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Unraveling gene expression: a beginner's guide from chromatin modifications to mRNA export in *Saccharomyces cerevisiae*

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ABSTRACT

Understanding gene expression requires grasping its multi-step processes, from chromatin remodeling to mRNA export. This manuscript provides an accessible entry point for PhD students and junior postdocs beginning research in this area, using yeast as a model organism. We present a beginner-friendly overview of gene expression, emphasizing the dynamic interplay between chromatin modifications, transcription, mRNA processing, and export. Key topics include chromatin organization, with a focus on H2B ubiquitylation and H3 methylation crosstalk; transcriptional control by RNA polymerase II, including initiation, elongation, and termination; and the export of mRNAs via Mex67–Mtr2, adaptor proteins, and the TREX and TREX-2 complexes at the nuclear pore complex. Relevant examples from yeast genetics, biochemistry, and structural biology illustrate each step. This overview aims to equip new researchers with foundational knowledge and provides references to key studies, current challenges, and open questions in the regulation of gene expression.

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Chromatin organization in *Saccharomyces cerevisiae*

The genomic sequence of budding yeast (*Saccharomyces cerevisiae*) was the first eukaryotic genome to be fully deciphered [1]. Its annotation was completed and made available two years later through the Saccharomyces Genome Database (https://yeastgenome.org [2]). Subsequent resequencing in 2014 revealed remarkably low genetic variation across strains, underscoring the stability of the yeast genome [3]. The haploid yeast genome spans approximately 12.1 megabase pairs (Mb) of DNA distributed across 16 chromosomes, each ranging from 250 to 2,500 kilobase pairs (kb). Of the ~ 6,500 annotated open reading frames (ORFs), approximately 6,000 are predicted to encode proteins. All this corresponds to a total DNA length of about 4.1 millimeters – roughly 400 times longer than the 10-micrometer yeast cell itself – highlighting the extraordinary degree of DNA compaction required to fit the genome into the nucleus. To achieve this level of compaction, the DNA is not left in a naked, linear state but is wrapped around histone proteins to form nucleosomes, the basic units of chromatin [4]. This chromatin structure not only condenses the genome to fit within the nuclear space but also plays a critical role in regulating access to genetic information and protecting DNA integrity.

The fundamental building block of chromatin is the nucleosome, in which 146 base pairs of DNA are coiled around an octameric complex composed of four histones. The histone octamer is composed of two copies each of histones H3, H4, H2A, and H2B, which are arranged into two H2A-H2B dimers and a tetramer of H3 and H4 [5,6]. In metazoans, the linker histone H1 binds to the DNA between nucleosomes – known as linker DNA – and helps stabilize the structure of chromatin by promoting its compaction. In budding yeast, Hho1 has a similar structure but does not play the same functional role. Although it can associate with chromatin, its absence has minimal effects on processes like transcription, DNA repair, and meiotic recombination, suggesting that Hho1 is not essential for organizing chromatin between nucleosomes in yeast [7,8].

Histones are present in all eukaryotic organisms and are relatively small proteins (~11 kDa), rich in

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arginine and lysine. This results in a positive charge, which enables them to interact with negatively charged phosphate groups in the DNA scaffold [9]. Histones contain a central 'histone-fold' domain composed of α-helices that mediate histone – histone interactions within the nucleosome core. Extending from this core, the flexible N-terminal tail is intrinsically disordered and serves as a key regulatory element. These tails are subject to post-translational modifications that modulate interactions among histones, DNA, and other chromatin-associated proteins [10,11].

Other processes also influence how histones interact with DNA, including the incorporation of histone variants into nucleosomes and the action of chromatin remodeling complexes that utilize energy derived from adenosine triphosphate (ATP) hydrolysis to reorganize nucleosomes, such as the SWItch/sucrose non-fermentable (SWI/SNF) complex [12,13].

Chromatin is a highly dynamic structure whose varying levels of compaction regulate DNA accessibility. Euchromatin, characterized by a more open conformation, permits access to the transcriptional machinery and is typically associated with active gene expression. In contrast, heterochromatin is more densely packed and often enriched in silenced or inactive genes. These structural states influence not only transcription, but also essential processes such as replication, DNA repair, and chromosome segregation. Consequently, the mechanisms that modulate chromatin compaction – by promoting transitions between relaxed and condensed states – are central to genome regulation and cellular function [14–16].

Histone post-translational modifications

Post-translational modifications (PTMs) of histones are covalent and reversible changes that alter chromatin structure by affecting the physical interactions within and between nucleosomes, as well as between histones and DNA. This can be achieved by changing the electrostatic charge of nucleosomes or by giving rise to new recognition or binding sites for various proteins [11].

Most histone modifications occur on the protruding N-terminal tails, although some also target the globular histone fold and the C-terminal domain [17]. The main PTMs include acetylation, ubiquitination, methylation, phosphorylation, proline isomerization, and SUMOylation [18]. In recent years, a number of new modifications, including acylation, crotonylation, serotonylation, propionylation, and glutarylation, among others, have been discovered [19].

Histone PTMs often act in a coordinated manner, with certain modifications influencing the establishment or removal of others [20]. Among these, histone ubiquitylation and methylation are particularly noteworthy – not only for their central roles in regulating transcription elongation but also for their hierarchical influence over other modifications, such as acetylation (as discussed later). In this review, we will therefore focus on these two PTMs to illustrate how chromatin structure and gene expression are dynamically coordinated through their interplay, and to provide a more targeted discussion.

Histone ubiquitination

Protein ubiquitination entails the covalent attachment of a 76-amino acid molecule (ubiquitin) to protein residues. Polyubiquitination operates as an identifier for targeting proteins for degradation by the proteasome, whereas monoubiquitination functions as a signal for diverse cellular processes and modifies protein function rather than targeting it for degradation [21]. Ubiquitin is unusually large for a histone modification, measuring approximately 8.5 kDa – nearly the size of an entire histone, which typically ranges from 11 to 15 kDa [22,23].

A remarkable example of the addition of ubiquitin to histones in *S. cerevisiae* is the monoubiquitination of histone H2B at lysine 123 (H2BK123ub;[1] lysine 120 in mammals), which is situated in its C-terminal helix. This modification has been linked to a variety of cellular processes, including cell growth, generation and repair of double strand breaks (DSBs) during meiosis, transcription initiation and elongation; and increased processivity of RNA polymerase II (RNAPolII) [24]. The function of H2BK123ub[1] in these processes has been proposed to involve the relaxation of chromatin caused by a disruption of internucleosomal interactions due to both the location

and size of the modification, which allows the binding of other molecules [25,26]. Other research also suggests that H2BK123ub[1] contributes to nucleosome stability, facilitating the association and progression of transcription factors [27,28].

In the yeast cell, H2BK123ub[1] is catalyzed *in vivo* by the E2-conjugating enzyme Rad6, together with the E3 ligase Bre1 and the accessory protein Lge1 [29–32] (Figure 1(a)).

In addition to Rad6, the presence of the RNAPolII-associated factor 1 (PAF1) complex [24,33]; is essential for H2BK123ub[1]. PAF1 complex is composed of five subunits: Rtf1 (which contains a histone modification domain (HMD) and is considered an essential unit for H2BK123ub[1]), Paf1, Ctr9, Cdc73, and Leo1 [34]. The recruitment of the PAF1 complex to chromatin is facilitated by FACT (which stands for FAcilitates Chromatin Transcription) as well as by interactions with elongating forms of RNAPolII and elongator factors such as Spt5, which will be explored in greater detail in subsequent sections [35–37]. It has been suggested that the Rtf1 subunit of PAF1 is a crucial cofactor in H2BK123ub[1] that promotes the catalytic activity of Rad6 in the presence of Bre1 [38,39].

The BUR complex, comprising Bur1 (a cyclin-dependent kinase, Cdk, Figure 1(a)) and Bur2 (its corresponding cyclin), plays a significant role in H2BK123ub[1] formation [34,40]. This complex affects H2BK123ub[1] in two ways: indirectly, by promoting recruitment of the PAF1 complex to chromatin and RNAPolII; and directly, by phosphorylating Rad6 at serine 120, thereby stimulating its catalytic activity ([36,41,42].

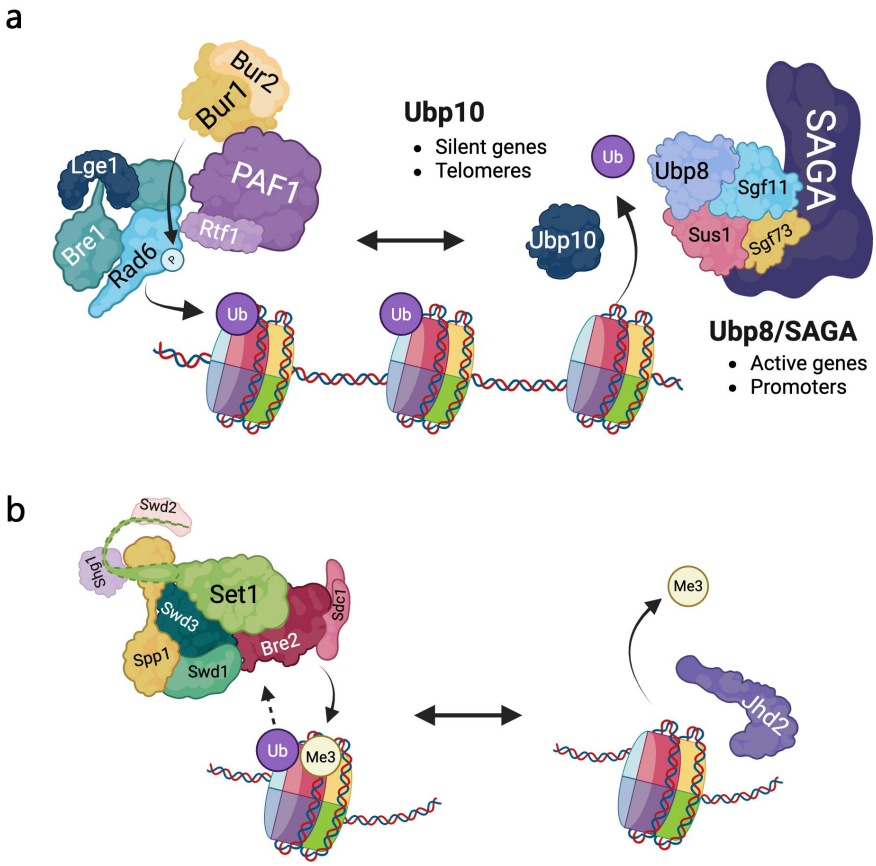

Figure 1. Dynamic mechanisms of H2B ubiquitination and H3K4 trimethylation. (a) The E2 conjugating enzyme Rad6 ubiquitinates H2B on its lysine 123 in association with the E3 ligase Bre1, and Lge1. Activation of Rad6 requires the phosphorylation of its serine 120 by the BUR complex, which is composed of Bur1/2. Additionally, this complex is necessary for the recruitment of PAF1 to chromatin. The subunit Rtf1 of PAF1 must make physical contact with Rad6 to stimulate its activity. H2B ubiquitination can be removed by either Ubp8 from SAGA or Ubp10. (b) The COMPASS complex trimethylates H3 on lysine 4 upon recognising H2B ubiquitination. This reaction is catalyzed by the enzyme Set1. This mark can be recognized by Jhd2, which can remove the moiety. Created in BioRender. https://biorender.com/5y8f2wx.

The incorporation of ubiquitin into H2B is a highly dynamic process, as ubiquitinated H2B constitutes less than 10% of the total H2B present in the cell [43]. Several studies have also shown that short cycles of ubiquitination and deubiquitination of H2B that occur at the onset of transcriptional elongation are essential for accurate gene activation [24,44].

The removal of ubiquitin is catalyzed by ubiquitin-specific proteases (USPs, Figure Figure 1(a)). In yeast, H2B deubiquitination is performed by two USPs, Ubp10 and Ubp8 [45]. Notably, Ubp10 operates as a monomeric enzyme, while Ubp8 belongs to a subcomplex of four proteins referred to as the deubiquitination module (DUBm) within the Spt-Ada-Gcn5 acetyltransferase (SAGA) complex, which is also known for its role in histone acetylation and transcriptional regulation. This subcomplex consists of Sgf73, Sgf11, Ubp8, and Sus1 [46–49].

Despite sharing substrate specificity for H2B monoubiquitylation at lysine 123, Ubp8 and Ubp10 fulfill non-overlapping roles due to differences in their molecular context and recruitment mechanisms. Ubp8 functions as part of the SAGA complex, specifically its deubiquitylating module (DUBm), which targets H2BK123ub^{1} at promoters and transcription start sites to facilitate transcription initiation by RNAPolII [20,50,51]. In contrast, Ubp10 acts independently of SAGA, is monomeric, and is recruited to repressive chromatin domains, such as subtelomeric regions, through interactions with silencing factors like Sir proteins or chromatin-associated regulators [52–54]. These differences explain their specialized functions in transcription activation and gene silencing, despite targeting the same histone mark.

Histone methylation

Methylation involves attaching methyl ($-CH_3$) groups to the side chains of lysine or arginine residues on histones. Specifically, lysine residues can receive one, two, or three methyl groups on their ε-amino groups, while arginine residues can be mono- or di-methylated on their terminal guanidinium group, with the dimethylation occurring in either a symmetric or asymmetric configuration [55,56].

Unlike other modifications, such as acetylation, methylation does not affect the charge of the lysine or arginine side chains nor does it impact chromatin folding through electrostatic mechanisms [57].

Different transcription factors can recognize and bind methylated histones, triggering distinct downstream transcription events. These methylation readers bear interfaces such as chromodomains [58] and plant homeodomains (PHD) that bind to methyl-lysines [59] or Tudor domains that recognize both methylated arginines and lysines [60].

The activity of histone methyltransferases (HMTs) is very specific, as each enzyme typically modifies only a single residue using S-adenosyl-L-methionine (SAM) as a donor [57,61]. HMTs can be classified into two families depending on their domains and targets; i) The SET domain (Su(var) 3–9, Enhancer of Zeste, Trithorax) is a conserved methyltransferase domain found in most histone lysine methyltransferases which facilitates methyl transfer to histone tails. In *S. cerevisiae* there are three Set1, Set2 and Set5 [62] and ii) Dot1, which lacks a SET domain and instead uses a completely different fold (seven-β-strand methyltransferase) to methylate lysine 79 on histone H3 [63,64]).

Transcriptionally activating histone methylations localize to histone H3 at positions 4 (H3K4), 36 (H3K36), and 79 (H3K79) [64–67]. In contrast, methylations of H2B in lysine 34 (H2BK34), of H3 in lysines 37 and 38 (H3K37, K38), and of H4 in lysine 5 (H4K5), 8 (H4K8), 12 (H4K12), or 20 (H4K20) have also been identified, but their functions remain partially unknown [57,68].

H3K4 methylations (H3K4me) and the COMPASS complex

In *S. cerevisiae*, all three methylation states of H3K4 (me^{1}, me^{2}, me^{3}) are catalyzed by Set1, the catalytic subunit of the eight-membered COMPASS complex [69,70]. COMPASS also includes Swd1, Swd3, Swd2, Sdc1, Bre2, Spp1, and Shg1, each contributing to complex assembly

and specific methylation outcomes [71]. Notably, Swd2 is essential for cell viability, not due to COMPASS, but because of its moonlighting role in the CPF complex [72,73].

Structural studies revealed that COMPASS functions as a dimer *in vivo*, a configuration driven by the Sdc1 subunit, which ensures symmetric H3K4 trimethylation on both histone H3 tails within a nucleosome [74,75]. This methylation is less dynamic than ubiquitination, but remains reversible through Jhd2, the only known H3K4 demethylase in yeast. Jhd2 preferentially removes asymmetric H3K4me^3 and thus collaborates with COMPASS to maintain symmetry [11,76]. Deletion of *JHD2* leads to widespread changes in gene expression due to altered H3K4 methylation dynamics. This supports the idea that Jhd2 plays a key role in regulating transcription through chromatin modification [77] (Figure 1(b)).

Genome-wide studies have shown distinct distributions and roles for each methylation state: H3K4me^3 is enriched at promoters and correlates with active RNAPolII and histone acetylation; H3K4me^2 occupies coding regions, and H3K4me^1 is found near transcript ends, possibly marking a transition to the unmodified state [57,78]. These patterns are responsive to transcriptional dynamics and environmental stress [79].

To highlight the complexity of these modifications, H3K4 methylation modulates chromatin accessibility *via* recruitment of effector proteins. H3K4me^3 recruits the NuA3 acetyltransferase through Yng1 to activate transcription, while H3K4me^2 is recognized by Set3 to repress cryptic transcription within gene bodies [80,81]. In contrast, H3K4 methylation can also participate in gene silencing at telomeric regions, where the Rpd3L histone deacetylase complex is recruited via the PHD finger of Pho23, which specifically recognizes H3K4me^3 [82,83].

Beyond transcription, H3K4 methylation also plays important roles in chromosome segregation, DNA damage response, and meiotic recombination, linking this modification to broader genome maintenance mechanisms [84–86].

H3K36 methylations (H3K36me)

H3K36 methylation in yeast is exclusively catalyzed by the SET-domain protein Set2 [87]. This mark is enriched in the bodies of actively transcribed genes and plays a central role in chromatin regulation during elongation. Its distribution is methylation-state specific: H3K36me^1 peaks near the 5' end, while me^2 and me^3 accumulate toward the 3' end [57,78]. H3K36me^3 is recognized by the Rpd3S histone deacetylase complex, which works with the chromatin remodeler Isw1b to suppress cryptic intragenic transcription and maintain transcriptional fidelity [88,89]. In parallel, the NuA4 acetyltransferase – involved in transcription activation – is also recruited to H3K36me sites, reflecting the dual regulatory potential of this mark [63,81].

The primary H3K36 demethylase is Rph1, which removes di- and tri-methyl groups, while Jhd1 and its paralog Gis1 target mono- and di-methylated forms [90,91].

Cells lacking H3K36 methylation exhibit growth defects, stress sensitivity, and accelerated aging due to transcriptional noise and chromatin instability [92,93].

H3K79 methylations (H3K79me)

Dot1 uniquely catalyzes the methylation of H3K79, a residue located in the structured globular core of histone H3—unlike other lysine methylations that occur on histone tails [94,95]. Dot1 lacks a SET domain and instead uses a 7β-strand fold to perform mono-, di-, and tri-methylation. To date, no demethylase for H3K79 has been identified in yeast [96].

H3K79 methylation is broadly distributed across active gene bodies and functions to block the binding of the SIR silencing complex within euchromatin, thus preserving transcriptional activity and safeguarding telomeric silencing boundaries [24,57,97].

Beyond its transcriptional role, H3K79me is involved in the DNA damage response and homologous recombination, particularly during meiosis, where it helps stabilize checkpoint activation and repair foci [68,98,99]. These functions position Dot1 as a key chromatin regulator linking gene expression and genome integrity.

The crosstalk of H2B ubiquitination and H3 methylations

The ubiquitination of H2B has been shown to influence other histone modifications that correlate with elevated transcriptional activity. In particular, H2BK123ub^{1} is required for di- and trimethylation (but not mono-methylation) of H3 in its lysines 4 and 79, catalyzed respectively by Set1 (in the context of COMPASS) and Dot1 [24,100]. Nonetheless, several studies have reported that H3K4me^{3} and H3K79me^{3} deposition are not entirely dependent on H2BK123ub^{1} under specific conditions [101]. This interdependence, in which a histone modification acts as a template for the deposition of another, is often referred to as 'histone crosstalk'.

One of the most widely studied cases of histone crosstalk is the interplay between H2B ubiquitination and H3K4 methylation [102]. The precise mechanism through which this connection is controlled has been the subject of extensive debate for over a decade.

Early models proposed that the COMPASS subunit Swd2 mediated crosstalk between H2BK123ub^{1} and H3K4me^{3} by recognizing ubiquitinated H2B [103]. Later, it was suggested that Swd2 itself might be ubiquitinated to recruit Spp1, a subunit essential for trimethylation [104]. An alternative model shifted the focus to the N-SET domain of Set1, suggesting it directly senses H2BK123ub^{1} [105], positioning Spp1 as the key effector.

Recent cryo-EM studies of reconstituted yeast COMPASS complexes revealed that H2B-linked ubiquitin contacts the Swd1 subunit, inducing a conformational change that activates the complex [75,106]. In parallel, an arginine-rich motif near the SET domain of Set1 folds into an α-helix upon H2BK123ub^{1} recognition, interacts with the H2A acidic patch, and further enhances methyltransferase activity [107,108].

Additional structural and biochemical studies confirm that ubiquitin functions as an allosteric regulator of COMPASS [105]. However, whether this mechanism is fully conserved *in vivo* remains uncertain. Notably, a direct interaction between H2BK123ub^{1} and either Spp1 or the SET domain has not been conclusively demonstrated under physiological conditions [57,109].

Transcription by RNA polymerase II

Transcription in eukaryotic organisms is performed by various DNA-dependent RNA polymerases, the number of which differs between species. Yeasts and animals possess three RNA polymerases, while plants have up to five [110]. They are mainly distinguished by their composition and structure, which regulate their activity, as well as by the types of genes transcribed [111].

RNA polymerase I is involved in the transcription of ribosomal RNA (rRNA) precursors.
RNA polymerase II (RNAPolII) is responsible for transcribing all protein-coding messenger RNAs (mRNAs), apart from some non-coding RNAs (ncRNAs).
RNA polymerase III transcribes transfer RNAs (tRNAs), some rRNAs, and a few ncRNAs.

To maintain a focused scope, this review will concentrate on RNAPolII-mediated transcription.

Yeast RNAPolII comprises 12 distinct subunits. The largest of these, Rpb1, is characterized by the presence of a large C-terminal domain (CTD), which presents in *S. cerevisiae* 26 repeats of the conserved consensus heptapeptide YSPTSPS [112]. This CTD is critical during all transcription steps, because it serves as a platform for the recruitment of proteins that regulate transcription. Among the modifications that occur within the CTD, phosphorylation and dephosphorylation of serine residues at positions 2, 5, and 7 are particularly important, as they ensure the precise recruitment of the transcriptional machinery in a sequential manner [113,114].

Initiation

The first prerequisite for transcription initiation is that chromatin acquires a relaxed conformation to facilitate access of transcription machinery to promoter regions. As mentioned earlier, this state can be achieved by several factors including chromatin remodeling enzymes or histone modifications, among others, which act as 'transcriptional coactivators' [12,13]. This name indicates the existence of 'transcriptional activators', which are factors that recognize specific DNA sequences to

assist in the onset of transcription [115]. Similarly, transcriptional 'repressors and corepressors' are vital to prevent unwanted initiation of transcription [116].

The coordinated action of all these activating factors allows for the assembly of RNAPolII and general transcription factors (GTFs), which are sequentially assembled, commencing with Transcription Factor II D (TFIID), that binds to the promoter and is rapidly stabilized by TFIIA. Subsequently, TFIIB, TFIIF, RNApolII, TFIIE, and TFIIH are also recruited, giving rise to the pre-initiation complex (PIC) [117,118].

A key element in many eukaryotic promoters is the 'TATA box', typically located 30–60 base pairs upstream of the transcription start site (TSS). However, true TATA boxes are relatively rare and are mainly found in stress-responsive genes regulated by the SAGA complex. In contrast, housekeeping genes – comprising about 80% of the genome – usually contain TATA-like elements and are primarily regulated by TFIID [119,120]. Despite this distinction, SAGA and TFIID have overlapping functions, share subunits, and both contribute to TATA-binding protein (TBP) recruitment during PIC formation [121].

Once the PIC assembly is completed, begins with the unwinding of the DNA double helix and the synthesis of a nascent mRNA, typically 20–60 nucleotides before entering productive elongation. In higher eukaryotes, the transcription apparatus comes to a halt near the promoter, though no such event has been described in yeast, as they lack the Negative Elongating Factor (NELF), a fundamental component involved in pausing [117,122]. Promoter-proximal pausing is a specific regulatory stage that occurs directly after initiation and before productive elongation carried out by RNAPolII. This phase is tightly regulated, and its disruption has been associated with a wide range of human disorders [113,123]. In yeast cells, RNAPolII can also stall due to various conditions, remaining inactive for a variable period until its fate is resolved – either advancing to productive elongation or undergoing premature termination [124].

Elongation

It is worth mentioning that the intricate biochemical regulation of transition to elongation displays a high level of complexity. On the one hand, it relies on the existence of specific histone modification patterns, with H2BK123ub[1] and H3 methylations playing a pivotal role. On the other hand, it requires the recruitment of several factors that display redundant functions, which may even appear contradictory, as they can vary in different organisms. These factors not only modify histone marks, but in many cases, also rely on them for timely recruitment. At this point, it becomes increasingly challenging to determine the precise sequence of events due to the extensive interdependencies and connections that involve many of the implicated factors.

The release from the promoter region occurs concurrently with the recruitment of TFIIH, the last GTF to be incorporated into the PIC. This transcription factor contains Kin28, the kinase responsible for phosphorylating the CTD of RNAPolII at serines 5 and 7 [125] (Figure 2(a)).

Phosphorylation of serine 5 is the most extensively studied, occurring immediately after transcriptional initiation and being removed as RNAPolII acquires its elongating state [126]. This modification disrupts the existing interactions between the CTD and PIC components, allowing RNAPolII to escape from the promoter region [113,127].

This CTD modification can also trigger the recruitment of capping enzymes responsible for the 5' modification of nascent RNAs, which consists of] the addition of a cap composed of the nucleotide 7-methylguanosine [128]. Interestingly, COMPASS is associated with high levels of phosphorylated serine 5, which facilitates H3K4 methylation around the promoter [57,129].

One of the most important factors in the transition into active elongation is the DRB Sensitivity-Inducing Factor (DSIF), which is composed of Spt5 and Spt4 [130]. Spt5 stands out for being the sole transcription elongation factor conserved across all forms of life, including archaea, bacteria, and eukaryotes [131]. Recent studies have shown that upon rapid depletion of Spt5, Rpb1 is ubiquitinated and

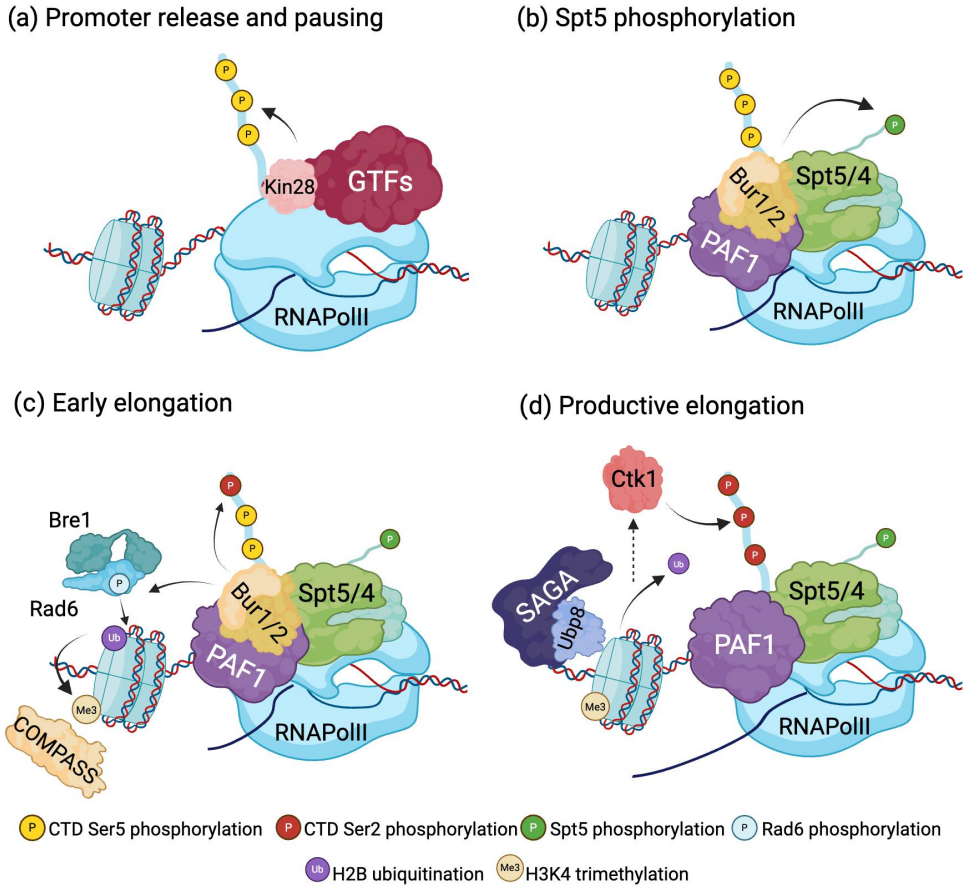

Figure 2. Different regulation steps during transcriptional elongation. (a) At the end of transcription initiation, Kin28, a subunit of TFIIH, phosphorylates the RNAPolII CTD at serine 5, allowing the enzyme to be released from the promoter region. (b) BUR phosphorylates the CTR of Spt5, turning DSIF into a positive elongation factor. (c) This phosphorylation facilitates the recruitment of PAF1. BUR partially phosphorylates RNAPolII CTD at serine 2, marking the early steps of transcription elongation. Additionally, BUR can phosphorylate Rad6, rendering it active and permitting H2B ubiquitination, which in turn enables the trimethylation of H3K4 by COMPASS. (d) To achieve maximum elongation productivity, the ubiquitin moiety must be removed from H2B by USP Ubp8 from the DUBm of SAGA. This allows the recruitment of the kinase Ctk1 which conducts the remaining phosphorylations of the CTD at serine 2, advancing to the final stages of productive elongation. Created in BioRender. https://BioRender.com/l83m241.

targeted for degradation, highlighting the important role of Spt5 in guaranteeing accurate gene expression [132]. Its carboxy-terminal region (CTR) is analogous to the CTD of RNAPolII, with 15 repeats of a hexapeptide with the consensus sequence S(A/T)WGG(A/Q), whose serine residues are targeted for phosphorylation by Bur1 from the BUR complex [41; 43]. The role of BUR is critical in attracting the PAF1 complex, which subsequently enables the interaction between Rad6, Bre1, and RNAPolII, resulting in H2BK123ub^{1} [36].

Proper entry into the elongating state also requires that Bur1 phosphorylates the CTR of Spt5. With this phosphorylation, DSIF (Spt5/4) is activated as a positive elongation factor that enables appropriate transit of the DNA template *via* the central groove of RNAPolII by stabilizing its clamp conformation [133] (Figure 2(b)). In metazoans, which display promoter-proximal pausing, DSIF has a double function, since its unphosphorylated form is responsible for maintaining that paused state, but upon phosphorylation it shifts into a functioning elongation factor. No such negative effect in elongation has been described for unphosphorylated Spt5 in yeast [113].

Similarly in metazoans, the PAF1 complex, is known to impede the discharge of stalled RNAPolII; however, it facilitates transcription elongation once RNAPolII is dislodged from pausing [134]. In yeasts, where it is only known as an elongation activator, PAF1 is attracted by the

phosphorylated version of Spt5 and acts as a platform that attracts nucleosome remodeling factors, histone chaperones, such as FACT or even histone-modifying factors, including the H3K4 and H3K79 methyltransferases COMPASS and Dot1 [113,135] (Figure 2(c)).

Another regulator of elongation worth mentioning is the carbon catabolite repression 4–negative on TATA-less (CCR4-Not) complex. This highly conserved complex influences not only transcription elongation but also various stages of mRNA metabolism, including transcription initiation, nuclear export, and mRNA decay [136]. One of its subunits, Not4, functions as an E3 ubiquitin ligase and regulates the activity of the demethylase Jhd2, thereby modulating H3K4me^3 levels through ubiquitination [137].

In a similar manner, the transcription factor Sub1 also plays multiple roles across the transcription cycle. It contributes to pre-initiation complex (PIC) formation, facilitates the transition from initiation to elongation, supports elongation by interacting with DSIF, and participates in transcription termination [138].

A defining feature of RNAPolII as it transitions from promoter-proximal pausing into productive elongation is the phosphorylation of its CTD on serine 2. This phosphorylation is initiated by Bur1, although it does not fully modify all repeats. The primary kinase responsible for widespread Ser2 phosphorylation is Ctk1. However, Ctk1 activity is influenced by chromatin state – specifically, it requires prior removal of ubiquitin from H2B, a mark that accumulates during early elongation. This deubiquitination step, carried out by the SAGA complex's DUBm, is necessary to relieve the chromatin barrier and allow Ctk1 recruitment or activation at the gene body. Thus, H2B deubiquitination functions as a prerequisite for maximal CTD-Ser2 phosphorylation during late elongation [139] (Figure 2(d)).

At the later stages of transcription elongation, phosphorylation of CTD on its serine 2 promotes the recruitment of Set2, which methylates H3K36. This modification is subsequently recognized by Rpd3S, which helps to reestablish the original nucleosome disposition, thereby preventing any further undesired transcription initiation [140,141].

Termination

Transcription termination by RNAPolII marks the final phase of the transcription cycle, ensuring that the enzyme is disengaged from the DNA template and reset for a new round of transcription, and that the nascent pre-mRNA is released and matured.

As with all the aforementioned steps in transcription, termination is not a mere passive conclusion but a highly regulated process that has to ensure that i) transcription concludes at the correct position, ii) the length and fate of the transcript are well defined, and iii) there is no undesired transcriptional interference with downstream genes or regulatory regions.

In *Saccharomyces cerevisiae*, transcription termination is tightly coupled to the 3'-end processing of mRNA, orchestrating the maturation of protein-coding mRNAs or the degradation of unstable non-coding RNAs. These two broad outcomes reflect the operation of two specialized termination pathways, each with distinct molecular machinery and regulatory logic.

(1) The Cleavage and Polyadenylation Factor (CPF) pathway terminates protein-coding genes, linking RNA cleavage, polyadenylation, and RNAPolII release.
(2) The Nrd1–Nab3–Sen1 (NNS) pathway serves non-coding RNAs, such as small nucleolar RNAs (snoRNAs) and cryptic unstable transcripts (CUTs), terminating transcription and routing RNAs into surveillance pathways.

CPF-mediated termination: coupling cleavage and RNAPolII release

In protein-coding genes, transcription termination is predominantly achieved through the coordinated action of the CPF complex together with Cleavage Factors (CF) IA and CF IB [142,143]. Recruitment of this machinery begins when RNAPolII transcribes into the 3' untranslated region (3' UTR), where polyadenylation signal (PAS)-like sequences and flanking auxiliary U- and A-rich elements are recognized within the

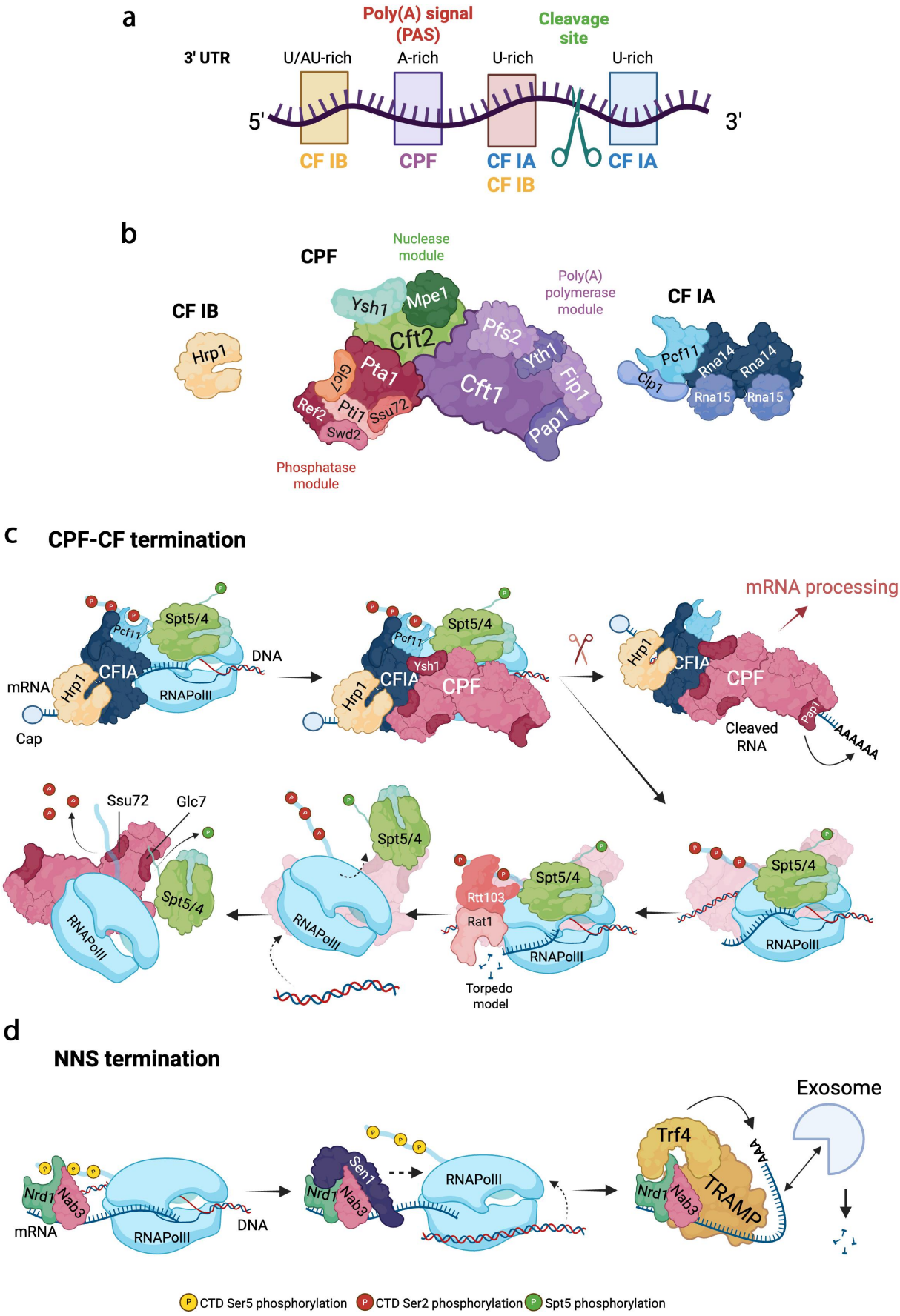

Figure 3. Termination and 3′ processing. (a) The 3′ UTR of the pre-mRNA has cis-regulatory regions that need to be recognized to ensure cleavage and polyadenylation, as well as termination. (b) Schematic of the complexes needed for both CPF/CF termination

mRNA by specific protein subunits (Figure 3(a)) [144]. Rna15 (CF IA) and Hrp1 (CF IB) bind these flanking motifs, positioning CPF for cleavage. CPF subunits such as Cft1, Cft2, Yth1, Mpe1, and Pfs2 stabilize the complex on the mRNA. Then, the CPF forms a modular cavity below the elongation complex, comprising an endonuclease module (Ysh1, Cft2, Mpe1), a polymerase module (Pap1, Fip1, Yth1, Cft1, Pfs2), and a scaffold-phosphatase module (Glc7, Ssu72, Ref2, Pti1, Pta1, Swd2) [72] (Figure 3(b)). This recruitment is reinforced by the CTD code: as Ser2 phosphorylation (Ser2-P) increases, Pcf11 (CF IA) binds Ser2-P *via* its CTD-interacting domain (CID) [145]. As illustrated in Figure 3(c), cleavage is catalyzed by Ysh1, which cuts 10–30 nt downstream of the PAS. The upstream fragment, bearing a 3'-OH, proceeds toward processing (see mRNA processing section) [146]. The downstream fragment is targeted by the torpedo pathway. Here, the 5'-monophosphate mRNA is degraded by Rat1, a 5'→3' exonuclease activated by its cofactor Rai1 [147]. Rat1 is recruited to the CTD *via* Rtt103, whose CTD-interacting domain binds Ser2-P [148], and whose Rai1-interacting segment (RIS) directly tethers the Rat1–Rai1 complex [149]. Cryo-EM studies have resolved pre- and post-termination states of Rat1 bound to RNAPolII, showing that Rat1 docks at the mRNA exit channel, threads mRNA into its active site, and, through degradation, shortens the RNA – DNA hybrid and collapses the bubble [150]. In post-termination states, Rat1 repositions inside the cleft of RNAPolII, potentially preventing its re-engagement with DNA. These findings substantiate the torpedo model, in which exonucleolytic degradation contributes directly to RNAPolII release, with Rtt103 and Rai1 coordinating Rat1 recruitment and activation.

Concurrently, the allosteric model posits that after transcription of the poly(A) site, binding of the termination complex results in a conformational change of the elongation complex due to the loss of elongation or anti-termination factors, which decreases processivity and ultimately leads to termination [151]. In support of this model, it has been shown that RNAPolII loses elongation factors such as Spt5 prior to release [152,153], and that Pcf11 alone can destabilize an elongation complex *in vitro* by simultaneously binding both the CTD and the nascent mRNA [154]. However, whether this mechanism operates *in vivo* in the context of the full CPF – CF complex remains to be determined.

Structural evidence now supports a unified model in which cleavage, allosteric weakening, and torpedo activity act in concert to dismantle the elongation complex at the 3' end [155].

Finally, the CPF phosphatase module plays a central role in transcriptional recycling. The subunits Ssu72 (which targets Ser5-P and Ser7-P) and Glc7 (which targets Tyr1-P and Spt5) from this module dephosphorylate the CTD of RNAPolII post-termination, resetting it to a hypophosphorylated state compatible with re-initiation at new promoters [156,157] (Figure 3(c)). Notably, Swd2—previously described in the COMPASS complex as playing a role in H3K4 methylation – now reappears in CPF where it helps position phosphatases on the CTD, thus linking chromatin cues to 3' end processing. In this way, CPF not only mediates termination but also actively prepares RNAPolII for the next transcription cycle.

Although initially introduced for its role in transcription elongation, the PAF1 complex has recently been shown to extend its influence beyond elongation. It contributes to efficient 3'

and 3' processing. (c) Mechanism of CPF/CF termination. CF IA and IB recognize motifs in the 3' UTR of the mRNA and bind to it, the recruitment is reinforced by the recognition of Ser2-P CTD by the CID of Pcf11. This allows the recruitment of CPF, whose subunit Ysh1 carries of cleavage. The 5' fragment is further processed and undergoes polyadenylation by the Pap1 polymerase from CPF. The 3' fragment remains attached to RNAPolII. Here, Rtt103 recognizes Ser2-P CTD and promotes the binding of the Rat1 exonuclease that upon removing the RNA strand, interferes with RNAPolII which gets released (torpedo model). Besides, the recruitment of termination factors gives rise to conformational changes that leads to the discharge of elongating factors and RNApolII from DNA (allosteric model). Finally, the phosphatases Glc7 and Ssu72 dephosphorylate the CTD and Spt5. (d) Mechanism of NNS termination. Nab1 and Nrd3 recognise specific sequences on the RNA, and Nrd1 recognises Ser5-P CTD with its CID. This recruits the helicase Sen1 that allows the release of RNAPolII. The CID of Nrd1 recruits Trf4 from TRAMP, which adenylates the RNA which is targeted to the exosome for surveillance. Created in BioRender. https://BioRender.com/ymgwsou.

end processing and termination of pervasive transcripts [158], and also facilitates RNAPolII recycling, ensuring prompt reinitiation of transcription at gene loci [159].

NNS-mediated termination: capturing and resolving non-coding transcription

In non-coding RNAs (ncRNAs), such as small nucleolar RNAs (snoRNAs) and cryptic unstable transcripts (CUTs), transcription termination is directed by the NNS complex (Figure 3(d)). This complex comprises the RNA-binding proteins Nrd1 and Nab3, along with the ATP-dependent helicase Sen1 [160–162]. Unlike the CPF pathway, NNS-mediated termination does not rely on RNA cleavage or recognition of polyadenylation signals and is therefore classified as PAS-independent [72].

Nrd1 and Nab3 are recruited to nascent RNA *via* their RNA recognition motifs (RRMs), which preferentially bind UGUA/G and UCUUG motifs, though broader sequence specificity has also been reported [163,164]. Their association with the transcription machinery is facilitated by the Ser5-P CTD, recognized by the CID of Nrd1 during early elongation [165,166]. As transcription progresses, increasing Tyr1 phosphorylation reduces Nrd1 binding, effectively restricting NNS activity to early transcriptional stages [167].

Although Sen1 can terminate transcription on its own *in vitro*, its low abundance in vivo means it relies on Nrd1 and Nab3 for efficient recruitment [168]. It contains a Nrd1-Interaction Motif (NIM), which mimics the CTD and enables direct binding to Nrd1 [169]. Recent cryo-EM data show that, after binding to RNA, Sen1 translocates toward elongating RNAPolII, interacting near the RNA exit channel. Its ATPase-driven activity exerts a pulling force on the transcript, shifting RNAPolII into a hypertranslocated state that destabilizes the RNA – DNA hybrid, rewinds the transcription bubble, and promotes RNA release [170]. Single-molecule studies further reveal that Sen1 May also push stalled RNAPolII, leading either to termination or reinitiation of elongation [171,172].

NNS termination is tightly integrated with nuclear RNA surveillance. Following termination, transcripts are handed off to the nuclear exosome with support from the TRAMP4 complex (Trf4, Air1/2, Mtr4), which oligoadenylates the RNA to facilitate processing or degradation [173–175]. The Nrd1–Nab3 heterodimer remains bound to the RNA and recruits TRAMP4 through a CTD-like NIM in Trf4 [176,177].

Ultimately, the choice between NNS- and CPF-dependent termination is not binary but shaped by a combination of CTD phosphorylation patterns, RNA sequence motifs, elongation kinetics, and chromatin context. Nrd1 and Pcf11 compete for CTD binding, and the transition from Ser5-P to Ser2-P, alongside the decline in Tyr1-P, defines a tunable window during which each pathway may be selectively engaged [178].

mRNA processing

In the previous section, we followed the trajectory of RNAPolII throughout the entire transcription cycle, from initiation to elongation and termination, with a primary focus on the mechanisms that govern enzyme recruitment, movement along chromatin, and eventual release. We now shift the focus from RNAPolII to its product, the emerging mRNA transcript. While transcription ensures the correct synthesis of the mRNA sequence, mRNA processing transforms this fragile intermediate into a stable, export-competent messenger.

This transformation proceeds through a tightly coordinated series of steps: 5’ capping, splicing (when applicable), 3’ end cleavage, polyadenylation, and assembly of the mature messenger ribonucleoprotein particle (mRNP). These processes are not isolated events but are physically and functionally linked to the transcription machinery, often occurring co-transcriptionally and serving as both signals and checkpoints for proper gene expression. Importantly, mRNA is not processed in isolation, from the moment it emerges from the mRNA exit channel, it is bound by a network of processing and packaging factors. This continuous interaction defines the formation of the mRNP, a dynamic and compositionally adaptable assembly whose composition evolves with each processing step, and whose proper assembly is essential for transcript stability, surveillance, and export.

5′ capping

The first modification undertaken by nascent pre-mRNA is the addition of a 5' cap. This reaction begins once RNAPolII has synthesized a strand of approximately 20–30 nucleotides. The cap, a 7-methylguanosine (m^7G) linked through an unusual 5'–5' triphosphate bond, is essential for transcript stability, efficient splicing, nuclear export, and translation. In *S. cerevisiae*, capping is tightly coordinated with early transcription and is mediated co-transcriptionally through interactions with the Ser5-P CTD of RNAPolII [179,180].

Capping involves three enzymatic steps, each catalyzed by a dedicated enzyme. First, Cet1, an RNA 5'-triphosphatase, removes the γ-phosphate from the 5' end of the nascent transcript. Next, Ceg1, a guanylyl-transferase, transfers GMP in a 5'–5' linkage to form the cap structure. This Cet1–Ceg1 complex, composed of two copies of each protein, is stabilized by the direct binding of Ceg1 to Ser5-P CTD repeats, ensuring timely recruitment [181]. Finally, Abd1, a guanine-N7 methyltransferase, methylates guanosine producing the mature m^7 G cap. These reactions occur sequentially on the surface of the transcription complex, securing early protection and defining the mRNA as RNAPolII-derived [182].

The capping process is further facilitated by the transcription elongation factor Spt5, which binds to the CTD and physically interacts with the capping enzyme Ceg1. Although yeast does not exhibit regulated promoter-proximal pausing, Spt5 is essential for anchoring capping enzymes to the elongating RNAPolII and coordinating their access to the emerging transcript. Disruption of Spt5 impairs proper capping, leading to defective mRNA maturation and degradation *via* nuclear surveillance mechanisms [183].

Once formed, the cap is immediately bound by the cap-binding complex (CBC) composed of Sto1 (or Cbp80) and Cbc2 (or Cbp20) in yeast. CBC protects nascent transcripts from 5' exonucleases and assists in early processing events such as the splicing of the first intron, when present, export factor recruitment, and proper mRNP formation [184]. Beyond its protective function, CBC acts as a central regulatory hub that coordinates transcription with downstream mRNA maturation steps. CBC serves as a molecular 'choreographer' by stabilizing interactions with processing factors, influencing spliceosome assembly, modulating RNAPolII dynamics, and ensuring the temporal order of transcript maturation. Altogether, CBC functions as an early molecular checkpoint tightly coupling capping to transcript fate, and its failure leads to mRNA degradation *via* nuclear quality control [185].

Splicing

Pre-mRNA splicing in *Saccharomyces cerevisiae* removes introns and joins exons to produce mature mRNAs. Although only ~ 5% of yeast genes contain introns, they include many essential and highly expressed transcripts, not only those for ribosomal proteins and RNA processing factors, but also those implicated in response to several stresses [186–188]. Splicing is largely co-transcriptional and intricately coupled to transcription through interactions between the spliceosome and RNAPolII CTD [189]. In addition to CTD interactions, histone acetylation by Gcn5, from the HAT module of SAGA, facilitates the co-transcriptional recruitment of U2 snRNP components such as Msl1 and Lea1 to the branchpoint region, thereby linking chromatin modification to early spliceosome assembly [190]. This highlights the importance of chromatin context in ensuring timely and efficient intron recognition during transcription.

Splicing is catalyzed by the spliceosome, a dynamic ribonucleoprotein complex composed of five small nuclear RNAs (snRNAs U1, U2, U4, U5, and U6) and dozens of associated proteins. These form snRNPs, which, along with protein-only subcomplexes like the NineTeen complex (NTC) – also involved in mRNA export (see later dedicated section) – assemble stepwise on each pre-mRNA transcript through ATP-dependent remodeling events and involve multiple spliceosome intermediates [191,192].

Spliceosome assembly begins with the recognition of three key intronic features: the 5' splice site (5'SS), branch site (BS), and 3' splice site (3'SS) (Figure 4(a))

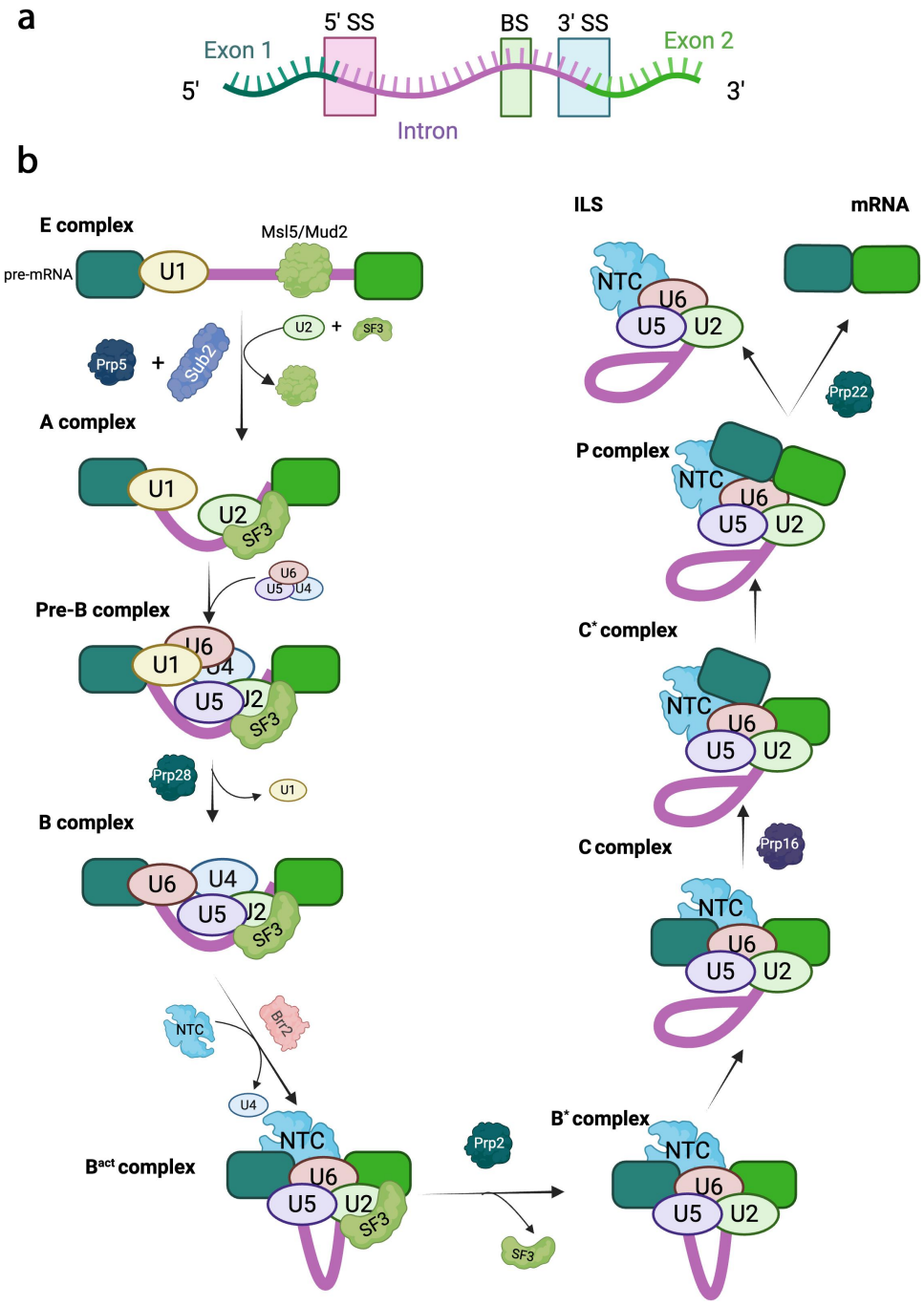

Figure 4. Splice site architecture and the dynamic assembly of the spliceosome. (a) Schematic representation of a pre-mRNA substrate in Saccharomyces cerevisiae, highlighting key intronic elements required for splicing: the 5′ splice site (5′SS), the branch point sequence (BS), and the 3′ splice site (3′SS). These conserved features are essential for spliceosome recognition and intron excision. (b) The spliceosome assembles in a stepwise manner on the pre-mRNA, undergoing extensive compositional and conformational changes. Small nuclear ribonucleoproteins (snRnps; U1, U2, U4/U6, and U5) and non-snRNP protein complexes such as the NineTeen complex (NTC) and SF3 are sequentially recruited and rearranged. Key transitions are driven by helicases and ATPases (shown in green), which facilitate spliceosome remodelling, catalytic activation, and disassembly following the two transesterification reactions. Created in BioRender. https://BioRender.com/mkvqaw0.

[193]. These elements are interpreted in a defined order by the different components that are recruited and released within the spliceosome (Figure 4(b)).

(1) Recruitment and A complex formation

- The U1 snRNP base-pairs with the 5′SS, whereas Msl5 and Mud2 bind to the BS, forming the E complex [194,195].

- The ATPases Sub2 and Prp5 displace Msl5/Mud2 and mediate U2 snRNP base pairing at the BS to form the A complex [196,197].

(2) Pre-B and B complex formation
- The tri-snRNP formed by U4, U5, and U6 joins to form the pre-B complex [198].
- Prp28, another ATPase, displaces U1 and transfers 5'SS to U6, forming the B complex [199].

(3) Activation and catalysis
- The Brr2 helicase unwinds U4/U6, enabling U2–U6 pairing and NTC recruitment to form the B^{act} complex [200,201].
- The Prp2 ATPase ejects a set of proteins associated with U2 (SF3), forming the B* complex [202].
- Factors such as Yju2, Cwc25, Isy1, and Prp16 assist in positioning the BS for the first transesterification reaction, where the 2' OH of the BS adenosine attacks the phosphate at the 5'SS. This leads to the separation of the 5' exon and generation of an intermediate intron lariat (Complex C) [203].
- Prp16 then remodels the spliceosome into the C* complex to promote exon ligation aided by Slu7 and Prp18 [204].
- At this step, the 3' OH at the 5'SS attacks the 3'SS, ligating both exons and producing the product (P) complex [205,206].

(4) Release and disassembly
- Prp22 promotes the release of mature mRNA [207,208].
- The Prp43 helicase, along with Ntr1 and Ntr2, disassembles the intron lariat spliceosome (ILS), whose components can be recycled and used for another pre-mRNA [209,210].

Although *S. cerevisiae* does not engage in alternative splicing, recent transcriptome-wide analyses have identified that not all splicing occurs co-transcriptionally. Some mRNAs retain unspliced introns even after polyadenylation and nuclear release, with delayed removal occurring in the cytoplasm or upon exposure to environmental stimuli. These observations suggest a level of temporal flexibility in yeast splicing and argue for a division of labor between the co- and post-transcriptional processing steps in different gene classes or physiological states [211].

Intriguingly, the genes encoding Yra1 and Sus1-two critical regulators of mRNA metabolism – contain one and two introns respectively, an uncommon feature in *S. cerevisiae*, where most genes are intronless. Yra1 serves as a key adaptor linking mature mRNPs to the Mex67–Mtr2 export receptor, while Sus1 connects transcription and export through its dual role in SAGA and TREX-2 complexes (see export section). The presence of introns in these genes, along with the use of non-canonical splice sites in *SUS1*, suggests an additional layer of post-transcriptional regulation for proteins that themselves coordinate multiple steps of gene expression [212–215].

3' end processing

Cleavage and polyadenylation represent the final and decisive steps in the maturation of most mRNAs in *Saccharomyces cerevisiae*. This phase overlaps substantially with transcription termination, as both are executed by Cleavage and Polyadenylation Factor (CPF) and associated factors. In fact, the endonucleolytic cleavage of the nascent transcript is a prerequisite not only for polyadenylation but also for the release of RNAPolII from the DNA, a principle underpinning the torpedo model of termination (see previous section) [72]. However, while the termination section of this review focused primarily on the fate of RNAPolII, the emphasis here shifts to the processing of the mRNA: how its 3' end is precisely defined, modified, and rendered competent for nuclear export and translation.

mRNA cleavage

Cleavage typically occurs 10–30 nucleotides downstream of a polyadenylation signal (PAS), often the canonical AAUAAA sequence or a variant. This signal is frequently accompanied by auxiliary upstream and downstream U-rich elements that further modulate the recognition efficiency [144]. In yeast, PAS recognition is primarily mediated by the polymerase module of CPF, particularly the Yth1 subunit, which engages the hexamer with its zinc fingers. Pfs2 stabilizes this interaction.

Notably, a conserved uridine at the – 1 position upstream of PAS is recognized by a specific pocket in Yth1, enhancing sequence selectivity [146].

mRNA cleavage is catalyzed by Ysh1, the endonuclease subunit of the CPF complex, which belongs to the metallo-β-lactamase/β-CASP family. Ysh1 cleaves the transcript 10–30 nucleotides downstream of the PAS, generating a 3'-hydroxyl group necessary for polyadenylation [216]. However, Ysh1 alone is inactive in isolation and requires cooperative assembly with other CPF modules and cleavage factors to acquire its activity. Among these, Mpe1 plays a pivotal role; it interacts directly with Ysh1 and acts as a molecular sensor for PAS through its pre-mRNA sensing region (PSR). This region forms weak contacts with the PAS, especially the second adenosine, and likely coordinates cleavage site recognition with catalytic activation. Disruption of the PSR leads to cleavage defects and unregulated polyadenylation [217].

Furthermore, two auxiliary complexes, Cleavage Factor IA (CF IA) and Cleavage Factor IB (CF IB), are required to stabilize the CPF complex on mRNA. CF IA (comprising Rna14, Rna15, Pcf11, and Clp1) positions CPF by bridging mRNA sequence elements and the CTD of RNAPolII (especially Ser2-P), whereas CF IB (Hrp1) enhances specificity by binding to upstream U-rich motifs. Only upon correct assembly of these components is Ysh1's active site positioned to cleave the nascent transcript [72].

mRNA Polyadenylation

Once cleavage occurs, the upstream cleavage product is rapidly polyadenylated by Pap1, which is a poly(A) polymerase. Pap1 is recruited and tethered to the CPF complex through Fip1, an intrinsically disordered protein that binds to both Pap1 and Yth1 [218,219]. This flexible arrangement enables the processive addition of adenosines. In isolation, Pap1 displays weak distributive activity, but CPF scaffolding and Fip1 tethering stimulate its function [220].

Polyadenylation is tightly controlled. In *S. cerevisiae*, poly(A) tails typically range from 60 to 80 nucleotides. Nab2 is the primary nuclear poly(A)-binding protein responsible for limiting tail length by displacing Pap1 upon saturation [221]. Under stress or Nab2 depletion, Pab1 can substitute, albeit resulting in slightly longer tails (~90 nt) [222]. Even in the absence of poly(A)-binding proteins, CPF intrinsically restricts the tail length to ~ 100–200 nucleotides, suggesting that kinetic regulation and structural feedback mechanisms limit excessive elongation [72].

Together, these findings establish cleavage and polyadenylation as highly regulated processes that interpret sequence elements, CTD phosphorylation patterns, and prior processing status to generate mature, export-competent mRNAs.

mRNP formation and export

mRNP assembly and surveillance

Once transcription and mRNA processing are underway, the transcript is not left unprotected or freely diffusing in the nucleoplasm. Instead, pre-mRNAs are rapidly and selectively assembled into messenger ribonucleoprotein particles (mRNPs), dynamic assemblies composed of the mRNA and a diverse array of proteins that influence every aspect of the mRNA's life – from maturation and export to localization and translation. In *S. cerevisiae*, mRNP formation begins co-transcriptionally and remodeling continues until it is exported to the cytoplasm. This process integrates all the steps described before which include transcription, 5' capping, splicing, 3' end processing, and packaging into a tightly regulated cascade that ensures mRNA quality and fidelity.

Unlike a simplistic 'last step' view of mRNA packaging, mRNP assembly is a central feature of mRNA biogenesis. Indeed, most RNA-binding proteins (RBPs) involved in this process are not recruited post-transcriptionally but rather co-transcriptionally. These proteins often recognize specific mRNA sequence elements, but they are also guided by CTD phosphorylation states, processing landmarks, and interactions with the transcription machinery itself [223]. One of the earliest components of the emerging mRNP is the CBC (see mRNA processing section), which binds to the 7-methylguanosine cap structure added shortly after transcription initiation and acts as a hub for downstream processing and export factor

recruitment [224,225]. CBC has been shown to promote co-transcriptional splicing, guide the assembly of export adaptors, and coordinate with TREX machinery for proper nuclear export. CBC-bound transcripts are typically retained in the nucleus until processing is complete, thereby acting as an early checkpoint in mRNP assembly [184,226,227].

In *S. cerevisiae*, several of the RBPs that make up the mRNP act as 'guardian' proteins – including Npl3, Nab2, Hrp1, Gbp2, and Hrb1 — that are co-transcriptionally recruited to nascent transcripts. These adaptor factors associate with the pre-mRNA as integral components of the mRNP, acting as both sensors and quality control elements (reviewed in [228].

Npl3 is one of the first RBPs to bind nascent pre-mRNAs and functions in both splicing and export. It responds to CTD Ser2 phosphorylation and can compete with other export factors when processing is incomplete [229,230]. Hrp1, while primarily a 3'-end processing factor part of the CPF-CF complex, also participates in quality control by recognizing aberrant cleavage events and preventing premature export [231]. Nab2, a key poly(A)-binding protein, regulates tail length and contributes to transcript surveillance by interacting with nuclear exosome cofactors under defective processing conditions [72].

Meanwhile, the SR-like proteins Gbp2 and Hrb1, act dually as adaptors and quality sensors. Their association with both mRNA and export machinery depends on the transcript context and is essential for coupling maturation to nuclear export [232,233].

Beyond these core RBPs, the composition of an mRNP is not fixed but varies depending on the features of the transcript and the physiological context. This compositional plasticity allows cells to tailor mRNP assembly to specific functional outcomes. For example, certain export adaptor proteins may be preferentially recruited in response to stress, specific mRNA sequence elements, or transcriptional programs, thereby modulating export efficiency and post-transcriptional fate [234,235]. Such dynamic assembly ensures that only properly processed and contextually appropriate transcripts are selected for export.

Among these specific adaptor proteins, we can find the RBP Mip6 which contributes to transcript-specific export regulation by binding to Mex67. Mip6 selectively limits the nuclear export of mRNAs induced by Msn2 and Msn4, two stress-responsive transcription factors that activate gene expression under adverse environmental conditions. By restricting the premature export of these transcripts under non-stress conditions, Mip6 helps maintain proper gene expression homeostasis and prevents unnecessary stress responses [236].

Together, guard proteins ensure that export competence is tightly coupled to splicing, 3' end formation, and polyadenylation, forming an additional surveillance layer that defines transcript fitness before export to the cytoplasm.

The TREX complex

In order to coordinate mRNP formation and transcription elongation, the THO complex, composed of Tho2, Hpr1, Mft1, Thp2, and Tex1, binds to elongating RNAPolII, particularly through interactions with its phosphorylated C-terminal domain (CTD) [237]. Additionally, the THO subunits Tho2 and Hpr1 have been shown to interact directly with chromatin [238,239]. reinforcing its strategic positioning at sites of transcription, as well as the coupling of nuclear events.

Through these interactions, THO engages with the nascent mRNA to form the Transcription-Export (TREX) complex. In yeast, TREX also includes the DEAD-box helicase Sub2 and the essential adaptor protein Yra1 [240,241]. Cryo-EM reconstructions have revealed that Tho2 serves as the central scaffold of the TREX complex, organizing the spatial configuration of the remaining subunits and shaping the overall architecture necessary for stable mRNA interactions [233]. Sub2 docks onto Tho2 *via* an extended interface, stabilizing it in an RNA-bound active conformation that facilitates mRNA remodeling (Figure 5).

In parallel, the adaptor proteins Gbp2 and Hrb1 are recruited to the transcript through coordinated contact with both THO and Sub2 [232]. Their arginine-serine-rich (RS) domains and RRMs enable dual binding to mRNA and export factors, thereby supporting transcript compaction and

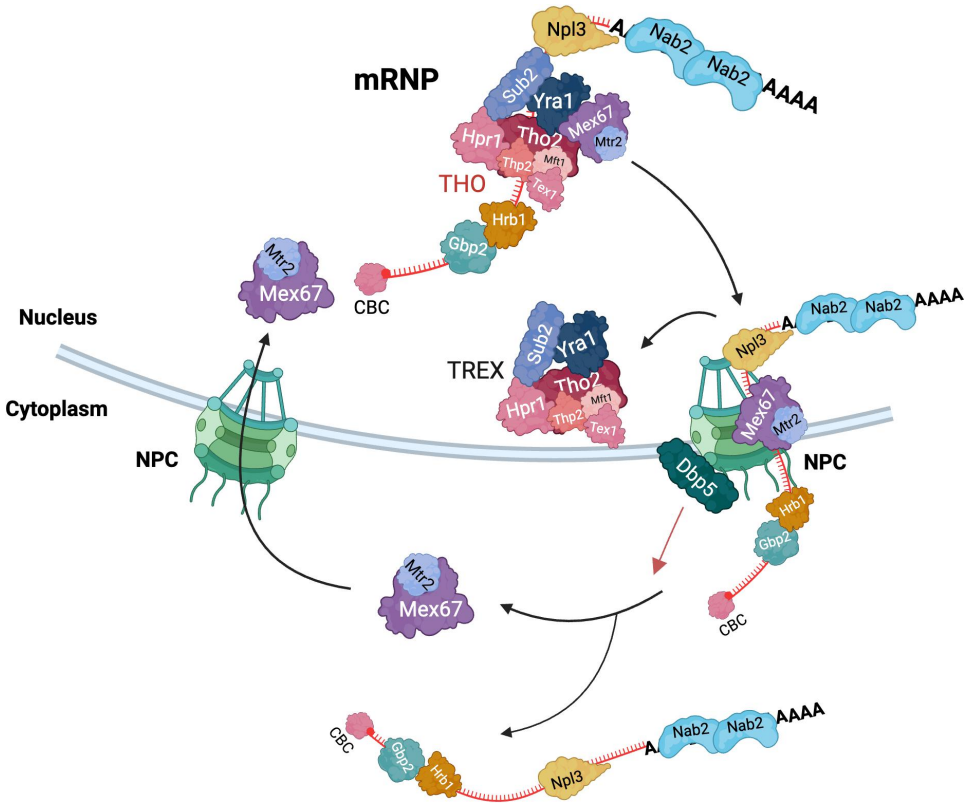

Figure 5. mRNP formation and export through the NPC. the mRNP particle forms co-transcriptionally. The CBC bind to the 5′ cap of the mRNA and serves to recruit a series of adaptor proteins, including, Gbp2, Hrb1, Npl3 and Nab2 which bind the poly-A tail. The TREX complex (made up by THO, Yra1 and Sub2) also binds and facilitates the recruitment of the export factors Mex67/Mtr2. These export factors enable the crossing through the NPC by contacts with FC-repeats, while TREX remains within the nucleus. The helicase Dbp5 displaces Mex67/Mtr2 – which traverses back the NPC – from the mRNA, to prevent its return to the nucleus. Created in BioRender. https://BioRender.com/r6gam3m.

quality control. The CBC also acts upstream in this cascade, facilitating Yra1 recruitment co-transcriptionally through its interaction with Sub2 and THO [240,242]. Once deposited, Sub2 and Yra1 coordinate the replacement of early processing factors with export factors, such as Mex67/Mtr2, licensing the mRNP for export.

TREX occupancy across both intron-containing and intronless genes also requires the NTC, a spliceosome-associated factor that plays a dual role in splicing (see Splicing section) and TREX recruitment [191,243]. Additionally, Yra1 recruitment is facilitated by mechanisms other than Sub2. It can also interact with Pcf11, a member of the CPF – CF complex [244], and is influenced by Dbp2, a second DEAD-box helicase distinct from Sub2 [245]. Yra1 also exhibits direct affinity for mRNA, implying multiple, redundant pathways for its stable mRNP association [237]. Together, these diverse interactions illustrate the complexity and redundancy of the TREX recruitment pathway to safeguard mRNA maturation and export readiness.

Mex67–Mtr2 export mechanism

As hinted in the previous sections, Mex67–Mtr2 is the principal mRNA export factor in yeast [246]. It is recruited to export-competent mRNPs through adaptor proteins, which recognize processing markers and help coordinate nuclear export readiness (Figure 5). Once associated with the mRNP, Mex67–Mtr2 ensures the transcript is routed correctly toward the NPC [247]. Importantly, Mex67 plays a role not only as a transport factor but also as a structural component of the export pathway.

In *S. cerevisiae*, the Mex67–Mtr2 heterodimer serves as the principal mRNA export receptor, orchestrating the translocation of mature mRNPs through the nuclear pore complex (NPC) into the

cytoplasm [246,247]. This complex forms the core of the general mRNA export machinery and is functionally conserved across eukaryotes. Mex67 is a modular protein comprising four domains: an RRM, a leucine-rich repeat (LRR) domain, a nuclear transport factor 2-like (NTF2L) domain, and a ubiquitin-associated (UBA) domain [248,249]. These domains collectively facilitate mRNA binding and interactions with phenylalanine – glycine (FG)-repeat nucleoporins within the NPC. Mtr2, characterized by its NTF2-like fold, forms a stable heterodimer with Mex67, enhancing the complex's structural integrity and export functionality [249].

Structural analyses have elucidated the spatial arrangement of these domains, revealing that the RRM and LRR domains of Mex67 are primarily responsible for RNA binding, while the NTF2L and UBA domains mediate interactions with FG-nucleoporins [248]. Notably, the NTF2L domain also contributes to mRNA binding, indicating a more intricate role in mRNP recognition than previously appreciated.

Functionally, Mex67–Mtr2 is recruited to export-competent mRNPs via adaptor proteins that recognize processing markers, ensuring that only properly processed transcripts are exported. Once associated with the mRNP, Mex67–Mtr2 interacts transiently with FG-repeat nucleoporins within the NPC, facilitating the directional export of mRNPs to the cytoplasm [250].

Beyond mRNA, Mex67–Mtr2 also partakes in the nuclear export of other RNA species, including pre-60S and pre-40S ribosomal subunits, as well as certain tRNAs. This versatility underscores its role as a general RNA export factor [251,252].

Intriguingly, recent studies have demonstrated that Mex67–Mtr2 exhibits a preferential binding affinity for double-stranded RNA (dsRNA) over single-stranded RNA (ssRNA). This preference suggests a role in the selective export of structured RNA molecules and may contribute to the regulation of gene expression under specific cellular conditions [253].

Recent quantitative fluorescence microscopy has demonstrated that Mex67 exhibits limited interaction with nuclear mRNA and primarily localizes to the NPC independently of its cargo [254]. This behavior implies that Mex67 functions as a 'mobile nucleoporin', dynamically integrating into the NPC scaffold *via* transient interactions with FG-repeat domains. In this capacity, it acts more like a gatekeeper stationed at the pore, receiving mRNP substrates at the central channel and facilitating their directional translocation to the cytoplasm. Supporting this spatial role, a fusion of Mex67 to the nucleoporin Nup116 is sufficient to rescue a deletion of *MEX67*, underscoring that its essential export function is spatially restricted to the NPC.

The TREX-2 complex

mRNA transcription and export are also facilitated by the TREX-2 complex (also known as the THSC complex), in addition to TREX. This complex is conserved in most eukaryotes [255–257]. In *S. cerevisiae*, TREX-2 is built with the scaffold subunit Sac3, which is associated with Sem1, Thp1, Cdc31, and two copies of the Sus1 protein, which is also a constitutive component of the DUBm of SAGA [214,258].

Regarding the structural disposition of TREX-2, the N-terminus of Sac3 has FG repeats similar to several nucleoporins, allowing it to bind to the Mex67/Mtr2 heterodimer [259,260]. The central part of Sac3 binds to Sem1 and Thp1 as well as to mRNA [261]. Additionally, the C-terminus contains a Cdc31-binding domain (CID) that serves as a binding site for Cdc31 and its two Sus1 subunits. This CID region enables tethering of TREX-2 to the NPC with the participation of the basket nucleoporins Nup1 and Nup60 [262] (Figure 6).

TREX-2 is functionally linked to 'gene gating', a process by which actively transcribed genes are positioned near the NPC to facilitate efficient mRNA export [263]. This tethering involves an interaction between TREX-2, SAGA, and the Mediator complex, a key coactivator that supports transcription initiation by bridging activators and RNAPolII. Through Mediator, TREX-2 helps recruit transcriptional machinery to promoters and anchors these loci at the nuclear periphery, thus coupling transcription activation with export readiness [264].

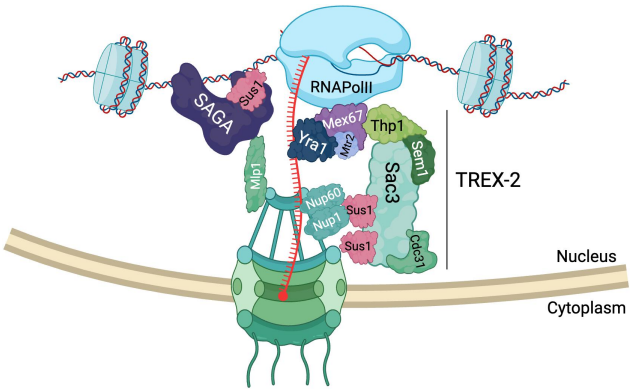

Figure 6. Interplay between SAGA and TREX-2: gene Gating. Localisation of actively transcribed genes to the NPC, so that they can be promptly exported to the cytoplasm. Created in BioRender. https://BioRender.com/r16q075.

Role of the nuclear pore complex

In order to reach the cytoplasm, the mRNP has to traverse the nuclear pore complex (NPC), a massive ~ 60 MDa channel embedded in the nuclear envelope.

As this review addresses broad aspects of the nuclear phases of gene expression, detailed descriptions of the NPC are available in other sources [265–267]. In brief, the yeast NPC consists of ~ 30 different proteins, termed nucleoporins, which assemble into an octagonally symmetric scaffold with functional and structural conservation across eukaryotes [266]. Although traditionally viewed as a passive conduit, recent data have challenged this idea. Single-molecule imaging and biochemical analyses have shown that the NPC is a heterogeneous and adaptable structure that selectively remodels or filters cargo [268]. Its FG-repeat regions, intrinsically disordered and densely packed in the central channel, create a selective barrier that only allows properly loaded export complexes to transit.

The translocation of mRNPs through the NPC is directional and energy-dependent, but rather driven by transport GTPases, as in protein or tRNA export, directionality arises from ATP-dependent remodeling of the cytoplasmic face of the pore. Recent live-cell imaging by Ashkenazy-Titelman et al. [269]. However, this binding must be reversed once the export is complete to prevent mRNP reentry and license translation.

This remodeling is catalyzed by the DEAD-box ATPase Dbp5, which is anchored to the cytoplasmic filaments of the NPC *via* the nucleoporin Nup159. Upon engagement, Dbp5 remodels the mRNP by displacing export factors, such as Mex67/Mtr2, which travel back to the nucleus, ensuring that the transcript is rendered translation-competent and cannot diffuse back into the nucleus [270,271] (Figure 5).

Cryo-EM and biochemical studies have recently visualized this step in greater detail. Bonneau et al. [272] showed that nuclear mRNPs are compact and modular particles stabilized by extensive RNA – RNA and protein – RNA networks. These compacted structures are likely shaped by the action of TREX and the cap-binding complex (CBC) during transcription and processing. As the mRNP exits the pore, these networks must be locally unwound by Dbp5 to permit cytoplasmic engagement with the translation machinery.

In support of this remodeling checkpoint model, structural work by Xie et al. [273], demonstrated how the TREX-2 complex, tethered to the nuclear basket through Nup1 and Nup60, facilitates the recruitment of Mex67 and orchestrates the release of earlier processing factors such as Sub2. By exposing the mRNA-binding surface of the mRNP at the NPC interface, TREX-2 ensures that only properly processed transcripts engage the export pathway, whereas Dbp5 remodeling at the cytoplasmic face provides the final handoff into translation.

Altogether, the formation and export of mRNPs constitute a highly orchestrated and selective process, marked by remarkable variability in composition and regulation. Each transcript assembles into a distinct mRNP, shaped by factors such as gene identity, intron content, transcriptional dynamics, and cellular conditions. This variability extends to the selective recruitment of RBPs, whose association with transcripts is influenced by both cis-acting RNA elements and co-transcriptional processing events. At the nuclear periphery, the NPC further contributes to this specificity, functioning not as a passive channel but as a dynamic and heterogeneous gatekeeper that selectively permits the transit of export-competent mRNPs. This multilevel surveillance and adaptability ensure that only properly matured and appropriately configured transcripts reach the cytoplasm – highlighting the essential

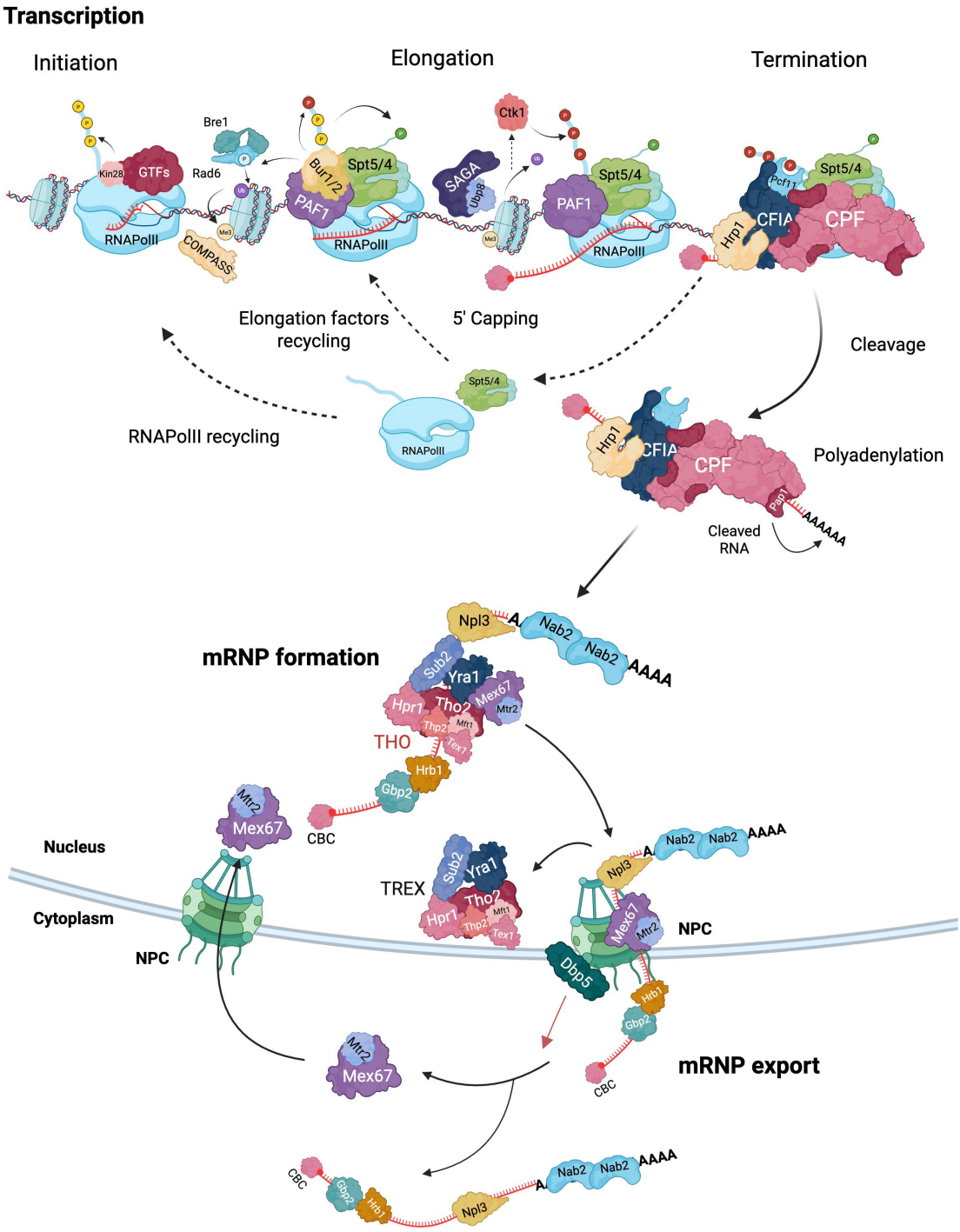

Figure 7. Integrated overview of mRNA life cycle: from transcription to Export. This schematic provides a summary of the key stages in the eukaryotic mRNA life cycle. Transcription by RNA polymerase II (RNAPolII) proceeds through initiation, elongation and termination, accompanied by co-transcriptional modifications such as 5′ capping, splicing (not shown), and 3′ end processing, including cleavage and polyadenylation. The resulting mature mRNA is packaged into a messenger ribonucleoprotein (mRNP) complex with the aid of multiple processing and export factors, including TREX, Mex67 and Nab2. The mRNP is then transported through the nuclear pore complex (NPC) into the cytoplasm for translation. This integrated depiction underscores the coordination between transcriptional, processing and export machineries to ensure accurate and efficient gene expression. Created in BioRender. https://biorender.com/o0pi0na.

role of mRNP diversity and NPC selectivity in maintaining accurate and responsive gene expression.

Concluding remarks and future questions

Over the past decades, studies in *Saccharomyces cerevisiae* have shaped our understanding of gene expression, offering a model system to dissect conserved mechanisms of chromatin modification, transcription, mRNA processing, and export. This review presents an integrative picture of these steps, emphasizing how gene expression is a dynamic, tightly regulated continuum – from nucleosome architecture to transcript delivery into the cytoplasm. In particular, the interplay between histone marks (such as H2BK123ub[1] and H3K4/36/79 methylation), transcriptional machinery (notably RNAPolII and its CTD modifications), and the coupling of mRNA biogenesis to nuclear export (Figure 7) underscores the complexity and coordination underlying gene regulation.

Importantly, we highlight how the so-called 'nuclear steps' of gene expression are not isolated but highly interconnected: chromatin marks modulate transcriptional kinetics; splicing influences export competence; and mRNP composition is tailored to transcript identity and cellular context. The final checkpoint at the nuclear pore – governed by TREX, TREX-2, and Mex67–Mtr2, and ultimately remodeled by Dbp5—ensures that only properly processed transcripts reach the cytoplasm. Together, these mechanisms underscore the selective and robust nature of gene expression surveillance.

Future advances in this field are likely to come from the integration of structural, imaging, genomic, and single-molecule approaches. Unresolved questions remain, including:

- To what extent are chromatin marks predictive of mRNA export/surveillance outcomes?
- How are mRNPs selectivity modulated under physiological or stress conditions?
- What is the spatial organization of these processes within the nucleus?

Moreover, advances in cryo-EM, proximity labeling, and high-resolution live-cell imaging will be pivotal in resolving the spatial and temporal order of events, revealing how gene expression is orchestrated in real time. By continuing to use yeast as a model system while expanding comparative analyses to metazoans, we will deepen our understanding of how cells maintain gene expression fidelity in health and disease.

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Susana Rodríguez-Navarro (SR-N): Writing – Review & Editing, Supervision, Conceptualization.

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Author contributions

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

No data were generated or analyzed in this study; therefore, data sharing does not apply.

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References

[1] Goffeau A, Barrell BG, Bussey H, et al. Life with 6000 genes. Science. 1996;274(5287):546–567. doi: 10.1126/science.274.5287.546

[2] Cherry JM, Adler C, Ball C, et al. SGD: Saccharomyces genome database. Nucleic Acids Res. 1998;26(1):73–79. doi: 10.1093/nar/26.1.73

[3] Engel SR, Dietrich FS, Fisk DG, et al. The reference genome sequence of Saccharomyces cerevisiae: then and now. G3 (Bethesda, Md). 2014;4(3):389–398. doi: 10.1534/g3.113.008995

[4] Martire S, Banaszynski LA. The roles of histone variants in fine-tuning chromatin organization and function. Nat Rev Mol Cell Biol. 2020;21(9):522–541. doi: 10.1038/s41580-020-0262-8

[5] Chen P, Li W, Li G. Structures and functions of chromatin fibers. Annu Rev Biophys. 2021;50(1):95–116. doi: 10.1146/annurev-biophys-062920-063639

[6] Kornberg RD. Chromatin structure: a repeating unit of histones and DNA. Science. 1974;184(4139):868–871. doi: 10.1126/science.184.4139.868

[7] Brush GS. Evidence that histone H1 is dispensable for proper meiotic recombination in budding yeast. BMC Res Notes. 2015;8(1):275. doi: 10.1186/s13104-015-1246-1

[8] Wang M, Li J, Wang Y, et al. Single-molecule study reveals Hmo1, not Hho1, promotes chromatin assembly in budding yeast. MBio. 2023;14(4):e0099323. doi: 10.1128/mbio.00993-23

[9] Lorch Y, Kornberg RD, Maier-Davis B. Role of the histone tails in histone octamer transfer. Nucleic Acids Res. 2023;51(8):3671–3678. doi: 10.1093/nar/gkad079

[10] Luger K, Dechassa ML, Tremethick DJ. New insights into nucleosome and chromatin structure: an ordered state or a disordered affair? Nat Rev Mol Cell Biol. 2012;13(7):436–447. doi: 10.1038/nrm3382

[11] Millán-Zambrano G, Burton A, Bannister AJ, et al. Histone post-translational modifications—cause and consequence of genome function. Nat Rev Genet. 2022;23(9):563–580. doi: 10.1038/s41576-022-00468-7

[12] Lin A, Du Y, Xiao W. Yeast chromatin remodeling complexes and their roles in transcription. Curr Genet. 2020;66(4):657–670. doi: 10.1007/s00294-020-01072-0

[13] Venkatesh S, Workman JL. Histone exchange, chromatin structure and the regulation of transcription. Nat Rev Mol Cell Biol. 2015;16(3):178–189. doi: 10.1038/nrm3941

[14] Kobayashi W, Kurumizaka H. Structural transition of the nucleosome during chromatin remodeling and transcription. Curr Opin Struct Biol. 2019;59:107–114. doi: 10.1016/j.sbi.2019.07.011

[15] Kornberg RD, Lorch Y. Primary role of the nucleosome. Mol Cell. 2020;79(3):371–375. doi: 10.1016/j.molcel.2020.07.020

[16] Morrison O, Thakur J. Molecular complexes at euchromatin, Heterochromatin and centromeric chromatin. Int J Mol Sci. 2021;22(13):6922. doi: 10.3390/ijms22136922

[17] Demetriadou C, Koufaris C, Kirmizis A. Histone N-alpha terminal modifications: genome regulation at the tip of the tail. Epigenet & Chromatin. 2020;13(1):29. doi: 10.1186/s13072-020-00352-w

[18] Smith E, Shilatifard A. The chromatin signaling pathway: diverse mechanisms of recruitment of histone-modifying enzymes and varied biological outcomes. Mol Cell. 2010;40(5):689–701. doi: 10.1016/j.molcel.2010.11.031

[19] Liu R, Wu J, Guo H, et al. Post-translational modifications of histones: mechanisms, biological functions, and therapeutic targets. MedComm. 2023;4(3):e292. doi: 10.1002/mco2.292

[20] Strahl BD, Briggs SD. The SAGA continues: the rise of cis- and trans-histone crosstalk pathways. Gcn5: Quintessential HAT. 2021;1864(2):194600. doi: 10.1016/j.bbagrm.2020.194600

[21] Magits W, Sablina AA. The regulation of the protein interaction network by monoubiquitination. Curr Opin Struct Biol. 2022;73:102333. doi: 10.1016/j.sbi.2022.102333

[22] Fuchs G, Oren M. Writing and reading H2B monoubiquitylation. Biochim et Biophys Acta (BBA) - Gene Regul Mech. 2014;1839(8):694–701. doi: 10.1016/j.bbagrm.2014.01.002

[23] Mattiroli F, Penengo L. Histone ubiquitination: an integrative signaling platform in genome stability. Trends Genet. 2021;37(6):566–581. doi: 10.1016/j.tig.2020.12.005

[24] Fetian T, Grover A, Arndt KM. Histone H2B ubiquitylation: connections to transcription and effects on chromatin structure. Biochim et Biophys Acta (BBA) - Gene Regul Mech. 2024;1867(2):195018. doi: 10.1016/j.bbagrm.2024.195018

[25] Fierz B, Chatterjee C, McGinty RK, et al. Histone H2B ubiquitylation disrupts local and higher-order chromatin compaction. Nat Chem Biol. 2011;7(2):113–119. doi: 10.1038/nchembio.501

[26] Sengupta B, Huynh M, Smith CB, et al. The effects of histone H2B ubiquitylations on the nucleosome structure and internucleosomal interactions. Biochemistry. 2022;61(20):2198–2205. doi: 10.1021/acs.biochem.2c00422

[27] Chandrasekharan MB, Huang F, Sun Z-W. Histone H2B ubiquitination and beyond. Epigenetics. 2010;5(6):460–468. doi: 10.4161/epi.5.6.12314

[28] Li Y, Ma T, Jiang J, et al. The dynamics and functional mechanisms of H2B mono-ubiquitination. Crop Health. 2024;2(1):1. doi: 10.1007/s44297-023-00022-9

[29] Deng Z-H, Ai H-S, Lu C-P, et al. The Bre1/Rad6 machinery: writing the central histone ubiquitin mark on H2B and beyond. Chromosome Res. 2020;28(3):247–258. doi: 10.1007/s10577-020-09640-3

[30] Hwang WW, Venkatasubrahmanyam S, Ianculescu AG, et al. A conserved RING finger protein required for histone H2B monoubiquitination and cell size control. Mol Cell. 2003;11(1):261–266. doi: 10.1016/S1097-2765(02)00826-2

[31] Robzyk K, Recht J, Osley MA. Rad6-dependent ubiquitination of histone H2B in yeast. Science. 2000;287 (5452):501–504. doi: 10.1126/science.287.5452.501

[32] Wood A, Krogan NJ, Dover J, et al. Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. Mol Cell. 2003;11 (1):267–274. doi: 10.1016/s1097-2765(02)00802-x

[33] Chen F, Liu B, Guo L, et al. Biochemical insights into Paf1 complex–induced stimulation of Rad6/Bre1-mediated H2B monoubiquitination. Proc Natl Acad Sci. 2021;118(33):e2025291118. doi: 10.1073/pnas.2025291118

[34] Francette AM, Tripplehorn SA, Arndt KM. The Paf1 complex: a keystone of nuclear regulation operating at the interface of transcription and chromatin. RNA Polymerase II Transcription. 2021;433(14):166979. doi: 10.1016/j.jmb.2021.166979

[35] Joo YJ, Ficarro SB, Chun Y, et al. In vitro analysis of RNA polymerase II elongation complex dynamics. Genes & Devel. 2019;33(9–10):578–589. doi: 10.1101/gad.324202.119

[36] Qiu H, Hu C, Gaur NA, et al. Pol II CTD kinases Bur1 and Kin28 promote Spt5 CTR-independent recruitment of Paf1 complex. Embo J. 2012;31 (16):3494–3505. doi: 10.1038/emboj.2012.188

[37] Van Oss SB, Cucinotta CE, Arndt KM. Emerging insights into the roles of the Paf1 complex in gene regulation. Trends Biochem Sci. 2017;42(10):788–798. doi: 10.1016/j.tibs.2017.08.003

[38] Fetian T, McShane BM, Horan NL, et al. Paf1 complex subunit Rtf1 stimulates H2B ubiquitylation by interacting with the highly conserved N-terminal helix of Rad6. Proc Natl Acad Sci. 2023;120(22):e2220041120. doi: 10.1073/pnas.2220041120

[39] Van Oss SB, Shirra MK, Bataille AR, et al. The histone modification domain of Paf1 complex subunit Rtf1 directly stimulates H2B ubiquitylation through an interaction with Rad6. Mol Cell. 2016;64(4):815–825. doi: 10.1016/j.molcel.2016.10.008

[40] Laribee RN, Krogan NJ, Xiao T, et al. BUR kinase selectively regulates H3 K4 trimethylation and H2B ubiquitylation through recruitment of the PAF elongation complex. Curr Biol. 2005;15(16):1487–1493. doi: 10.1016/j.cub.2005.07.028

[41] Liu Y, Warfield L, Zhang C, et al. Phosphorylation of the transcription elongation factor Spt5 by yeast Bur1 kinase stimulates recruitment of the PAF complex. Mol Cell Biol. 2009;29(17):4852–4863. doi: 10.1128/MCB.00609-09

[42] Wood A, Schneider J, Dover J, et al. The Bur1/Bur2 complex is required for histone H2B monoubiquitination by Rad6/Bre1 and histone methylation by COMPASS. Mol Cell. 2005;20(4):589–599. doi: 10.1016/j.molcel.2005.09.010

[43] Zhou K, Kuo WHW, Fillingham J, et al. Control of transcriptional elongation and cotranscriptional histone modification by the yeast BUR kinase substrate Spt5. Proc Natl Acad Sci. 2009;106(17):6956–6961. doi: 10.1073/pnas.0806302106

[44] Bonnet J, Wang C-Y, Baptista T, et al. The SAGA coactivator complex acts on the whole transcribed genome and is required for RNA polymerase II transcription. Genes & Devel. 2014;28(18):1999–2012. doi: 10.1101/gad.250225.114

[45] Schulze JM, Hentrich T, Nakanishi S, et al. Splitting the task: Ubp8 and Ubp10 deubiquitinate different cellular pools of H2BK123. Genes & Devel. 2011;25 (21):2242–2247. doi: 10.1101/gad.177220.111

[46] Grant PA, Winston F, Berger SL. The biochemical and genetic discovery of the SAGA complex. Gcn5: Quintessential HAT. 2021;1864(2):194669. doi: 10.1016/j.bbagrm.2020.194669

[47] Henry KW, Wyce A, Lo WS, et al. Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. Genes Devel. 2003;17(21):2648–2663. doi: 10.1101/gad.1144003

[48] Köhler A, Pascual-García P, Llopis A, et al. The mRNA export factor Sus1 is involved in Spt/Ada/Gcn5 acetyltransferase-mediated H2B deubiquitinylation through its interaction with Ubp8 and Sgf11. Mol Biol Cell. 2006;17(10):4228–4236. doi: 10.1091/mbc.E06-02-0098

[49] Samara NL, Datta AB, Berndsen CE, et al. Structural insights into the assembly and function of the SAGA deubiquitinating module. Science. 2010;328 (5981):1025–1029. doi: 10.1126/science.1190049

[50] Batta K, Zhang Z, Yen K, et al. Genome-wide function of H2B ubiquitylation in promoter and genic regions. Genes & Devel. 2011;25(21):2254–2265. doi: 10.1101/gad.177238.111

[51] Daniel JA, Torok MS, Sun Z-W, et al. Deubiquitination of histone H2B by a yeast acetyltransferase complex regulates transcription. J Biol Chem. 2004;279 (3):1867–1871. doi: 10.1074/jbc.C300494200

[52] Emre NCT, Ingvarsdottir K, Wyce A, et al. Maintenance of low histone ubiquitylation by Ubp10 correlates with telomere-proximal Sir2 association and gene silencing. Mol Cell. 2005;17(4):585–594. doi: 10.1016/j.molcel.2005.01.007

[53] Gardner RG, Nelson ZW, Gottschling DE. Ubp10/Dot4p regulates the persistence of ubiquitinated histone H2B: distinct roles in telomeric silencing and general chromatin. Mol Cell Biol. 2005;25 (14):6123–6139. doi: 10.1128/MCB.25.14.6123-6139.2005

[54] Nune M, Morgan MT, Connell Z, et al. FACT and Ubp10 collaborate to modulate H2B deubiquitination

and nucleosome dynamics. Elife. 2019;8:e40988. doi: 10.7554/eLife.40988
[55] Husmann D, Gozani O. Histone lysine methyltransferases in biology and disease. Nat Struct & Mol Biol. 2019;26(10):880–889. doi: 10.1038/s41594-019-0298-7
[56] Zhang J, Jing L, Li M, et al. Regulation of histone arginine methylation/demethylation by methylase and demethylase (review). Mol Med Rep. 2019;19 (5):3963–3971. doi: 10.3892/mmr.2019.10111
[57] Separovich RJ, Wilkins MR. Ready, SET, go: post-translational regulation of the histone lysine methylation network in budding yeast. J Biol Chem. 2021;297(2):100939. doi: 10.1016/j.jbc.2021.100939
[58] Yap KL, Zhou M-M. Structure and mechanisms of lysine methylation recognition by the chromodomain in gene transcription. Biochemistry. 2011;50 (12):1966–1980. doi: 10.1021/bi101885m
[59] Jain K, Fraser CS, Marunde MR, et al. Characterization of the plant homeodomain (PHD) reader family for their histone tail interactions. Epigenet & Chromatin. 2020;13(1):3. doi: 10.1186/s13072-020-0328-z
[60] Lu R, Wang GG. Tudor: a versatile family of histone methylation 'readers'. Trends Biochem Sci. 2013;38 (11):546–555. doi: 10.1016/j.tibs.2013.08.002
[61] Guo H-B, Guo H. Mechanism of histone methylation catalyzed by protein lysine methyltransferase SET7/9 and origin of product specificity. Proc Natl Acad Sci. 2007;104(21):8797–8802. doi: 10.1073/pnas.0702981104
[62] Lanouette S, Mongeon V, Fi Geys D, et al. The functional diversity of protein lysine methylation. Mol Syst Biol. 2014;10(4):724. doi: 10.1002/msb.134974
[63] Hamey JJ, Wilkins MR. The protein methylation network in yeast: a landmark in completeness for a eukaryotic post-translational modification. Proc Natl Acad Sci. 2023;120(23):e2215431120. doi: 10.1073/pnas.2215431120
[64] Ng HH, Feng Q, Wang H, et al. Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and sir protein association. Genes & Devel. 2002;16(12):1518–1527. doi: 10.1101/gad.1001502
[65] Briggs SD, Xiao T, Sun Z-W, et al. Gene silencing: trans-histone regulatory pathway in chromatin. Nature. 2002;418(6897):498. doi: 10.1038/nature00970
[66] Liu CL, Kaplan T, Kim M, et al. Single-nucleosome mapping of histone modifications in S. cerevisiae. PLOS Biol. 2005;3(10):e328. doi: 10.1371/journal.pbio.0030328
[67] van Leeuwen F, Gafken PR, Gottschling DE. Dot1p modulates silencing in yeast by methylation of the nucleosome core. Cell. 2002;109(6):745–756. doi: 10.1016/s0092-8674(02)00759-6
[68] Chou KY, Lee J-Y, Kim K-B, et al. Histone modification in Saccharomyces cerevisiae: a review of the current status. Comput Struct Biotechnol J. 2023;21:1843–1850. doi: 10.1016/j.csbj.2023.02.037
[69] Miller T, Nj K, Dover J, et al. COMPASS: a complex of proteins associated with a trithorax-related SET domain protein. Proc Natl Acad Sci U S A. 2001;98 (23):12902–12907. doi: 10.1073/pnas.231473398
[70] Roguev A, Schaft D, Shevchenko A, et al. The Saccharomyces cerevisiae Set1 complex includes an Ash2 homologue and methylates histone 3 lysine 4. Embo J. 2001;20(24):7137–7148. doi: 10.1093/emboj/20.24.7137
[71] Deshpande N, Bryk M. Diverse and dynamic forms of gene regulation by the S. cerevisiae histone methyltransferase Set1. Curr Genet. 2023;69(2):91–114. doi: 10.1007/s00294-023-01265-3
[72] Boreikaitė V, Passmore LA. 3'-end processing of eukaryotic mRNA: machinery, regulation, and impact on gene expression. Annu Rev Biochem. 2023;92 (1):199–225. doi: 10.1146/annurev-biochem-052521-012445
[73] Cheng H, He X, Moore C. The essential WD repeat protein Swd2 has dual functions in RNA polymerase II transcription termination and lysine 4 methylation of histone H3. Mol Cell Biol. 2004;24(7):2932–2943. doi: 10.1128/mcb.24.7.2932-2943.2004
[74] Choudhury R, Singh S, Arumugam S, et al. The Set1 complex is dimeric and acts with Jhd2 demethylation to convey symmetrical H3K4 trimethylation. Genes & Devel. 2019;33(9–10):550–564. doi: 10.1101/gad.322222.118
[75] Hsu PL, Li H, Lau H-T, et al. Crystal structure of the COMPASS H3K4 methyltransferase catalytic module. Cell. 2018;174(5):1106–1116.e9. doi: 10.1016/j.cell.2018.06.038
[76] Huang F, Ramakrishnan S, Pokhrel S, et al. Interaction of the Jhd2 histone H3 lys-4 demethylase with chromatin is controlled by histone H2A surfaces and restricted by H2B ubiquitination. J Biol Chem. 2015;290(48):28760–28777. doi: 10.1074/jbc.M115.693085
[77] Ramakrishnan S, Pokhrel S, Palani S, Pflueger C, Parnell T J, Cairns B R, Bhaskara S and Chandrasekharan M B. (2016). Counteracting H3K4 methylation modulators Set1 and Jhd2 co-regulate chromatin dynamics and gene transcription. Nat Commun, 7(1). doi: 10.1038/ncomms11949
[78] Pokholok DK, Harbison CT, Levine S, et al. Genome-wide map of nucleosome acetylation and methylation in yeast. Cell. 2005;122(4):517–527. doi: 10.1016/j.cell.2005.06.026
[79] Soares LM, He PC, Chun Y, et al. Determinants of histone H3K4 methylation patterns. Mol Cell. 2017;68 (4):773–785.e6. doi: 10.1016/j.molcel.2017.10.013
[80] Kim T, Buratowski S. Dimethylation of H3K4 by Set1 recruits the Set3 histone deacetylase complex to 5' transcribed regions. Cell. 2009;137(2):259–272. doi: 10.1016/j.cell.2009.02.045
[81] Martin BJE, McBurney KL, Maltby VE, et al. Histone H3K4 and H3K36 methylation independently recruit

the NuA3 histone acetyltransferase in Saccharomyces cerevisiae. Genetics. 2017;205(3):1113–1123. doi: 10.1534/genetics.116.199422

[82] Kleinschmidt RA, Lyon LM, Smith SL, et al. Genetic screen for suppressors of increased silencing in rpd3 mutants in Saccharomyces cerevisiae identifies a potential role for H3K4 methylation. G3 genes|genomes|genet. 2021;11(11):jkab309. doi: 10.1093/g3journal/jkab309

[83] Krogan NJ, Dover J, Khorrami S, et al. COMPASS, a histone H3 (lysine 4) methyltransferase required for telomeric silencing of gene expression. J Biol Chem. 2002;277(13):10753–10755. doi: 10.1074/jbc.C200023200

[84] Beilharz TH, Harrison PF, Miles DM, et al. Coordination of Cell cycle progression and mitotic spindle assembly involves histone H3 lysine 4 methylation by Set1/COMPASS. Genetics. 2017;205(1):185–199. doi: 10.1534/genetics.116.194852

[85] Faucher D, Wellinger RJ, Copenhaver GP. Methylated H3K4, a transcription-associated histone modification, is involved in the DNA damage response pathway. PLOS Genet. 2010;6(8):e1001082. doi: 10.1371/journal.pgen.1001082

[86] Serrano-Quílez J, Roig-Soucase S, Rodríguez-Navarro S. Sharing marks: H3K4 methylation and H2B ubiquitination as features of meiotic recombination and transcription. Int J Mol Sci. 2020;21(12):4510. doi: 10.3390/ijms21124510

[87] Strahl BD, Grant PA, Briggs SD, et al. Set2 is a nucleosomal histone H3-selective methyltransferase that mediates transcriptional repression. Mol Cell Biol. 2002;22(5):1298–1306. doi: 10.1128/MCB.22.5.1298-1306.2002

[88] Li B, Jackson J, Simon MD, et al. Histone H3 lysine 36 dimethylation (H3K36me2) is sufficient to recruit the Rpd3s histone deacetylase complex and to repress spurious transcription. J Biol Chem. 2009;284(12):7970–7976. doi: 10.1074/jbc.M808220200

[89] Venkatesh S, Li H, Gogol MM, et al. Selective suppression of antisense transcription by Set2-mediated H3K36 methylation. Nat Commun. 2016;7(1):13610. doi: 10.1038/ncomms13610

[90] Kim T, Buratowski S. Two Saccharomyces cerevisiae JmjC domain proteins demethylate histone H3 Lys36 in transcribed regions to promote elongation. J Biol Chem. 2007;282(29):20827–20835. doi: 10.1074/jbc.M703034200

[91] Sharda A, Humphrey TC. The role of histone H3K36me3 writers, readers and erasers in maintaining genome stability. DNA Repair (amst). 2022;119:103407. doi: 10.1016/j.dnarep.2022.103407

[92] McDaniel SL, Hepperla AJ, Huang J, et al. H3K36 methylation regulates nutrient stress response in Saccharomyces cerevisiae by enforcing transcriptional fidelity. Cell Rep. 2017;19(11):2371–2382. doi: 10.1016/j.celrep.2017.05.057

[93] Sen P, Dang W, Donahue G, et al. H3K36 methylation promotes longevity by enhancing transcriptional fidelity. Genes & Devel. 2015;29(13):1362–1376. doi: 10.1101/gad.263707.115

[94] Farooq Z, Banday S, Pandita TK, et al. The many faces of histone H3K79 methylation. Mutat Res/Rev Mutat Res. 2016;768:46–52. doi: 10.1016/j.mrrev.2016.03.005

[95] Ng HH, Xu R-M, Zhang Y, et al. Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine 79. J Biol Chem. 2002;277(38):34655–34657. doi: 10.1074/jbc.C200433200

[96] Wood K, Tellier M, Murphy S. DOT1L and H3K79 methylation in transcription and genomic stability. Biomolecules. 2018;8(1):11. doi: 10.3390/biom80100118(1).

[97] Norris A, Boeke JD. Silent information regulator 3: the goldilocks of the silencing complex. Genes & Devel. 2010;24(2):115–122. doi: 10.1101/gad.1865510

[98] Ontoso D, Acosta I, van Leeuwen F, et al. Dot1-dependent histone H3K79 methylation promotes activation of the Mek1 meiotic checkpoint effector kinase by regulating the Hop1 adaptor. PLOS Genet. 2013;9(1):e1003262. doi: 10.1371/journal.pgen.1003262

[99] Wysocki R, Javaheri A, Allard S, et al. Role of Dot1-dependent histone H3 methylation in G1 and S phase DNA damage checkpoint functions of Rad9. Mol Cell Biol. 2005;25(19):8430–8443. doi: 10.1128/MCB.25.19.8430-8443.2005

[100] Fields JK, Hicks CW, Wolberger C. Diverse modes of regulating methyltransferase activity by histone ubiquitination. Curr Opin Struct Biol. 2023;82:102649. doi: 10.1016/j.sbi.2023.102649

[101] Fiorucci A-S, Bourbousse C, Concia L, et al. Arabidopsis S2Lb links AtCOMPASS-like and SDG2 activity in H3K4me3 independently from histone H2B monoubiquitination. Genome Biol. 2019;20(1):100. doi: 10.1186/s13059-019-1705-4

[102] Soares LM, Buratowski S. Histone crosstalk: H2Bub and H3K4 methylation. Mol Cell. 2013;49(6):1019–1020. doi: 10.1016/j.molcel.2013.03.012

[103] Lee J-S, Shukla A, Schneider J, et al. Histone crosstalk between H2B monoubiquitination and H3 methylation mediated by COMPASS. Cell. 2007;131(6):1084–1096. doi: 10.1016/j.cell.2007.09.046

[104] Vitaliano-Prunier A, Menant A, Hobeika M, et al. Ubiquitylation of the COMPASS component Swd2 links H2B ubiquitylation to H3K4 trimethylation. Nat Cell Biol. 2008;10(11):1365–1371. doi: 10.1038/ncb1796

[105] Kim J, Kim JA, McGinty RK, et al. The n-SET domain of Set1 regulates H2B ubiquitylation-dependent H3K4 methylation. Mol Cell. 2013;49(6):1121–1133. doi: 10.1016/j.molcel.2013.01.034

[106] Qu Q, Takahashi Y, Yang Y, et al. Structure and conformational dynamics of a COMPASS histone H3K4 methyltransferase complex. Cell. 2018;174(5):1117–1126.e12. doi: 10.1016/j.cell.2018.07.020

[107] Hsu PL, Shi H, Leonen C, et al. Structural basis of H2B ubiquitination-dependent H3K4 methylation by COMPASS. Mol Cell. 2019;76(5):712–723.e4. doi: 10.1016/j.molcel.2019.10.013

[108] Worden EJ, Hoffmann NA, Hicks CW, et al. Mechanism of cross-talk between H2B ubiquitination and H3 methylation by Dot1L. Cell. 2019;176(6):1490–1501.e12. doi: 10.1016/j.cell.2019.02.002

[109] Anderson CJ, Baird MR, Hsu A, et al. Structural basis for recognition of Ubiquitylated nucleosome by Dot1L methyltransferase. Cell Rep. 2019;26(7):1681–1690.e5. doi: 10.1016/j.celrep.2019.01.058

[110] Garrido-Godino AI, Gutiérrez-Santiago F, Navarro F. Biogenesis of RNA polymerases in yeast. Front Mol Biosci. 2021;8:669300. doi: 10.3389/fmolb.2021.669300

[111] Cramer P. Organization and regulation of gene transcription. Nature. 2019;573(7772):45–54. doi: 10.1038/s41586-019-1517-4

[112] Corden JL. Tails of RNA polymerase II. Trends Biochem Sci. 1990;15(10):383–387. doi: 10.1016/0968-0004(90)90236-5

[113] Chen FX, Smith ER, Shilatifard A. Born to run: control of transcription elongation by RNA polymerase II. Nat Rev Mol Cell Biol. 2018;19(7):464–478. doi: 10.1038/s41580-018-0010-5

[114] Jasnovidova O, Stefl R. The CTD code of RNA polymerase II: a structural view. Wiley Interdiscip Rev RNA. 2013;4(1):1–16. doi: 10.1002/wrna.1138

[115] Hahn S, Young ET. Transcriptional regulation in Saccharomyces cerevisiae: transcription factor regulation and function, mechanisms of initiation, and roles of activators and coactivators. Genetics. 2011;189(3):705–736. doi: 10.1534/genetics.111.127019

[116] Liang Z, Brown KE, Carroll T, et al. A high-resolution map of transcriptional repression. Elife. 2017;6:e22767. doi: 10.7554/eLife.22767

[117] Chen X, Qi Y, Wu Z, et al. Structural insights into preinitiation complex assembly on core promoters. Science. 2021;372(6541):eaba8490. doi: 10.1126/science.aba8490

[118] Schier AC, Taatjes DJ. Structure and mechanism of the RNA polymerase II transcription machinery. Genes & Devel. 2020;34(7–8):465–488. doi: 10.1101/gad.335679.119

[119] de Jonge WJ, O'Duibhir E, Lijnzaad P, et al. Molecular mechanisms that distinguish TFIID housekeeping from regulatable SAGA promoters. Embo J. 2017;36(3):274–290. doi: 10.15252/embj.201695621

[120] Timmers HTM. SAGA and TFIID: friends of TBP drifting apart. Gcn5: Quintessential HAT. 2021;1864(2):194604. doi: 10.1016/j.bbagrm.2020.194604

[121] Donczew R, Warfield L, Pacheco D, et al. Two roles for the yeast transcription coactivator SAGA and a set of genes redundantly regulated by TFIID and SAGA. Elife. 2020;9:e50109. doi: 10.7554/eLife.50109

[122] Roeder RG. 50+ years of eukaryotic transcription: an expanding universe of factors and mechanisms. Nat Struct & Mol Biol. 2019;26(9):783–791. doi: 10.1038/s41594-019-0287-x

[123] Hu S, Peng L, Xu C, et al. SPT5 stabilizes RNA polymerase II, orchestrates transcription cycles, and maintains the enhancer landscape. Mol Cell. 2021;81(21):4425–4439.e6. doi: 10.1016/j.molcel.2021.08.029

[124] Shao W, Zeitlinger J. Paused RNA polymerase II inhibits new transcriptional initiation. Nat Genet. 2017;49(7):1045–1051. doi: 10.1038/ng.3867

[125] Jeronimo C, Collin P, Robert F. The RNA polymerase II CTD: the increasing complexity of a low-complexity protein domain. Macromol Complexes Transcription co-Transcriptional RNA Process. 2016;428(12):2607–2622. doi: 10.1016/j.jmb.2016.02.006

[126] Harlen KM, Churchman LS. The code and beyond: transcription regulation by the RNA polymerase II carboxy-terminal domain. Nat Rev Mol Cell Biol. 2017;18(4):263–273. doi: 10.1038/nrm.2017.10

[127] Allen BL, Taatjes DJ. The Mediator complex: a central integrator of transcription. Nat Rev Mol Cell Biol. 2015;16(3):155–166. doi: 10.1038/nrm3951

[128] Bentley DL. Coupling mRNA processing with transcription in time and space. Nat Rev Genet. 2014;15(3):163–175. doi: 10.1038/nrg3662

[129] Ng HH, Robert F, Ra Y, et al. Targeted recruitment of Set1 histone methylase by elongating pol II provides a localized mark and memory of recent transcriptional activity. Mol Cell. 2003;11(3):709–719. doi: 10.1016/s1097-2765(03)00092-3

[130] Decker T-M. Mechanisms of transcription elongation factor DSIF (Spt4–Spt5). RNA Polymerase II Transcription. 2021;433(14):166657. doi: 10.1016/j.jmb.2020.09.016

[131] Viktorovskaya OV, Appling FD, Schneider DA. Yeast transcription elongation factor Spt5 associates with RNA polymerase I and RNA polymerase II directly. J Biol Chem. 2011;286(21):18825–18833. doi: 10.1074/jbc.M110.202119

[132] Aoi Y, Takahashi Y-H, Shah AP, et al. SPT5 stabilization of promoter-proximal RNA polymerase II. Mol Cell. 2021;81(21):4413–4424.e5. doi: 10.1016/j.molcel.2021.08.006

[133] Grohmann D, Nagy J, Chakraborty A, et al. The initiation factor TFE and the elongation factor Spt4/5 compete for the RNAP clamp during transcription initiation and elongation. Mol Cell. 2011;43(2):263–274. doi: 10.1016/j.molcel.2011.05.030

[134] Krogan NJ, Kim M, Tong A, et al. Methylation of histone H3 by Set2 in Saccharomyces cerevisiae is linked to transcriptional elongation by RNA polymerase II. Mol Cell Biol. 2003;23(12):4207–4218. doi: 10.1128/mcb.23.12.4207-4218.2003

[135] Klein BJ, Bose D, Baker KJ, et al. RNA polymerase and transcription elongation factor Spt4/5 complex structure. Proc Natl Acad Sci U S A. 2011;108(2):546–550. doi: 10.1073/pnas.1013828108

[136] Chalabi Hagkarim N, Grand RJ. The regulatory properties of the Ccr4–not complex. Cells. 2020;9(11):2379. doi: 10.3390/cells9112379

[137] Collart MA. The Ccr4-not complex is a key regulator of eukaryotic gene expression. WIREs RNA. 2016;7(4):438–454. doi: 10.1002/wrna1332

[138] Garavís M, Calvo O. Sub1/PC4, a multifaceted factor: from transcription to genome stability. Curr Genet. 2017;63(6):1023–1035. doi: 10.1007/s00294-017-0715-6

[139] Maudlin IE, Beggs JD. Conditional depletion of transcriptional kinases Ctk1 and Bur1 and effects on co-transcriptional spliceosome assembly and pre-mRNA splicing. RNA Biol. 2021;18(sup2):782–793. doi: 10.1080/15476286.2021.1991673

[140] Keogh M-C, Kurdistani SK, Morris SA, et al. Cotranscriptional Set2 methylation of histone H3 lysine 36 recruits a repressive Rpd3 complex. Cell. 2005;123(4):593–605. doi: 10.1016/j.cell.2005.10.025

[141] Wagner EJ, Carpenter PB. Understanding the language of Lys36 methylation at histone H3. Nat Rev Mol Cell Biol. 2012;13(2):115–126. doi: 10.1038/nrm3274

[142] Gross S, Moore C. Five subunits are required for reconstitution of the cleavage and polyadenylation activities of Saccharomyces cerevisiae cleavage factor I. Proc Natl Acad Sci U S A. 2001;98(11):6080–6085. doi: 10.1073/pnas.101046598

[143] Hill CH, Boreikaitė V, Kumar A, et al. Activation of the endonuclease that defines mRNA 3' ends requires incorporation into an 8-subunit core cleavage and polyadenylation factor complex. Mol Cell. 2019;73(6):1217–1231.e11. doi: 10.1016/j.molcel.2018.12.023

[144] Tian B, Graber JH. Signals for pre-mRNA cleavage and polyadenylation. Wiley Interdiscip Rev RNA. 2012;3(3):385–396. doi: 10.1002/wrna.116

[145] Noble CG, Hollingworth D, Martin SR, et al. Key features of the interaction between Pcf11 CID and RNA polymerase II CTD. Nat Struct & Mol Biol. 2005;12(2):144–151. doi: 10.1038/nsmb887

[146] Casañal A, Kumar A, Hill CH, et al. Architecture of eukaryotic mRNA 3'-end processing machinery. Science. 2017;358(6366):1056–1059. doi: 10.1126/science.aao6535

[147] Kim M, Krogan NJ, Vasiljeva L, et al. The yeast Rat1 exonuclease promotes transcription termination by RNA polymerase II. Nature. 2004;432(7016):517–522. doi: 10.1038/nature03041

[148] Nemec CM, Yang F, Gilmore JM, et al. Different phosphoisoforms of RNA polymerase II engage the Rtt103 termination factor in a structurally analogous manner. Proc Natl Acad Sci U S A. 2017;114(20):E3944–E3953. doi: 10.1073/pnas.1700128114

[149] Chu H-F, Tong L. Molecular basis for the interaction between Saccharomyces cerevisiae Rtt103 and the Rat1-Rai1 complex. Nat Commun. 2025;16(1):3266. doi: 10.1038/s41467-025-58671-z

[150] Yanagisawa T, Murayama Y, Ehara H, et al. Structural basis of eukaryotic transcription termination by the Rat1 exonuclease complex. Nat Commun. 2024;15(1):7854. doi: 10.1038/s41467-024-52157-0

[151] Epshtein V, Dutta D, Wade J, et al. An allosteric mechanism of rho-dependent transcription termination. Nature. 2010;463(7278):245–249. doi: 10.1038/nature08669

[152] Hazelbaker DZ, Marquardt S, Wlotzka W, et al. Kinetic competition between RNA polymerase II and Sen1-dependent transcription termination. Mol Cell. 2013;49(1):55–66. doi: 10.1016/j.molcel.2012.10.014

[153] Mayer A, Heidemann M, Lidschreiber M, et al. CTD tyrosine phosphorylation impairs termination factor recruitment to RNA polymerase II. Science. 2012;336(6089):1723–1725. doi: 10.1126/science.1219651

[154] Zhang Z, Fu J, Gilmour DS. CTD-dependent dismantling of the RNA polymerase II elongation complex by the pre-mRNA 3'-end processing factor, Pcf11. Genes Dev. 2005;19(13):1572–1580. doi: 10.1101/gad.1296305

[155] Eaton JD, Francis L, Davidson L, et al. A unified allosteric/torpedo mechanism for transcriptional termination on human protein-coding genes. Genes & Devel. 2020;34(1–2):132–145. doi: 10.1101/gad.332833.119

[156] Fidler E, Dwyer K, Ansari A. Ssu72: a versatile protein with functions in transcription and beyond. Front Mol Biosci. 2024;11:1332878. doi: 10.3389/fmolb.2024.1332878

[157] Schreieck A, Easter AD, Etzold S, et al. RNA polymerase II termination involves C-terminal-domain tyrosine dephosphorylation by CPF subunit Glc7. Nat Struct & Mol Biol. 2014;21(2):175–179. doi: 10.1038/nsmb.2753

[158] Liu X, Guo Z, Han J, et al. The PAF1 complex promotes 3' processing of pervasive transcripts. Cell Rep. 2022;38(11):110519. doi: 10.1016/j.celrep.2022.110519

[159] Chen Z, Hankey W, Zhao Y, et al. Transcription recycling assays identify PAF1 as a driver for RNA pol II recycling. Nat Commun. 2021;12(1):6318. doi: 10.1038/s41467-021-26604-1

[160] Arigo JT, Eyler DE, Carroll KL, et al. Termination of cryptic unstable transcripts is directed by yeast RNA-Binding proteins Nrd1 and Nab3. Mol Cell. 2006;23(6):841–851. doi: 10.1016/j.molcel.2006.07.024

[161] Carroll KL, Pradhan DA, Granek JA, et al. Identification of cis elements directing termination of yeast nonpolyadenylated snoRNA transcripts. Mol Cell Biol. 2004;24(14):6241–6252. doi: 10.1128/MCB.24.14.6241-6252.2004

[162] Han Z, Libri D, Porrua O. Biochemical characterization of the helicase Sen1 provides new insights into the mechanisms of non-coding transcription termination. Nucleic Acids Res. 2017;45(3):1355–1370.

[163] Carroll KL, Ghirlando R, Ames JM, et al. Interaction of yeast RNA-binding proteins Nrd1 and Nab3 with RNA polymerase II terminator elements. RNA. 2007;13(3):361–373. doi: 10.1261/rna.338407

[164] Chaves-Arquero B, Pérez-Cañadillas JM. The Nrd1-Nab3-Sen1 transcription termination complex

from a structural perspective. Biochem Soc Trans. 2023;51(3):1257–1269. doi: 10.1042/BST20221418

[165] Kubicek K, Cerna H, Holub P, et al. Serine phosphorylation and proline isomerization in RNAP II CTD control recruitment of Nrd1. Genes & Devel. 2012;26 (17):1891–1896. doi: 10.1101/gad.192781.112

[166] Vasiljeva L, Kim M, Mutschler H, et al. The Nrd1-Nab3-Sen1 termination complex interacts with the Ser5-phosphorylated RNA polymerase II C-terminal domain. Nat Struct Mol Biol. 2008;15 (8):795–804. doi: 10.1038/nsmb.1468

[167] Collin P, Jeronimo C, Poitras C, et al. RNA polymerase II CTD tyrosine 1 is required for efficient termination by the Nrd1-Nab3-Sen1 pathway. Mol Cell. 2019;73 (4):655–669.e7. doi: 10.1016/j.molcel.2018.12.002

[168] Kulak NA, Pichler G, Paron I, et al. Minimal, encapsulated proteomic-sample processing applied to copy-number estimation in eukaryotic cells. Nat Methods. 2014;11(3):319–324. doi: 10.1038/nmeth.2834

[169] Han Z, Jasnovidova O, Haidara N, et al. Termination of non-coding transcription in yeast relies on both an RNA pol II CTD interaction domain and a CTD-mimicking region in Sen1. Embo J. 2020;39(7): e101548. doi: 10.15252/embj.2019101548

[170] Rengachari S, Hainthaler T, Oberthuer C, et al. Mechanism of polyadenylation-independent RNA polymerase II termination. Nat Struct & Mol Biol. 2025;32(2):339–345. doi: 10.1038/s41594-024-01409-0

[171] Aiello U, Porrua O, Libri D. Sen1: the varied virtues of a multifaceted helicase. Controlling Transcription Elongation Termination. 2025;437(1):168808. doi: 10.1016/j.jmb.2024.168808

[172] Xiong Y, Han W, Xu C, et al. Single-molecule reconstruction of eukaryotic factor-dependent transcription termination. Nat Commun. 2024;15(1):5113. doi: 10.1038/s41467-024-49527-z

[173] Falk S, Weir JR, Hentschel J, et al. The molecular architecture of the TRAMP complex reveals the organization and interplay of its two catalytic activities. Mol Cell. 2014;55(6):856–867. doi: 10.1016/j.molcel.2014.07.020

[174] Vasiljeva L, Buratowski S. Nrd1 interacts with the nuclear exosome for 3' processing of RNA polymerase II transcripts. Mol Cell. 2006;21(2):239–248. doi: 10.1016/j.molcel.2005.11.028

[175] Villa T, Barucco M, Martin-Niclos M-J, et al. Degradation of non-coding RNAs promotes recycling of termination factors at sites of transcription. Cell Rep. 2020;32(3):107942. doi: 10.1016/j.celrep.2020.107942

[176] LaCava J, Houseley J, Saveanu C, et al. RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. Cell. 2005;121(5):713–724. doi: 10.1016/j.cell.2005.04.029

[177] Vaňáčová Š, Wolf J, Martin G, et al. A new yeast Poly(A) polymerase complex involved in RNA quality control. PLOS Biol. 2005;3(6):e189. doi: 10.1371/journal.pbio.0030189

[178] Porrua O, Libri D. Transcription termination and the control of the transcriptome: why, where and how to stop. Nat Rev Mol Cell Biol. 2015;16(3):190–202. doi: 10.1038/nrm3943

[179] Bharati AP, Singh N, Kumar V, et al. The mRNA capping enzyme of Saccharomyces cerevisiae has dual specificity to interact with CTD of RNA polymerase II. Sci Rep. 2016;6(1):31294. doi: 10.1038/srep31294

[180] Cho EJ, Rodriguez CR, Takagi T, et al. Allosteric interactions between capping enzyme subunits and the RNA polymerase II carboxy-terminal domain. Genes & Devel. 1998;12(22):3482–3487. doi: 10.1101/gad.12.22.3482

[181] Gu M, Rajashankar KR, Lima CD. Structure of the Saccharomyces cerevisiae Cet1-Ceg1 mRNA capping apparatus. Struct (lond, Engl: 1993). 2010;18 (2):216–227. doi: 10.1016/j.str.2009.12.009

[182] Mao X, Schwer B, Shuman S. Yeast mRNA cap methyltransferase is a 50-kilodalton protein encoded by an essential gene. Mol Cell Biol. 1995;15(8):4167–4174. doi: 10.1128/MCB.15.8.4167

[183] Lindstrom DL, Squazzo SL, Muster N, et al. Dual roles for Spt5 in pre-mRNA processing and transcription elongation revealed by identification of Spt5-associated proteins. Mol Cell Biol. 2003;23 (4):1368–1378. doi: 10.1128/MCB.23.4.1368-1378.2003

[184] Gonatopoulos-Pournatzis T, Cowling VH. Cap-binding complex (CBC). Biochem J. 2014;457 (2):231–242. doi: 10.1042/BJ20131214

[185] Rambout X, Maquat LE. The nuclear cap-binding complex as choreographer of gene transcription and pre-mRNA processing. Genes & Devel. 2020;34(17–-18):1113–1127. doi: 10.1101/gad.339986.120

[186] Morgan JT, Fink GR, Bartel DP. Excised linear introns regulate growth in yeast. Nature. 2019;565 (7741):606–611. doi: 10.1038/s41586-018-0828-1

[187] Parenteau J, Maignon L, Berthoumieux M, et al. Introns are mediators of cell response to starvation. Nature. 2019;565(7741):612–617. doi: 10.1038/s41586-018-0859-7

[188] Sales-Lee J, Perry DS, Bowser BA, et al. Coupling of spliceosome complexity to intron diversity. Curr Biol. 2021;31(22):4898–4910.e4. doi: 10.1016/j.cub.2021.09.004

[189] Maita H, Nakagawa S. What is the switch for coupling transcription and splicing? RNA polymerase II C-terminal domain phosphorylation, phase separation and beyond. Wiley Interdiscip Rev RNA. 2020;11(1): e1574. doi: 10.1002/wrna.1574

[190] Gunderson FQ, Johnson TL, Madhani HD. Acetylation by the transcriptional coactivator Gcn5 plays a novel role in Co-transcriptional spliceosome assembly. PLOS Genet. 2009;5(10):e1000682. doi: 10.1371/journal.pgen.1000682

[191] Chanarat S, Seizl M, Strässer K. The Prp19 complex is a novel transcription elongation factor required for TREX occupancy at transcribed genes. Genes & Devel. 2011;25(11):1147–1158. doi: 10.1101/gad.623411

[192] Plaschka C, Newman AJ, Nagai K. Structural basis of nuclear pre-mRNA splicing: lessons from yeast. Cold Spring Harb Perspect Biol. 2019;11(5):a032391. doi: 10.1101/cshperspect.a032391

[193] Senn KA, Hoskins AA. Mechanisms and regulation of spliceosome-mediated pre-mRNA splicing in Saccharomyces cerevisiae. Wiley Interdiscip Rev RNA. 2024;15(4):e1866. doi: 10.1002/wrna.1866

[194] Hansen SR, White DS, Scalf M, et al. Multi-step recognition of potential 5' splice sites by the Saccharomyces cerevisiae U1 snRNP. Elife. 2022;11:11. doi: 10.7554/eLife.70534

[195] Li X, Liu S, Zhang L, et al. A unified mechanism for intron and exon definition and back-splicing. Nature. 2019;573(7774):375–380. doi: 10.1038/s41586-019-1523-6

[196] Zhang M, Green MR. Identification and characterization of yUAP/Sub2p, a yeast homolog of the essential human pre-mRNA splicing factor hUAP56. Genes & Devel. 2001;15(1):30–35. doi: 10.1101/gad.851701

[197] Zhang Z, Rigo N, Dybkov O, et al. Structural insights into how Prp5 proofreads the pre-mRNA branch site. Nature. 2021;596(7871):296–300. doi: 10.1038/s41586-021-03789-5

[198] Boesler C, Rigo N, Anokhina MM, et al. A spliceosome intermediate with loosely associated tri-snRNP accumulates in the absence of Prp28 ATPase activity. Nat Commun. 2016;7(1):11997. doi: 10.1038/ncomms11997

[199] Staley JP, Guthrie C. An RNA switch at the 5' splice site requires ATP and the DEAD box protein Prp28p. Mol Cell. 1999;3(1):55–64. doi: 10.1016/s1097-2765(00)80174-4

[200] Raghunathan PL, Guthrie C. RNA unwinding in U4/U6 snRnps requires ATP hydrolysis and the DEIH-box splicing factor Brr2. Curr Biol. 1998;8(15):847–855. doi: 10.1016/s0960-9822(07)00345-4

[201] Yan C, Wan R, Bai R, et al. Structure of a yeast activated spliceosome at 3.5 Å resolution. Science. 2016;353(6302):904–911. doi: 10.1126/science.aag0291

[202] Wlodaver AM, Staley JP. The DExD/H-box ATPase Prp2p destabilizes and proofreads the catalytic RNA core of the spliceosome. RNA. 2014;20(3):282–294. doi: 10.1261/rna.042598.113

[203] Galej WP, Wilkinson ME, Fica SM, et al. Cryo-EM structure of the spliceosome immediately after branching. Nature. 2016;537(7619):197–201. doi: 10.1038/nature19316

[204] Wilkinson ME, Fica SM, Galej WP, et al. Structural basis for conformational equilibrium of the catalytic spliceosome. Mol Cell. 2021;81(7):1439–1452.e9. doi: 10.1016/j.molcel.2021.02.021

[205] Bai R, Yan C, Wan R, et al. Structure of the post-catalytic spliceosome from Saccharomyces cerevisiae. Cell. 2017;171(7):1589–1598.e8. doi: 10.1016/j.cell.2017.10.038

[206] Liu S, Li X, Zhang L, et al. Structure of the yeast spliceosomal postcatalytic P complex. Science. 2017;358(6368):1278–1283. doi: 10.1126/science.aar3462

[207] Mayas RM, Maita H, Staley JP. Exon ligation is proofread by the DExD/H-box ATPase Prp22p. Nat Struct & Mol Biol. 2006;13(6):482–490. doi: 10.1038/nsmb1093

[208] Wagner JD, Jankowsky E, Company M, et al. The DEAH-box protein PRP22 is an ATPase that mediates ATP-dependent mRNA release from the spliceosome and unwinds RNA duplexes. Embo J. 1998;17(10):2926–2937. doi: 10.1093/emboj/17.10.2926

[209] Martin A, Schneider S, Schwer B. Prp43 is an essential RNA-dependent ATPase required for release of lariat-intron from the spliceosome. J Biol Chem. 2002;277(20):17743–17750. doi: 10.1074/jbc.M200762200

[210] Wan R, Yan C, Bai R, et al. Structure of an intron lariat spliceosome from Saccharomyces cerevisiae. Cell. 2017;171(1):120–132.e12. doi: 10.1016/j.cell.2017.08.029

[211] Choquet K, Patop IL, Churchman LS. The regulation and function of post-transcriptional RNA splicing. Nat Rev Genet. 2025;26(6):378–394. doi: 10.1038/s41576-025-00836-z

[212] Cuenca-Bono B, García-Molinero V, Pascual-García P, et al. SUS1 introns are required for efficient mRNA nuclear export in yeast. Nucleic Acids Res. 2011;39(19):8599–8611. doi: 10.1093/nar/gkr496

[213] Hossain MA, Rodriguez CM, Johnson TL. Key features of the two-intron Saccharomyces cerevisiae gene SUS1 contribute to its alternative splicing. Nucleic Acids Res. 2011;39(19):8612–8627. doi: 10.1093/nar/gkr497

[214] Rodríguez-Navarro S, Fischer T, Luo MJ, et al. Sus1, a functional component of the SAGA histone acetylase complex and the nuclear pore-associated mRNA export machinery. Cell. 2004;116(1):75–86. doi: 10.1016/S0092-8674(03)01025-0

[215] Rodríguez-Navarro S, Strässer K, Hurt E. An intron in the YRA1 gene is required to control Yra1 protein expression and mRNA export in yeast. EMBO Rep. 2002;3(5):438–442. doi: 10.1093/embo-reports/kvf091

[216] Zhelkovsky A, Tacahashi Y, Nasser T, et al. The role of the Brr5/Ysh1 C-terminal domain and its homolog Syc1 in mRNA 3'-end processing in Saccharomyces cerevisiae. RNA. 2006;12(3):435–445. doi: 10.1261/rna.2267606

[217] Rodríguez-Molina JB, O'Reilly FJ, Fagarasan H, et al. (Directors). Mpe1 senses the binding of pre-mRNA and controls 3' end processing by CPF. Mol Cell. 2022 July 7;82(13):2490–2504.e12. doi: 10.1016/j.molcel.2022.04.021 Video recording.

[218] Ezeokonkwo C, Zhelkovsky A, Lee R, et al. A flexible linker region in Fip1 is needed for efficient mRNA

polyadenylation. RNA. 2011;17(4):652–664. doi: 10.1261/rna.2273111
[219] Meinke G, Ezeokonkwo C, Balbo P, et al. Structure of yeast poly(A) polymerase in complex with a peptide from Fip1, an intrinsically disordered protein. Biochemistry. 2008;47(26):6859–6869. doi: 10.1021/bi800204k
[220] Zhang Y, Sun Y, Shi Y, et al. Structural insights into the human pre-mRNA 3'-end processing machinery. Mol Cell. 2020;77(4):800–809.e6. doi: 10.1016/j.molcel.2019.11.005
[221] Soucek S, Corbett AH, Fasken MB. The long and the short of it: the role of the zinc finger polyadenosine RNA binding protein, Nab2, in control of poly(A) tail length. Biochim et Biophys Acta (BBA) - Gene Regul Mech. 2012;1819(6):546–554. doi: 10.1016/j.bbagrm.2012.03.006
[222] Turtola M, Manav MC, Kumar A, et al. Three-layered control of mRNA poly(A) tail synthesis in Saccharomyces cerevisiae. Genes & Devel. 2021;35(17–18):1290–1303. doi: 10.1101/gad.348634.121
[223] Wende W, Friedhoff P, Sträßer K. Mechanism and regulation of Co-transcriptional mRNP assembly and nuclear mRNA export. Adv Exp Med Biol. 2019;1203:1–31. doi: 10.1007/978-3-030-31434-7_1
[224] Das B, Guo Z, Russo P, et al. The role of nuclear cap binding protein Cbc1p of yeast in mRNA termination and degradation. Mol Cell Biol. 2000;20(8):2827–2838. doi: 10.1128/MCB.20.8.2827-2838.2000
[225] Lewis JD, Görlich D, Mattaj IW. A yeast cap binding protein complex (yCBC) acts at an early step in pre-mRNA splicing. Nucleic Acids Res. 1996;24(17):3332–3336. doi: 10.1093/nar/24.17.3332
[226] Sen R, Barman P, Kaja A, et al. Distinct functions of the cap-binding complex in stimulation of nuclear mRNA export. Mol Cell Biol. 2019;39(8). doi: 10.1128/MCB.00540-18
[227] Viphakone N, Sudbery I, Griffith L, et al. Co-transcriptional loading of RNA export factors shapes the human transcriptome. Mol Cell. 2019;75(2):310–323.e8. doi: 10.1016/j.molcel.2019.04.034
[228] Querl L, Krebber H. Defenders of the transcriptome: Guard protein-mediated mRNA quality control in Saccharomyces cerevisiae. Int J Mol Sci. 2024;25(19):10241. doi: 10.3390/ijms251910241*25*(19
[229] Dermody JL, Dreyfuss JM, Villén J, et al. Unphosphorylated SR-like protein Npl3 stimulates RNA polymerase II elongation. PLOS One. 2008;3(9):e3273. doi: 10.1371/journal.pone.0003273
[230] Hackmann A, Gross T, Baierlein C, et al. The mRNA export factor Npl3 mediates the nuclear export of large ribosomal subunits. EMBO Rep. 2011;12(10):1024–1031. doi: 10.1038/embor.2011.155
[231] Li J, Querl L, Coban I, et al. Surveillance of 3' mRNA cleavage during transcription termination requires CF IB/Hrp1. Nucleic Acids Res. 2023;51(16):8758–8773. doi: 10.1093/nar/gkad530
[232] Hurt E, Luo M-J, Röther S, et al. Cotranscriptional recruitment of the serine-arginine-rich (SR)-like proteins Gbp2 and Hrb1 to nascent mRNA via the TREX complex. Proc Natl Acad Sci U S A. 2004;101(7):1858–1862. doi: 10.1073/pnas.0308663100
[233] Xie Y, Clarke BP, Kim YJ, et al. Cryo-EM structure of the yeast TREX complex and coordination with the SR-like protein Gbp2. Elife. 2021;10:10. doi: 10.7554/eLife.65699
[234] Hentze MW, Castello A, Schwarzl T, et al. A brave new world of RNA-binding proteins. Nat Rev Mol Cell Biol. 2018;19(5):327–341. doi: 10.1038/nrm.2017.130
[235] Mitchell SF, Parker R. Principles and properties of eukaryotic mRnps. Mol Cell. 2014;54(4):547–558. doi: 10.1016/j.molcel.2014.04.033
[236] Martín-Expósito M, Gas M, Mohamad N, et al. Mip6 binds directly to the Mex67 UBA domain to maintain low levels of Msn2/4 stress-dependent mRNAs. EMBO Rep. 2019;20(12):e47964. doi: 10.15252/embr.201947964
[237] Meinel DM, Burkert-Kautzsch C, Kieser A, et al. Recruitment of TREX to the transcription machinery by its direct binding to the phospho-CTD of RNA polymerase II. PLOS Genet. 2013;9(11):e1003914. doi: 10.1371/journal.pgen.1003914
[238] Li Y, Wang X, Zhang X, et al. Human hHpr1/p84/thoc1 regulates transcriptional elongation and physically links RNA polymerase II and RNA processing factors. Mol Cell Biol. 2005;25(10):4023–4033. doi: 10.1128/MCB.25.10.4023-4033.2005
[239] Peña A, Gewartowski K, Mroczek S, et al. Architecture and nucleic acids recognition mechanism of the THO complex, an mRNP assembly factor. Embo J. 2012;31(6):1605–1616. doi: 10.1038/emboj.2012.10
[240] Sträßer K, Masuda S, Mason P, et al. TREX is a conserved complex coupling transcription with messenger RNA export. Nature. 2002;417(6886):304–308. doi: 10.1038/nature746
[241] Valkov E, Dean JC, Jani D, et al. Structural basis for the assembly and disassembly of mRNA nuclear export complexes. Nucl Transp RNA Process. 2012;1819(6):578–592. doi: 10.1016/j.bbagrm.2012.02.017
[242] Heath CG, Viphakone N, Wilson SA. The role of TREX in gene expression and disease. Biochem J. 2016;473(19):2911–2935. doi: 10.1042/BCJ20160010
[243] Chanarat S, Burkert-Kautzsch C, Meinel DM, et al. Prp19C and TREX: interacting to promote transcription elongation and mRNA export. Transcription. 2012;3(1):8–12. doi: 10.4161/trns.3.1.19078
[244] Johnson SA, Cubberley G, Bentley DL. Cotranscriptional recruitment of the mRNA export factor Yra1 by direct interaction with the 3' end processing factor Pcf11. Mol Cell. 2009;33(2):215–226. doi: 10.1016/j.molcel.2008.12.007
[245] Ma WK, Paudel BP, Xing Z, et al. Recruitment, duplex unwinding and protein-mediated inhibition of the dead-box RNA helicase Dbp2 at actively transcribed chromatin. J Mol Biol. 2016;428(6):1091–1106. doi: 10.1016/j.jmb.2016.02.005

[246] Segref A, Sharma K, Doye V, et al. Mex67p, a novel factor for nuclear mRNA export, binds to both poly(A)+ RNA and nuclear pores. Embo J. 1997;16(11):3256–3271. doi: 10.1093/emboj/16.11.3256

[247] Santos-Rosa H, Moreno H, Simos G, et al. Nuclear mRNA export requires complex formation between Mex67p and Mtr2p at the nuclear pores. Mol Cell Biol. 1998;18(11):6826–6838. doi: 10.1128/MCB.18.11.6826

[248] Aibara S, Valkov E, Lamers M, et al. Domain organization within the nuclear export factor Mex67: Mtr2 generates an extended mRNA binding surface. Nucleic Acids Res. 2015;43(3):1927–1936. doi: 10.1093/nar/gkv030

[249] Senay C, Ferrari P, Rocher C, et al. The Mtr2-Mex67 NTF2-like domain complex. Structural insights into a dual role of Mtr2 for yeast nuclear export. J Biol Chem. 2003;278(48):48395–48403. doi: 10.1074/jbc.M308275200

[250] Strässer K, Bassler J, Hurt E. Binding of the Mex67p/Mtr2p heterodimer to FXFG, GLFG, and FG repeat nucleoporins is essential for nuclear mRNA export. J Cell Biol. 2000;150(4):695–706. doi: 10.1083/jcb.150.4.695

[251] Faza MB, Chang Y, Occhipinti L, et al. Role of Mex67-Mtr2 in the nuclear export of 40S pre-ribosomes. PLOS Genet. 2012;8(8):e1002915. doi: 10.1371/journal.pgen.1002915

[252] Yao W, Roser D, Köhler A, et al. Nuclear export of ribosomal 60S subunits by the general mRNA export receptor Mex67-Mtr2. Mol Cell. 2007;26(1):51–62. doi: 10.1016/j.molcel.2007.02.018

[253] Coban I, Lamping J-P, Hirsch AG, et al. dsRNA formation leads to preferential nuclear export and gene expression. Nature. 2024;631(8020):432–438. doi: 10.1038/s41586-024-07576-w

[254] Derrer CP, Mancini R, Vallotton P, et al. The RNA export factor Mex67 functions as a mobile nucleoporin. J Cell Biol. 2019;218(12):3967–3976. doi: 10.1083/jcb.201909028

[255] Fischer T, Rodríguez-Navarro S, Pereira G, et al. Yeast centrin Cdc31 is linked to the nuclear mRNA export machinery. Nat Cell Biol. 2004;6(9):840–848. doi: 10.1038/ncb1163

[256] García-Oliver E, García-Molinero V, Rodríguez-Navarro S. mRNA export and gene expression: the SAGA – TREX-2 connection. bba - Gene Regul Mech. 2012;1819(6):555–565. doi: 10.1016/j.bbagrm.2011.11.011

[257] González-Aguilera C, Tous C, Gómez-González B, et al. The THP1-SAC3-SUS1-CDC31 complex works in transcription elongation-mRNA export preventing RNA-mediated genome instability. Mol Biol Cell. 2008;19(10):4310–4318. doi: 10.1091/mbc.e08-04-0355

[258] Stewart M. Structure and function of the TREX-2 complex. Subcell Biochem. 2019;93:461–470. doi: 10.1007/978-3-030-28151-9_15

[259] Dimitrova L, Valkov E, Aibara S, et al. Structural characterization of the Chaetomium thermophilum TREX-2 complex and its interaction with the mRNA nuclear export factor Mex67: Mtr2. Structure. 2015;23(7):1246–1257. doi: 10.1016/j.str.2015.05.002

[260] Fischer T, Sträßer K, Rácz A, et al. The mRNA export machinery requires the novel Sac3p–Thp1p complex to dock at the nucleoplasmic entrance of the nuclear pores. Embo J. 2002;21(21):5843–5852. doi: 10.1093/emboj/cdf590

[261] Ellisdon AM, Dimitrova L, Hurt E, et al. Structural basis for the assembly and nucleic acid binding of the TREX-2 transcription-export complex. Nat Struct & Mol Biol. 2012;19(3):328–336. doi: 10.1038/nsmb.2235

[262] Jani D, Valkov E, Stewart M. Structural basis for binding the TREX2 complex to nuclear pores, GAL1 localisation and mRNA export. Nucleic Acids Res. 2014;42(10):6686–6697. doi: 10.1093/nar/gku252

[263] Cabal GG, Genovesio A, Rodriguez-Navarro S, et al. SAGA interacting factors confine sub-diffusion of transcribed genes to the nuclear envelope. Nature. 2006;441(7094):770–773. doi: 10.1038/nature04752

[264] Schneider M, Hellerschmied D, Schubert T, et al. The nuclear pore-associated TREX-2 complex employs Mediator to regulate gene expression. Cell. 2015;162(5):1016–1028. doi: 10.1016/j.cell.2015.07.059

[265] Akey CW, Singh D, Ouch C, et al. Comprehensive structure and functional adaptations of the yeast nuclear pore complex. Cell. 2022;185(2):361–378.e25. doi: 10.1016/j.cell.2021.12.015

[266] Beck M, Hurt E. The nuclear pore complex: understanding its function through structural insight. Nat Rev Mol Cell Biol. 2017;18(2):73–89. doi: 10.1038/nrm.2016.147

[267] Stewart M. Function of the nuclear transport machinery in maintaining the distinctive compositions of the nucleus and cytoplasm. Int J Mol Sci. 2022;23(5):2578. doi: 10.3390/ijms23052578

[268] Bensidoun P, Zenklusen D, Oeffinger M. Choosing the right exit: how functional plasticity of the nuclear pore drives selective and efficient mRNA export. WIREs RNA. 2021;12(6):e1660. doi: 10.1002/wrna.1660

[269] Ashkenazy-Titelman A, Atrash MK, Boocholez A, et al. RNA export through the nuclear pore complex is directional. Nat Commun. 2022;13(1):5881. doi: 10.1038/s41467-022-33572-7

[270] Lund MK, Guthrie C. The DEAD-Box protein Dbp5p is required to dissociate Mex67p from exported mRnps at the nuclear rim. Mol Cell. 2005;20(4):645–651. doi: 10.1016/j.molcel.2005.10.005

[271] Stewart M. Polyadenylation and nuclear export of mRNAs. J Biol Chem. 2019;294(9):2977–2987. doi: 10.1074/jbc.REV118.005594

[272] Bonneau F, Basquin J, Steigenberger B, et al. Nuclear mRnps are compact particles packaged with a network of proteins promoting RNA-RNA interactions. Genes & Devel. 2023;37(11–12):505–517. doi: 10.1101/gad.350630.123

[273] Xie Y, Clarke BP, Xie D, et al. Structures and mRNP remodeling mechanism of the TREX-2 complex. Structure. 2025;33(3):566–582.e6. doi: 10.1016/j.str.2024.12.019