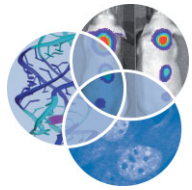
Advanced Review



Cajal bodies: where form meets function

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The cell nucleus contains dozens of subcompartments that separate biochemical processes into confined spaces. Cajal bodies (CBs) were discovered more than 100 years ago, but only extensive research in the past decades revealed the surprising complexity of molecular and cellular functions taking place in these structures. Many protein and RNA species are modified and assembled within CBs, which have emerged as a meeting place and factory for ribonucleoprotein (RNP) particles involved in splicing, ribosome biogenesis and telomere maintenance. Recently, a distinct structure near histone gene clusters—the Histone locus body (HLB)—was discovered. Involved in histone mRNA 3′-end formation, HLBs can share several components with CBs. Whether the appearance of distinct HLBs is simply a matter of altered affinity between these structures or of an alternate mode of CB assembly is unknown. However, both structures share basic assembly properties, in which transcription plays a decisive role in initiation. After this seeding event, additional components associate in random order. This appears to be a widespread mechanism for body assembly. CB assembly encompasses an additional layer of complexity, whereby a set of pre-existing substructures can be integrated into mature CBs. We propose this as a multi-seeding model of CB assembly.

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INTRODUCTION

In the process of evolution, eukaryotic cells developed a highly complicated system of compartments to separate and regulate different biochemical processes. Extensive research in the past decades revealed a remarkable level of compartmentalization imparted by internal membranes: the nuclear envelope as well as internal membranes enclosing the cytoplasmic organelles. Yet the nucleus and cytoplasm are filled with a variety of small organelle-like structures, commonly referred to as bodies (Box 1). Bodies are often composed of multiple protein and RNA types held together by many weak interactions. When appropriately stained, their appearance ranges from spherical to interconnected, irregular foci. Because their shapes are not maintained by lipid membranes, bodies are in direct contact with their surrounding environment. Thus, molecular constituents tend to exchange rapidly with cytoplasm or nucleoplasm, and the bodies themselves are highly dynamic. This raises two questions: How do bodies arise and persist? What are the likely functions of such transient structures?

The Cajal body (CB) is one of the most studied nuclear bodies and the information gathered about its components, behavior, and dynamics makes it a good model for understanding other types of bodies.[1] CBs appear in the light or electron microscope (EM) as spherical objects of 0.5–1 μm in diameter, found inside the nucleus of higher eukaryotes. However, their number and size varies between different tissues and organisms as well as during different stages of differentiation and development. Generally, there tend to be more CBs in early rather than late embryos, with CB numbers decreasing upon differentiation. In somatic tissues, a smaller number of large CBs are prominent in high-metabolically active cells such as muscle, neurons or cancer cells.[1–4]

The most concentrated protein in CBs seems to be the p80 coilin protein, which is still widely considered as a marker and essential component

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of CBs.[5–10] However, since the discovery of coilin, the proteome of CBs has greatly expanded and now comprises dozens of structurally and functionally diverse members (Table 1). Many are known to interact with themselves or with other CB components, thus creating a potentially dense network of interactions, which may hold the entire structure together. This has been demonstrated for cytoplasmic granules *in vitro*, where interactions between low-complexity domains of RNA-binding proteins polymerize them into amyloid-like fibers, which are then able to undergo a phase transition into a hydrogel-like state.[11,12] Because of the absence of a phospholipid membrane barrier, all CB components are in constant flux between the body and the surrounding nucleoplasm.[13] Weak interactions within CBs even allow slow diffusion of the components in the CB itself, which altogether keep the whole CB in steady-state equilibrium.[13–15] It has been proposed that these weak interactions have the same effect as hydrophobic interactions of lipid molecules in water environment. Interactions between lipids are sufficiently strong to separate them from water but are still weak enough to maintain the liquid behavior of the droplet. Such phase separation was observed *in vivo* for P-granules and nucleoli, which behave as droplets of viscous liquid surrounded by more fluid cytoplasm or nucleoplasm, respectively.[16–18] At the EM level, CBs appear to be composed of coiled threads separated by what seems to be an extension of the surrounding nucleoplasm.[19] The porous structure of CBs might explain the fact that these bodies are penetrable to 15 MDa dextran particles, and absolute protein concentration, viscosity and density inside the body is very similar to the nucleoplasm.[14,16] Phase separation and liquid-like behavior is now a leading model used to explain physical properties and the shape of these bodies, possibly including CBs.

CBs are packed with RNA (Table 2). CBs, like nucleoli, are well-stained by silver ions, which Santiago Ramón y Cajal used to image sections of vertebrate brain; this led to his discovery of 'accessory bodies' that were subsequently renamed in his honor.[65] The spliceosomal snRNAs (U2, U4/U6, and U4/U6•U5 snRNAs) can also be considered markers whose concentration in CBs depends on coilin.[5,66] Other CB components, such as scaRNAs and Survival Motor Neuron protein (SMN), have the tendency to form 'residual bodies' in the absence of coilin,[5] but most researchers in the field would agree that these are not CBs. However, defining nuclear bodies based on the presence of certain constituents can be rather difficult, since many of them can be

TABLE 1 | Known Protein Components of Cajal Bodies (CBs)

Function	Protein Name	Organism	Reference
snRNP Maturation and Splicing	SMN	Human	20
	Gemins (2,3,4,6,7)	Human	21
	SART3	Human, Mouse	22
	TGS1	Human	23,24
	WRAP53/WDR79	Human	25,26
	PHAX	Human	27
	CRM1	Human	27
	Snurportin1	Human	28
	Sm	Mouse	6
	Prp4	Human	29
	Prp3	Human	29
	Snu114	Human	29
	SF3a60	Human	30
	SF3a66	Human	30
	FRG1P	Human, Green monkey, Mouse	31
	ZPR1	Human	32
	U2AF	Human	33
	AIDA-1c	Human	34
snoRNP Maturation	Nopp140	Human, Green monkey, Rat, Mouse	35
	Fibrillarin	Human, Rat, Mouse	36
	Gar1	Human	37
	Nop10	Human	37
	NHP2	Human	37
	Dyskerin	Rat	35
	Nop56	Human	24
	Nop58	Human	24
	15.5 kD	Human	24
Transcription and RNA Stability	CGI-55	Human	38
	Topoisomerase I	Human	6
	Pol II	Xenopus, Newt	39
	Pol III	Xenopus	40
	ELL	Human	41
	EAF1	Human	41

TABLE 1 | *Continued*

Function	Protein Name	Organism	Reference
	ISG20	Human	42
	TAFII70	Newt	43
	TFIIS	Xenopus, Newt	44
	AGO4	Arabidopsis	45
	Pol IV (NRPD1b)	Arabidopsis	45
Signaling	FGF-2	Human	46
	SUMO-1	Rat	47
	PIASy	Human, Mouse	48
	PARP	Drosophila	49
	PARG	Drosophila	49
	PP1γ	Human	50
	P99/PNUTS	Human	50
	CDK2-cyclinE	Human, Rat	51
	USPL1	Human, Zebrafish	52
Other	Coilin	Human, Mouse, Rat, Xenopus, Zebrafish, Drosophila, Arabidopsis	36,53–56
	hCINAP	Human	57
	Profilin I	Human	58
	TERT	Human	59
	p220(NPAT)	Human	60
	FLASH	Human, Mouse	61
	SLBP	Xenopus	62
	Lsm10	Human	63
	Lsm11	Drosophila	64

found in other types of bodies as well (Figure 1, Box 1). An example is coilin, which is always present in CBs and sometimes present in HLBs.[53] Here we contrast the properties of these two highly related bodies in order to fully discuss current models of CB assembly and function.

RESOLVING THE CB–HLB CONTROVERSY

As mentioned above, CBs harbor a diverse pool of protein and RNA components (Tables 1 and 2). Many of those CB constituents were initially discovered in studies conducted in the germinal vesicles (GVs) of amphibian oocytes. The *Xenopus* GV contains up to 100 enormous (10 μm in diameter) coilin-positive bodies, previously referred to as 'spheres'.[9,10] Spheres seemed to be a larger version of the CBs found in tissue culture cells. One of the earliest identified sphere/CB components was the U7 snRNA,[80,81] which directs 3′-end cleavage of canonical histone transcripts after a conserved stem-loop.[63] In addition, a fraction of mammalian CBs were shown to be located near histone gene clusters by DNA *in situ* hybridization.[80] It seemed that CBs in somatic cells might be a place of histone mRNA maturation. However, further examination revealed that components of the histone gene expression machinery did not always colocalize with CBs but rather resided in separate foci in some mammalian cells.[82–84] This heterogeneity was perplexing until a study in *Drosophila melanogaster* identified a distinct nuclear body localized at histone genes; this U7 snRNP-containing nuclear body was named the histone locus body.[53] Since then, HLBs and CBs have been recognized as distinct nuclear bodies in *Danio rerio* embryos, *Xenopus* early oocytes, human embryonic stem cells and certain cancer cells.[54,85–87] The HLB harbors many additional components of the machinery for histone gene expression, including transcriptional regulators (Table 3), suggesting that HLBs arise at active sites of histone gene transcription.

Given the presence of shared components between these two bodies, are HLBs distinct from CBs? As described above, HLBs are characterized by components of the histone gene expression machinery, such as U7 snRNP. Paradoxically, coilin is a prominent component of spheres (see above), which are packed with U7 snRNP in amphibian GVs; therefore, the current thought is that HLB and CB contents can in some instances mix and colocalize. In immortalized mammalian cells, for example, HLB and CB markers colocalize about half of the time.[61,84,87] Colocalization occurs in a cell-cycle regulated manner in mammalian cell-culture, with increased colocalization during S-Phase when histone gene transcription takes place.[86,87] In developing organisms, the separability of these nuclear bodies seems to depend on progression through development.[85,105] HLBs are unaffected by coilin depletion;[61,64] yet coilin localization is altered if the HLB components NPAT or FLASH are depleted.[61,105,106] Whether CBs and HLB mixing reflects simply an unspecific affinity between the two bodies or whether it has physiological importance remains unclear.

FORMATION AND FUNCTION OF HLBS

The mechanism of how HLBs and other nuclear bodies are assembled has been tackled multiple times (Box 2).

TABLE 2 | RNA Species Enriched Within Cajal Bodies

RNA	Family	Class	Promotor	Cap	Reference
U1[1]	snRNA	Sm	Pol II	TMG	33
U2	snRNA	Sm	Pol II	TMG	33
U4	snRNA	Sm	Pol II	TMG	33
U5	snRNA	Sm	Pol II	TMG	33
U6	snRNA	Lsm	Pol III	Met-pppG (MPG)	33
U7	snRNA	Sm/Lsm	Pol II	TMG	56
U3	snoRNA	C/D	Pol II	TMG	56
U8	snoRNA	C/D	Pol II	TMG	56
U13[2]	snoRNA	C/D	Pol II	TMG	
Telomerase RNA	scaRNA	H/ACA	Pol II	TMG	59
Poly(A) RNA[1]	mRNA	—	Pol II	m^7G	67
U4atac[2]	snRNA	Sm	Pol II	TMG	22
U6atac[2]	snRNA	Lsm	Pol III	Met-pppG (MPG)	22
U11	snRNA	Sm	Pol II	TMG	68
U12	snRNA	Sm	Pol II	TMG	68
U14	snoRNA	C/D	Pol II (intronic)	—	69
U64[3]	snoRNA	H/ACA	Pol II (intronic)	—	70
SCARNA10 (U85)	scaRNA	C/D, H/ACA	Pol II (intronic)	—	71
SCARNA5 (U87)	scaRNA	C/D, H/ACA	Pol II (intronic)	—	72
SCARNA6 (U88)	scaRNA	C/D, H/ACA	Pol II (intronic)	—	72
SCARNA12 (U89)	scaRNA	C/D, H/ACA	Pol II (intronic)	—	72
SCARNA7 (U90)	scaRNA	C/D	Pol II (intronic)	—	72
SCARNA17 (mgU12-22/U4-8, U91)	scaRNA	C/D or 2xC/D	Pol II	TMG	72,73
SCARNA8 (U92)	scaRNA	H/ACA	Pol II (intronic)	—	72
SCARNA13 (U93)	scaRNA	2xH/ACA	Pol II (intronic)	—	74
SCARNA4 (ACA26)	scaRNA	H/ACA	Pol II (intronic)	—	75
SCARNA1 (ACA35)	scaRNA	H/ACA	Pol II (intronic)	—	75
SCARNA11 (ACA57)	scaRNA	H/ACA	Pol II (intronic)	—	75
SCARNA23 (ACA12)[2]	scaRNA	H/ACA	Pol II (intronic)	—	75
SCARNA15 (ACA45)[2]	scaRNA	H/ACA	Pol II (intronic)	—	75
SCARNA16 (ACA47)[2]	scaRNA	H/ACA	Pol II (intronic)	—	75
SCARNA22 (ACA11)[2]	scaRNA	H/ACA	Pol II (intronic)	—	75
SCARNA20 (ACA66)[2]	scaRNA	H/ACA	Pol II (intronic)	—	76
SCARNA21 (ACA68)[2]	scaRNA	H/ACA	Pol II (intronic)	—	76
SCARNA3 (HBI-100)[2]	scaRNA	H/ACA	Pol II (intronic)	—	77
SCARNA2 (HBII-382, mgU2-25/61)[2]	scaRNA	C/D or 2xC/D	Pol II	TMG	73,77
SCARNA9 (mgU2-19/30)[2]	scaRNA	2xC/D	Pol II (intronic)	—	73
SCARNA14 (U100)	scaRNA	H/ACA	Pol II (intronic)	—	78
SCARNA18 (U109)[2]	scaRNA	H/ACA	Pol II (intronic)	—	79

[1]Evidence only in plants.
[2]Expected in CBs but not experimentally verified.
[3]In CBs only if overexpressed.

Two pathways of nuclear body formation have been considered. In one scenario, bodies follow an ordered assembly pathway, where all components are added in predefined sequence. Alternatively, stochastic self-organization, in which components assemble in random order, may occur.[107] The formation of HLBs has been studied in mammalian cell culture, *Xenopus* oocyte maturation and during *D. melanogaster* embryogenesis. Supporting evidence for the stochastic assembly model comes from a cell culture study, where individual HLB components were tethered via Lac repressor to a genome integrated LacO-array and the *de novo* formation of HLBs studied.[108] Tethering of histone mRNA as well as transcriptional regulators and RNA processing factors involved in histone mRNA 3′-end formation resulted in recruitment of NPAT and coilin to the array. This initiation of HLB assembly by any component supports an intrinsic stochastic self-organization property of HLBs, showing that tethered RNA–protein interaction can support body formation. The challenge is to understand the circumstances in which bodies do form *in vivo*.

Restricted availability of components and/or signaling cues *in vivo* during phases of cell cycle or development impact HLB formation. Support for ordered HLB assembly comes from observations in mammalian cells, suggesting that the HLB component stem-loop binding protein (SLBP) joins HLBs during the cell cycle only after NPAT and LSM10 protein are already present.[88] Embryogenesis offers a unique chance to study the formation of nuclear bodies in transcriptional on and off states, since initial stages of development are transcriptionally silenced. Recently, White and colleagues showed that Mxc (*Drosophila* ortholog of NPAT) and FLASH are present on *Drosophila* histone gene clusters one cell-cycle prior to the start of histone gene transcription and prior to the recruitment of other components (Figure 2). Importantly, knockdown of Mxc or FLASH prevented the assembly of other HLB components, suggesting that initial events follow a hierarchical order and NPAT and FLASH seem to seed the body formation.[89] Contrary to the ordered assembly model, further assembly of the full HLB seems to occur then in a stochastic fashion after this initial ordered phase. This has led to the proposal of a seeding model for body formation (Box 2).[109,110] Some proteins, such as Spt6 and Pol II, are only detected within HLBs during ongoing transcription.[85,89] Consistent with their transcription-dependence, HLBs also undergo repeated steps of assembly and disassembly in cycling mammalian cells.[82,83,88]

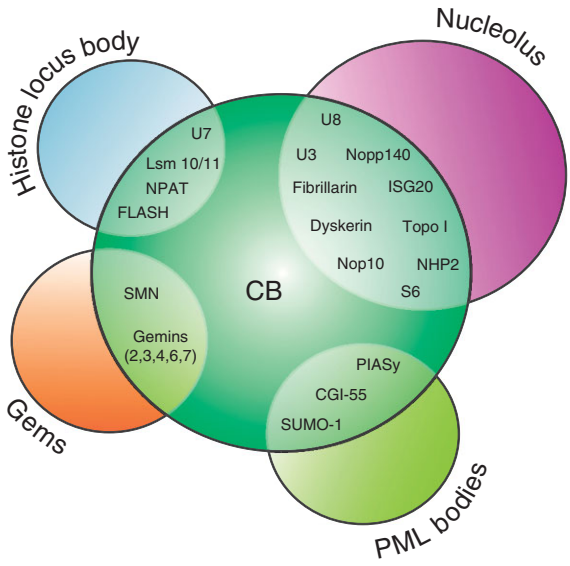

FIGURE 1 | Shared components between Cajal bodies (CB) and other known types of nuclear bodies. Many protein and RNA types accumulate within CBs. Some of them have been found also in other nuclear subcompartments either because they are in different functional complexes, or shuttle between the two compartments.

Taken together, studies of HLB formation in multiple systems point to two phases of assembly (Figure 2). In the first phase, histone gene repeats prepare for transcription, through recruitment of transcriptional regulators. In the second phase, the local production of histone transcripts recruits 3′-end processing factors such as U7 snRNP and SLBP. Thus, HLBs are localized at the histone gene clusters and harbor the machinery for canonical histone gene expression and 3′-end formation[53] (see Table 3). This scenario is reminiscent of nucleolar structure and function, in which rRNA processing factors accumulate at the sites of transcription of the rDNA repeats.[111] One clear possibility is that the accumulation of 3′-end processing factors in HLBs localized to histone gene repeats may increase the efficiency of histone gene expression, in terms of both 3′-end cleavage and preparation for nucleo-cytoplasmic export.

CB FUNCTIONS IN RNA METABOLISM AND RNP BIOGENESIS

snRNP Biogenesis

Shortly after realizing that CBs can be visualized by immunostaining for coilin, CBs were shown to contain snRNAs (Table 2), which are components

TABLE 3 | Known Protein Components of Histone Locus Bodies (HLBs)

Function	Protein name	Organism	Reference
Transcription	NPAT / Mxc	Human, Mouse, Drosophila	88–91
	NELF	Human	92
	Spt6	Drosophila	89
	TBP	Drosophila	93
	TRF2	Drosophila	93
	Pol II	Xenopus, Drosophila	85
	FLASH	Human, Mouse, Drosophila	61,89–91,94
	HiNF-P	Human	95
3′-End Processing	GAPDH	Drosophila	96
	U7 snRNP (U7 snRNA, LSM11, LSM10)	Human, Xenopus, Drosophila	84,85,88,97–100
	SLBP	Human	88
	Symplekin	Xenopus, Drosophila	85,101
	ZFP100	Human	102
Other	ARS2	Human	103
	Mute	Human, Drosophila	98
	Coilin	Xenopus, Drosophila	85
	hCINAP	Human	104
	PARP	Drosophila	49

of spliceosomal particles (snRNPs) essential for pre-mRNA splicing.[33] Spliceosomal snRNPs undergo a complicated maturation pathway, during which they visit CBs several times[112] (Figure 3). The first encounter already happens at the stage of transcription, as CBs can often be found in close proximity to snRNA gene loci.[113,114] Association of CBs with U2 genomic loci was shown to be dependent on active transcription and mediated by nascent pre-U2 RNA.[66,115] Further, deletion of 3′ half of a U2 gene disrupts the association completely.[116] Newly transcribed snRNAs may enter the CB for the purpose of proper loading of the export factors PHAX and CRM1, which then transport snRNAs to the cytoplasm.[117] In the cytoplasm, snRNAs undergo 3′-end trimming and hypermethylation of their 5′ caps and acquire Sm-rings. These modifications are necessary for re-import into the nucleus. These newly imported immature snRNPs are again directed to CBs[118] where they undergo final steps of their maturation, including the addition of snRNP-specific proteins[30] and snRNA modification by scaRNPs, which guide methylation and pseudouridylation of specific nucleotides.[119]

An exception to this process is the U6 snRNA, which is transcribed by RNA polymerase III and not exported to the cytoplasm. CBs are not concentrated at U6 genes,[113] possibly because U6 genes do not occur in tandem repeats and may not attract sufficient CB components to produce CBs. On the other hand, other single-copy snRNA genes such as U4, U11, U12 associate with CBs, suggesting polymerase identity and/or sequence differences are important.[113] U6 snRNP is brought to CBs by the SART3 protein and assembled into functional U4/U6.U5 tri-snRNPs necessary for efficient splicing reaction.[22] First, U6 is joined with U4 by SART3 to form the U4/U6 di-snRNP intermediate,[120] followed by addition of U5 by the Prp31 assembly factor to yield final tri-snRNP ready for splicing.[29] During splicing, snRNPs go through dramatic reorganization leading to the separation of U4, U5, and U6 snRNPs. In order to perform another round of splicing, they enter CBs to be reassembled into tri-snRNP again.[121] Similarly, U2 snRNP maturation has also been observed to occur in CBs.[30] Thus, CBs are sites of snRNP assembly and recycling.

Because coilin knockout mice and flies as well as cell lines lacking CBs appear to have normal levels of splicing, CBs are not the exclusive place of snRNP formation.[5,122] Instead, CBs have been proposed to act like a catalyst, which speeds up snRNP assembly by concentrating necessary components as predicted by *in silico* modeling.[123] This model was recently experimentally verified and it was inferred that CBs can produce 3.8 tri-snRNPs per second, which is roughly 10-fold faster than in surrounding nucleoplasm.[124]

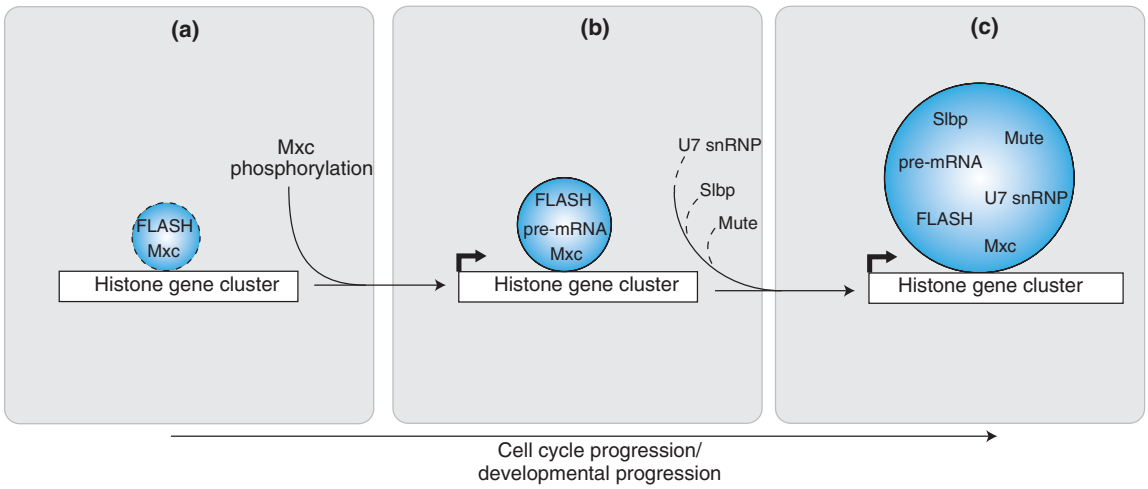

FIGURE 2 | Model of histone locus body (HLB) formation in *Drosophila melanogaster*. (a) Histone gene cluster locus with transcriptional regulators (FLASH, Mxc), which initially assemble on the locus shortly before activation of transcription. Phosphorylation of Mxc during cell-cycle progression promotes active histone transcription in (b). Upon active transcription, other components of the HLB assemble on the locus; here, non-ordered assembly is shown. (c) Fully assembled HLB with the major components.

Although, the increase may not seem to be extraordinarily high, it can make a huge change when cells are highly metabolically active. This is supported by observations in zebrafish, where embryos depleted of coilin and lacking CBs are unable to complete embryogenesis, display splicing defects and reduced numbers of mature snRNPs.[54] In comparison, coilin knockout mice show severe fertility and fecundity defects reflecting both, increased *in utero* death of pups and underdeveloped reproductive organs.[125]

snoRNP and scaRNP Biogenesis

Another abundant group of RNPs found in CBs are snoRNPs and scaRNPs, which are closely related in structure and function. snoRNAs can contain either box C/D or box H/ACA motifs, which determine the protein constituents of the snoRNP and the function it performs. While box C/D snoRNPs perform guided methylation of their RNA targets, box H/ACA snoRNPs direct pseudouridilation of specific nucleotides of rRNA and U6 snRNA. In contrast, scaRNAs can contain either of the boxes or both at the same time. The scaRNAs are concentrated in CBs and modify spliceosomal snRNAs during their final maturation inside the CBs (see above). To properly localize in CBs, scaRNAs contain a conserved CAB box sequence, which serves as a CB-localization signal.[70] This sequence is specifically recognized by WRAP53/WDR79/TCAB1 protein that is, in turn, necessary for scaRNA accumulation in CBs.[25,26]

Both snoRNAs and scaRNAs can be transcribed from separate pol II promoters; more commonly, they are excised from introns of other pol II genes (Table 2). Similar to snRNAs, active genomic loci carrying snoRNA genes and genes containing snoRNAs in introns are closely associated with CBs in the nucleus.[126,127] Since both sno- and scaRNAs and their associated proteins were found to colocalize inside CBs, it was proposed that CBs might also play a role in maturation of this type of RNPs. In the case of snoRNAs transcribed from a separate promoter, the m^7G cap is bound by CBC and PHAX proteins and transported to CBs. Here, they seem to undergo several steps of maturation including loading of specific proteins, trimming of the 3′-end and hypermethylation of the cap by Tgs1. CRM1 then displaces Tgs1 from the complex, releasing mature snoRNPs for transport to the nucleolus.[27,128] In addition, box C/D and H/ACA snoRNAs of intron origin were found associated with Tgs1 and detected in CBs, suggesting that uncapped snoRNAs may undergo maturation steps in CBs.[69,70,128,129] However, since they lack the 5′ m^7G cap structures, the means of their transport in and out of CBs remains unknown. ScaRNAs may mature in CBs as well; in this case, their CB localization is maintained by WDR79/WRAP53 protein.

Telomerase Maturation and Telomere Maintenance

Telomerase is an RNP responsible for telomere length maintenance and plays a role in aging and pluripotency. Mature telomerase RNP consists of telomerase RNA (TR), telomerase reverse transcriptase (TERT) and other associated proteins. Although mainly found

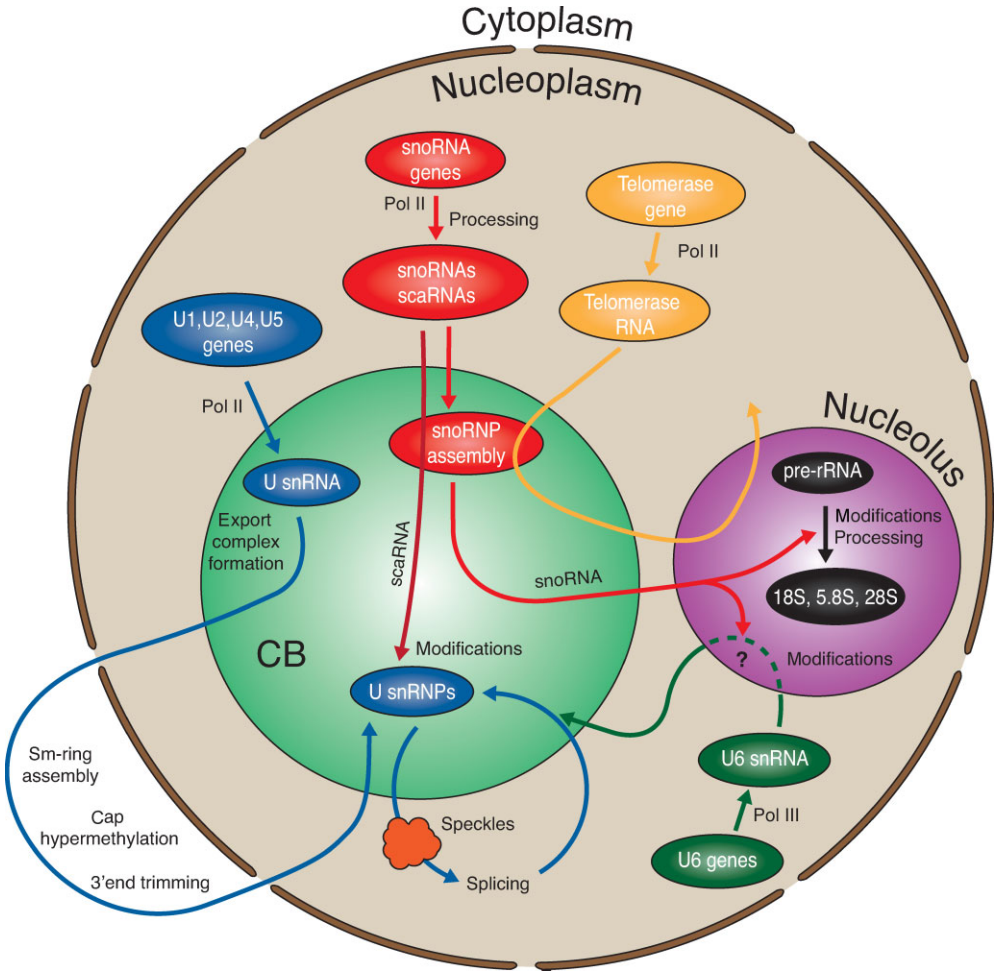

FIGURE 3 | Overview of Cajal body (CB) functions in different steps of maturation and assembly of ribonucleoprotein complexes (RNPs). After transcription of pre-snRNAs (U1, U2, U4, U5) by RNA-polymerase II, m^7G caps are loaded with cap-binding complex (CBC). SnRNAs then enter CBs where PHAX and CRM1 are loaded to form export-competent RNP complexes. After reaching the cytoplasm, the heptameric Sm-ring is assembled on snRNAs, m^3G is formed and 3′-ends are trimmed. Snurportin then brings these still immature snRNPs back to nucleus where they move to CBs for modification by scaRNPs and addition of snRNP-specific proteins. Mature snRNPs then move to the nucleoplasm where splicing occurs. After splicing, snRNPs return to CBs for reassembly into active forms. SnoRNAs and scaRNAs are transcribed by Pol II, either as individual genes or harbored within introns. They mature in CBs by associating with specific proteins and then traffic to the nucleolus to process and modify pre-rRNA and U6 snRNA. U6 snRNA is transcribed by Pol III and, after modification possibly in the nucleolus, enters CBs to be incorporated into U4/U6.U5 tri-snRNPs. Telomerase follows a maturation pathway similar to snoRNAs but may leave CBs to elongate chromosomal telomeres.[112]

in stem cells, telomerase RNA has been found to be localized to CBs in human cancer cells.[59,130] TR is transcribed by Pol II thus possesses m^7G cap. Recently, it has been shown that the cap structure is bound by PHAX protein and thus TR is transported to CBs, similarly to snoRNAs.[27] Furthermore, vertebrate TR contains a conserved snoRNA-like H/ACA domain in its 3′-end. This strongly suggests that TR matures inside CBs, like snoRNAs.[131] Since RNA in the mature telomerase RNP contains TMG cap, one can speculate that this modification is done by CB-localized Tgs1 methyltransferase.[130] Indeed, fluorescently labeled and injected TR accumulates first in CBs and later in nucleoli in *Xenopus* oocyte, which could mean that TR undergoes maturation steps in each of these compartments.[132] In addition, mature TR localizes to CBs throughout the cell cycle thanks to the presence of the CAB box and WDR79/WRAP53 protein.[130,133] Vertebrate TR is therefore *de facto* a scaRNA; however, since its target sequence is not complementary to any known RNA, it is not functional for guiding RNA modifications. Interestingly, localization to CBs peaks in S phase. During S phase, TERT is also recruited to CBs. Both components associate to form functional telomerase in separate foci adjacent to CBs and then relocalize to telomeres.[134,135]

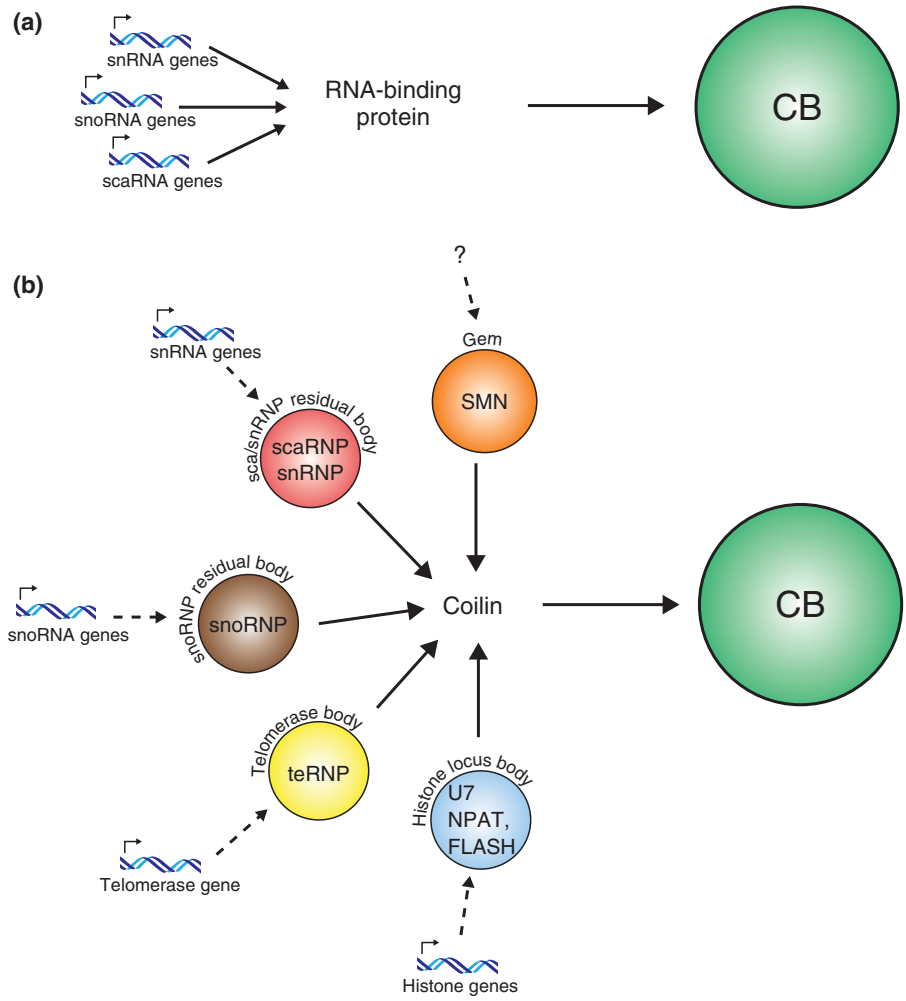

FIGURE 4 | Models for Cajal body (CB) formation. (a) CB self-assembly via RNA seed. Upon transcriptional activation of specific loci, nascent RNA nucleates the formation of the body by recruiting specific RNA-binding protein(s). Later, other proteins are added in non-ordered fashion to create complete canonical CBs. (b) Self-assembly via coilin. Each of the different RNA species nucleates its own 'sub-Cajal body' (sub-CB). Note that there is no known RNA component of gems. Sub-CBs are then integrated into canonical CBs via binding properties of coilin protein. It is not known whether CB formation occurs by different sub CB-components coming together simultaneously or whether sub-CBs are pre-formed and then fuse together, analogous to lipid droplets fusing and possibly taking advantage of proposed phase transitions.

It is not known whether CBs are essential for proper telomerase RNP assembly or whether CBs act as catalysts. However, inability of TR to localize to CBs causes inefficient recruitment to telomeres and thus impaired telomere elongation in human cells.[133,136,137] According to recent reports, this telomerase pathway may be unique for human TR, since mouse TR does not require CBs for recruitment to telomeres.[138]

DYNAMICS AND FORMATION OF CBS

From the images of stained cell nuclei, one might assume that CBs are static structures inside the nucleoplasm. However, imaging of live cells revealed that CBs are very motile. CBs can travel within nucleoplasm at rates up to ~1 μm/min and fuse or split.[139] CB movement has been shown to be spatially restricted and alternating between association with chromatin or free movement in the nucleoplasm.[140] Their size and numbers are also variable and can differ in different stages of the cell cycle. The most dramatic change happens during M-phase, when CBs completely disassemble. CBs re-appear again, starting as multiple small foci, after mitosis in early G1-phase.[141] Also, CBs have been seen to form during cell differentiation or disappear upon different stress conditions. This implies that cells tightly regulate CB number and size.

Two models for nuclear body formation are widely considered (see above and Box 2).[110] Recent experiments from the Dundr lab showed that by tethering different CB components to a specific site inside the nucleus, one can induce nucleation of CBs.[142] Because different tethered RNAs (e.g., scaRNAs and snRNAs) also function in this assay, CBs may be nucleated at particular active sites of transcription.[108,143] These data lend strong support to seeded assembly of CBs that proceeds stochastically. The fact that almost any CB component is capable of seeding CBs also argues against ordered assembly. However, this work does not address how assembly of CBs proceeds *in vivo* from endogenous components/seeds.

Several observations support the notion that CB integrity depends on RNA. First, the detection of CBs depends on ongoing transcription as shown in cells treated with chemical inhibitors of transcription or DNA damage, which causes transcription to shut off.[144,145] Coilin was thereby found to relocalize to nucleolar caps. One interpretation of these data is that CBs depend on splicing.[33] Splicing is reduced in cells when transcription is inhibited such that fewer snRNPs undergo recycling in CBs; in this model, the reduced flux of snRNPs through CBs leads to their disassembly. However, an alternative model is that nascent RNA nucleates CBs. This second model is supported by the observation that CB mobility is restricted by association with chromatin and increases upon transcription inhibition.[140] Transcription shuts off naturally during mitosis and resumes in early G1-phase which coincides with appearance of CBs as multiple small foci.[141] These foci might then coalesce due to the self-interacting properties of the RNA-binding protein(s) or other factors to create larger CBs. In a similar way, tiny nucleoli that form in early G1 coalesce to form fewer, larger nucleoli later in the cell cycle.[17] A variation of this second model is that transcriptionally active genomic loci might be recruited to already nucleated CBs and cause them to grow.[146] Since chromosomal movement in nucleoplasm is spatially restricted, only a subset of active loci might actually associate with a given CB. Indeed, not all U1 loci were associated with CBs and only a subset of those CBs was also associated with U2 loci at the same time.[114] Taken together, it seems likely that RNA seeds nucleate CB assembly, and these seeds may include distinct subsets of RNA species arising from separate genomic loci.

Consistent with the role of RNA in nucleating CB assembly at active sites of transcription *in vivo*, CBs have been detected at different gene loci (e.g. tandem repeats of U2 snRNA genes) in a manner dependent on ongoing transcription and immature RNA.[66,113–116,126] In such a scenario, seeding could in principle be provided by different snRNAs, snoRNAs, and/or scaRNAs transcribed from their respective genes (Figure 4(a)), though this possibility has not been comprehensively addressed to date. These other RNA seeds would nucleate CB formation by recruiting RNA-binding proteins to their transcription sites. Such proteins could well be one of the known CB components (Table 1) or might still await discovery. Additional components would then randomly join to create full canonical CBs.

One implication of the notion that multiple RNAs and RNA types can act as CB seeds is that nucleation may not be mediated by a single RNA binding protein, but instead by a set of proteins specific for particular RNA species. In that case, a group of sub-CBs would be created as independent entities and later on 'glued' together by an organizing protein, most probably coilin (Figure 4(b)). This follows from the key observation that, upon coilin knockout or depletion, CB components disperse into numerous types of residual bodies with snRNPs redistributing to the nucleoplasm.[5,7,54,55] We favor a multiseeding model that is based on a combination of standard seeding model with a model of CB organization presented by Matera and Shpargel.[147] The fact that CBs consist of heterologous bodies has been demonstrated in coilin KO mouse cells and in the fly. Such cells lack canonical CBs but contain several subtypes of so-called residual bodies: gems containing the SMN protein, another enriched in scaRNAs with associated proteins, and a third containing snoRNA and fibrillarin.[5,119] This could also explain the observation that two daughter CBs resulting from a splitting event are often found to contain an equal amount of coilin but largely different amounts of other proteins.[139] The idea that nucleation of sub-CBs might be upstream of CB assembly has also been proposed by Matera and colleagues.[105] The ability of coilin to bring all sub-CBs together is therefore crucial for CB assembly. At the same time, it appears that multiple interactions among CB compartments are important for CB integrity; for example SMN, Tgs1, WRAP53, and USPL1 SUMO isopeptidase knockdown lead to mislocalization of coilin.[7,26,52,148–150]

The observed dynamics of CBs throughout the cell cycle and during development and differentiation suggests that CB assembly and maintenance may be tightly regulated. In living systems, the most common means of regulating protein activity is by post-translational modifications. Given the central role of coilin in CB integrity, it is interesting that coilin is post-translationally modified in several ways.

BOX 1

WHAT IS A BODY?

Recent research in the field of nuclear structure, taking advantage of increased sensitivity and resolution in fluorescence light microscopy as well as emerging molecular markers, has led to the identification of an increasing number of 'bodies'. However, the lack of an exact definition sometimes creates confusion. The most common signature is the existence of punctate staining after immunofluorescent labeling of protein or RNA components. For the puncta to be considered bodies, an unwritten rule is that the apparent diameter of the object be greater than the wavelength of light (i.e., >250 nm). Bodies lack membranes and—unlike other assembled cellular structures, like filaments—tend to be round. Because of limits of resolution, fluorescent images of bodies can be hard to interpret; it is not clear whether the signal comes from a real subcompartment or is just a result of component accumulation in space without any tight link. Transcription factors binding to gene promoters, for example, might produce a very similar punctate pattern, especially if genes are present in dense clusters such as histone or Hox genes. It is therefore hard to discern the point of body origin in time or space, especially if the cell is caught in the act of assembling a body. For example, does the HLB already exist as a body when only NPAT and FLASH are localized? If other components are missing, is this still an HLB? Detection of bodies with higher resolution methods, such as EM, is helpful for defining the body's detailed morphology. EM has been a powerful method for defining the nature of Cajal bodies and nucleoli. It would be therefore wise to continue using EM as a gold standard for precise ultrastructural definition of a body. Sometimes differential interference contrast (DIC) microscopy can also reveal the presence of bodies, as in the case of spheres in *Xenopus* oocytes.

BOX 2

A LEXICON OF BODY LANGUAGE

Assembly: a general term to describe series of events leading to body formation, according to a morphological definition of the body (see Box 1). Different bodies appear and disappear in response to different stimuli, for example, phase of cell cycle, differentiation, stress conditions, and signaling. Such events are referred to as assembly and disassembly.

Self-assembly: a mode of assembly in which the structure forms according to the inherent tendency of components to associate with one another. A simple example of self-assembly is the aggregation of prion proteins. In self-assembly, the notion that the kinetics of assembly is shifted toward assembled product is implicit; as the association of components reaches equilibrium, an apparently static structure with a defined morphology (e.g. size and shape) is attained.

Self-organization: However, most of the assembly processes in cells are far from equilibrium. Therefore the resulting structures are in steady-state, where individual components constantly enter and leave the structure (e.g., microtubule treadmilling).

Ordered assembly: is a mode of assembly, in which every component of the structure is added in strictly predefined order. An example is the assembly of the spliceosomal snRNPs, which mature in an ordered fashion (see main text).

Stochastic assembly: In contrast to ordered assembly, body components may be added in a random order.

Seeded assembly: One component, such as a protein or RNA may act as a seed for body formation. This is compatible with either ordered or stochastic assembly, given that the spatial localization of the seed (e.g., pre-rRNA or histone pre-mRNA at their respective transcription units) is the event that nucleates further assembly, similarly to crystallization centers during crystallization.

Fusion and splitting: Bodies can also grow or shrink from the fusion of two or more smaller bodies or alternatively by division of an existing larger body.

First, coilin is symmetrically dimethylated on several arginine residues, which is crucial for interaction with SMN protein. In cells containing the hypomethylated version of coilin, SMN is not localized in CBs but present in separate sub-CBs called gems.[151] Interestingly, the nuclear form of FGF-2 might play a crucial role in this process, as different levels of FGF-2 effect gems but not CBs.[46] Gems were observed as separate bodies in certain cell lineages and fetal tissues but are apparently always integrated into CBs in adult tissues.[152] This suggests that coilin is always fully methylated *in vivo*, at least at interphase, but this still needs to be experimentally verified.

Coilin is also a known phosphoprotein and its phosphorylation status influences its interactions with other CB proteins.[153] Coilin phosphorylation actively changes during the cell cycle, where the highest level is reached in M phase, which correlates with absence of CBs in this part of the cell cycle.[153] Primary cells that weakly express serine/threonine phosphatase PPM1G and have hyperphosphorylated coilin have fewer CBs in comparison to transformed cells.[154] Therefore, telomerase RNP is found as separate body in primary cells,[59] suggesting that coilin phosphorylation status governs integration of telomerase into CBs. Taken together, coilin modification by arginine dimethylation and phosphorylation seems to provide important mechanisms by which cells regulate CB assembly and maintenance.

CONCLUSION

There is now good evidence that numerous steps of snRNP and snoRNP biogenesis take place in CBs. This colocalization of function with CBs does not imply that CBs are necessary for the biogenesis of any of the RNP components. However, recent experiments indicate that coilin is essential for embryogenesis in mouse and zebrafish and correlated with the loss of CBs in coilin-depleted cells. In the zebrafish embryo, lethality due to coilin depletion was attributable to defects in snRNP biogenesis and splicing, consistent with the proposal that RNP assembly in CBs is more efficient due to the concentration of components in the body. Similarly, telomerase trafficking through CBs may enhance telomere length maintenance. Although CB function can formally be seen as a property that may be separable from CB assembly, form and function is very likely linked. None of the small RNAs contained within CBs—snRNAs, snoRNAs, scaRNAs—can function without processing, modification, and assembly into their respective RNPs. Because CB formation and maintenance depend on the same small RNAs that will eventually undergo maturation and RNP assembly within CBs, it is reasonable to propose that CB formation and function represents a cellular mechanism to ensure the expression of functional RNPs following transcription. The example of mixing between CBs and HLBs in specific biological contexts, like oogenesis, provides a precedent for cooperation among RNA processing systems. An outstanding question is how the CB integrates the biogenesis of multiple RNP species and whether cross-regulation occurs.

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