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Mediator roles going beyond transcription

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Keywords

Mediator complex, chromatin architecture, phase separation, transcription-coupled DNA repair, human diseases

Abstract

Dysfunctions of nuclear processes including transcription and DNA repair lead to severe human diseases. Gaining an understanding of how these processes operate in the crowded context of chromatin can be particularly challenging. Mediator is a large multiprotein complex conserved in eukaryotes with a key coactivator role in the regulation of RNA polymerase II transcription. Despite intensive studies, the molecular mechanisms underlying Mediator function remain to be fully understood. Novel findings have provided insights into the relationship between Mediator and chromatin architecture, revealed its role in connecting transcription with DNA repair and proposed an emerging mechanism of phase separation involving Mediator condensates. Recent developments in the field suggest multiple functions of Mediator going beyond transcriptional processes *per se* that would explain its involvement in various human pathologies.

A multisubunit complex to mediate transcription regulation

In eukaryotic cells, all DNA-related processes including transcription and DNA repair occur in the crowded context of chromatin. The detailed understanding of these processes is particularly challenging, and their dysfunctions constitute the basis of many human pathologies.

The discovery of the Mediator complex nearly 30 years ago completely changed our vision of transcriptional regulation (reviewed in [1]). Mediator is a large multiprotein complex conserved in eukaryotes that plays an essential **coregulator** role in RNA polymerase (Pol) II transcription. This complex is recruited to regulatory regions called **enhancers** in metazoans or upstream activating sequences (UASs) in yeast. It serves as a functional link between **specific transcription factors (TFs)** bound to these regions and the basal transcriptional machinery at **core promoters**. Together with general transcription factors (GTFs), Mediator promotes the assembly of the **preinitiation complex (PIC)** including Pol II recruitment. It also stimulates the phosphorylation of the Pol II **C-terminal domain (CTD)** by the Kin28/CDK7 kinase subunit of TFIIF, one of the GTFs. Despite intensive studies, it remains to be deciphered how the complexity and multiple physical connections of Mediator contribute to its functions. Moreover, recent studies suggest that the spectrum of Mediator roles in eukaryotic cells could be wider than previously established. In this Opinion article, we focus on three specific aspects of Mediator function in transcription-associated events beyond its roles in transcriptional processes *per se* that, in our view, would help to understand its involvement in diverse human pathologies [2, 3] (*see Box 1*). We will discuss novel findings on the involvement of Mediator in chromatin architecture, phase-separated condensate formation and DNA repair.

Mediator and chromatin architecture

One of the key questions on the mechanism of Mediator function is related to chromatin context. The Mediator-chromatin interplay occurs at many levels, from nucleosomal organisation to 3D chromatin structure. Indeed, its coactivator function is linked to chromatin architecture at promoter regions and enhancer-promoter contacts. Moreover, Mediator binding to nucleosomes and its functional interactions with chromatin-acting complexes were previously reported [4-9].

In eukaryotic cells, nucleosomes are structural components of chromatin. Their positioning, dynamics and modifications are thus integral to the regulation of nuclear processes. Their presence can hide specific DNA sequences and disturb the binding of different factors to DNA. Consequently, gene promoters contain **nucleosome-depleted regions (NDRs)** ensuring that DNA is accessible to proteins, including chromatin regulators and the transcriptional machinery [10]. The role of Mediator in PIC assembly was proposed to be related to chromatin architecture in terms of nucleosome occupancy and dynamics [1, 11]. Indeed, a detailed analysis of the gene-specific effect of a mutation in the Med10 Mediator subunit on PIC formation in *S. cerevisiae* showed that the most strongly affected genes had a low nucleosome occupancy around the transcription start site (TSS) and dynamic first nucleosomes upstream and downstream from the NDR, so-called hot nucleosomes -1 and +1 with shorter residence time [1, 11].

To transmit regulatory signals, Mediator is recruited to enhancers via direct interactions with specific TFs and then contacts the PIC components including Pol II at respective core promoters [1]. In metazoans, it was proposed that Mediator participates in chromatin looping between enhancers and core promoters through a physical interaction with cohesin [5, 12]. In support of a model involving chromatin looping, in yeast, a single Mediator complex is known to bind to UASs and transiently contact core promoters [13, 14]. A recent study sheds new light on this question proposing that Mediator together with Pol II serve as a functional rather than just a physical bridge between enhancers and promoters and that architectural proteins like cohesin are required to establish these enhancer-promoter contacts [15]. Indeed, the depletion of Mediator from mouse embryonic stem (mES) cells does not impair promoter-enhancer interactions, suggesting that neither Mediator nor Pol II alone are absolutely required for loop formation [15]. Moreover, Pol II inhibition that leads to full depletion of Mediator and Pol II from chromatin has little or no effect on cohesin binding or promoter-enhancer interactions. In contrast, depletion of cohesin impairs these contacts [15]. These data challenge the previous model of a physical Mediator bridge between enhancers and promoters [15, 16]. Interestingly, a model in which Mediator condensates on enhancers interact transiently with the transcription machinery at promoters would further question the definition of a physical chromatin contact and suggest that an effective distance required for functional interactions between enhancers and

promoters could be of the same order as the size of condensates and therefore larger than a direct contact distance (*see next section for **phase separation** discussion*).

Large clusters of enhancers called **super-enhancers (SEs)** involved in the regulation of cell identity gene and oncogene expression are particularly enriched for Mediator, chromatin-acting complexes and specific TFs [17, 18]. Physical and functional interactions between Mediator and chromatin-modifying complexes were proposed, providing further evidence for Mediator-chromatin interplay [1, 4, 8, 19, 20]. Recent work in mouse neural stem cells (NSCs) confirmed known physical interactions between Mediator and Ep300/CBP or cohesin and led to the identification of novel contacts, including the chromatin modifiers Carm1 and Jmjd1c [8].

Next, Mediator has also been suggested to play a role in global chromatin structure. Short- and long-range interactions between nucleosomes contribute to the overall genome organisation. Not all genomic loci have the same level of compaction, and the genome is organised into self-associating domains named **Chromosomal Interacting Domains (CIDs)** in yeast and **Topologically Associating Domains (TADs)** in mammals [21-23]. In budding yeast, these domains encompass one to five genes whose compaction is anticorrelated with transcription rate and active epigenetic marks [22]. Using the **Micro-C approach**, changes in 3D-genome organisation at nucleosomal resolution were recently observed in a yeast deletion mutant for the Med1 Mediator subunit with a decompaction of most genes compared to the wild type [22]. The CIDs are separated by boundaries that are enriched for NDRs, transcription-related histone marks such as H3K4me3 and H3K18ac and correlate with promoter locations [22]. A recent analysis of yeast Mediator ChIP-Seq data showed that the majority of Mediator enrichment peaks overlapped with these boundaries, in particular with the strongest ones that are characterised by few or no interactions across the boundaries [4]. Moreover, the purification of chromatin-bound Mediator revealed its interactions with proteins involved in chromatin architecture regulation such as chromatin remodelers and gene-looping factors including **SWI/SNF** and Ssu72 [4].

In addition to being present at regulatory regions essential for gene transcription and engaged in interactions with transcription initiation factors, Mediator is bound to regulatory architectural regions and interacts with chromatin-modifying proteins. It could therefore participate in the establishment of subnuclear structures dedicated to transcription and related processes. Further work is needed to understand the exact molecular mechanisms

involved in these key aspects of Mediator function [4-9, 11, 15, 19, 20, 22]. Its own recruitment and dynamics are strongly related to chromatin organisation. Moreover, this complex might influence recruitment of chromatin-modifying and architectural proteins or their activity, thus shaping chromatin structure.

Mediator in phase separation

To understand how chromatin-related processes are organised within the nucleus, a model of protein condensates or membraneless compartments driven by phase separation has emerged. The capacity of Mediator to undergo phase separation makes it an active player in these mechanisms, as proposed for its activity at super-enhancers (SE) (**Figure 1**) [24]. As SEs are large clusters of enhancers, they allow for the *in vivo* visualisation of the condensates that potentially also occur on classical enhancers. Liquid-liquid phase separation, a process of a fluid phase formation, is thought to be driven by dynamic interactions involving proteins with **intrinsically disordered regions (IDR)** [25]. Condensate formation in cells was assumed to rely on these phase separation phenomena that were initially observed *in vitro*.

Recent studies showed that Mediator, together with master TFs, the BRD4 coactivator and Pol II, forms condensates through disordered protein regions to compartmentalise and concentrate transcriptional machinery for regulation of key cell identity genes in mammals [26-29]. Indeed, the MED1 Mediator subunit and the BRD4 coactivator were visualised in mES cells as discrete bodies at SEs exhibiting features of biomolecular liquid-like condensates [29]. Moreover, the IDR domains of MED1 and BRD4 behave as phase-separated liquid droplets *in vitro* and this domain of MED1 is important for the formation of condensates in cells [29]. Different TFs including mammalian OCT4 and yeast GCN4 form condensates with Mediator through their activation domains (AD), further emphasising the importance of phase separation mechanisms in Mediator action [26].

Mediator and Pol II clusters that have properties of phase-separated condensates were directly visualised in mES cells by live cell super-resolution imaging [27]. The majority of stable Mediator clusters colocalised with those of Pol II in living mES cells. Based on the analysis of one SE-controlled gene, a model has been proposed in which large Mediator clusters on enhancers can transiently interact with the transcription machinery at

promoters, suggesting potential contacts with multiple promoters and raising the possibility of a larger distance requirement between enhancer and promoter elements [27].

Mediator condensates on SEs can encompass many different proteins including signalling factors that transmit extracellular stimuli to these enhancers [30]. Signalling factors of WNT, TGF- β and JAK/STAT pathways can enter and concentrate in Mediator condensates through their IDRs both *in vitro* and *in vivo*. In particular, WNT coactivator β -catenin integrates into nuclear condensates in response to WNT pathway stimulation in living mES cells [30]. Moreover, selective occupancy of β -catenin on SEs depends on its IDR along with its condensate- and TF-interactions [30]. These results suggest that signalling factors can be incorporated into SE condensates established by Mediator and master TFs and that this mechanism contributes to the cell-type specificity of signalling.

Transcription is correlated with different nuclear processes, and RNA splicing represents one of the well-known co-transcriptional events associated with nuclear condensates. Pol II phosphorylation was recently proposed to regulate the transition from transcription to splicing in the context of nuclear condensates, suggesting a functional link between different membraneless compartments of the nucleus [28]. Phosphorylation of the Pol II CTD influences its interactions with transcriptional and splicing machineries. Recent work has established that Pol II CTD containing an IDR contributes to its interaction with condensates formed by purified human Mediator or MED1-IDR *in vitro* [28]. At SE-driven active genes, splicing-factor condensates were observed in mES cells [28]. Analysis of hypo- or hyperphosphorylated forms of Pol II suggests a model in which Pol II CTD phosphorylation leads to its eviction from Mediator condensates and contributes to formation of splicing-factor condensates at actively-transcribed genes associated to SEs [28]. These results thus suggest that phosphorylation could contribute to partitioning of Pol II between Mediator and splicing-factor condensates (**Figure 1**).

The latest literature clearly illustrates the complexity and rapid development of the field. The links between transcription and other nuclear functions that involve phase-separation phenomena could be rather complex and unexpected, possibly including the mechanisms of chromatin organisation or the DNA damage response. For example, chromatin on its own can undergo liquid-liquid phase separation *in vitro* [31] and the synthesis of long non-coding RNAs from functional promoters which are assembled on double-strand breaks stimulates the formation of DNA-damage-response foci [32]. The

capacity of Mediator to participate in multiple protein-protein interactions and undergo phase separation is believed to be related to the presence of disordered regions in many subunits of this complex [28, 29, 33-35]. The fact that the location of these regions is similar within Mediator subunits across different eukaryotes may reflect their importance for Mediator function in transcriptional regulation and potentially even more broadly in chromatin-related processes. It should be noted that the capacity of the Mediator to undergo phase separation was mainly analysed for MED1 subunit that is dispensable in many mammalian cell lines [15], emphasising the importance of extending the work to different Mediator subunits.

Mediator in DNA repair

Transcription influences genetic integrity by affecting DNA repair, replication, recombination and mutagenesis. Particularly, in order to ensure a rapid removal of DNA damage from transcribed regions, transcription is coupled with DNA repair. DNA lesions are deleterious for transcription as they impede Pol II progression and can lead to its arrest, affecting gene expression and thereby cell growth and survival [36-41]. Interestingly, recent findings propose that Mediator, in addition to its essential coactivator activity, links transcription with DNA repair. This novel role was proposed via physical and functional contact of Mediator with Rad2 endonuclease, the yeast homolog of human XPG protein involved in **nucleotide-excision repair (NER)** [42, 43]. This interaction was later confirmed *in vitro* with human XPG and MED17 proteins [44]. Mediator was proposed to function in **transcription-coupled repair (TCR)**, one of the two NER subpathways (*see Box 2*). The principal components of the TCR machinery are well known, but much is left to be understood on the coupling of transcription and DNA repair *in vivo*.

Two hypotheses were proposed for the physiological role of the physical interaction observed between Mediator and Rad2: Rad2 may be implicated in transcription through this interaction with Mediator and/or Mediator may play a role in DNA repair by loading Rad2 to active genes [42]. No growth phenotypes, except UV sensitivity, or transcriptional effects were observed in the *rad2* deletion context suggesting that Rad2 does not play a major role in transcription in yeast. Conversely, genome-wide analysis revealed that Mediator and Rad2 occupancies correlated on the UASs of Pol II-transcribed genes [42]. Importantly, specific point mutations in the Med17 Mediator subunit led to a reduced Rad2 occupancy on Pol II-

transcribed genes and a decreased Rad2-Mediator interaction. Moreover, these mutations led to UV sensitivity in a global genome repair (GGR)-deficient context where only the TCR pathway is active, suggesting the implication of this complex in TCR [42, 43]. Synthetic genetic interactions between Mediator and the TCR-related proteins (Rpb9 Pol II subunit or Rad26/CSB) further support a functional link between Mediator and the TCR pathway [43]. Furthermore, deletions of genes encoding the Med12, Med15 and Med2 Mediator subunits cause UV sensitivity [45] suggesting that Mediator may be involved in DNA repair beyond the functional role of its Med17 subunit.

The interplay between Mediator, Rad2 and Pol II has been investigated using yeast genetic and genomic approaches [43]. Notably, in a yeast genetic context deficient for Pol II phosphorylation led to Mediator stabilisation on core promoters [13, 14, 46, 47], Rad2 genome-wide occupancy underwent major changes, following that of Mediator, but decreased on transcribed regions together with Pol II. This work demonstrates that the loading of Rad2 on transcribed regions is strongly affected by changes in Mediator/Rad2/Pol II interfaces. Several mechanisms could be proposed for how Mediator may contribute to Rad2 recruitment to DNA. One of the interesting possibilities would be that the loading of Rad2 to transcribed regions is connected to its recruitment to UASs by Mediator and that this could involve transient Mediator-Pol II intermediates on core promoters (**Figure 2**). These findings put forward a role for Mediator linking transcription to DNA repair through the recruitment of Rad2/XPG. The proposed model further supports the idea that Mediator recruited to regulatory regions may act remotely through its transient contacts with core promoters.

Future work will determine if other NER factors intervene in the functional dynamics between Mediator, Pol II and Rad2/XPG, and more generally in linking Mediator to DNA repair. One of the best-known factors implicated in both transcription and NER is TFIIF [48]. Interestingly, both Mediator and Rad2/XPG are engaged in interactions with Pol II and TFIIF [49-59]. Moreover, a recent proteomic analysis in human cells identified several Mediator subunits in the CSB (TCR-specific protein) and Pol II interactomes upon UV irradiation [60]. The functional arrangement of these contacts within the TCR pathway remains to be investigated.

Mediator could therefore act as an assembly platform or as a regulatory element beyond transcriptional processes *per se*, linking transcription and DNA repair. The complete

orchestration of these contacts remains to be fully understood. The questions on how chromatin organisation and phase separation phenomena could contribute to the role of Mediator in DNA repair will also need to be addressed.

Concluding Remarks

Many years of Mediator studies significantly improved our understanding of the essential functions of this complex in transcription regulation. With its complicated subunit composition, modular organisation and multiple connections to different components of the nucleus, Mediator represents a key source of interesting and unexpected findings. Recent advances in elucidating the molecular mechanisms of Mediator action led to a significant expansion of our knowledge of its functions in transcriptional regulation and transcription-coupled events, suggesting more novel findings to come.

Mediator might be considered as one of the ‘integrating platforms’ of the nucleus. With its extraordinary structural flexibility and interacting capacities, this complex may be more generally involved in chromatin and nuclear organisation. Many aspects remain to be addressed in order to provide a complete picture of Mediator action within different levels of chromatin architecture, from nucleosomal distribution and dynamics to short- and long-range chromatin interactions [4, 8, 11, 15, 22] (*see Outstanding Questions*). As it is recruited to regulatory regions, Mediator can act from a distance on other chromatin regions, with its role in DNA repair also possibly relying on its transient contacts with core promoters [43]. Moreover, phase separation mechanisms would suggest that the effective distance required for functional interactions between regulatory and core promoter regions could be larger than the direct contact distance. This model of phase separation as an emerging mechanism for nuclear functions is certainly a significant milestone in our understanding of Mediator function. It remains to be shown if these phenomena observed for Mediator and its partners on super-enhancers [24, 26-30] could be extended to classical enhancers. Since Pol II was found to be partitioned between transcriptional and splicing condensates, Mediator could take part in different membraneless compartments through protein interactions with its partners to participate in various nuclear processes.

The mechanisms by which Mediator acts in transcriptional regulation might also be important for its broader roles in eukaryotic cells. It may be involved in a number of processes including RNA metabolism or DNA repair that occur co-transcriptionally or in

relation with transcription serving as an assembly platform or as a regulatory element. In this regard, interesting findings have identified connections between Mediator and the nuclear pore complex that contribute to mRNA export or transcriptional memory mechanisms [1, 61, 62]. Future studies should elucidate the molecular mechanisms of Mediator role in DNA repair as a link between transcription and NER [42, 43] and their potential expansion to different DNA repair components and pathways. These findings also raise an important question as to how a single Mediator complex could share its activities between various nuclear processes and how all of these functions may be coordinated.

Given the central role of Mediator in cell physiology, many subunits of this complex were implicated in human diseases including cancers or neurodevelopmental diseases (*see Box 1*). In many cases, the precise mechanisms involving Mediator and biological events in transcription and beyond remain to be uncovered. Moreover, novel aspects of Mediator function will help to understand pathologies in which the involvement of this complex was not previously suspected, such as in chromatin or DNA repair-related disorders. The fundamental biological questions raised by Mediator studies are highly relevant to human diseases and will potentially lead to innovative therapeutic strategies.

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Figure legends

Figure 1. Mediator in phase-separated condensates on super-enhancers.

Nuclear condensates that are proposed to be driven in part by interactions of intrinsically disordered regions (IDR) within proteins and rely on liquid-liquid phase separation are schematised for transcription activation (in pink on the left) and splicing (in light blue-green on the right). Mediator forms condensates together with master TFs, the BRD4 coactivator (in dark green) and Pol II (in purple) at super-enhancers for regulation of key cell identity genes. Mediator is represented in modular organisation with subunits from the head module (in red), middle module (in yellow), tail module (in blue) and Med14 subunit linking these 3 main modules (in orange). The Cdk8 kinase module (in green) dissociates from the complex when Mediator transiently contacts Pol II and GTFs (not shown) within the PIC on core

promoters. Signalling factors (in orange) from the WNT, TGF- β and JAK/STAT pathways that transmit extracellular stimuli to super-enhancers can concentrate in Mediator condensates. The C-terminal domain (CTD) of the largest Pol II subunit that contains an IDR is phosphorylated (P) by cyclin-dependent kinases for transition from transcription initiation to elongation. Pol II CTD phosphorylation can regulate the transition from transcription to splicing in the context of condensates formed by Pol II, transcription elongation factors (in light brown) associated with Pol II and splicing factors (in blue) that interact with Pol II CTD and RNA (in blue).

Figure 2. Mediator in a model for Rad2 DNA repair protein recruitment to Pol II transcribed genes via dynamic Mediator/Rad2/Pol II interactions.

Recent work shows that the functional interplay between Mediator and Pol II is involved in Rad2 loading on chromatin and proposes that the presence of this DNA repair protein on transcribed regions could be linked to its recruitment to the UAS. In this model, Rad2 is first recruited to the UAS by Mediator and then transferred to transcribed regions through interactions with Pol II and a transient Mediator/Pol II intermediate(s) (shown in brackets) formed at core promoters. Additional PIC components assembled at core promoters may influence Rad2 binding. Alternatively, Rad2 may be recruited to the UAS and transcribed regions through two independent events. Several mechanisms might co-exist to explain Rad2 recruitment to transcribed regions. The colour code used is as follows: Activator (Act) in blue; Rad2 in pink; Pol II in purple. Mediator subunits are coloured in light blue/red/orange/yellow/green as in Figure 1 according to the modules. The dissociation of the Cdk8 kinase module (in green) when Mediator transiently contacts Pol II and GTFs (not shown) within the PIC on core promoters is indicated. Double-headed arrows show interactions between Mediator, Rad2 and Pol II.

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Box 1 - Mediator and diseases.

Having key functions in the cell, Mediator is involved in a broad range of human diseases such as neurodevelopmental, cardiovascular, and behavioural disorders along with cancers [2, 3]. Indeed, mutations in the MED12, MED13 and CDK8 genes that encode subunits of the Mediator kinase module are associated with neurodevelopmental disorders including X-linked mental retardation, developmental delay and/or intellectual disability [63-68]. A mutation in MED17 leads to infantile cerebral atrophy [69] and a mutation in MED23 co-segregates with intellectual disability [70]. Some genes that encode Mediator subunits are either considered as oncogenes or tumour suppressors or both. Whereas CDK8 is a colorectal cancer-specific oncogene, MED29 has both pro- and antitumoral functions in pancreatic cancer [71, 72]. Consequently, targeting Mediator in this context disturbs the disease process: in prostate cancer cells, inhibition of MED1 and MED17 through RNA interference blocked the proliferation of cancer cells and increased apoptosis [73]. In the same way, lentivirus-mediated inhibition of MED19 in breast cancer cells blocked the cell cycle and cell proliferation [74]. A knockdown of MED30 by siRNA in renal cell carcinoma resulted in a decrease in the proliferation, migration and invasion [75].

Cell identity genes along with oncogenes are under the control of super-enhancers (SE) enriched for Mediator, specific transcription factors and other coregulator complexes. The inhibition of the BRD4 coactivator, a Mediator interactor, led to the preferential loss of both complexes at SEs and impaired transcription of their associated genes [17]. Moreover, specific inhibition of Mediator kinases in cases of acute myeloid leukaemia led to the upregulation of SE-associated tumour suppressor genes and subsequent cell growth arrest [76].

Targeting Mediator interactions with specific transcription factors was proposed as a potential therapeutic treatment in fungal infections [77]. The pleiotropic drug resistance transcription factor (Pdr1) orthologues are key regulators of antifungal drug resistance in the human pathogen *Candida glabrata*. The inhibition of the Pdr1-Med15 interaction perturbed Pdr1-dependent gene activation and sensitised yeasts to azole antifungals [77].

Although Mediator has been found to be involved in many pathologies and so its targeting is studied in diseased cells, its molecular pathophysiology is mostly unknown. With its multiple connections to nuclear components and key cell functions, one can suggest a future development of therapeutic potential for the Mediator complex based on the accurate understanding of its dysregulation.

Box 2 - Nucleotide excision repair subpathways.

Nucleotide Excision Repair (NER) is an evolutionarily conserved pathway that removes bulky base adducts, like pyrimidine dimers induced by UV light, which induce severe helix distortion [38, 39]. Two NER subpathways recognise the damages in different genomic conditions (*see Figure, note that only selected NER components are shown*): Global Genome Repair (GGR) removes DNA lesions in the entire genome, whereas Transcription-Coupled Repair (TCR) works on lesions located on the transcribed strand allowing Pol II to proceed through actively-transcribed genes [36-41]. The first step of NER is the recognition of the DNA lesion. This detection is distinct in two subpathways, as different factors participate in this step. In GGR, the lesion-induced DNA distortion triggers the recruitment of a specific protein complex. In TCR, it is Pol II blocked by the lesion that creates the initial signal leading to the assembly of the NER machinery. The CSB factor (and its yeast homolog Rad26) was proposed to participate in the recognition of the lesion [78-80]. Rad26/CSB and the Rpb9 Pol II subunit appear to be essential for efficient TCR repair [81]. Once the damage is recognised, DNA is opened by TFIIH activities in order to make it accessible to the NER machinery including RPA and Rad14/XPA. Dual excision is performed on the 3' side by XPG (Rad2 in yeast) and by a dimer of XPF-ERCC1 (Rad1-Rad10) on the 5' side. Interestingly, *in vitro* experiments with purified human proteins show that XPG binds to CSB and enhances its binding to the DNA bubble. XPG could therefore have a non-enzymatic role in DNA damage recognition [54]. After DNA excision, DNA is re-synthesised by a specific DNA polymerase and ligated by a DNA ligase. Mediator was proposed to be involved in TCR for Rad2/XPG recruitment in cooperation with Pol II [42, 43].

NER is essential for DNA repair, and mutations in this pathway lead to severe diseases including Xeroderma Pigmentosum (XP) and Cockayne Syndrome (CS). Patients affected by these diseases present overlapping symptoms like UV sensitivity. First, patients with XP suffer from hypersensitivity to sunlight, predisposition to skin cancer and internal

tumours associated with mutation accumulation [82]. XP patients possess mutations in genes involved in the GGR subpathway or steps common to both TCR and GGR. Secondly, patients with CS present UV sensitivity and developmental disorders [83, 84]. CS patients have mutations in the genes specifically involved in the TCR subpathway. It should be noted that mutations in the XPG gene lead to XP that is sometimes associated with CS diseases.

Glossary

Coregulator: protein or multisubunit protein complex that interacts with specific transcription factors and serves as a bridge to basal transcription machinery or acts on chromatin structure for transcription regulation. Mediator is one of these essential complexes.

Enhancer: regulatory region in metazoans bound by specific transcription factors and located at different distances from the core promoter. Transcription of enhancers produces non-coding enhancer RNAs that contribute to transcription regulation. In yeast, these regulatory regions are named Upstream Activating Sequences (UAS).

Super enhancer (SE): large cluster of enhancers highly occupied by Mediator along with other coregulators and specific transcription factors. These enhancers are essential for transcriptional regulation of key cell identity genes and tumour oncogenes.

Core promoter: DNA region for RNA polymerase recruitment and preinitiation complex assembly that is located close to Transcription Start Site (TSS) and is sufficient to direct a precise transcription initiation.

Specific transcription factor (TF): sequence-specific DNA-binding activator or repressor protein essential for regulated transcription.

Preinitiation complex (PIC): protein complex assembled on core promoter for transcription initiation. It is composed of six general transcription factors (TFIIA, TFIIB, TFIID, TFIIF, TFIIE and TFIIH), RNA polymerase II and Mediator.

C-terminal domain (CTD): domain of the largest RNA polymerase II subunit containing multiple heptapeptide repeats. Phosphorylation and other post-translational modifications of this domain are important for different steps of the transcription cycle and for RNA processing.

Nucleosome-depleted region (NDR): DNA region in core promoters depleted for nucleosomes to ensure the accessibility of DNA to different proteins, including the transcription machinery.

Chromosomal Interacting Domain (CID): genome-organising and self-associating domain identified by **Micro-C** in yeast with DNA sequences interacting with each other.

Topologically Associating Domain (TAD): genome-organisation domain identified by Hi-C in metazoans characterised by a high frequency of interactions within a TAD but insulation between TADs. Architectural proteins including CTCF and cohesin are important for genome organisation in TADs.

Micro-C approach: Hi-C-based method for 3D chromatin analysis with nucleosomal resolution in which micrococcal nuclease is used instead of restriction enzymes to fragment chromatin.

SWI/SNF (SWItch/Sucrose Non-Fermentable): ATP-dependent chromatin remodeling complex.

Phase separation (or liquid-liquid phase separation): local concentration and fluid phase formation driven by multivalent, cooperative and dynamic macromolecular interactions for organisation of biomolecular condensates or membraneless compartments with dynamic internal re-organisation and rapid exchange kinetics. Intrinsically disordered regions (IDR) within the proteins are particularly involved in these interactions.

Intrinsically Disordered Region (IDR): protein region lacking a defined 3D structure and often containing repeated sequence elements, providing a basis for multivalent weak

intermolecular interactions. IDR-containing proteins can phase separate under physiological conditions and are enriched in many biological condensates.

Nucleotide Excision Repair (NER): evolutionarily conserved DNA repair pathway that removes bulky and/or helix-distorting DNA lesions including pyrimidine dimer photoproducts induced by UV light (*see Box 2*).

Transcription coupled repair (TCR): subpathway of nucleotide excision DNA repair that removes DNA lesions on the transcribed strand that interfere with the progression of RNA polymerase II (*see Box 2*).

Figure 1

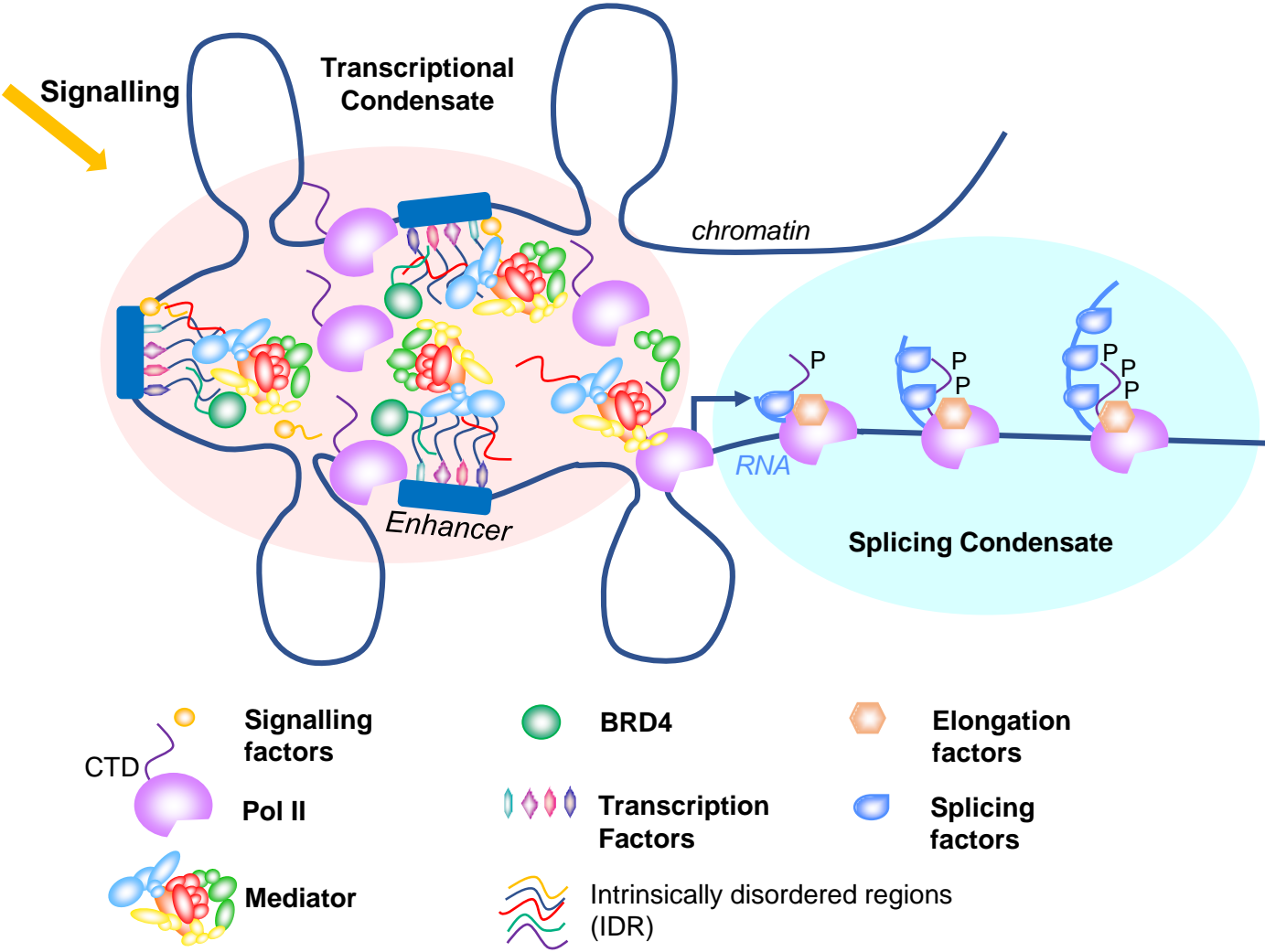


Figure 2

