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The Promyelocytic (PML) Nuclear Compartment and Transcription Control

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ABSTRACT. Wild-type promyelocytic leukemia (PML) protein and an increasingly documented number of cellular proteins are localized within discrete nuclear structures known as PML nuclear bodies or PODs (*p*otential *o*ncogenic *d*omains). Even though POD function remains elusive, the integrity, topology, and molecular composition of these nuclear compartments have been associated with certain human diseases, including cancer, autoimmunity, neurodegenerative disorders, and viral propagation. At the molecular level, PML protein has been shown to be a coactivator of nuclear hormone receptors, whereas its oncogenic counterpart PML-retinoic acid receptor α, which promotes POD disaggregation, has been found to activate activator protein-1 transcription in a retinoic acid-dependent manner. Recently, we demonstrated that the CREB-binding protein (CBP) associates with PML protein *in vitro* and is recruited to the PODs *in vivo* in a signal-dependent manner. In exploring the consequence of this association, we proposed that POD nuclear bodies are regulatory cellular domains where proteins such as the CBP and CBP-interacting molecules may be activated or inactivated to coordinate signal-activated cellular response. This paper discusses the association of PML nuclear bodies with transcription control and underscores the pharmacological aspects of such an observation. BIOCHEM PHARMACOL **60**;8:1197–1201, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. PML; CBP; nuclear compartmentalization; signal transduction; transcription control; viral propagation; cancer

THE PROMYELOCYTIC NUCLEAR COMPARTMENTS

Increasing evidence suggests that a major role in the regulation of cellular activities, such as signal transduction and DNA transcription, could be attributed to the spatial organization of the nucleus. Research on nuclear compartments has uncovered evidence for transcription-related proteins in nuclear substructures and has supported their potential relevance to transcriptional regulation [1–3]. The cell nucleus contains a variety of morphologically distinct substructures called nuclear bodies, which include the sphere organelles, coiled bodies [4–6], and the PML‡ nuclear bodies or PODs [7]. The PODs (also known as ND 10 or Kr bodies) are macromolecular multiprotein complexes that are present in all cultured cell lines as well as *in vivo*. Immunofluorescence studies have demonstrated that PODs can vary in number and size depending on cell type, hormonal exposure, and cell cycle [7]. A major component of the POD is the PML protein, which was originally identified as the fusion partner of RARα in the chromsomal translocation t(15;17) [8–13]. The leukemic fusion protein contains all but the amino-terminus of the RARα and includes both the DNA-binding and ligand-binding domains. The PML–RAR fusion protein contains most of the amino-terminal portion of PML protein, which includes the homology regions most likely to encode functional domains such as the cysteine-rich motif also known as the RING finger [14]. In addition, PML protein contains a long putative α-helical region including a leucine zipper that is required for PML homodimer formation as well as downstream PML function [1, 3, 8]. The presence of two protein–protein interaction motifs in PML protein, the RING finger and the α-helical region, implies that PML protein possesses a self-polymerizing feature that permits efficient packaging and/or transfer of bound target molecules into a round vesicle-like structure [7].

UNDERSTANDING FUNCTION THROUGH DISRUPTION AND INTEGRATION

In leukemic cells from patients with APL that carry the translocation t(15;17), the expression of the PML-RARα fusion protein disrupts the structural integrity of PODs. In

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‡ *Abbreviations:* POD, potential oncogenic domains; APL, acute promyelocytic leukemia; PML, promyelocytic leukemia; CBP, CREB-binding protein; NR, nuclear receptors; GR, glucocorticoid receptor; RXR, retinoid X receptor; RARα, retinoic acid receptor alpha; and AP-1, activator protein-1.

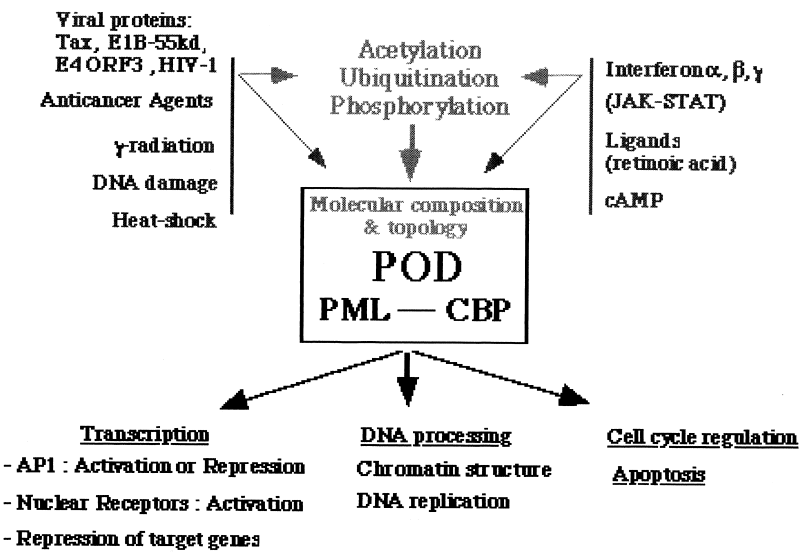

FIG. 1. Major signaling pathways associated with POD integrity. Extracellular signals, such as activation of DNA damage, γ-radiation, heat shock and interferon treatment of the cells (activation of Janus tyrosine kinase [JAK] and signal transducers and activators of transcription family [STAT proteins], ligands including adenosine 3′,5′-cyclic monophosphate (cAMP) and RA, cell cycle, viral infection, and overexpression of viral proteins have been directly or indirectly associated with POD remodeling [2, 3, 19]. Even though direct protein–protein interaction between viral or cellular activators and POD-localized proteins could mediate the POD remodeling, it is also plausible that signal-activated posttranslational modifications of POD-localized proteins, such as acetylation, phosporylation and ubiquitination, might control POD molecular composition and topology. We speculate that POD might play a coordinate regulatory role at the transcriptional level and in DNA processing and cell cycle control.

fact, PML-RARα forms homodimers and PML:PML–RARα heterodimers *in vitro*, which results in disruption of normal POD integrity and may contribute to the oncogenic state in APL patients. The POD structure is reformed in leukemic cells following treatment with all-*trans* retinoic acid (RA), a process that is associated with RA therapy [15–18]. The integrity of the compartment is also altered during adenovirus infection, where it appears that POD-associated proteins are released to viral replication and transcription domains [19]. Indeed, two early transcribed adenoviral proteins, E4-open reading frame (ORF)3 11kD and E1B 55kD, are targeted to the POD and trigger its dissociation. A third viral protein, the E4-ORF6, cooperates with E4-ORF3 in promoting POD dissociation, even though E4-ORF6 is negative for POD localization in immunofluorescence studies [19]. At the functional level, inhibition of viral-induced POD disaggregation considerably reduces adenoviral replication, suggesting that PODs contain cellular factors that might play a key role in viral propagation [19]. Indeed, this observation linked for the first time the integrity and molecular composition of a nuclear domain (POD) to adenoviral propagation. More recently, the PODs have been shown to be a target of herpes, hepatitis, papillomavirus, human immunodeficiency virus type-1 (HIV-1), and human T-cell leukemia virus type-1 (HTLV-1) viral proteins [2, 7, 20–22].

The viral–host interaction at the level of PML bodies suggested that PODs may represent a depot that sequesters regulatory proteins whose activities or soluble levels must be tightly controlled. Recently, the spinocerebellar ataxia 1 neurodegenerative disorder-associated protein (SCA1) has also been shown to colocalize with PML protein and alter POD morphology through a glutamine track motif carried by the mutant SCA1 protein [23, 24]. Finally, the adenosine 3′,5′-cyclic monophosphate response element binding protein (CREB)-binding protein (CBP) has been shown to associate the PML protein *in vitro* and to be recruited to the PODs *in vivo* in a signal-dependent manner [22, 3]. The results demonstrate that a fraction of CBP is compartmentalized to the POD through its association with PML protein, thus suggesting that PML and PML-interacting proteins could play an unexpected role in transcription control. The plethora of biochemical events of cellular and viral origin that associate with POD topology and composition (Fig. 1) promote the PML bodies to an integrator of multiple extra cellular signals. Ultimately, an analysis of each pathway will be necessary to understand POD functioning. However, the diversity of these events suggests that PODs might play a coordinate role in elaborating signal-activated cellular response.

THE PML-CBP ASSOCIATION

Immunofluorescence studies with anti-CBP antibodies have suggested a uniform or diffuse nuclear distribution for the endogenous CBP protein. It has been shown that anti-CBP antibodies may also reveal punctuate CBP staining that was virtually identical to the POD speckled pattern observed in immunofluorescence studies (Fig. 2) [3, 22]. Indeed, we demonstrated that the intensity of the visualization of CBP in the POD can vary in an asynchronous cell population and depends strongly on cell growth conditions [3]. Taken together, these observations suggest that CBP may be found in at least two different physical states, i.e. randomly diffused in the nucleus or compartmentalized to PML nuclear bodies. In exploring the consequence of PML–CBP colocalization in the POD, PML protein was shown to associate with CBP *in vitro* and *in vivo*. Indeed, it has been shown that amino acid (aa) 216–331 of PML protein are dispensable for CBP association and that a minimal 321–521-aa domain of CBP is sufficient for PML interaction [3, *]. Furthermore, PML protein was shown to

* Doucas V and Nakajima T, unpublished data.

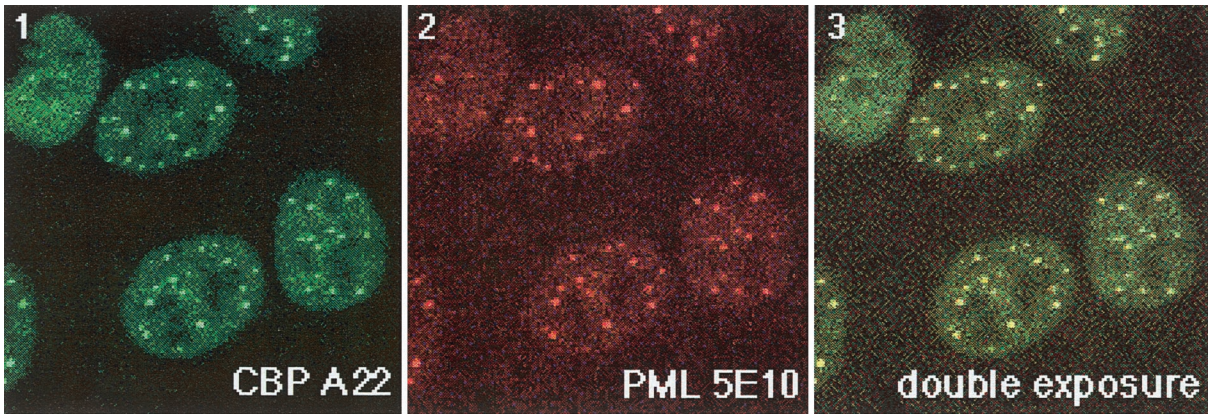

FIG. 2. CBP localization in PML nuclear bodies. Immunohistochemistry of CV-1 cells, fixed at 80% confluence and analyzed by confocal microscopy. Primary antibodies are used, as indicated. Green corresponds to the CBP staining revealed with fluorescein isothiocyanate-conjugated secondary antibody, red corresponds to the PML staining revealed with the Texas red-conjugated secondary antibody, and the yellow color in the double-exposure image shows the PML and CBP colocalization in the PODs.

promote increased localization of CBP to the POD and to function as potent NR coactivator.

PML, A TRANSCRIPTIONAL COFACTOR

Although NR-dependent transcriptional activation relies on protein–DNA interactions, nuclear receptors are able to modulate the activity of other classes of trans-activators through DNA-binding-independent mechanism(s). This process of regulation is known as transcriptional cross-talk or cross-coupling [25, 26]. In recent years, the biochemical basis of transcriptional cross-talk has been under intense investigation. For example, RARα has been shown to repress AP-1 transcription and vice versa [27]. In exploring the consequence of PML–RARα fusion in cross-coupling, it has been shown that PML protein changes RARα from an RA-dependent inhibitor to an RA-dependent activator of AP-1 transcriptional activity [1]. Furthermore, in the absence of RA, a circumstance in which RARα has no major effect on AP-1 at low levels of expression, PML–RARα was observed to be an inhibitor [1]. In view of the association between AP-1 activity and hemopoietic differentiation, these results suggested that PML–RARα could contribute to the APL phenotype and its response to RA therapy [1]. Since unfused wild-type PML protein neither interfered with nor enhanced AP-1 activity in these experiments, we suggest that the reorganization of POD, triggered by PML–RAR fusion in the presence of RA, redistributes POD-associated «activity» and thus promotes AP-1 transcription.* Thus, PML–RAR activity on AP-1 transcription constitutes the first observation linking POD integrity to transcription control. Furthermore, the discovery of the PML–CBP association linked POD integrity to transcription control at the biochemical level and thus established that PML functions as potent nuclear receptor coactivator. Although PML protein does not directly associate with NRs, PML was found to be a potent activator of GR and RXR transcription [3]. In conclusion, the recruitment of CBP in the POD by PML is associated with enhanced NR-dependent transcription, whereas the PML–RAR-induced dissociation of POD integrity is linked to activation of AP-1 (Fig. 3). These observations do not establish that POD integrity is an essential intermediate in signal transmission at the transcriptional level, but do raise this as a distinct possibility.

* Doucas V, unpublished data.

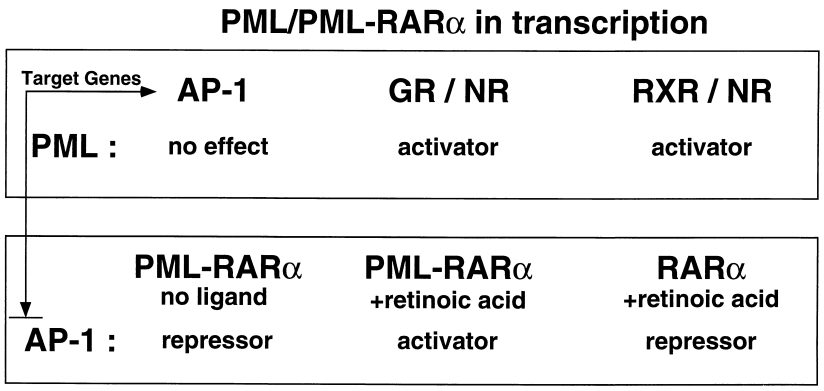

FIG. 3. Schematic representation of PML and PML–RARα effects on AP-1- and NR-mediated transcription. PML–RARα was shown to be an AP-1 transcriptional activator or repressor functioning in a ligand-dependent manner [1], while PML was shown to be a GR and RXR coactivator [3].

COMPARTMENTALIZATION AND TRANSCRIPTION CONTROL

Extracellular signaling interpretation has been found to associate with complex molecular interactions in the target cells mediating rapid and accurate response to the nucleus at the transcriptional level. The extracellular signal is transmitted through different pathways, such as the translocation of activated protein kinases from the cytoplasm to the nucleus or by the nuclear translocation of activated transcription factors [28]. Initiation of gene transcription by RNA polymerase II is dependent on general initiation and specific transcription factors [29]. Although general initiation factors such as the TATA box-binding protein are in close proximity to the polymerase holoenzyme and mediate basal gene activity, efficient gene transcription requires the binding of «activated» specific transcription factors to DNA promoter/enhancer elements. Specific transcription factors may establish contacts with the basal transcription machinery on their own, but they often need bridging coactivators such as CBP and p300 [30]. CBP binds to a variety of transcription factors including adenosine 3′,5′-cyclic monophosphate response element binding protein (CREB), Jun, Fos, NRs, nuclear factor-kappa B, signal transducers and activators of transcription family (STAT proteins), as well as viral proteins such as the human T-cell leukemia virus type-1 (HTLV-1) Tax protein or adenoviral E1A/E1B [31–34]. Subsequently, a variety of different DNA-binding transcription factors and coactivators have been shown to rely on CBP for their function in *in vivo* and *in vitro* transcription assays [31, 35]. In addition, CBP interacts with the tumor suppressor p53 as a coregulatory factor [36–38]. CBP contains an intrinsic histone acetyltransferase activity and also associates with other coactivators such as the p300/CBP-associated factor histone acetylase (P/CAF), the steroid receptor coactivator-1 (SRC-1), the transcriptional intermediary factor 2 (TIF2 or SRC-2), and the activator of thyroid and retinoic acid receptors (ACTR or SRC-3) [39, 40]. Recently, CBP was shown to associate with Smad3 in a phosphorylation-dependent manner, and was thereby found to link transforming growth factor-beta (TGF-β) and activin signaling pathways [41]. The plethora of cellular and viral proteins that interact with CBP suggests that CBP may serve as a transcriptional integrator of multiple signaling pathways involved in cell growth, cell differentiation, and viral pathogenesis. The elucidation of the biochemical pathway responsible for the signal-dependent specificity of CBP recruitment at the transcriptional level would be essential both in basic and applied biological research. The demonstration that a fraction of CBP is compartmentalized to the POD in a signal-dependent manner suggests that PML nuclear bodies may play an unexpectedly broad role in aspects of transcriptional regulation and human disease.

CONCLUSION

Recent advances in the understanding of human carcinogenesis have united apparently incompatible hypotheses: viral-transforming principles, covalent DNA damage as a primary cause of cancer, and inherited traits. Our challenge is to elaborate the regulatory role of nuclear space, as illustrated by the PODs and POD-related domains, in disease control, cancer promotion, and viral propagation. The biochemical characterization of PODs and associated domains should enable us to establish novel molecular interactions and to understand how the relative molecular density of biological molecules such as proteins, RNA, and ligands may contribute to the regulation of signal transduction. These findings could promote the PML domain to a dynamic integrator of multiple extracellular signals and thus link POD molecular composition and topology to human carcinogenesis and disease control.

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