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GUEST EDITORIAL

Special focus on the Cajal Body

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The Cajal body (CB) is a nuclear compartment enriched in specific protein and RNA components without the barrier of a lipid bilayer. Like the nucleolus in the nucleus and stress granules in the cytoplasm, the CB likely forms through a process of liquid-liquid demixing or phase separation of these components. A hallmark of such cellular compartments is the involvement of proteins with intrinsically disordered regions coupled to specific, often multivalent binding domains that recognize RNA and/or protein partners. The CB marker protein, coilin, appears to qualify, because it binds RNA and contains low complexity and intrinsically disordered regions in combination with an N-terminal domain that mediates coilin-coilin "self" interaction and is predicted to be structured. Similarly, the protein deficient in spinal muscular atrophy (SMA), spinal motor neuron protein (SMN), contains a tudor domain that binds dimethyl arginine as well as intrinsically disordered regions. Depletion of either of these proteins abolishes CB formation and maintenance. The RNAs enriched in CBs include a host of small non-coding RNAs, namely the small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs). CBs are the sites of snRNA assembly into mature snRNPs, and the concentration of immature snRNPs and transient intermediates in the snRNP assembly pathway makes snRNP assembly more efficient. Loss of coilin and therefore CBs is lethal for mouse and zebrafish embryos, and lethality is due to insufficient production of spliceosomal snRNPs needed for splicing zygotic pre-mRNAs expressed in embryos at the time of genome activation. SnoRNPs are present in CBs to guide RNA modifications on snRNAs (these snoRNAs are specifically named scaRNAs, because they localize to the CB and not nucleoli), and true nucleolar snoRNAs traffic through CBs en route to nucleoli, likely to undergo snoRNP assembly steps. Current research into the mysteries of CBs and their activities is reflected in the 12 contributions to this special issue of *RNA Biology*.

Much of the depth of understanding regarding CB composition and function has emerged since the publication of Joe Gall's classic review in 2000 ("Cajal bodies: the first 100 years" in the Annual Review of Cell and Developmental Biology), which renamed the "coiled body" for its discoverer, Ramon y Cajal. Using silver staining of sections of vertebrate brains, Cajal first documented the appearance of small round structures adjacent to nucleoli in 1903. Perhaps because much subsequent work on CBs has been performed in amphibian oocytes and aneuploid tissue culture cells like HeLa, many cell biologists labor under the misapprehension that CBs are not present in normal somatic cells. Nothing can be further from the truth. Cajal's discovery in human pyramidal neurons and further work by many investigators – e.g. Gall working in *Drosophila melanogaster*, Matera in mouse, Shaw in Arabidopsis and my laboratory in zebrafish – has abundantly documented the presence of CBs in normal cells and tissues throughout the organism. The review byMiguel LaFarga and colleagues, "Cajal bodies in neurons," provides both historical perspective and an insightful outlook on modern approaches to understanding the role of CBs in nervous tissue, in the context of health and human neurologic disease. We are alerted to the probable disruption of CBs in SMA and amyotrophic lateral sclerosis (ALS). Given the preponderance of intrinsically disordered proteins involved in neurodegenerative disease, it is remarkable that so little is known about CBs. This is clearly a rich avenue for future study.

In contrast to other nuclear and cytoplasmic membraneless organelles, CBs owe their formation and maintenance to a protein that has no known function other than scaffolding the CB: coilin. This is an advantage to studying the structure and function of CBs, as depletion and mutation does not impact other cellular functions. It is important to understand how coilin acts as a scaffold, since CBs are dynamic structures that disassemble at mitosis. The review by Hebert and Poole exhaustively discusses post-translational modifications of coilin and other CB proteins to illuminate their role in CB assembly, maintenance and disassembly. For example, coilin is phosphorylated, methylated, acetylated, sumoylated, and ubiquitinated at different stages of the CB life cycle. Another modified CB protein is SMN, the nuclear function of which is further explored by Matera and colleagues in their review; here, we learn that SMN – with vital cytoplasmic functions in snRNP assembly – likely cooperates with coilin to maintain CB integrity. Recent insights into the role of SMN in recognizing and resolving R-loops at transcription termination sites raise the question of whether CBs may also regulate transcription termination. As CBs are concentrated at sites of snRNA transcription and sometimes at histone loci, the snRNA genes would be prime candidates for transcription regulation by SMN present in CBs.

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CBs play important roles in the biogenesis of snRNAs and snoRNAs, including their assembly into RNPs. The review by Meier shows the prevalence of site-specific pseudouridylation and 2′-O-methylation of snRNAs, accomplished by snoRNA guides (scaRNAs) that meet them in the CB. Stanek's review details the body of evidence supporting the model that snRNP assembly occurs in CBs. Because spliceosomal snRNPs are disassembled by the process of spliceosome assembly, snRNPs assembling in CBs include both new snRNPs arising from newly transcribed snRNAs as well as "used" snRNPs as they are recycled from prior splicing activity to splicing-competent snRNPs once again. These activities are crucial for maintaining enough snRNPs for ongoing splicing, thereby contributing to cellular homeostasis. Moreover, CBs perform a surveillance function, retaining defective snRNPs; the latter observation suggests that the concentration of splicing-incompetent snRNPs may protect gene expression from the deleterious effects of "used" snRNPs potentially interfering with productive splicing. Here the plot thickens, as explained in the review by Verheggen and colleagues: splicing releases pre-snoRNAs from the introns of pre-mRNAs, and the new snoRNAs assemble with chaperone complexes during splicing. Pre-snoRNPs then traffic to CBs for final maturation before reaching the nucleolus. Both snRNA and snoRNA trafficking among nuclear compartments is accomplished by intricate targeting mechanisms that seem to ensure assembly occurs before function. On the other hand, Meier points out, there are many open questions regarding targeting of the hundreds of distinct snoRNAs expressed by cells. Different snRNAs and snoRNAs display unique maturation and localization pathways, suggesting that these classes of short non-coding RNAs should not be lumped together into 2 or 3 arbitrary herds.

CBs form at the active sites of transcription of snRNA genes, which often occur in tandem repeats, and moreover cluster these genes together in the 3D space of the nucleus. Thus, CBs organize not only the molecules within them; CBs contribute to chromosome topology, as discussed in the review by Dundr and colleagues. Remarkably, these loci occur on several different chromosomes in human, meaning CBs are capable of mediating long range interactions between chromosomes. Dundr discusses how nucleation at active transcription sites together with liquid phase separation phenomena can help to accomplish this topological feat, which may modulate gene expression. CB association with chromatin may also reflect activities on transcription (see above) and DNA metabolism. The research paper by Farnebo and colleagues demonstrates that the protein WRAP53 – also essential for CB integrity – accumulates at sites of double-stranded DNA breaks triggered by DNA damaging agents. The missing link appears to be WRAP53 phosphorylation by the protein kinase ATM upon UV exposure. Indeed, CBs number and integrity are affected by a variety of stressors including UV, heat shock, starvation, etc. As discussed by Taliansky and colleagues in their review, CBs modulate stress responses in plants through coilin and fibrillarin interactions with poly-ADP ribose-polymerase (PARP). PARylated target proteins then shuttle from CBs to the nucleolus where PAR recycling occurs, suggesting that CBs sense cellular stress and signal to multiple cellular processes through PARylation.

As we have seen, trafficking of components between CBs and nucleoli result in some overlaps in composition. The review by Trinkle-Mulcahy and Sleeman tackles the give and take relationship between these 2 nuclear bodies, speculating that coilin interactions with snoRNAs and/or nascent rRNA could account for coilin relocalization to nucleoli under stress. Another nuclear body that often overlaps with/is contained by CBs is the histone locus body (HLB), which forms on histone gene arrays and concentrates the factors required for histone mRNA 3′ end formation: U7 snRNP, SLBP, and other factors. The review by Duronio and Marzluff focus on changes in histone gene expression, which is highest at S-phase. NPAT, a transcription factor that may scaffold the HLB, has the potential to coordinate both transcription and HLB assembly during cell cycle. In a related research paper from my laboratory, we see that histone gene transcription turns on for the first time in the zebrafish embryo at the 256-cell stage, exactly coinciding with the localization of HLB components. Inhibition of transcription leads to U7 snRNP relocalization to CBs, presumably because coilin can bind U7 and again emphasizing the potential for mixing. Consistent with the role of transcription in the formation of discrete compartments, fibrillarin segregates into CBs and nucleoli hours later when rDNA transcription finally turns on.

In contrast to cytoplasmic RNP granules lacking membrane boundaries, for which there is currently no rationale for the location of each body, nuclear bodies are therefore profoundly dependent on transcription. The resulting proximity of nuclear bodies to DNA thereby could facilitate additional functions in DNA repair, transcription termination, and/or transcriptional output as we have seen above. In this regard, transcription of the snRNA and histone loci could therefore also contribute to chromosome topology and the 3D organization of the nucleus, through the formation of CBs that either cluster these loci through CB-CB fusion events or alternatively through formation of discrete CBs capable of capturing new loci as they become transcriptionally active. Surely, we will not have to wait another 100 y for the answers to these intriguing questions about the formation and function of CBs.