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Minireview

A role for Cajal bodies in assembly of the nuclear transcription machinery

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Abstract Cajal bodies (CBs) are small nuclear organelles that contain the three eukaryotic RNA polymerases and a variety of factors involved in transcription and processing of all types of RNA. A number of these factors, as well as subunits of polymerase (pol) II itself, are rapidly and specifically targeted to CBs when injected into the cell. It is suggested that pol I, pol II, and pol III transcription and processing complexes are preassembled in the CBs before transport to the sites of transcription on the chromosomes and in the nucleoli.

Key words: Cajal body; Nucleus; Oocyte; RNA polymerase; Transcription

1. Introduction

In 1903 the Spanish neurobiologist Ramón y Cajal found a new structure in nuclei of vertebrate neurons, which, because of its close association with the nucleolus, he named the accessory body (cuerpo accesorio) [1]. Structures that are now known to share essential features with Cajal's accessory body were described during the following decades from a variety of cell types and organisms, ranging from insect and amphibian oocytes to mammalian somatic tissues, cultured cells, and plants. They were named spheres, Binnenkörper, endobodies, and coiled bodies. To recognize their essential homology and to honor the person who first noted their existence, these structures are now called Cajal bodies (CBs) [2]. Details of CB structure and composition are considered in several recent reviews [3–7].

2. Composition of Cajal bodies

CBs cannot be defined unambiguously by their structure or by a unique macromolecular component. Instead they contain a remarkable assemblage of components involved in transcribing and processing all major types of nuclear RNA (mRNA, rRNA, and polymerase (pol) III transcripts). Chromosomes, nucleoli, and nuclear speckles each contain a subset of these components, but no other structure contains the total constellation of factors found in CBs. The first indication of this heterogeneity came from immunostaining studies carried out about 10 years ago by Tan and his collaborators at the Scripps Institute [8,9]. These investigators found autoimmune sera that stained CBs specifically, the CBs in this case being defined by their characteristic fine structure in electron micrographs. Using the autoimmune sera they isolated a human gene clone that encoded a novel protein responsible for the strong staining of the CBs. They named this protein *coilin* because of its location in *coiled bodies*, the term then used for CBs in mammalian cells. As soon as it became possible to identify CBs by rapid immunofluorescent staining with antibodies against coilin, rather than by cumbersome electron micrographic techniques, quick progress was made in identifying other components.

Splicing factors were among the first macromolecules to be identified in CBs. All five of the major splicing snRNPs (U1, U2, U4, U5, and U6) were demonstrated by a combination of immunofluorescent staining and in situ hybridization [10–14]. This finding came as somewhat of a surprise, since it was already well known that splicing snRNPs were prominent components of the chromosomes and the regions known as speckles (by light microscopy) or interchromatin granule clusters (by electron microscopy). The absence of poly(A) RNA and non-snRNP essential splicing factors such as SC35 and U2AF suggested that splicing itself did not occur in CBs. For this reason speculation focussed on the possibility that CBs were involved in aspects of snRNP biogenesis. Targeting of snRNAs and their associated proteins to CBs provided circumstantial evidence in favor of such a model. When fluorescein-labeled snRNAs were injected into *Xenopus* oocytes, they appeared first in the CBs [2]. Similarly, when cultured mammalian cells were injected with plasmids encoding the Sm proteins B, D1, or E, the proteins were first detected in the CBs and nucleoli and only later in the speckles [15]. Important support for the idea that CBs play a role in snRNP biogenesis came from experiments on the survival motor neurons (SMN) protein [16–18]. SMN and several associated proteins are complexed with spliceosomal proteins in the cytoplasm, where they are involved in snRNP assembly. SMN protein also occurs in CBs, suggesting that newly formed snRNPs may travel from the cytoplasm to CBs in the nucleus for some additional assembly step(s) before taking up residence in the speckles.

From the beginning there was evidence linking CBs not just with splicing but with the nucleolus and rRNA processing. Morphological studies showed that CBs could be physically attached to nucleoli or even embedded within them [19,20]. In

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their original studies the Scripps group demonstrated fibrillarin in CBs. Fibrillarin is a highly conserved protein first identified in the dense fibrillar region of the nucleolus [21]. It is essential for pre-rRNA processing in yeast and is complexed with the box C/D snoRNAs that are required for processing and site-specific 2′-*O*-methylation of pre-rRNA. Similarly, CBs contain the nucleolar proteins Nopp140 and NAP57, which almost certainly are associated with the box H/ACA snoRNAs that are involved in pseudouridinylation of pre-rRNA. SnoRNAs themselves have been demonstrated in CBs by in situ hybridization [22,23] and at least some fluorescent snoRNAs are rapidly targeted to CBs when injected into the nucleus [24,25]. Since the snoRNAs and their associated proteins presumably act on pre-rRNA in the nucleolus itself, it is possible that they are transient components of CBs on their way to the nucleolus.

CBs are uniquely associated with the processing of histone pre-mRNAs. Unlike typical pol II transcripts, most histone pre-mRNAs do not contain introns and therefore are not spliced. However, they carry a 3′ extension beyond the coding region, which is cleaved in a reaction that requires the specific U7 snRNP [26]. U7 snRNA was originally demonstrated in CBs of *Xenopus* oocytes by in situ hybridization, and subsequently it was shown that U7 is rapidly and specifically targeted to CBs when injected into the oocyte cytoplasm [27,28]. The close association between CBs and histone pre-mRNA processing is further underscored by the fact that some CBs are physically attached to the chromosomes at the histone gene loci. This attachment was first seen in the giant lampbrush chromosomes of amphibian oocytes [29,30] and later demonstrated in HeLa nuclei [31]. It presumably represents a mechanism by which the U7 snRNP and other processing factors are brought into proximity with the actual sites of histone pre-mRNA transcription.

3. A model of Cajal body function

Any comprehensive model for the role of CBs in nuclear physiology must account for the large number and diverse nature of their macromolecular components. Because these components are in a state of flux, CBs must be more than simple storage particles. As already mentioned, it is likely that they are involved in some steps in assembly of the RNA processing machinery (splicing, pre-rRNA processing, and histone pre-mRNA processing). However, recent studies indicate that CBs also contain the three eukaryotic RNA polymerases and additional factors involved in transcription and processing beyond those already discussed. From these observations emerges a model in which CBs are major sites within the nucleus for assembly of the entire transcription machinery, not just the RNA processing machinery (Fig. 1). The existence of the three polymerases in CBs and their movement into and out of CBs is crucial to this model and will be considered next.

The first evidence for pol II in CBs was provided by Schul et al. [32], who showed staining of somatic CBs with monoclonal antibody (mAb) 8WG16, which recognizes the unphosphorylated C-terminal domain (CTD) of the largest subunit of pol II (RPB1). CBs in the *Xenopus* germinal vesicle are also stained by this antibody [2], and by two other antibodies against RPB1: mAb ARNA, which recognizes an epitope outside of the CTD, and mAb H14, which recognizes the CTD when serine-5 is phosphorylated. The staining of CBs with mAb H14 is somewhat surprising, because phosphorylation of the CTD is thought to take place after pol II binds to the chromatin template but before elongation [33]. Nevertheless, experiments with the transcription inhibitor 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) support the view that the epitope recognized in CBs is, indeed, the CTD of

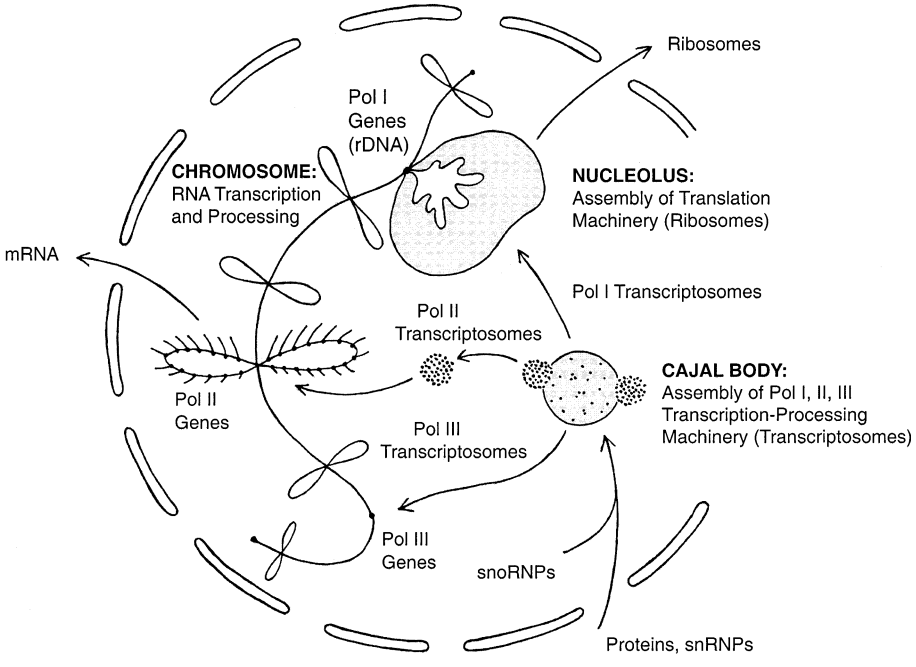

Fig. 1. A proposed model of CB function. The basic assumption is that complexes of RNA polymerases I, II, and III along with specific transcription and processing factors are preassembled in the CBs as unitary particles (transcriptosomes) before transport to their target genes on the chromosomes.

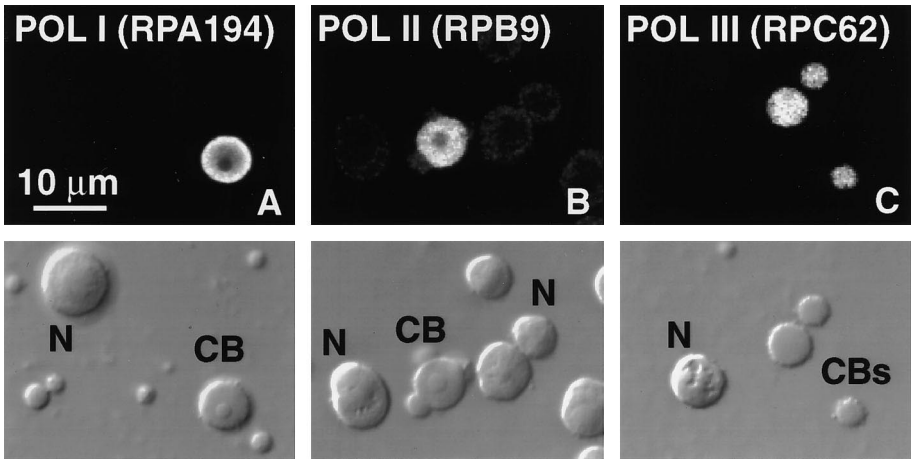

Fig. 2. Evidence for RNA polymerases I, II, and III in CBs from oocytes of *Xenopus*. Each panel shows a small fraction of the material from a single oocyte nucleus, whose contents were spread on a microscope slide before immunofluorescent staining. Vertical pairs of panels show immunofluorescent stain (top) and corresponding differential interference contrast image (bottom). A: Stained with a polyclonal serum against the largest subunit of rat pol I (RPA194). B: Distribution of a myc-tagged subunit of human pol II (RPB9), 4.5 h after injection of in vitro transcripts into the cytoplasm of an oocyte. Stained with an antibody against the myc tag. C: Stained with a polyclonal serum against a subunit of human pol III (RPC62). N, nucleolus.

pol II. When oocytes are treated with DRB, the ability of CBs to stain with mAb H14 is abolished within 2–3 h, but staining returns when the inhibitor is removed [34]. DRB inhibits a number of protein kinases and prevents phosphorylation of the CTD both in vivo and in vitro. One interpretation of the DRB experiment is that pol II is continuously entering and leaving the CB. In the presence of DRB, the phosphorylated form leaves the CB but is replaced only by the unphosphorylated form.

Support for the idea that pol II transits through the CB was provided by targeting experiments with two smaller subunits of the pol II core enzyme, RPB6 and RPB9. Epitope-tagged transcripts of these two proteins were injected into the cytoplasm of *Xenopus* and *Triturus* oocytes. Within a few hours the newly translated proteins had entered the nucleus and were detectable by immunofluorescent staining in the CBs (Fig. 2B) [34]. Together the targeting and DRB experiments suggest that pol II subunits may pass through CBs with a transit time of no more than a few hours.

In addition to the snRNPs involved in splicing and in histone pre-mRNA cleavage, other factors required for transcribing and processing pol II transcripts are demonstrable within or immediately adjacent to CBs by immunostaining. These include the TATA-binding protein TBP, PTF-γ, and TFIIF (RAP74) [32], as well as TFIIH [35,36]. The cleavage and polyadenylation factors CstF77 and CPSF100 are present in oocyte CBs [2] and in bodies closely associated with CBs in cultured cells [37].

The evidence for pol I and pol III in CBs is based primarily on antibody staining. Antibodies against the two largest subunits of pol I (RPA194 and RPA127), two subunits of pol III (RPC62 and RPC53), and a subunit shared by pol I and pol III (RPC19) all stain CBs in *Xenopus* oocytes (Fig. 2A,C). Furthermore the transcription factor TFIIIA is readily demonstrable in oocyte CBs [2].

4. Conclusion

In summary, CBs contain the three eukaryotic RNA polymerases and a variety of factors, both protein and RNA, involved in transcription and processing of all types of RNA. Targeting experiments show that many of these factors are rapidly and specifically directed to CBs when injected into the cell, even though at steady state most are more abundant in other parts of the nucleus, specifically the chromosomes and speckles in the case of pol II factors and the nucleoli in the case of pol I factors. Although one can imagine a number of reasons for the accumulation of so many disparate factors in the CBs, these observations are consistent with CBs playing a role in preassembly of the transcription machinery. The model presented in Fig. 1 provides a cell biological correlate to the increasing evidence for the existence of preassembled holoenzymes in both yeast and mammalian systems [38–43]. One might question why such preassembly, if it does occur, need involve all three polymerases and their associated factors in the same cytological organelle. A plausible answer to this question is provided by molecular features of the polymerases themselves. The three eukaryotic polymerases contain two large subunits that are related to the two subunits of bacterial polymerase. Furthermore, there is considerable sequence similarity or identity among the remaining 10–15 subunits, strongly suggesting that the three present-day polymerases are derived from a single primitive eukaryotic polymerase. One can imagine that the assembly of this early polymerase into a complex with its various transcription and processing factors required chaperones and some type of specialized cellular machinery. Over evolutionary time the gradual differentiation of the present-day polymerases could have taken place while the common assembly machinery in the CBs was maintained.

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