadonaga JT. The MTE, a new core promoter element for transcription by RNA polymerase II. Genes & Development. 2004;**18**: 1606-1617. DOI: 10.1101/gad.1193404

[32] Deng W, Roberts S. A core promoter element downstream of the TATA box that is recognized by TFIIB. Genes & Development. 2005;**19**:2418-2423. DOI: 10.1101/gad.342405

[33] Tokusumi Y, Ma Y, Song X, Jacobson R, Takada S. The new core promoter element XCPE1 (X Core Promoter Element 1) directs activator-, mediator-, and TATA-binding protein-dependent but TFIID-independent RNA polymerase II transcription from TATA-less promoters. Molecular and Cellular Biology. 2007;**27**:1844-1858. DOI: 10.1128/MCB.01363-06

[34] Anish R, Hossain M, Jacobson R, Takada S. Characterization of transcription from TATA-less promoters: Identification of a new core promoter element XCPE2 and analysis of factor requirements. PLoS One. 2009;**4**:e5103. DOI: 10.1371/journal.pone.0005103

[35] Parry TJ, Theisen JW, Hsu JY, Wang YL, Corcoran DL, Eustice M, et al. The TCT motif, a key component of an RNA polymerase II transcription system for the translational machinery. Genes & Development. 2010;**24**:2013-2018. DOI: 10.1101/gad.1951110

[36] Witt I, Straub N, Käufer NF, Gross T. The CAGTCACA box in the fission yeast *Schizosaccharomyces pombe* functions like a TATA element and binds a novel factor. The EMBO Journal. 1993;**12**:1201-1208

[37] Witt I, Kwart M, Gross T, Käufer NF. The tandem repeat AGGGTAGGGT is, in the fission yeast, a proximal activation sequence and activates basal transcription mediated by the sequence TGTGACTG. Nucleic Acids Research. 1995;**23**:4296-4302

[38] Gross T, Käufer NF. Cytoplasmic ribosomal protein genes of the fission yeast *Schizosaccharomyces pombe* display a unique promoter type: A suggestion for nomenclature of cytoplasmic ribosomal proteins in databases. Nucleic Acids Research. 1998;**26**:3319-3322

[39] Rojas DA, Moreira-Ramos S, Zock-Emmenthal S, Urbina F, Contreras-Levicoy J, Käufer NF, et al. Rrn7 protein, an RNA polymerase I transcription factor, is required for RNA polymerase II-dependent transcription directed by core promoters with a HomolD box sequence. The Journal of Biological Chemistry. 2011;**286**: 26480-26486. DOI: 10.1074/jbc.M111.224337

[40] Witt I, Kivinen K, Käufer NF. Core promoters in *S. pombe*: TATA and HomolD boxes. In: Egel R, editor. The Molecular Biology of *Schizosaccharomyces pombe*. Berlin/Heidelberg: Springer-Verlag; 2004. pp. 343-351. DOI: 10.1007/978-3-662-10360-9

[41] Contreras-Levicoy J, Moreira-Ramos S, Rojas DA, Urbina F, Maldonado E. Transcription directed by human core promoters with a HomolD box sequence requires DDB1, RECQL and RNA polymerase II machinery. Gene. 2012;**505**:318-323. DOI: 10.1016/j.gene.2012.05.059

[42] Tanay A, Regev A, Shamir R. Conservation and evolvability in regulatory networks: The evolution of ribosomal regulation in yeast. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**:7203-7208. DOI: 10.1073/pnas.0502521102

[43] Li B, Nierras CR, Warner JR. Transcriptional elements involved in the repression of ribosomal protein synthesis. Molecular and Cellular

Biology. 1999;**19**:5393-5404. DOI: 10.1128/MCB.19.8.5393

[44] Knutson BA, Hahn S. Yeast Rrn7 and human TAF1B are TFIIB-related RNA polymerase I general transcription factors. Science. 2011;**333**:1637-1640. DOI: 10.1126/science.1207699

[45] Moreira-Ramos S, Rojas DA, Montes M, Urbina F, Miralles VJ, Maldonado E. Casein kinase 2 inhibits HomolD-directed transcription by Rrn7 in *Schizosaccharomyces pombe*. The FEBS Journal. 2015;**282**:491-503. DOI: 10.1111/febs.13157

[46] Montes M, Moreira-Ramos S, Rojas DA, Urbina F, Käufer NF, Maldonado E. RNA polymerase II components and Rrn7 form a preinitiation complex on the HomolD box to promote ribosomal protein gene expression in *Schizosaccharomyces pombe*. The FEBS Journal. 2017;**284**:615-633. DOI: 10.1111/febs.14006

[47] Nischt R, Thüroff E, Küfer NF. Molecular cloning of a ribosomal protein gene from the fission yeast *Schizosaccharomyces pombe*. Current Genetics. 1986;**10**:365-370

[48] Nischt R, Gross T, Gatermann K, Swida U, Käufer NF. Sequence and regulatory responses of a ribosomal protein gene from the fission yeast *Schizosaccharomyces pombe*. Nucleic Acids Research. 1987;**15**:1477-1492

[49] Gross T, Nischt R, Gatermann K, Swida U, Käufer NF. Primary structure of the ribosomal protein gene S6 from *Schizosaccharomyces pombe*. Current Genetics. 1988;**13**:57-63

[50] Liebich I, Köhler G, Witt I, Gross T, Käufer NF. Two genes encoding ribosomal protein L3 of *Schizosaccharomyces pombe* and their proximal promoter regions. Gene. 1994;**142**:119-122. DOI: 10.1016/0378-1119(94)90365-4

[51] Schawalder SB, Kabani M, Howald I, Choudhury U, Werner M, Shore D. Growth-regulated recruitment of the essential yeast ribosomal protein gene activator Ifh1. Nature. 2004;**432**:1058-1061. DOI: 10.1038/nature03200

[52] Wade JT, Hall DB, Struhl K. The transcription factor Ifh1 is a key regulator of yeast ribosomal protein genes. Nature. 2004;**432**:1054-1058. DOI: 10.1038/nature03175

[53] Diao LT, Xiao ZD, Leng XM, Li B, Li JH, Luo YP, et al. Conservation and divergence of transcriptional coregulations between box C/D snoRNA and ribosomal protein genes in Ascomycota. RNA. 2014;**20**:1376-1385. DOI: 10.1261/rna.042309.113

[54] Rudra D, Mallick J, Zhao Y, Warner JR. Potential interface between ribosomal protein production and pre-rRNA processing. Molecular and Cellular Biology. 2007;**27**:4815-4824. DOI: 10.1128/MCB.02062-06

[55] Rudra D, Zhao Y, Warner JR. Central role of Ifhl1p-Fhl1p interaction in the synthesis of yeast ribosomal proteins. The EMBO Journal. 2005;**24**:533-542. DOI: 10.1038/sj.emboj.7600553

[56] Hall DB, Wade JT, Struhl K. An HMG protein, Hmo1, associates with promoters of many ribosomal protein genes and throughout the rRNA gene locus in *Saccharomyces cerevisiae*. Molecular and Cellular Biology. 2006;**26**:3672-3679. DOI: 10.1128/MCB.26.9.3672-3679.2006

[57] Perry RP. The architecture of mammalian ribosomal protein promoters. BMC Evolutionary Biology. 2005;**5**:15. DOI: 10.1186/1471-2148-5-15

[58] Maldonado E. Transcriptional functions of a new mammalian TATA-binding protein-related factor. The Journal of Biological Chemistry. 1999;**274**:12963-12966. DOI: 10.1074/jbc.274.19.12963

[59] Moore PA, Ozer J, Salunek M, Jan G, Zerby D, Campbell S, et al. A human TATA binding protein-related protein with altered DNA binding specificity inhibits transcription from multiple promoters and activators. Molecular and Cellular Biology. 1999;**19**:7610-7620. DOI: 10.1128/MCB.19.11.7610

[60] Wang YL, Duttke SH, Chen K, Johnston J, Kassavetis GA, Zeitlinger J, et al. TRF2, but not TBP, mediates the transcription of ribosomal protein genes. Genes & Development. 2014;**28**: 1550-1555. DOI: 10.1101/gad.245662.114

[61] Kedmi A, Zehavi Y, Glick Y, Orenstein Y, Ideses D, Wachtel C, et al. Drosophila TRF2 is a preferential core promoter regulator. Genes & Development. 2014;**28**:2163-2174. DOI: 10.1101/gad.245670.114