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Review

The many roles of the conserved eukaryotic Paf1 complex in regulating transcription, histone modifications, and disease states ☆

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ABSTRACT

The Paf1 complex was originally identified over fifteen years ago in budding yeast through its physical association with RNA polymerase II. The Paf1 complex is now known to be conserved throughout eukaryotes and is well studied for promoting RNA polymerase II transcription elongation and transcription-coupled histone modifications. Through these critical regulatory functions, the Paf1 complex participates in numerous cellular processes such as gene expression and silencing, RNA maturation, DNA repair, cell cycle progression and prevention of disease states in higher eukaryotes. In this review, we describe the historic and current research involving the eukaryotic Paf1 complex to explain the cellular roles that underlie its conservation and functional importance. This article is part of a Special Issue entitled: RNA polymerase II Transcript Elongation.

1. Introduction

Through the regulation of transcription, cells are able to mount proper responses to exogenous stimuli, initiate signaling pathways involved in development and differentiation, and proliferate in complex environments. Regulation of RNA polymerase II (pol II) transcription can occur at each of the four general steps in the transcription cycle: promoter binding by RNA pol II and initiation of transcript synthesis, promoter clearance, transcription elongation, and termination. Proteins that interact with RNA pol II can control its activity to facilitate or repress transcription at one or more of these steps. The organization of eukaryotic DNA into chromatin, the basic element of which is a nucleosome containing ~147 basepairs of DNA wrapped around an octamer of histone proteins, presents a barrier to DNA accessibility during transcription. However, alterations to nucleosomes also provide an opportunity for carefully orchestrated levels of transcriptional regulation. Given the fundamental importance of transcription and chromatin regulatory factors, intensive research in a variety of organisms has focused on identifying these proteins and elucidating their interactions, molecular activities, and gene target specificities.

This review focuses on the eukaryotic Polymerase-Associated Factor 1 (Paf1) complex (Paf1C), which is a conserved protein complex that acts globally in multiple aspects of RNA pol II transcriptional regulation. First identified and characterized in *Saccharomyces cerevisiae* through an interaction with RNA pol II, Paf1 complexes have now been found in many eukaryotes. The overlapping functions shared by these complexes demonstrate the functional significance of the Paf1C. Here we describe the functions of the Paf1C in promoting histone modifications and regulating transcription elongation and gene expression. We also discuss less well-understood functions of the Paf1C in RNA 3′-end formation and non-histone processes. Lastly, we address the importance of the complex in regulating development and protecting against various diseases. As further research enhances our understanding of the molecular and cellular functions of the Paf1C, we hope that the involvement of Paf1C components in disease progression in higher eukaryotes will be more fully explained.

2. Subunit composition and genetic properties of the Paf1C

To isolate proteins associated with RNA pol II, an antibody against the conserved C-terminal repeat domain (CTD) of the largest *S. cerevisiae* RNA pol II subunit, Rpb1, was used for affinity purification [1]. These studies revealed a novel protein interacting with RNA pol II, which was termed Paf1 [2]. In addition, Cdc73 (Cell Division Cycle 73), a protein that had been previously shown to have connections to mating signaling pathways and cell division, was found to co-purify with Paf1 and RNA pol II in these studies [3,4]. Cdc73 was subsequently shown to interact directly with RNA pol II *in vitro* [3]. Three more proteins were later identified as being part of the budding yeast Paf1C (yPaf1C): Ctr9, Leo1, and Rtf1 [5,6]. Ctr9/Cdp1 (Cln Three Requiring 9) was genetically identified through its connections to the cell cycle, including effects on expression of the G1 cyclin genes *CLN1* and *CLN2* as well as microtubule formation [7–9]. Whereas the gene encoding Leo1 (Left Open Reading Frame 1) was sequenced but not characterized, Rtf1 (Restores TBP Function 1) was first identified in a yeast genetic screen for mutations that suppress the transcriptional

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effects of a defective TATA-binding protein and was later found to have extensive genetic interactions with transcription elongation factors [10–12]. Like yeast, the human and *Drosophila* Paf1 complexes have been shown to interact with RNA pol II; however, Rtf1 is less tightly associated with the rest of the Paf1C components in higher eukaryotes [13–16]. The human Paf1 complex (hPaf1C) also contains another protein, Ski8/Wdr61, which has been shown to have a role in mRNA decay as part of the Ski complex [13]. To date, structural data have been obtained for both the Ras-like C-domain of yCdc73 and the Plus-3 domain of hRtf1 [17,18]. Extensive sequence similarity exists between Paf1C subunits in yeast and humans, suggesting conservation of structure and function (Fig. 1).

Information on the interactions among members of the Paf1C is emerging. In yeast, the overexpression or deletion of individual subunits can influence the levels of other complex members. For example, the overexpression of Cdc73 or Paf1 increases the cellular levels of the other protein by enhancing protein stability [3]. Jaehning and coworkers subsequently showed that deletion of *PAF1* decreases the cellular levels of Rtf1, Cdc73, and Ctr9 more than ten-fold, deletion of *CDC73* decreases the levels of Rtf1, Paf1, and Leo1 at least three-fold, and deletion of *CTR9* also decreases the levels of Paf1, Rtf1, and Leo1 [19]. Consistent with these findings, knockdown of hCdc73 lowers hPaf1 protein levels in human cells [20]. With respect to inter-subunit interactions, the association of Rtf1 with the rest of the Paf1C is significantly reduced in yeast cells lacking *CDC73* [21]. However, in *rtf1*Δ or *cdc73*Δ cells, Paf1 still interacts with Ctr9 and Leo1, and in *rtf1*Δ cells, Cdc73 remains associated with Ctr9, Leo1, and Paf1 [21]. The interactions between human Paf1C components have been defined *in vitro*, revealing an extensive set of binary interactions between individual subunits reinforced by the function of the Paf1 component as a likely scaffold [16].

Deletion of genes encoding individual members of the yPaf1C causes mutant phenotypes that vary in their severity. The loss of Paf1 or Ctr9 is the most detrimental to cellular growth, correlating with the importance of these two subunits for overall complex integrity [22]. While none of the five Paf1C subunits are essential for *S. cerevisiae* viability, their functional importance is evident from the range of phenotypes caused by mutations in individual genes. These phenotypes include the Spt$^-$ (suppression of Ty) phenotype, which is indicative of defects in chromatin and transcription, sensitivity to the base analog 6-azauracil, which is frequently used as an indicator of transcription elongation defects, and sensitivity to compounds that elicit cellular stress responses, including caffeine, cycloheximide, rapamycin, hygromycin, and high temperature [5,11,12,22,23]. Loss of individual yPaf1C components can also alter the phenotypes associated with loss of other complex members [22]. For example, the deletion of *RTF1* can partially rescue the caffeine and hydroxyurea sensitivity and restore proper mRNA levels of selected genes in *paf1*Δ cells, indicating that the reduced levels of Rtf1 in *paf1*Δ cells retain some activity outside the normal complex [6]. Analysis of the gene expression profiles of yeast cells lacking individual Paf1C components shows some overlap and some differences [24]. Although these observations indicate that the subunits of the Paf1C may have distinct functions, more experiments are needed to assign definitive functional classifications to individual Paf1C components. To date, specific separation-of-function alleles have only been reported for yeast *RTF1*, with regions identified as being important for histone modifications, interactions with other Paf1C subunits, Paf1C association with chromatin, and recruitment of transcription co-factors [23]. Unlike in *S. cerevisiae*, Paf1C components in higher eukaryotes are essential for viability, suggesting that the complex acquired additional functional roles over the course of evolution or that the recognized functions of the complex have essential consequences in these organisms [25,26]. The characterization of the Paf1C in many eukaryotes has highlighted its conserved roles in transcriptional processes.

3. Connections between the Paf1C and the RNA pol II transcription elongation machinery

The initial identification of Paf1 as an RNA pol II-interacting protein implicated the Paf1C in transcription. Beyond this physical interaction, genetic studies in yeast further suggested a connection between the Paf1C and RNA pol II transcription. For example, strong synthetic growth defects were observed in yeast strains simultaneously mutated in a component of the Paf1C and other important transcriptional regulatory proteins, including subunits of the

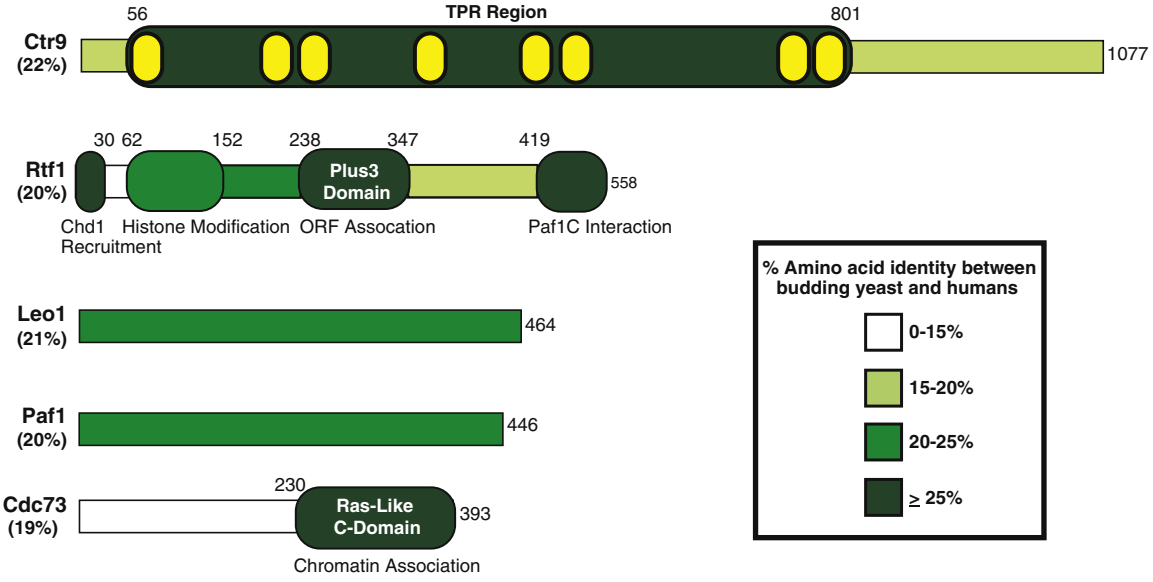

Fig. 1. Conservation of the yeast Paf1C subunits. The five proteins that comprise the Paf1C in budding yeast are depicted. The overall percent amino acid identity between *S. cerevisiae* and *H. sapiens* is listed under each protein name. The amino acid identity was determined using a global pairwise alignment algorithm within EMBOSS [177]. Using published literature, regions with defined structures (Rtf1 Plus3 and Cdc73 C-domain) are indicated within each protein and areas of assigned functions are listed below. The predicted TPR motifs found within Ctr9 (depicted internally in yellow) were defined by the SMART domain server [178]. The percent amino acid identity within defined functional and/or structural domains is depicted by color (see legend). Information on domains of the human Paf1C components have been described previously [168].