Archives of Biochemistry and Biophysics 496 (2010) 69–76

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Review

Phosphorylation and the Cajal body: Modification in search of function

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ARTICLE INFO

Article history:
Received 28 January 2010
Available online 1 March 2010

Keywords:
Coilin
Kinase
Phosphatase
Signal transduction
SMN
Spinal muscular atrophy

ABSTRACT

The Cajal body (CB) is a subnuclear domain that contains proteins and factors involved in a diverse range of activities including ribonucleoprotein maturation, histone gene transcription and telomerase assembly. Among these activities, the CBs' role in small nuclear ribonucleoprotein (snRNP) biogenesis is best characterized. Although CBs are found in plants, flies and mammals, not all cell types contain CBs. Rather, CBs are most prominent in transcriptionally active cells, such as cancer and neuronal cells. Many CB components, including the CB marker protein coilin, are phosphorylated in humans. The functional consequence of phosphorylation on CB assembly, activity and disassembly is largely unknown. Also unknown are the signaling pathways, kinases and phosphatases that act upon proteins which localize in the CB. The goal of this review is to demonstrate the need for a concerted effort towards elucidating the functional consequence of phosphorylation on CB formation and activity.

Introduction

The eukaryotic nucleus contains an astonishing array of compartments, foci, domains and bodies. These subnuclear structures presumably allow for vital nuclear processes, such as RNA synthesis and processing, to occur in the most efficient manner for a given cell type. One of these structures is the Cajal body (CB).[1] The initial discovery of CBs was made in 1903 by Ramon y Cajal, who called these subnuclear domains "nucleolar accessory bodies" due to their frequent association with nucleoli in neurons [1]. There are several excellent reviews that discuss major advances in the study of CBs, and the reader is encouraged to examine this literature for a comprehensive overview about CB function [2–7]. This review will focus on the role of phosphorylation on CB formation and function, as well as explore some of the signaling pathways that may impact phosphorylation of CB components. In the first section of the review, CB composition and activity will be described in brief. This will be followed by an examination of conditions that alter CBs, and signaling pathways triggered by these conditions. Phosphoproteins that accumulate in CBs will be discussed, as will the kinases and phosphatases known to modify these proteins. The review will end with an analysis of important questions that need resolution in order to better understand the influence of phosphorylation on CB activity.

The Cajal body: what have we learned in the last 107 years?

A cursory PubMed search demonstrates that around 90% of publications on CBs have come since the identification in 1991 of antibodies that recognize coilin, the CB signature protein [8,9]. Interestingly, CBs are heterogeneous (described below) and some factors will co-localize with coilin in CBs during specific conditions, such as cell-cycle phase. Knockout and knockdown studies have shown that coilin is essential for proper CB formation and composition [10–14]. No obvious growth phenotype in mutants lacking coilin and CBs in *Arabidopsis* or *Drosophila* is detected [11,12]. However, HeLa cells depleted of coilin by RNAi proliferate slower than control treated cells [13,14], and knockout mice display reduced viability, fertility and fecundity [10,15]. Coilin and CBs, therefore, are not essential for survival, but their conservation from vertebrates to plants to flies strongly suggests that their presence must be of some benefit.

Many cell types do not have CBs [16,17]. Thus cells with CBs must have a physiological need for their existence, although the activities that take place within the CB likely can also occur in the nucleoplasm. Additionally, most (if not all) components found in the CB also localize to other cellular compartments such as the cytoplasm, nucleoplasm and nucleolus [18]. For some proteins that accumulate in the CB, it is not clear if their function in the CB is the same function found for these proteins in other cellular compartments, causing further complications in understanding the role of CBs in the cell. Moreover, some proteins initially thought to reside in CBs (e.g. NPAT and FLASH) have recently been shown to localize in histone locus bodies (HLBs) [19–23]. Replication-dependent histone gene clusters have been shown to associate with HLBs. This association allows for components in the HLB to orchestrate

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[1] *Abbreviations used:* CB, Cajal body; HLBs, histone locus bodies; snRNPs, small nuclear ribonucleoproteins; snRNA, small nuclear RNA; SMA, spinal muscular atrophy; PML, promyelocytic leukemia; snoRNAs, small nucleolar RNAs; snoRNP, small nucleolar ribonucleoprotein; HSV-1, herpes simplex virus type 1; ICP0, infected cell protein 0; iCDR, interphase centromere damage response.

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doi:10.1016/j.abb.2010.02.012

replication-dependent histone gene transcription and 3′-end pre-mRNA processing (reviewed in [22]). Interestingly, HLBs and CBs fuse during the S-phase of the cell cycle [24]. Thus CB composition changes during the cell cycle. Collectively, the lack of CBs in some cell types, the localization of CB proteins to other cellular compartments, and the heterogeneity of CBs all conspire to make elucidation of CB function a challenge.

A key to understanding CB function stems from the finding that CBs contain the highest concentration of small nuclear ribonucleoproteins (snRNPs) in the nucleus [25]. Although snRNPs play a vital role in pre-mRNA splicing, active splicing does not take place within the CB [18]. Rather, the CB participates in snRNP modification and assembly. Evidence in support of this idea comes from detailed studies into snRNP biogenesis. Splicing snRNPs are comprised of a small nuclear RNA (snRNA), a septet of Sm proteins and additional snRNP-specific proteins [26]. U1, U2, U4 and U5 snRNA (but not U6 snRNA) are transported to the cytoplasm for processing and Sm protein assembly under the control of the survival of motor neuron (SMN) protein [27,28]. Mutations in SMN cause most cases of spinal muscular atrophy (SMA), the leading genetic cause of infant mortality (reviewed in [27]). Upon nuclear re-entry, newly assembled snRNPs target first to the CB before they accumulate in speckles, another subnuclear domain [29]. In the CB, the snRNA moiety of the nascent snRNP is subject to modification reactions guided by small Cajal body specific RNAs (scaRNAs) [30,31]. When certain scaRNAs are misdirected to the nucleolus, their cognate snRNAs are not properly modified [31]. SnRNA modifications are necessary for proper snRNP function [32–34]. Interestingly, overexpression of one Sm protein (SmB) in a cell line that normally lacks CBs induces the formation of this subnuclear domain [35].

Taken together, the above findings strongly argue for a role of the CB in the efficient maturation of snRNPs. Again, however, cell types without CBs still have sufficient snRNP resources to splice pre-mRNA, so CBs and the activities therein are not absolute requirements for cell function. ScaRNAs, for example, have been shown to properly modify snRNAs in flies lacking CBs [12]. Rather, it is thought that CBs facilitate snRNP biogenesis by providing a locally high concentration of the requisite materials. For example, elegant studies by Neugebauer and colleagues have shown that U4/U6 di-snRNP assembly is increased 11-fold in cells with CBs compared to the assembly rate in cells lacking CBs [36]. Since CBs are most prominent in cells that are transcriptionally active, such as neuronal and cancer cells [25,37–40], it is logical to assume that reactions facilitated by the CB contribute to the overall physiological robustness of these cell types.

It is notable that SMN, in most cell lines and adult tissues, is found in CBs in addition to the cytoplasm [41,42]. The exact role of SMN in the CB is not clear. Since SMN is critical for the nuclear import of nascent snRNPs to the CB [43], the accumulation of SMN in CBs may be reflective of this function. Support for this hypothesis comes from data showing that coilin can directly interact with SMN and Sm proteins [44–47]. Alternatively, or additionally, the SMN in CBs may facilitate the regeneration or recycling of snRNPs after a splicing reaction has taken place [46,48]. Curiously, in some cell lines and fetal tissues, nuclear SMN is found in Gems (for Gemini of CBs), which are nuclear bodies often found adjacent to CBs [49,50]. The function of Gems, which contain SMN and associated proteins known as Gemins but lack snRNPs, remains obscure. Interestingly, cells that display Gems still have CBs. These CBs are indistinguishable from CBs found in cells without Gems except that they are not enriched for the nuclear SMN complex proteins. In this review, we refer to 'canonical CBs'. Canonical CBs are those found in most cell lines and adult tissues and contain, among other factors, coilin, SMN, and snRNPs.

The composition of the CB and its nuclear dynamics suggest other functions besides those centered upon snRNP maturation and maintenance. CBs are mobile and associate with chromatin in an ATP- and transcription-dependent manner [51]. Furthermore, CBs have been shown to associate with snRNA genes (e.g. U2 genes) and histone gene clusters, as discussed above (reviewed in [7]), suggesting that CBs play a role in the transcription of these genes. Support for this hypothesis comes from the observation that basal transcription factors, such as RNA polymerase I and II, TFIIF, TFIIH, TBP and PTFγ, are enriched within the CB, possibly indicating that these factors are assembled or processed in the CB before transport to their site of action [7,52,53]. The association of CBs with certain genes may therefore indicate that these genes require locally high concentrations of these factors. Alternatively, the fact that snRNPs are highly enriched in the CB supports the idea that CB association with U snRNA genes is the manifestation of some type of feedback regulatory control on the transcription of these genes [18].

Besides Gems and certain gene loci, CBs have been shown to occasionally associate with PML bodies with a high degree of statistical significance [54]. PML (promyelocytic leukemia) bodies contain numerous factors implicated in a variety of cellular functions, such as transcriptional regulation [18,55,56], and have been shown to associate with transcriptionally active gene loci [57,58]. We have demonstrated that a CB can simultaneously associate with an U2 gene locus and a PML body, possibly indicating that these two structures may work in concert to provide a hitherto unknown mechanism of regulatory control [59]. Additionally, CBs associate with telomeres [60,61]. This is an interesting result given that telomerase RNA has a structure similar to scaRNAs and is found within the CB. Telomerase RNA and scaRNAs require the WDR79/TCAB1 protein for localization to CBs [62,63]. In the CB, the telomerase RNA is most likely subject to modification reactions and holoenzyme assembly [64–66]. Thus CBs participate in the biogenesis and delivery of telomerase to telomeres.

CB dynamics: from cold carp to KO'ed flies

In order to better understand CB function, it is helpful to consider circumstances that either disrupt or promote canonical CBs assembly (Table 1). Strikingly, 13 of the 47 conditions listed increase coilin and/or CB component association with the nucleolus. Considering that Cajal initially called CBs "nucleolar accessory bodies" because he often observed them near nucleoli [1], the existence of biochemical dialog between CBs and nucleoli should not be surprising. Exactly why this happens, however, and what benefit it confers, is unclear. CBs and nucleoli share some components, Nopp140 and fibrillarin for example. Both of these proteins interact with snoRNAs (small nucleolar RNAs), which suggests that their presence in CBs may facilitate snoRNP (small nucleolar ribonucleoprotein) maturation [6]. Furthermore, Nopp140 and fibrillarin interact with CB proteins coilin and SMN, respectively [67–69], providing a direct link between the CB and nucleolus. Another link between CBs and nucleoli comes from studies in *Drosophila* that show that poly(ADP-ribose) polymerase 1 (PARP1) is important for CB integrity and may facilitate the localization of nucleolar proteins, like fibrillarin, to the CB [70]. An ancestral connection between CBs and nucleoli may be evident in budding yeast, which contains a structure known as the nucleolar body [71]. The nucleolar body contains components that are found within CBs, so it is possible that the CB assumed the nucleolar body capacity in higher eukaryotes. Situations listed in Table 1 that mis-localize CB or CB components to nucleoli, therefore, may be indications that the connection between CBs and nucleoli has been evolutionarily conserved, but not fully understood.

Viral infection by adenovirus or herpes simplex virus type 1 (HSV-1) has also been shown to alter typical CB localization. For