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Review

Splicing and beyond: The many faces of the Prp19 complex

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ABSTRACT

The conserved Prp19 complex (Prp19C) — also known as NineTeen Complex (NTC) — functions in several processes of paramount importance for cellular homeostasis. NTC/Prp19C was discovered as a complex that functions in splicing and more specifically during the catalytic activation of the spliceosome. More recent work revealed that NTC/Prp19C plays a role in transcription elongation in *Saccharomyces cerevisiae* and in genome maintenance in higher eukaryotes. In addition, mouse PRP19 might ubiquitylate proteins targeted for degradation and guide them to the proteasome. Furthermore, NTC/Prp19C has been implicated in lipid droplet biogenesis. In the future, the molecular function of NTC/Prp19C in all of these processes needs to be refined or elucidated. Most of NTC/Prp19C's functions have been shown in only one or few organisms. However, since this complex is highly conserved it is likely that it has the same functions across all species. Moreover, one NTC/Prp19C or different subcomplexes could function in the above-mentioned processes. Intriguingly, NTC/Prp19C might link these different processes to ensure an optimal coordination of cellular processes. Thus, many important questions about the functions of this interesting complex remain to be investigated. In this review we discuss the different functions of NTC/Prp19C focusing on the novel and emerging ones as well as open questions.

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1. Introduction

Cellular homoeostasis requires the finely tuned regulation and correct interplay of all metabolic processes. For example, gene expression, protein degradation and genome maintenance are of major importance for a correctly expressed proteome. Interestingly, one protein complex is involved in a variety of these cellular processes: The Prp19 complex (Prp19C) or NineTeen Complex (NTC), named after its founding member Prp19. In this review we will refer to the complex as NTC/Prp19C and to its subunit as Prp19. NTC/Prp19C is a large protein complex consisting of eight core proteins and up to 19 associated proteins in *Saccharomyces cerevisiae* and more than 30 proteins in higher eukaryotes including animals and plants (see Table 1; [1,2] and references therein).

The best described role of NTC/Prp19C is its function in splicing (Fig. 1A; [3–5] and references therein; also see Section 3.1). Splicing is a critical step in gene expression as introns are removed by this process with an amazing precision. Prp19C NTC/Prp19C function is crucial for the splicing reaction by regulating the formation and progression of essential spliceosome conformations (see Section 3.1). Prp19 was also identified in screens for mutations that lead to increased sensitivity to DNA damage [6,7], and a direct function of NTC/Prp19C in genome maintenance has now been shown (Fig. 1B; see also Section 3.2). Furthermore, first evidence suggests that NTC/Prp19C has a direct function in the degradation of proteins by the proteasome (Fig. 1C; [8,9]; see also Section 3.3). Interestingly, one report found a function for murine Prp19 in yet another metabolic process, lipid droplet formation ([10]; see also Section 3.4). Last but not the least, NTC/Prp19C functions in transcription elongation and is thus important for two steps of gene expression (Fig. 1D; [11,12]; also see Section 3.5).

Taken together, NTC/Prp19C functions in diverse processes including splicing, transcription, DNA repair, protein degradation and lipid droplet formation (Fig. 1; [13]). It is thus of fundamental importance for cellular survival. In this review we illuminate the role of NTC/Prp19C in these processes and discuss the many open questions about this multitasking protein complex.

2. Identification, composition and conservation of Prp19C

The precursor RNA processing 19 (Prp19) protein was first identified as *PSO4* in a screen for mutants conferring sensitivity to X-rays and *pso*ralen as well as other interstrand cross-link (ICL) inducing reagents [6] and thus implicated in DNA repair (see Section 3.2). In addition, Prp19 was first identified as a splicing factor based on its tight interaction with the spliceosome ([14], see Section 3.1). Using genetic and biochemical approaches more components of NTC/Prp19C were identified for their ability to complement the splicing

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Table 1
Composition and conservation of Prp19 complexes. In *S. cerevisiae*, one NTC/Prp19C has been identified so far that consists of eight core subunits (indicated in bold) and at least up to 19 associated proteins in total. Essential proteins are marked with an "E". In humans, at least three different protein complexes have been isolated: the PRP19/CDC5L complex, the PRP19-associated complex and the XAB2 complex. Known domains of each protein are listed in the rightmost column.

Organism	*S. cerevisiae*	*H. sapiens*	*H. sapiens*	*H. sapiens*	Known domains
Complex	S.c. Prp19C [16]	PRP19/CDC5L [17]	PRP19-associated [17]	XAB2 [11]	
Known function(s)	Splicing, transcription, transcription coupled DNA repair (TCR)	Splicing	Splicing	Splicing, transcription, transcription coupled DNA repair (TCR)	
Components	**Prp19/Pso4**[E]	**PRP19/PSO4/SNEV**	–	PRP19/PSO4/SNEV	U-box and WD40
	Syf1/Ntc90[E]	–	XAB2/SYF1/HCRN	XAB2/HCRN	TPR
	Isy1/Ntc30	–	ISY1/FSAP33	ISY1/FSAP33	–
	Cef1/Ntc85[E]	**CDC5L**	–	–	c-Myb DNA binding motif
	Prp46/Ntc50[E]	**PRL1/PRP46/CWC1**	–	–	WD40
	Snt309/Ntc25	**SPF27/SNT309/ DAM1**	–	–	–
	Cwc15	AD002/CWF15	–	–	–
	Clf1/Ntc77/Syf3[E]	–	SYF3/CRNKL1/CLF	–	TPR
	Syf2/Ntc31	–	SYF2/fSAP29/p29	–	–
	Cwc2/Ntc40[E]	–	RBM22/fSAP47/CWC2	–	Zn finger and RRM
	Ntc20	–	–	–	–
	Yju2[E]	–	–	–	–
	–	–	PPIE/CypE/Cyclophilin-33	PPIE/Cyclophilin-33	PPIase
	–	–	hAquarius/fSAP164	hAquarius/fSAP164	–
	–	–	–	CCDC16/ZNF830	Zn finger and coiled-coil
	–	CTNNB1	–	–	Beta-catenin like
	–	HSP73	–	–	a member of HSP70 family; nucleotide binding
	–	–	PRP45/SKIP/SNW1	–	SKIP homology
	–	–	G10/CWC14/fSAP17/ BUD31	–	–
	–	–	CCDC12	–	coiled-coil
	–	–	PPIL1/CYPL1	–	PPIase
	–	–	GCIP	–	–
	–	–	MGC23918	–	–
	–	–	KIAA0560	–	–

E: essential.

defect of *prp19* mutant cells. Tandem affinity purification (TAP) of NTC/Prp19C from budding and fission yeast using TAP-tagged Cef1 and Cdc5 suggested a conservation of at least 26 Prp19-associated proteins [15]. When active spliceosomes (B^{act}, also see below) were isolated by centrifugation and affinity purification from yeast, nine yeast NTC/Prp19C were identified by mass spectrometry: Prp19, Cef1, Snt309, Prp46, Syf1, Syf2, Syf3/Clf1, Isy1 and Ntc20 (Table 1; [16]). All other NTC/Prp19C associated proteins appear to have a more dynamic

Fig. 1. The Prp19 complex (Prp19C) functions in many different cellular processes. (A) NTC/Prp19C was first identified as a non-snRNP splicing complex. Recent studies, however, have revealed that the complex also plays critical roles in (B) genome maintenance, (C) protein degradation by the proteasome and (D) transcription elongation. In addition, NTC/Prp19C functions in lipid droplet biogenesis. See text for details.

interaction with the NTC/Prp19C core subunits and the spliceosome (see Section 3.1).

Human cells show an even more heterogeneous complex formation of NTC/Prp19C as at least three different Prp19-like complexes exist (Table 1). The Prp19 complex itself consists of PRP19, CDC5L, HSP73, β-catenin-like-1 CTNNBL1, PRL1, AD002 and SPF27 [17]. Stringent purification conditions identified a core complex comprised of PRP19, CDC5L, PRL1 and SPF27 [18]. It is interesting to note that all four of these core subunits of hNTC/Prp19C are conserved and also present in the core complex of yeast NTC/Prp19C (Table 1). There are dramatic alterations in protein content during the splicing reaction as revealed by mass spectrometric analyses of spliceosome complexes of different stages of the splicing reaction, the human A [19], B [20], B^{act} [21], and C [20] complexes. At each step of splicing the NTC/Prp19C components associated with the spliceosome vary greatly. For instance, during the transition from complex A to complex B the U4/U6.U5 tri-snRNP and more than 35 non-snRNP proteins are recruited to the spliceosome. Among these 35 proteins are components of the PRP19/CDC5L complex as well as the so-called PRP19-associated complex, such as XAB2, ISY1, CRNKL1, SYF2, PPIE, hAquarius, SKIP, CCDC12 and PPIL1. These join the spliceosome during formation of the B complex. When complex B is activated to B^{act}, several further Prp19-associated factors associate with the spliceosome indicating a connection between the dynamics of NTC/Prp19C formation and RNP remodeling events during spliceosome activation (see also Section 3.1; [16,17]).

Interestingly, hPRP19 was also found in a mass spectrometry analysis of affinity-purified XAB2, a protein interacting with the DNA repair factor Xeroderma pigmentosum group A (XPA). The XAB2 complex is a multi-functional protein complex comprised of five core components, *i.e.* PRP19, ISY1, the peptidyl-prolyl *cis-trans* isomerase PPIE, CCDC16, a component of spliceosome complex B, and hAquarius (Table 1). It is worth noting that half of the complex components, namely PPIE, CCDC16 and hAquarius, are found only in higher eukaryotes [11]. Even though PRP19 is the only subunit that both PRP19/CDC5L and XAB2 complexes share, all subunits of the XAB2 complex can also be detected during spliceosome assembly. It is still unclear, however, what the exact functions of both complexes are as well as whether they function differently, similarly or redundantly in splicing.

In contrast to human, only one NTC/Prp19C has been identified to date in *S. cerevisiae*. This could reflect the fact that splicing control in human cells is more complex than it is in yeast. However, NTC/Prp19C plays a role in many different processes in yeast, including splicing, transcription and transcription coupled DNA repair. It is thus tempting to speculate that distinct subcomplexes of NTC/Prp19C might also exist in yeast. If this were the case, different subcomplexes could function in different pathways or have overlapping activities.

3. Cellular functions of Prp19C

3.1. Function of NTC/Prp19C in splicing

The best characterized function of NTC/Prp19C is splicing [3–5]. Most mRNAs in eukaryotes contain exons and introns. These pre-mRNAs are processed to generate mature mRNA *via* splicing, a critical process of gene expression that removes introns and ligates exons precisely together. The splicing reaction is catalyzed by the spliceosome and occurs by two sequential transesterification reactions (see below). For each splicing event the spliceosome newly assembles on the pre-mRNA (Fig. 2; also see below). Thus, both the composition and the conformation of the spliceosome during splicing are very dynamic, resulting in its high fidelity and flexibility required for the splicing of a wide variety of pre-mRNAs [2].

The spliceosome is a large macromolecular machine consisting of five small nuclear RNP (snRNPs) and several non-snRNP factors. Each snRNP is composed of a uridine-rich snRNA, *i.e.* U1, U2, U4, U5 or U6, and a specific set of interacting proteins. The snRNPs assemble dynamically on the

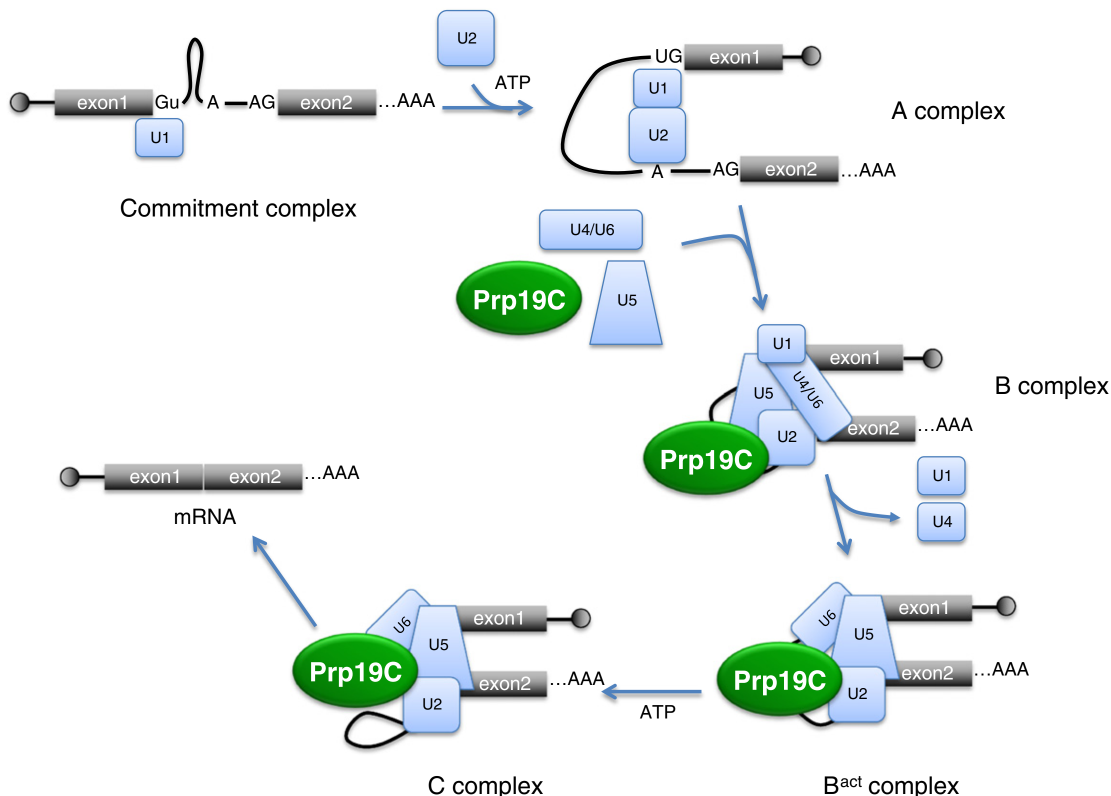

Fig. 2. NTC/Prp19C is a non-snRNP splicing complex crucial for multiple steps in splicing. NTC/Prp19C associates with the assembling spliceosome during or after the dissociation of the U4 snRNP, stabilizes the U5/U6 snRNP in the activated spliceosomal complex (B^{act}) and remains associated with the spliceosome during the second step of splicing.

pre-mRNA together with the non-snRNP protein factors, catalyze the two transesterification reactions producing the mature mRNA by excision of the intron(s). First, the U1 snRNP recognizes the intron by base-pairing of the U1 snRNA with the 5′ splice site within the intron (Fig. 2). Second, the U2 snRNP interacts with the conserved branch-point sequence forming complex A. The U2 snRNA hybridizes with the branch-point sequence protruding a conserved intronic adenosine residue required for the first step of splicing. This branch point adenosine serves as the nucleophile which attacks the 5′ splice site during the first transesterification reaction. A preformed tri-snRNP particle composed of the U4, U5 and U6 snRNPs joins complex A to form complex B. To form the active spliceosome (B^{act}), the pre-assembled U4/U6 base-pairing interaction is unwound, permitting the U6 snRNA to form two new associations: One with the U2 snRNA and the other one with the 5′ splice site, provoking the destabilization of U1 and U4. Then the branch-point adenosine performs the nucleophilic attack on the first nucleotide of the intron at the 5′ splice site forming the lariat intermediate. Subsequently, several rearrangements occur resulting in complex C that catalyzes the second transesterification reaction, in which the two exons are ligated and the intron is removed [2].

In addition to the snRNPs, non-snRNP splicing factors also play critical roles in the splicing process. One of them is the Prp19 complex and Prp19-associated proteins (Table 1 and [17]). Prp19-associated proteins either physically interact with Prp19 or are present in the human 35S U5 snRNP, a complex that is released from the spliceosome together with the U5 snRNP [17]. NTC/Prp19C and Prp19-associated proteins join the spliceosome together with the U4/U6.U5 tri-snRNP before or during unwinding of U4 from U6 and remain associated with the spliceosome during both steps of splicing [16]. The U4/U6.U5 tri-snRNP is extensively remodeled throughout splicing. During catalytic activation of the spliceosome U4/U6 base-pairing is disrupted leading to hybridization of the U6 snRNA with the pre-mRNA and the U2 snRNA. It has been shown in yeast that NTC/Prp19C is essential for a stable interaction of the U5 and U6 snRNPs with the spliceosome after activation [4]. In addition, the U5 snRNP is remodeled during activation, an event necessary for its stable association with NTC/Prp19C and Prp19-associated proteins. Taken together, NTC/Prp19C is crucial for catalytic activation of the spliceosome and thus for both splicing reactions by facilitating rearrangements within the spliceosome.

Interestingly, Prp19 contains an N-terminal U-box/RING finger domain and has an E3 ubiquitin ligase activity [22]. Its first — and so far only — identified substrate is the splicing factor hPrp3. hPrp19 adds a non-proteolytic K63-linked ubiquitin chain to hPrp3, a U4 snRNP subunit, enhancing its interaction with hPrp8, a core protein of the tri-snRNP, which is important for stabilization of the U4/U6.U5 tri-snRNP [23]. Ubiquitylated hPrp3 is recognized by hPrp24, a U4/U6 snRNP component, and deubiquitylated by hUsp4 before spliceosome activation [23]. As mentioned above, the spliceosome undergoes extensive conformational changes during the splicing reaction. Since protein ubiquitylation is considered to be an important requirement for these conformational changes, the function of Prp19C in facilitating rearrangements within the spliceosome could be mediated by its ubiquitylation activity. Another domain essential for Prp19 function is its central coiled-coil domain, which is crucial for its tetramerization and thus for NTC/Prp19C and spliceosome assembly [18,24].

In addition to Prp19, other components of NTC/Prp19C play crucial roles in splicing. Snt309 interacts genetically and physically with Prp19. Yeast *prp19* and *snt309* mutant cells accumulate free U4 snRNA, whereas U6 snRNA is downregulated in the *snt309* mutant strain. Since overexpression of the U6 snRNA suppresses the growth defect of *snt309* cells NTC/Prp19C might also function in recycling of spliceosomal snRNPs [25]. Snt309 is also required for a stable association of Prp19 with Cef1, another component of Prp19C [26]. Mutation of *CEF1* in yeast causes an accumulation of retained introns in partially spliced pre-mRNAs and leads to a G2 cell cycle arrest [27]. Syf1/Ntc90/XAB2, a tetratricopeptide-repeat (TPR) containing protein, is essential in yeast and interacts with most NTC/Prp19C components except Prp19 and Snt309 [28]. Syf1 is crucial for recruitment of Yju2, a coiled-coil protein transiently associated with NTC/Prp19C before the first step of splicing [29,30]. Yju2 is in turn required for splicing subsequent to spliceosome activation [30]. Another TPR containing protein of the Prp19 complex is Syf3. This essential protein is thought to be one of the first NTC/Prp19C subunits important for tri-snRNP loading during complex A to B transition [31].

Interestingly, in human cells at least three different Prp19 (sub) complexes exist (Table 1), all of which are crucial for splicing [2]. As mentioned above, NTC/Prp19C, Prp19-associated proteins and the XAB2 complex are crucial for splicing by mediating early spliceosome assembly, complex activation as well as recycling. However, it still remains to be determined, how each of the three Prp19 subcomplexes function in splicing mechanistically, whether subcomplex formation is conserved during evolution and/or how dynamics of complex composition influence each step of splicing.

Taken together, splicing is probably the main function of NTC/Prp19C since splicing is the best characterized role of NTC/Prp19C, subunits are shared by NTC/Prp19C and XAB2 complexes, and many of the XAB2 complex proteins are involved in different splicing complexes.

3.2. Function of NTC/Prp19C in genome maintenance

Since DNA is the repository of genetic information of each cell, its integrity and stability is crucial. However, the genome is subject to constant damage caused by several sources such as UV radiation, free radicals generated during cellular metabolism, DNA replication errors and exogenous carcinogenic reagents. The accumulation of DNA damage results in cell cycle arrest, apoptosis and a number of diseases in multicellular organisms. Therefore, cells have developed elaborate mechanisms for tolerating and repairing DNA damage to maintain the integrity of the genome.

The cell cycle and DNA repair defects observed in yeast *prp19* mutants were originally explained by Prp19C's function in splicing in combination with the fact that many proteins involved in genome maintenance are encoded by intron-containing genes (for recombination *MEI4*, *MER2*, *REC114* and *DMC1*, for repair *MMS2*, *RFA2*, *RAD14* and *KIN28* and for chromosome segregation *UBC9*, *GLC7*, *HOP2* and *CIN2*). In addition, many components of the XAB2 complex, the Prp19 subcomplex mainly involved in genome maintenance (see below), participate in splicing, underlining the possibility that the function of NTC/Prp19C in DNA repair might be indirect. However, several studies suggest that NTC/Prp19C might directly function in these processes.

Several lines of evidence indicate a connection between NTC/Prp19C and genome stability. Prp19 was first identified in *S. cerevisiae* in a genetic screen for mutations conferring sensitivity to the DNA damaging reagent *pso*ralen as well as other interstrand cross-link (ICL) inducing reagents and thus termed Pso4 [7,32]. Interestingly, the human homolog of Prp19 has been purified from human cell extracts as a component of the nuclear matrix and was named NMP200 [33]. NMP200/hPRP19 interacts with the terminal deoxynucleotidyl transferase (TdT) and is involved in mediating cell survival after DNA damage [34]. In addition, loss of hPso4/hPRP19 results in accumulation of double-strand breaks (DSBs) [34]. hPso4 also interacts with metnase, a protein which contributes to DSB repair and restart of stalled replication forks, and is necessary to bring metnase to DSB sites (Beck et al., 2008, JBC, 9023). In addition, the human Pso4 complex, consisting of hPso4/hPrp19, Cdc5L, Plrg1, and Spf27, is required for repair of DNA interstrand crosslinks [35]. These functions of NTC/Prp19C in genome maintenance might explain its implication in cell cycle progression [36] and ultimately in aging and life span [37,38].

Furthermore, another component of NTC/Prp19C was identified as a protein interacting with the DNA repair factor XPA in a yeast two hybrid screen and thus named XAB2 for Xeroderma pigmentosum group A (XPA)-binding protein 2 [39]. Xeroderma pigmentosum is a rare

human genetic disease characterized by hypersensitivity to sunlight, accelerated skin cancer and neurodegeneration [40]. XPA is central to both nucleotide excision repair pathways, transcription-coupled repair (TCR) and global genome repair (GGR) [41]. XAB2 interacts with the TCR-specific proteins Cockayne syndrome group A (CSA) and B (CSB) proteins, RNA polymerase II (RNAPII) and the nucleotide excision repair machinery [42,43]. Both *in vitro* and *in vivo* XAB2 plays a crucial role not only in pre-mRNA splicing, but also in TCR [42]. Knock-down of XAB2 by siRNAs conferred UV sensitivity and microinjection of anti-XAB2 antibodies into wild-type cells resulted in reduced TCR activity [11]. Thus, accumulating evidence suggests multiple functions of NTC/Prp19C components in DNA repair.

Independent lines of evidence show that not only Prp19 and Syf1, but also other components of NTC/Prp19C are involved in DNA repair. p29, the human homologue of Syf2 and a component of NTC/Prp19C that is not present in the human XAB2 complex, has been implicated in DNA replication in mammalian cells [44]. p29 interacts with DNA replication licensing factor MCM3 and localizes to proliferating cell nuclear antigen (PCNA) foci during S phase. p29 knock-down leads to reduced DNA synthesis rates and renders cells more sensitive to UV irradiation [44]. Furthermore, in Fanconi anemia (FA) defective cells monoubiquitylation of the FA ID complex, a heterodimer of Fanconi anemia I and Fanconi anemia D2 proteins, is reduced but can be rescued by constitutively expressing p29. This finding indicates an involvement of p29 in the Fanconi anemia pathway [45]. In addition, Clf1, which is also absent from the human XAB2 complex, might play a role in DNA replication in budding yeast [46].

Interestingly, NTC/Prp19C also interacts with the THO complex in yeast, which plays a crucial role in genome maintenance [12,47]. THO is a subcomplex of TREX, a conserved complex coupling *tr*anscription to nuclear mRNA *ex*port in *S. cerevisiae* (see Section 3.4). In budding yeast, deletion of *HPR1* or *THO2*, two THO subunits, increases UV sensitivity, causes hyper-recombination and impairs TCR [48]. Moreover, THO complex depletion in human cells increases genome instability associated with DNA breaks resulting in hyper-recombination. This phenotype is also manifested by an increase in the number of foci containing histone γH2AX as well as the DNA-damage checkpoint protein 53BP1 [49]. As NTC/Prp19C stabilizes the recruitment of the THO/TREX complex to transcribed genes in yeast [12], it is likely that the association of these two complexes is also essential for their function in genome maintenance.

Moreover, NTC/Prp19C and THO are involved in antibody diversification, a crucial process of protection from a variety of pathogens. A key player in this process is a B cell-specific enzyme called activation-induced deaminase (AID), which removes the amino group from a cytidine on single-stranded DNA (ssDNA) and converts it to uridine [50]. Interestingly, THO mutants generate R-loops, which contain ssDNA targeted by AID and show strong hypermutation [51]. Besides, AID interacts with CTNNBL1, a member of human NTC/Prp19C. AID alleles that cannot bind to CTNNBL1 show reduced hypermutation and antibody class switching [52]. Moreover, hPrp19 interacts with TdT, which adds nucleotides to DNA ends generated during V(D)J recombination [34]. Thus, NTC/Prp19C most likely functions in antibody diversification, its precise role in this process, however, remains to be determined.

In addition, many other questions remain. It is still unclear how NTC/Prp19C mechanistically functions in genome maintenance. It is possible that NTC/Prp19C serves as a scaffold for the recruitment of essential DNA repair factors, *e.g.* XPA-related complexes or THO-associated TCR pathway proteins, to the site of damage. Another intriguing question is whether the E3 ubiquitin ligase activity of Prp19 is important for its function in genome maintenance. If so, the substrates and deubiquitylating enzymes have yet to be determined.

3.3. Function of NTC/Prp19C in protein degradation by the proteasome

The most prominent function of the ubiquitin–proteasome system (UPS) is to conjugate ubiquitin to lysine residues within substrate proteins, targeting them for degradation by the proteasome, a large multicatalytic proteinase complex [53]. The 76-amino acid ubiquitin protein is linked to a substrate through the activity of three enzymes, *i.e.* the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2), and the ubiquitin ligase (E3). First, the E1 enzyme forms a thioester bond with the C-terminus of ubiquitin in an ATP-dependent manner and transfers the ubiquitin to an E2 enzyme. The E2 then either transfers ubiquitin to the E3 or binds the E3 and transfers the ubiquitin directly to the substrate. In either case, substrate specificity is determined by the E3 enzyme. This process is repeated several times to conjugate multiple ubiquitin molecules to the substrate, which is essential for protein degradation by the 26S proteasome [54,55].

Prp19 contains a U-box domain at its N-terminus, which is structurally related to the RING finger motif found in certain E3 ubiquitin ligases, and exhibits E3 ligase activity *in vitro* and *in vivo* [22]. Therefore, it is tempting to speculate that Prp19 also participates in the canonical ubiquitin–proteasome pathway as many E3 ligases do. It has been shown that *in vivo* and *in vitro* mouse Prp19 (mPrp19) interacts with mouse SUG1 (mSUG1), a subunit of 19S regulatory part of the proteasome. Inhibition of the proteasome led to accumulation of mPrp19 together with mSUG1 in speckle-like structures in the cytoplasm. Interestingly, the activity of the proteasome was increased in cells overexpressing mPrp19 [9]. In addition, hPRP19 as well as its yeast homolog interact with PSMB4/Pre4/beta7, a component of the 20S catalytic core of the proteasome, is thought to function in recruitment of ubiquitylated proteins to the proteasome [8]. These data suggest that Prp19 functions in protein degradation and may transport its substrates to the proteasome by binding to proteasomal subunits. As proteins play critical roles in all biological processes, a finely tuned equilibrium between synthesis and degradation and thus NTC/Prp19C function is essential.

Although likely, it is still unclear whether the E3 ligase activity of the Prp19 U-box domain is necessary for its function in protein degradation and what the substrates of mPrp19 are. In addition, Prp19 might not function in protein ubiquitylation alone but as part of a complex. Thus, it will be interesting to determine whether other components of the NTC/Prp19C also function in protein degradation and whether — yet another — NTC/Prp19 complex exists.

3.4. Function of NTC/Prp19C in the biogenesis of lipid droplets

Lipid droplets are ubiquitous organelles found in most eukaryotic cells including unicellular organisms such as yeast. They vary remarkably in size and consist of a phospholipid monolayer surrounding a neutral lipid core, *e.g.* sterol esters or triacylglycerols. A number of proteins are present on the surface of lipid droplets and play a critical role in lipid droplet biology. These proteins are structurally similar to plasma lipoproteins secreted by cells, which transport lipids to different regions of the body in higher eukaryotes. Therefore, the biogenesis of lipid droplets is crucial for cellular homeostasis and needs to be highly controlled [56,57].

Interestingly, Prp19 is not only present in the nucleus, but also in the cytoplasm, where it is associated with lipid droplets and functions in lipid droplet biogenesis. Knock-down of mouse Prp19 repressed lipid droplet formation concomitantly with the reduction of structural proteins of lipid droplets and the level of triacylglycerols. Moreover, Prp19 is necessary for the maturation of lipid droplets and fat storage in differentiating preadipocytes [10]. However, as only one report so far has shown the localization of mouse Prp19 to lipid droplets and a function in their biogenesis, this function of Prp19 needs to be corroborated. Alternatively, Prp19 could be "stored away" in lipid

droplets without a function in their biogenesis. By analogy, histones, which are toxic in their DNA-unbound state, are sequestered to lipid droplets during early embryogenesis of fruit flies when not used [58]. Thus, it needs to be determined if and if so how Prp19 regulates lipid droplet biogenesis and whether other complex components (and if so which ones) play a role in this process.

3.5. Function of NTC/Prp19C in transcription elongation and mRNA export

NTC/Prp19C functions in transcription and is thus important for a second process of major importance for gene expression in addition to splicing. In higher eukaryotes, XAB2 not only interacts with XPA, CSA and CSB (see Section 3.2) but also with RNAPII [39]. Consistently, micro-injection of anti-XAB2 antibodies not only inhibits TCR (see Section 3.2) but also transcription [39]. Interestingly, XAB2 co-localizes with the hyperphosphorylated RNAPII [11] suggesting a function of XAB2 in transcription elongation. XAB2 is part of a protein complex comprising hAquarius (IBP160), hPRP19, CCDC16, hISY1, and PPIE [11]. Thus, the whole XAB2 complex might function in transcription elongation in higher eukaryotes. In addition, two NTC/Prp19C components, SNT309 and SKIP, are transcriptional co-activators of nuclear receptors and are directly recruited to promoters of protein-coding genes in human cells [59–61]. Thus, in addition to the most likely general function of the XAB2 complex in transcription elongation NTC/Prp19C plays a role in activated transcription initiation of specific genes.

In yeast NTC/Prp19C functions in transcription elongation by ensuring the occupancy of the so-called TREX complex at transcribed genes (Fig. 3, [12,62]). TREX is a protein complex coupling *tr*anscription to mRNA *ex*port [12,62–66]. In yeast, TREX consists of the subcomplex THO (comprised of Tho2, Hpr1, Mft1, Thp2 and Tex1), the mRNA export factors Sub2 and Yra1 and the mRNA-binding proteins Gbp2 and Hrb1 [63]. TREX is recruited to transcribed genes and moves along genes together with the transcription machinery [63]. It is thought to bind co-transcriptionally to the synthesized mRNA — thereby preventing R-loops formed by RNA–DNA hybrids leading to hyper-recombination (see also Section 3.2). Thus, TREX functions in co-transcriptional assembly of the mRNA into an mRNP. NTC/Prp19C interacts with TREX as well as RNAPII and is recruited to intron-containing and intronless genes [12]. In cells expressing a C-terminal truncated Syf1 NTC/Prp19C still interacts with TREX, but its interaction with RNAPII and its recruitment to genes is strongly diminished. Concomitantly, TREX occupancy at genes is decreased. The lower TREX levels at genes are probably the reason for the transcription defect observed in the *syf1* mutant. Since TREX occupancy in the *syf1* mutant mainly decreases at the 3′ end of genes NTC/Prp19C most likely stabilizes TREX at genes rather than recruiting it. Thus, it remains to be shown which factor(s) is/are responsible for TREX recruitment (indicated by a "?" in Fig. 3). Taken together, yeast NTC/Prp19C functions in transcription elongation by ensuring the occupancy of TREX (Fig. 3, [12]).

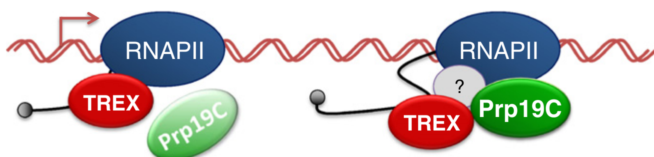

Fig. 3. NTC/Prp19C functions in transcription elongation. NTC/Prp19C stabilizes the occupancy of TREX, a conserved multisubunit protein complex coupling *tr*anscription to nuclear mRNA *ex*port, at transcribed genes. The question mark indicates the fact that further proteins might be involved in recruiting TREX to and stabilizing its interaction with the transcription machinery.

At least for the nuclear export of intron-less mRNAs NTC/Prp19C's function is probably conserved in higher eukaryotes. Indirect evidence for this model provides the findings that drosophila U2AF50, the homolog of the splicing factor U2AF65, functions in the export of intronless mRNAs, *i.e.* independent of splicing [67] and that NTC/Prp19C interacts with U2AF65 [68,69]. More direct evidence is provided by the analysis of cytoplasmic accumulation region elements (CAR-Es) in intronless mRNAs that are essential for their nuclear export [70]. In human cells, both TREX and NTC/Prp19C associate with CAR-Es. Importantly, knock down of NTC/Prp19C components leads to the nuclear retention of intronless mRNAs [70]. Thus, NTC/Prp19C and TREX most likely function in the nuclear export of intronless mRNAs. But also in case of intron-containing mRNAs NTC/Prp19C might be involved: In higher eukaryotes TREX is recruited to the mRNP during splicing [71] and NTC/Prp19C joins the spliceosome before TREX does [72]. Thus, NTC/Prp19C might be an early factor linking transcription or splicing to nuclear mRNA export by recruitment of TREX to the mRNA.

A function of NTC/Prp19C in transcription has been shown, but many open questions for Prp19C's role in transcription and mRNA export remain: First, it remains to be explored, how NTC/Prp19C interacts with TREX and with the transcription machinery. Recently, it has been shown in higher eukaryotes that cotranscriptional splicing is enhanced by the interaction of NTC/Prp19C with the splicing factor U2AF65 [68]. In yeast, the NTC/Prp19C subunit Clf1 also interacts with U2AF65 homolog, Mud2 [69]. U2AF65 in turn binds directly to the serine 2 phosphorylated C-terminal domain (CTD) of Rpb1, the largest subunit of RNAPII [68]. This U2AF65-mediated interaction of NTC/Prp19C with RNAPII might also be essential for the function of NTC/Prp19C in transcription. Alternatively, but not mutually exclusive, NTC/Prp19C could be recruited to the transcription machinery by P-TEFb, the positive transcription elongation factor phosphorylating the CTD at serine 2, since P-TEFb interacts with human SKIP, a component of the Prp19-related complex [73]. NTC/Prp19C components also co-purified with the TREX components UAP56 [74]. Although this interaction could be indirect it is likely that it is important for the interaction of NTC/Prp19C with TREX.

So far, it is unknown whether the E3 ligase activity of Prp19 is important for its function for TREX occupancy. Interestingly, ubiquitylation of proteins with a function in mRNA export is crucial for mRNA export, even though the mechanism is not yet clearly understood [75]. Two ubiquitin ligases of the HECT family, Rsp5 and Tom1, have been shown to regulate mRNA export in *S. cerevisiae* [76–78]. Rsp5 ubiquitylates the THO/TREX component Hpr1, targeting Hpr1 for degradation [79]. However, the UBA-domain of Mex67, a subunit of the mRNA exporter Mex67-Mtr2 that transports mRNPs through the nuclear pore complex, interacts with polyubiquitin chains on Hpr1 protecting Hpr1 from degradation [80]. Ubiquitylation of the TREX component Yra1 by Tom1 is necessary for the release of Yra1 from the mRNA before nuclear export [81]. It is, however, likely that Rps5 and Tom1 have more substrates. In addition, the F-box protein Mdm30, which facilitates the interaction between the E2 ubiquitin conjugating enzyme and its substrate, promotes recruitment of Yra1 to genes and thus mRNA export [82]. In addition to mRNA export this protein is also involved in turnover of the yeast transcription activator Gal4 and in co-transcriptional mRNA processing ([83]; see also Section 4). Thus, it is clear that ubiquitylation plays a role in mRNA export as well as transcription but many details remain to be discovered. Here, the analysis of NTC/Prp19C might add pieces to the puzzle.

4. Perspectives

NTC/Prp19C is a large, dynamic and well conserved protein complex that functions in many processes essential for cellular survival. Thus, in order to better understand the intricate network necessary for proper cell function a detailed understanding of NTC/Prp19C function is of major importance.

One NTC/Prp19C or different subcomplexes could function in the different cellular pathways. Thus, it will be important to determine, which subunits of NTC/Prp19C function in each of these processes. Interestingly, different NTC/Prp19C components might interact with different proteins of the other complexes involved in splicing, transcription elongation, genome maintenance, protein ubiquitylation and degradation and lipid droplet biogenesis.

The function of NTC/Prp19C in splicing is well established. It is clear that NTC/Prp19C is required for the correct conformational changes of the spliceosome during the splicing reaction. However, how this is achieved remains an open question. Furthermore, it is not known how NTC/Prp19C interacts with the spliceosome and how NTC/Prp19C function is integrated with the function of spliceosomal ATPases and GTPases that facilitate the structural transitions required for splicing. Furthermore, even though the first substrate of the E3 ligase activity of Prp19 in humans has been identified in splicing, more substrates might exist that are ubiquitylated by Prp19 during splicing.

In addition to its function in splicing, novel functions of NTC/Prp19C are emerging such as in genome maintenance, protein degradation, lipid droplet biogenesis and transcription. Although first hints for the function of NTC/Prp19C in genome maintenance, protein degradation and lipid droplet formation are emerging, the precise function(s) and the molecular mechanism of NTC/Prp19C in these processes need to be unraveled.

In contrast to genome maintenance, protein degradation and lipid droplet biogenesis, the function of NTC/Prp19C in transcription elongation is quite well understood — at least in yeast. Nevertheless, many questions remain (see also Section 3.5): The molecular mechanism of NTC/Prp19C function in transcription needs to be unraveled in higher eukaryotes as well as in yeast. Furthermore, it will be crucial to determine whether the E3 ligase activity of Prp19 is essential for its function in transcription and to identify the substrate(s). Interestingly, it has been known for years that the UPS is involved in transcription — in a proteolytic as well as a non-proteolytic manner ([84] and references therein). The UPS controls the function of transcriptional activators mainly by degradation [84,85]. Its most prominent function, however, is probably the regulation of gene activity by ubiquitylation of histones in a non-proteolytic manner [86,87]. In addition, at least the 19S proteasome is involved in transcription elongation [87]. Intriguingly, also some proteins of the transcription machinery belong to the UPS system: TAF_I, a core component of the basal transcription initiation factor TFIID, has a combined E1 and E2 activity [88]. TFIIH, another basal transcription initiation factor, acts as a ubiquitin ligase and also modulates the activity of other ligases [89,90]. SAGA, a chromatin remodeling complex, has a dequbiquitylase activity that deubiquitylates histone H2B [91]. In addition, also the proteasome has been shown to play a role in transcription and is implicated in almost every step of transcription from initiation over elongation and termination to covalent histone modifications and repressing cryptic transcription through its proteolytic but also non-proteolytic activity ([84] and references therein). It remains to be determined, how Prp19 and its E3 ligase activity fit into the intricate connection between the UPS and transcription. Furthermore, first evidence suggests that NTC/Prp19C also functions in mRNA export. Here, it needs to be determined whether NTC/Prp19C functions in nuclear export of intron-containing mRNAs in higher eukaryotes and whether the E3 ligase activity of NTC/Prp19C is important for its function in mRNA export (also see Section 3.5).

In addition to the elucidation of NTC/Prp19C functions in each individual process at a molecular level it remains to be shown whether different processes and their regulation might be linked by NTC/Prp19C. For example, protein synthesis (at the level of transcription and splicing) could be connected to protein degradation by NTC/Prp19C. As one scenario, when aberrant proteins accumulate, NTC/Prp19C could down-regulate transcription elongation while activating protein degradation. Also, gene expression could be linked to genome maintenance, *i.e.* NTC/Prp19C — maybe in conjunction with THO — could down-regulate transcription until the damaged DNA that would lead to the synthesis of aberrant proteins is repaired. Interestingly, another DNA repair protein also functions in RNA metabolism: Apurinic/apyrimidinic endonuclease 1 (APE1) is the major abasic endonuclease in NER and also functions as a redox factor to maintain critical cysteine residues of several transcription factors in their active reduced state. APE1 also plays a role in rRNA and mRNA quality control processes ([92] and references therein). It was thus speculated that APE1 might link DNA and RNA surveillance mechanisms. NTC/Prp19C might also link the different cellular processes it is involved in. One hint that splicing and DNA repair might be connected by NTC/Prp19C comes from the finding that Prp19 is ubiquitylated upon DNA damage, which in turn stimulates the release of CDC5 and PRL1. This dissociation might lead to a switch in NTC/Prp19C function from splicing to DNA repair [93]. Thus, modification of NTC/Prp19C components might switch its activity from one process to another depending on cellular conditions.

Taken together, NTC/Prp19C plays a role in many different cellular processes. Its function in these processes still needs to be illuminated as well as the question, whether different processes are co-regulated by NTC/Prp19C. And who knows: This complex might surprise us with a function in yet another important cellular process.

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