

REVIEW

Finding a role for PML in APL pathogenesis: a critical assessment of potential PML activities

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In normal mammalian cells the promyelocytic leukemia protein (PML) is primarily localized in multiprotein nuclear complexes called PML nuclear bodies. However, both PML and PML nuclear bodies are disrupted in acute promyelocytic leukemia (APL). The treatment of APL patients with all-*trans* retinoic acid (ATRA) results in clinical remission associated with blast cell differentiation and reformation of the PML nuclear bodies. These observations imply that the structural integrity of the PML nuclear body is critically important for normal cellular functions. Indeed, PML protein is a negative growth regulator capable of causing growth arrest in the G₁ phase of the cell cycle, transformation suppression, senescence and apoptosis. These PML-mediated, physiological effects can be readily demonstrated. However, a discrete biochemical and molecular model of PML function has yet to be defined. Upon first assessment of the current PML literature there appears to be a seemingly endless list of potential PML partner proteins implicating PML in a variety of regulatory mechanisms at every level of gene expression. The purpose of this review is to simplify this confusing field of research by using strict criteria to deduce which models of PML body function are well supported.

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Introduction

Acute promyelocytic leukemia (APL) is associated with a variety of chromosomal translocations.¹ The most common translocation (approximately 98% of APL cases) involves the promyelocytic leukemia protein (PML) gene on chromosome 15 and the retinoic acid receptor alpha (RAR α) gene on chromosome 17, resulting in the expression of the PMLRAR α fusion protein.^{1–5} The block in myeloid differentiation associated with APL apparently results from the combined disruption of the normal activities of both PML and RAR α .^{1,6–9} PML is a negative growth regulator capable of causing growth arrest in the G₁ phase of the cell cycle, transformation suppression, senescence and apoptosis.^{8,10–15} In contrast, RAR α induces differentiation upon binding retinoic acid (RA).⁹

Unfortunately, the task of further implicating PML in APL pathogenesis is hindered by the fact that although the physiological outcomes of PML overexpression have been established, the molecular and biochemical mechanisms regulated by the PML protein remain under characterized. PML is ubiquitously expressed in normal mammalian cells,^{16,17} forming multiprotein PML nuclear bodies (PML-NBs) also known as PML oncogenic domains (PODs), nuclear domain 10 (ND10) or Kremer bodies (Kr).^{1,18,19} In APL cells these bodies

are disrupted and the treatment of APL patients with all-*trans* retinoic acid (ATRA) leads to disease remission correlated with PML-NB reformation.^{1,2,7,20,21} Therefore, the structural integrity of the PML-NB could potentially be important for normal cellular functions. However, when determining both PML protein and PML-NB activities, it is important to remember that PML gene expression is not required for viability, as PML^{–/–} mice develop normally and do not get spontaneous cancers.²² In addition, the PML gene appears limited to higher eukaryotes and is absent from the *Drosophila melanogaster*, *Saccharomyces cerevisiae* and *Arabidopsis thaliana* genomes.²³

PML protein contains a set of zinc finger domains known as the RING and B-boxes.^{24,25} Although early studies proposed that PML utilized these zinc fingers to directly bind DNA and subsequently alter gene expression,²⁶ it is now clear that the RING and B-box domains do not bind nucleic acids.²⁴ These domains are actually used for interacting with partner proteins that preserve both the structural integrity and subsequent physiological activities of the PML-NB.^{8,10,12,27,28} Upon first assessment of current PML literature there appears to be a seemingly endless list of potential PML partner proteins implicating PML in a variety of functions at every level of gene expression.^{1,29} Indeed, the sheer number of potential partners has led some researchers to propose that the PML-NB is a storage site or nuclear depot.^{19,30} Several review articles have listed and characterized a variety of possible PML partner proteins,^{1,18,29} but assigning functions to PML based on the activities of every potential partner would be beyond the scope of this review. Instead, we will critically assess the PML literature in order to highlight potential biochemical and molecular activities that may explain PML's established physiological functions. Strict criteria will be used to evaluate a variety of intriguing PML-NB functions with the eventual goal of further understanding APL pathogenesis.

What is the biological significance of PML expression?

The PML protein contains a number of distinct domains. The RING, B1 B-box and B2 B-box are cysteine-rich, zinc-binding domains primarily involved in protein–protein interactions and do not appear to directly bind nucleic acid.²⁴ These three domains, together with the adjacent leucine-rich coiled coils form the RBCC motif of the PML protein. Studies with PML proteins containing mutations within this motif show that an intact RBCC motif is required for PML's physiological function as a negative growth regulator.^{10,12,14,31} Instead of manipulating global gene expression PML appears to only regulate the expression of specific genes. Total protein synthesis, as indicated by ³⁵S-methionine incorporation into cell cultures, is unaffected by PML overexpression.³¹ However, overexpressed PML has been shown by two independent groups to specifi-

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cally regulate the expression of cyclin D1, a gene involved in cellular growth.^{13,14,31} Consistent with its role as a negative growth regulator, PML specifically decreases the expression of cyclin D1, a potent growth stimulatory protein, but does not alter either actin or GAPDH protein levels.^{13,14,31}

PML gene expression is not essential for survival and PML^{-/-} mice are, in essence, morphologically normal.²² However, PML^{-/-} mice are more susceptible to tumor formation when exposed to carcinogens and PML^{-/-} cells are less likely to undergo apoptosis under certain types of cellular stress.^{15, 22} PML gene expression appears limited to higher eukaryotes and may only be found in mammals.²³ No PML gene has been observed in *Saccharomyces cerevisiae* (<http://genome-www.stanford.edu/Saccharomyces>), *Drosophila melanogaster* (<http://www.fruitfly.org>) or *Arabidopsis thaliana* (<http://www.arabidopsis.org>). In fact, although RING domain-containing proteins can be found in *S. cerevisiae* and *A. thaliana* genomes, no RBCC motifs, or even combined RING/B-boxes are present in either genome (<http://smart.embl-heidelberg.edu>).²³

The PML nuclear body (PML-NB)

In addition to mediating the physiological functions of the PML protein, the RBCC motif is required for the organization of PML into multiprotein nuclear complexes (PML-NBs).^{8,10,12,27,28} There are approximately 10–20 bodies per nucleus, ranging in size from 0.1–1 μm .¹ PML-NBs are associated with the nuclear matrix and do not rely on nucleic acid for structural integrity as treatment with either RNase or DNase does not alter their morphology.³² PML-NB morphology does vary during the cell cycle, especially during mitosis.^{11,33} Here, PML-NBs become disrupted, reforming upon commencement of the G₁ phase. In early G₁ some PML protein may remain outside the developing nuclear envelope and form distinct, cytoplasmic bodies.³³ The molecular composition of these cytoplasmic bodies needs further investigation since there are certain isoforms of the PML protein that reside predominantly in the cytoplasm.^{29,34} The alterations in PML-NB morphology observed during the cell cycle may result, at least in part, from a variety of post-translational modifications of certain PML-NB proteins, including PML and Sp100.³³ Both PML and Sp100 are tightly co-localised in PML-NBs and conjugated with the small ubiquitin modifier SUMO-1 (discussed in more detail later in this review).¹⁹ In addition, both proteins have a diffuse nuclear component.¹ It has been proposed that at least some PML and Sp100 protein lose their SUMO-1 moiety during mitosis, with PML becoming phosphorylated.³³ These events may contribute to the mitosis-specific, disruption of PML-NBs. PML-NB morphology is also altered by a variety of environmental stresses including heat shock, cadmium exposure, interferon treatment and viral infection.^{35–37,150} Cadmium exposure causes PML-NBs to disperse while interferon treatment induces PML gene expression causing PML-NBs to increase in number and size.³⁵ It is possible that these exogenous stresses alter PML-NB structure by causing discrete biochemical changes to the PML protein. These changes may be related to those occurring during the cell cycle.³³

PML-NBs are disrupted in APL patients expressing PMLRAR α , but tend to reform following ATRA-mediated, PMLRAR α degradation.^{1,2,7,21} PML-NB reformation correlates with ensuing blast cell differentiation and subsequent disease remission.^{1,2,7,20,21} These phenotypic changes can also be observed following ATRA treatment of the APL patient-derived NB4 cell line.^{7,38} ATRA treatment leaves wild-type PML protein intact, but the extent to which PMLRAR α degradation cor-

relates with PML-NB reformation is debatable.^{39,40} For example, the ATRA-resistant NB4 cell line (NB4-R1) responds transcriptionally to ATRA and degrades PMLRAR α , becoming competent for maturation, but PML-NB reformation and subsequent differentiation requires an additional cAMP dependent event.^{41,42}

Arsenic trioxide (As₂O₃) also stimulates PMLRAR α degradation in NB4 cells, but these cells subsequently undergo apoptosis.^{43,44} Unlike ATRA, As₂O₃ also induces the degradation of wild-type PML.⁴⁵ It is possible that the additional, As₂O₃-mediated loss of wild-type PML, may direct APL cells to undergo apoptosis instead of differentiation, but alternatively As₂O₃ may just be more nonspecific in its action than ATRA. Future studies are needed to adequately address this issue.

When assessing PML partners, reject the foreigners?

Potential PML partner proteins function at all levels of gene expression, including DNA repair and replication, transcription, translation, RNA stability and RNA transport (Table 1).^{1,29,31,46–48} This makes assigning specific functions to PML protein, and subsequently associating these functions with the PML-NB, a daunting task. The potential for PML to interact with another protein must be clearly established in a variety of cell types, without the need for either protein to be overexpressed. The importance of detecting PML interactions using endogenous proteins is highlighted by the following study. Tsukamoto *et al*⁴⁹ developed an elegant, green fluorescent protein (GFP)-based system to visualize both the location and transcriptional status of a specific gene within the genome of a living cell. Specifically, both tetracycline and Lac operator-repressor systems were used to visualize the gene locus and regulate transcription. For these systems to operate, the exogenous expression of various VP16 and Lac repressor-based accessory proteins is required. Intriguingly, PML-NBs associated with the accessory proteins used to visualize gene loci and regulate transcription. Localization was based on the overexpression of VP16 and the Lac repressor. This association was independent of the transcriptional status of the visualized gene.⁴⁹ Therefore, since PML-NBs appear to detect and surround foreign protein, any data obtained showing colocalization with an overexpressed protein must be interpreted with caution and subjected to further, more rigorous analyses involving both endogenous PML, as well as endogenous putative partner protein. In addition, only purified components should be used to definitively prove a direct interaction between PML and a potential partner protein. Many studies attempt to show direct interactions using proteins expressed in rabbit reticulocyte lysates. While a direct interaction is entirely possible, lysates are potentially loaded with a variety of factors and/or cofactors capable of mediating indirect interactions. These various problems represent common pitfalls in the characterization of PML partner proteins.

PML partner proteins with the right credentials

To date, only four members of a growing PML partner protein family have been shown, through the use of purified components, to directly interact with PML (Table 1).^{14,31,50–52} From this small group of proteins, the ubiquitin-conjugating enzyme (Ubc) 9, and SUMO-1, are functionally related.⁵¹ Elegant studies demonstrate that Ubc9 directly interacts with the RING domain of PML, leading to an Ubc9-mediated, conjugation of SUMO-1 to the PML protein.⁵¹ A variety of other, unrelated

Table 1 Grouping potential PML activities based on current knowledge of a cross-section of PML partner proteins

<i>Nature of interaction with PML protein</i>	<i>PML partner(s)</i>	<i>Potential functions assigned to PML/PML-NB</i>	<i>Refs</i>
Direct (validated with purified components)	Ubc9/SUMO-1	SUMO-1 modification of PML by Ubc9 important for recruiting PML partners	51, 55
	eIF4E	Negative regulation of eIF4E functions, eg nuclear export of growth stimulatory mRNAs and protein synthesis	14, 31, 56
	PRH	Role in myeloid differentiation	52
	Z (viral protein)	Z relocates PML to cytoplasm, possible role in protein synthesis and disabling host cell apoptosis	116, 132
Indirect (colocalization, coimmuno-precipitation studies only)	Ribosomal P proteins, eIF3/Int6, L7a, EF1	Protein synthesis (cytoplasmic or nuclear translation)	48, 50, 101, 102
	GAPDH (RNA-dependent PML interaction)	Regulate novel GAPDH functions, eg tRNA shuttling, binding to AU-rich RNA	104
	Bloom's syndrome protein (BLM) (cell cycle-dependent PML-NB association)	DNA repair	46, 114
	NCoR	Mediating transcriptional repression	72
	p53, Rb, CBP (Ras-induced PML-NB association)	Regulating transcription, DNA damage response and apoptosis	47, 67–71
	Isg20 (IFN-induced PML-NB association)	Possible involvement in antiviral response/IFN-inducible RNase	103

proteins are similarly conjugated with SUMO-1, including RanGAP and I κ B.^{51,53} This modification, which does not appear to trigger degradation, may instead be an important determinant of subcellular localization.⁵³ The question of whether or not SUMO-1 modification is required for PML-NB formation is debatable. PML protein contains a number of specific lysine residues required for SUMO-1 modification.⁵¹ However, if these residues are mutated, preventing attachment of the SUMO-1 moiety or association of Ubc9, the resultant mutant PML protein is still able to form PML-NBs.⁵⁴ It is possible that SUMO-1 modification may be necessary for PML to associate with a variety of potential PML partner proteins including Sp100 and Daxx.⁵⁵ The two other proteins meeting the strict criteria required to be classified as direct PML partners are the eukaryotic translation initiation factor 4E (eIF4E) and the proline-rich homeodomain protein (PRH) (Refs 14, 31, 52; unpublished observations, KLBB and M Sharma). Bacterially expressed proteins purified to homogeneity were subjected to a battery of biochemical and biophysical assays to establish that these interactions were direct. These tests included GST pulldown assays and limited proteolysis in conjunction with electrospray mass spectrometry.^{14,56} One of the few distinct biochemical activities assigned to the PML protein has been derived from a functionally relevant interaction between eIF4E and the RING of PML.^{14,31,56} PML has the ability to reduce the affinity of eIF4E for its substrate 5'm⁷G capped mRNA, leading to reduced RNA export.^{14,31,56} This functionally relevant interaction is described in greater detail later in this review. PRH also directly and functionally interacts with both the RING of PML and eIF4E (our unpublished results and Ref. 52). PRH is known to play a role in myeloid development and may therefore contribute to the block in myeloid differentiation characteristic of APL.

What can the neighbors tell us about PML and PML-NB function?

In the nucleus there are a variety of structures possessing defined functions. Indeed, PML-NBs are often found near

Cajal or coiled bodies, cleavage bodies and speckles (as identified by splicing factor Sc35).^{1,57,58} Cleavage bodies are involved in the 3' end processing of mRNA and Sc35 is involved in specific RNA splicing events.^{59,60} Although clues to potential PML activities may be obtained from these frequent neighbors, difficulty may be encountered when determining functional relationships in the absence of a direct colocalization. For example, in T24 cells at least one Cajal body is always adjacent to a PML-NB.⁵⁷ Functionally, Cajal bodies are believed to be involved in assembling both spliceosomes and the transcriptosome.⁶¹ But the role that PML-NBs would play in pre-mRNA splicing is unclear since the key splicing factors, including U2-snRNP and U1-snRNP, are absent from PML-NBs.⁵⁷ Future work is needed to determine if there is any functional significance to PML-NBs being near Sc35 speckles, cleavage bodies, and Cajal bodies.

The great debate: does PML directly regulate transcription?

The current PML literature provides volumes of evidence to suggest that PML participates in transcription and could even be a bona fide transcription factor.^{62–65} PML was initially labeled as a direct, DNA-binding, transcription factor because of an early misconception that the zinc-finger RING and B-box domains of PML were DNA binding domains.²⁶ However, PML does not directly bind to, and PML-NBs do not localize with, nascent DNA.^{1,57} The RING and B-box domains were shown to mediate protein–protein interactions instead.^{24,27,28} PML may only exhibit direct transcriptional activity when artificially fused to a Gal4-DNA binding domain.^{1,66} If PML has a role in transcription, then it would not be unreasonable to find PML-NBs surrounding transcriptionally active genomic loci. Freemont and co-workers made a statistically significant observation that at least one PML-NB per cell is adjacent to a specific, gene-rich major histocompatibility complex (MHC) locus on chromosome 6.⁵⁸ However, consistent with observations regarding integrated plasmid loci (as outlined earlier in this review and Ref. 49), the association of PML-NBs with endogenous loci is independent of both the transcriptional

status of the cell and cell cycle phase.^{49,58} Furthermore, various general transcription factors including TFIID and RNA polymerase II, as well as nascent DNA, do not co-localize with PML-NBs.⁵⁷ Thus, it appears unlikely that PML protein would play a direct role in transcription.

More recent data indicate that some transcription and chromatin-modifying factors are associated with PML-NBs, implicating the PML protein in a number of classical transcriptional mechanisms.¹ PML-NBs have been proposed to colocalize with such potent and prominent transcription regulators as Rb and p53, and the transcriptional co-activator CBP.^{47,62,67–69}

However, there are some caveats to these observations. The potential interaction between PML and CBP is somewhat controversial since an organized CBP nuclear structure can only be observed with certain CBP antibodies.^{47,70} These organized structures colocalize with PML. However, other anti-CBP antibodies indicate that CBP is distributed uniformly throughout the nucleoplasm with no evidence of nuclear bodies.^{47,70} In addition, two independent studies using the same commercial CBP antibody (A22, Santa Cruz Biotechnology, Santa Cruz, CA, USA) have revealed conflicting results. In one study, CBP is demonstrated to colocalize with PML-NBs under normal conditions,⁷⁰ while in another study the same CBP antibody shows that CBP is only a significant component of PML nuclear bodies in the presence of oncogenic Ras (Figure 5 in Ref. 71). In this second study, PML-NBs are present prior to the introduction of oncogenic Ras (RasV12), but CBP is dispersed throughout the nucleus with no organization into bodies.⁷¹ Similarly, p53 protein is normally dispersed throughout the nucleus and only colocalizes with PML-NBs following the introduction of oncogenic Ras (Ref 68, Figure 5 in Ref. 71). In fact, prior to the introduction of oncogenic Ras, approximately 1% of endogenous Rb associates with roughly 1% of endogenous PML-NBs as observed by confocal and coimmunoprecipitation studies.^{67,68} The introduction of oncogenic Ras results in approximately 14% of endogenous PML bodies associating with roughly 14% of endogenous Rb and *visa versa*.⁶⁸

Recent findings suggest that upon transfection, the nuclear co-repressor NcoR localizes with endogenous PML.⁷² However, these findings need further evaluation due to the ability of endogenous PML to 'sense' transfected foreign proteins (discussed above, and Refs 49, 58). Intriguingly, it has been proposed that NcoR requires PML to form nuclear structures and subsequently function as a transcriptional repressor.⁷² Therefore, PML-NBs would effectively be sites for co-repressor assembly. However, the NcoR knockout is embryonic lethal at day 15.5,⁷³ while the PML^{-/-} mice are perfectly viable and, in essence, morphologically normal.²² If PML acts as the critical organizer of co-repressor complexes, one would expect that a much more severe phenotype would be observed for the PML^{-/-} mice. More work clearly needs to be done in this area to resolve these issues.

Later in this review, evidence will be presented showing that PML can regulate gene expression at the post-transcriptional level. It is essential that these mechanisms be considered when assigning transcriptional functions to PML based on chloramphenicol acetyl-transferase (CAT) and luciferase (Luc) reporter-driven assays. Most CAT and Luc reporter-driven assays tend to be interpreted using measurements from indirect enzyme reactions. CAT or Luc protein yield could potentially be affected by any number of post-transcriptional mechanisms including alterations in transcript stability, mRNA transport, translation and/or protein stability. Therefore the

amount of reporter protein produced in these assays is not necessarily a true reflection of transcriptional activity. Further complications arise from the potential for PML to indirectly affect transcription itself. For example, overexpressing PML causes a nuclear retention of cyclin D1 mRNA, reducing cyclin D1 protein levels without altering total cyclin D1 transcript levels.^{14,31} Cyclin D1 protein is known to recruit the p300/CBP associated factor (PCAF) to the estrogen response element (ERE).⁷⁴ Previous studies have shown that the ERE could potentially be controlled by PML.⁷⁵ So it is possible that PML, through cyclin D1, indirectly regulates the transcription of genes possessing EREs. A similar scenario may explain how overexpressing PML can cause a two-fold decrease in the ability of the progesterone receptor (PR) to transcribe a CAT gene possessing a progesterone response element (PRE).⁷⁵ In this study, the transcriptional decrease was monitored by CAT protein production and no interactions (direct or indirect) could be detected between PR and PML. Intriguingly, in these studies PML overexpression resulted in a significant accumulation of PML not only in PML-NBs, but also in the cytoplasm.⁷⁵ Therefore, if overexpression of cytoplasmic proteins such as translation factors can have the eventual effect of inducing DNA synthesis,⁷⁶ the potential for PML to indirectly effect transcription cannot be ignored, but again it is clearly difficult to assign a direct transcriptional role for PML.

Although multiple groups have shown that RNA polymerase II, TFIID, E2F and DNA are not found at the body,^{1,57} the possibility still exists that PML can modulate transcription indirectly and/or that PML's functions could be linked to transcriptional activity within the cell. Furthermore, the nucleoplasmic diffuse population of PML,¹⁶ and not the PML-NB, could be acting directly in the activation and/or repression of transcription. Clearly, much more research is needed before PML can be viewed as a direct actor in classical transcriptional processes.

An alternative point of view: PML regulates post-transcriptional gene expression

PML protein physically and functionally interacts with the translation factor eIF4E.^{14,31,56} In the cytoplasm, eIF4E functions in the rate limiting step of cap-dependent protein synthesis.^{77,78} Both eIF4E and eIF4A, a DEAD-box RNA helicase that acts to unwind RNA, bind to eIF4G to form the eIF4F complex. The initiation phase of protein synthesis begins when eIF4E interacts with the 5' m⁷G cap of mRNA. Subsequently, eIF4G serves to position the 40S ribosomal subunit at the 5' end of an mRNA through its ability to bind to multiple components of the translation machinery including, eIF4E, the 40S ribosomal subunit and mRNA.^{77,78} However, a substantial fraction of eIF4E (33–68%) localizes to discrete structures in the nucleus called eIF4E nuclear bodies.^{14,31,79,80} PML and eIF4E have been shown by us to co-localize and co-immunoprecipitate in several mammalian cell types, including NIH 3T3 mouse and 551 human fibroblasts, and a variety of leukemic cell lines including U937, K562 and ATRA treated NB4 cells.^{14,31} The PML-eIF4E interaction has also been shown independently (H Seker and C Harris, unpublished observations). Biochemical studies using purified proteins indicate that PML and eIF4E directly interact.^{14,56} These studies reveal that for interaction purposes PML uses regions around the first zinc-binding site of its RING domain and eIF4E uses a region including tryptophan 73 (W73).^{14,56} This region of eIF4E also binds eIF4G and the 4E-BPs.⁷⁸ As outlined

above, the RING is required for several physiological functions of PML including formation of nuclear bodies, growth suppression and apoptosis.^{8,10,12,27,28} If W73 of eIF4E is mutated to alanine (W73A), the resulting W73A mutant is still able to bind the m⁷G cap structure with wild-type affinity, but is unable to form either an active translation initiation complex with eIF4G, or inactive complexes with negative regulators including the 4E-BPs.^{14,81,82} Therefore, PML and eIF4E interact through functionally important regions.

In direct contrast to PML-NBs, eIF4E nuclear bodies appear conserved in eukaryotic cells including *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Xenopus laevis*, as well as mammalian cells.^{14,83,84} Some PML partner proteins have been shown to require PML to be organized into body structures.¹⁹ From an evolutionary standpoint, this is clearly not the case for the formation of eIF4E nuclear bodies. In fact, eIF4E nuclear bodies are clearly visible in a PML^{-/-} cell line.^{14,150} If PML is reintroduced into PML^{-/-} cells, it colocalizes with eIF4E nuclear bodies and intriguingly, PML's ability to form nuclear bodies is at least in part dependent on eIF4E.¹⁴ PML-NBs and eIF4E nuclear bodies have similar biophysical characteristics. They are both nuclear matrix-associated and their structural integrities do not rely on nucleic acid since both bodies are unaffected by either DNase or RNase treatment.^{14,32,79,85} In addition, when permeabilized cells are incubated with the cap analogue m⁷GpppG, both PML-NBs and eIF4E nuclear bodies are almost completely dispersed causing a significant release of both PML and eIF4E from the nucleus. In contrast, both nuclear bodies are unaffected following treatment with an unmethylated GpppG cap analogue shown previously not to bind eIF4E.^{14,85} These m⁷GpppG effects are specific as both speckles (as identified by Sc35 and Sm) and the nucleolus, which are morphologically distinct nuclear structures, are unaffected.^{14,85} Such a complete dispersal of both PML-NBs and eIF4E nuclear bodies following m⁷GpppG treatment is striking considering that RNase treatment suggests that mRNA is not essential for the morphology of either body.^{14,85} It is not unreasonable to assume that RNA and m⁷GpppG association are intimately linked for eIF4E since mRNA is the only cellular component possessing the m⁷GpppG cap structure. However, it is possible to reconcile these apparently conflicting m⁷GpppG and RNase treatment results. Previous studies indicate that cap binding alters the conformation of eIF4E protein.^{14,86,87} Consequently, the m⁷GpppG cap analogue may bind to the eIF4E present in eIF4E nuclear bodies, causing a significant structural change within the eIF4E protein and leading to body disruption. Consistent with this explanation, the GpppG analogue, which is unable to bind eIF4E, and cannot induce conformational changes, does not alter eIF4E nuclear body morphology.^{14,85,88} The GpppG analogue is also unable to alter PML-NB morphology. However, unlike eIF4E, PML protein is unable to directly bind the m⁷GpppG cap analogue.⁵⁶ Therefore, the complete dispersal of PML and other PML-NB components such as Sp100, following m⁷GpppG treatment suggests that at least a portion of PML protein relies on the presence of eIF4E protein for its organization into PML-NBs. The additional fact that PML protein is also restricted to mammalian cells suggests that PML is a mammalian regulator of evolutionarily older, eIF4E nuclear functions.

In contrast to PML, eIF4E expression is necessary for active cellular growth.^{77,78} An excess of eIF4E, rather than elevating global translation rates, leads to selective increases in the synthesis of a variety of potent growth stimulatory proteins including vascular endothelial growth factor (VEGF), ornithine decarboxylase (ODC) and cyclin D1.⁸⁹⁻⁹⁴ Accordingly, transi-

ent overexpression of eIF4E increases cellular proliferation rates and rescues serum-starved fibroblasts from apoptosis.^{78,95} The prolonged overexpression of eIF4E in NIH 3T3 mouse fibroblast cells and Chinese hamster ovary (CHO) cells leads to oncogenic transformation and an invasive/metastatic phenotype is observed in animal models.^{89,96,97} At the molecular level, eIF4E apparently transforms cells by both selectively up-regulating the translation of transcripts encoding growth stimulatory proteins and, in some cases, facilitating the transport of a subset of transcripts from the nucleus to the cytoplasm.^{14,31,77,92,98} For example, although overexpressing eIF4E results in unaltered total cyclin D1 mRNA levels, increased nuclear export of cyclin D1 mRNA is observed, with a resultant increase in cyclin D1 protein levels.^{92,98} Further studies show that PML protein negatively regulates the eIF4E-dependent nuclear export of select growth regulatory mRNAs.³¹

In direct contrast to the effects of excess eIF4E and consistent with PML being a negative growth regulator, PML overexpression results in a decrease in endogenous cyclin D1 protein levels due to the nuclear retention of cyclin D1 transcripts.^{31,150} The PML-induced down-regulation of cyclin D1 protein synthesis can be reverted by subsequent overexpression of eIF4E. Furthermore, analyses of specific PML RING domain and eIF4E mutant proteins reveal that the ability of PML to suppress cyclin D1 mRNA transport is linked to its ability to bind eIF4E.^{14,31} The RING of PML appears to modulate eIF4E function by decreasing eIF4E's affinity for the 5' m⁷G cap of mRNA. *In vitro* data indicate that the m⁷G cap affinity of eIF4E is decreased over 100-fold by PML.^{14,56} This activity is specific to the PML RING and a specific, RING-containing, arenaviral protein (Z protein), since other RING domains from unrelated proteins such as Cbl, BRCA1 and TIF1 α do not have this effect on eIF4E.^{14,56} These findings are again consistent with previous observations that PML induces G₁ arrest while eIF4E is mitogenic.^{11,13,78} In contrast to PML, the eIF4E partner protein eIF4G enhances the cap-binding affinity of eIF4E in the cytoplasm and thereby stimulates eIF4E's translational activities.^{87,99} Both PML and eIF4G bind to the same region of eIF4E protein suggesting that this region of eIF4E can be used to positively or negatively regulate eIF4E activity. Therefore, the ability to modulate eIF4E activity by altering its affinity for the m⁷G cap appears to be conserved between the cytoplasm and the nucleus. The extent to which nuclear eIF4E is involved in mRNA export is unknown as eIF4E nuclear bodies appear to be relatively discrete, static entities. It is entirely possible that instead of shuttling the mRNA out of the nucleus, eIF4E protein and/or eIF4E nuclear bodies may be involved in the specific processing required for transport.

There are probably additional negative regulators of nuclear eIF4E function in mammals since PML^{-/-} mice are viable and do not get spontaneous cancers at higher rates than littermate controls.²² During the ATRA-induced differentiation of NB4 cells, expression of the negative regulator 4E-BP1 is decreased and expression of the negative regulator 4E-BP2 is increased.¹⁰⁰ These changes, combined with the potential restoration of PML-mediated negative regulatory mechanisms due to PML-NB reformation, may further contribute to the subsequent growth arrest and maturation response of APL cells.

There are several other independent results that support the idea that PML could function in post-transcriptional regulation of gene expression. As discussed above, PML-NBs are adjacent to known sites of RNA processing including splicing speckles, cleavage bodies and Cajal bodies.⁵⁷ Furthermore,

the identification of eIF3/Int6, Isg20, GAPDH and the ribosomal P-proteins as PML-NB components suggests the potential for some intriguing post-transcriptional functions.^{48,101–104} Isg20 protein is an interferon and estrogen-regulated RNase/DNase¹⁰⁵ and GAPDH, outside of glycolysis, is able to bind to AU-rich segments of RNA and also shuttle specific tRNAs into the nucleus.^{106,107} Accordingly, GAPDH is found in nuclear structures that associate with PML-NBs in an RNA-dependent manner.¹⁰⁴ In addition to eIF4E, several components of the translation machinery associate with PML including eIF3/Int6, ribosomal P-proteins, L7a and elongation factor EF-1.^{48,50}

Intriguingly, recent reports have demonstrated the potential for eIF4E-dependent protein synthesis to occur in the nucleus as a mechanism for proofreading transcripts prior to export to the cytoplasm.⁸⁰ Sites for transcription and translation appear to at least partially overlap within the nucleus. Electron microscopic studies reveal that nascent polypeptides (labeled with biotin-lysine-tRNA), and nascent RNA (labeled with Br-UTP) are often found together within the nucleus. Immunogold labeling further reveals that the newly made biotin-peptides in nuclei also partially colocalize with eIF4E nuclear bodies and phosphorylated SR proteins.⁸⁰ It is not unreasonable to assume that newly made biotin-peptides also colocalize with PML-NBs, since previous observations show that nuclear eIF4E protein co-localizes and co-immunoprecipitates with PML. Since transcription and nuclear translation appear to be coupled, it is possible that both PML-NBs and eIF4E nuclear bodies participate in both intermediate, RNA processing steps such as pre-mRNA splicing and/or the modulation of transcript stability, as well as subsequent cap-dependent protein synthesis.^{80,85} A number of these processes would be required for RNA transport. Intriguingly, *in vitro* translation studies indicate that PML protein can inhibit eIF4E-mediated translation in a RING-dependent manner,⁵⁶ indicating that PML would be ideally situated to negatively regulate this m⁷G cap-dependent nuclear eIF4E activity *in vivo*.⁵⁶ Much more work needs to be done before these issues can be resolved and the precise biochemical function of PML and eIF4E in the nucleus is understood.

Potential roles for PML in DNA replication and repair

Newly synthesized DNA has a distinct spatial distribution throughout the nucleus.⁵⁷ During the entire DNA replication process, PML-NBs are excluded from domains containing newly synthesized DNA, arguing against a direct role for PML-NBs in DNA replication.⁵⁷ Furthermore, only during the middle to late S-phase ~50% of PML-NBs are found adjacent to, but not overlapping with, these domains.⁵⁷ A variety of other studies provide evidence against the direct involvement of PML-NBs in DNA replication. For example, Parathymosin structures, which are localized to sites of early DNA replication, do not localize with PML-NBs.¹⁰⁸ In addition, during SV40 infection, SV40 replication domains are often adjacent to, but do not overlay with, PML-NBs.¹⁹

PML-NBs do not normally contain nascent DNA.^{1,57} However, a special class of PML-NBs, termed ALT-associated PML bodies (APBs) may be the exception to this rule.¹⁰⁹ Approximately 5% of telomerase-negative cells develop a mechanism of telomere length maintenance that bypasses the need for telomerase activity. This mechanism is called alternative lengthening of telomeres (ALT). Recent studies have shown that in ALT cells, the telomere binding proteins hTRF1 and hTRF2 co-

localize with PML in nuclear bodies (APBs).¹⁰⁹ Telomeric DNA repeats are also present in APBs, together with replication factor A and Rad52. This may be the only time that an association between DNA and PML-NBs has been reported. In contrast, non-ALT cells (which can be either telomerase negative or telomerase positive), do not contain APBs and hTRF1, hTRF2, Rad52 and replication factor A exist in discrete subnuclear domains that do not overlap with, or have any apparent spatial relationship to, PML-NBs.^{109–111}

The Bloom's syndrome protein, BLM is a RecQ DNA helicase family member important in DNA repair⁴⁶ and is mainly expressed in lymphocytes and proliferating tissues.^{112,113} PML protein co-localizes with BLM in cells with BLM nuclear bodies, implicating PML in DNA repair.¹¹⁴ The cellular distribution of BLM is highly dependent on the cell cycle. In the S-phase, the majority of BLM is found in the nucleolus.¹¹⁵ However, BLM forms nuclear foci in approximately 40% of fibroblast or HeLa cells in the G1/S phase of the cell cycle, whereas only 6% of these cells possess BLM bodies in other cell cycle phases.¹¹⁵ Disrupting BLM expression results in a high level of genomic instability, leading to an increased potential for tumorigenesis.¹¹² BLM-null cells have attenuated DNA damage-activated and p53-mediated apoptosis, yet PML-NB morphology is normal in BLM^{-/-} cells.⁴⁶ Intriguingly, BLM does not form nuclear bodies in PML^{-/-} cells and further studies show that the association between PML and BLM depends on p53, since in p53^{-/-} cells PML-NBs appear normal whereas BLM is no longer localized to bodies.⁴⁶ However, studies on Bloom syndrome cells indicate that both the induction of p53 protein expression and subsequent p53-mediated transcription of target genes such as Gadd45, Bax and p21^{waf1/cip1} proceed normally.⁴⁶ Obviously, the potential for functional interactions between PML, BLM and p53 is an exciting area of research which undoubtedly will receive much more attention.

The forgotten fraction: potential functions for cytoplasmic PML

Normally, a small percentage of PML is found in cytoplasmic bodies, especially during the early G₁ phase of the cell cycle.^{29,33,34} Indeed, there are several isoforms of PML that lack a nuclear localization signal but retain functional RBCC domains.^{29,34} Furthermore, in several pathogenic viral infections, including HIV and arenavirus infection, PML is translocated to the cytoplasm potentially leading to a gain of function in the cytoplasm as well as a loss of function in the nucleus.^{116,117} Cytoplasmic PML has also been observed in APL cells prior to ATRA treatment and in hepatocellular carcinoma cells.^{1,17} Fractionation studies reveal that endogenous PML interacts with endogenous eIF4E in the cytoplasm, as well as the nucleus.³¹ As discussed above, *in vitro* translation studies indicate that PML inhibits eIF4E-mediated translation in a RING-dependent manner.⁵⁶ This effect can be reversed by additional eIF4E.⁵⁶ However, translation inhibition is not reversed by treatment with protease or proteasomal inhibitors suggesting that PML does not regulate the ubiquitin-mediated degradation of translation machinery components.^{56,118} Instead, PML may potentially inhibit eIF4E-dependent protein synthesis by inducing a decrease in the m⁷G cap affinity of eIF4E. In addition, since the binding sites for both PML and eIF4G reside in the same region of eIF4E protein, it may be possible that PML, like the 4E-BPs, also acts as a negative regulator by competing with eIF4G for binding to

eIF4E.^{56,81,82,119,120} To date, no other potential cytoplasmic functions have been attributed to PML.

So are PML-NBs storage compartments?

The sheer number of potential PML partner proteins, each possessing divergent activities, has made it increasingly difficult to assign distinct functions to the PML-NB. It is not surprising that an alternative theory has arisen stating that the bodies themselves are not functionally active compartments, but instead are storage facilities or nuclear depots for the cell.^{19,30} This theory is supported by the observations that overexpressed proteins, such as the lac repressor, and a variety of viral proteins associate with the body.^{1,49} Furthermore, the pathogenic form of Huntingtin protein also accumulates at PML-NBs, making the bodies abnormally larger and inclusion-like in nature.¹²¹ It has been proposed that PML bodies could be sensors for foreign or inappropriately expressed proteins, acting like a potential nuclear immune system.^{19,49} Indeed, recent data have shown that some PML-NBs are adjacent to proteosomal components.^{122,123} The frequency of colocalization with the proteosome is greatly increased by treatment of cells with As₂O₃.¹²³

In addition to foreign or overexpressed proteins, it has been proposed that PML-NBs are storage areas for normal functional components.¹⁹ PML-NBs would be able to modulate biochemical processes by adjusting the levels of active components in the nucleoplasm.¹⁹ In fact, several other nuclear bodies, including Cajal bodies, could also potentially be storage areas for certain types of components and not sites of active biochemistry. An alternative, but complimentary, theory suggests that these nuclear bodies form catalytic surfaces, where the types of surface activities are dependent on body protein composition.^{24,25,124} This catalytic surface mechanism has been suggested for other bodies with associated RING domain-containing proteins.^{24,25,124} Whether PML-NBs and/or other subnuclear structures are active organelles or subnuclear storage facilities remains hotly contested.

PML-NBs as an antiviral defense force

It has been well established that members of the IFN family trigger a variety of cellular defense mechanisms against viral infection by stimulating the transcription of genes containing IFN-inducible gene promoters.³⁷ Both the 2'5' oligoadenylate (2'5'A) synthetase and the double-stranded RNA-dependent protein kinase (PKR) genes contain such promoters and mediate potential antiviral pathways.³⁷ The PML gene also contains an IFN-inducible promoter and all IFNs (α , β , and γ) strongly induce PML expression leading to an increase in PML-NB number and size.^{35,37} However, the list of genes stimulated by IFN is very large and includes many genes encoding proteins of unknown function.³⁷ Therefore assigning a definitive role for PML in the regulation of the IFN-mediated, antiviral response has remained a difficult task. In the absence of IFN, PML overexpression does confer resistance to infection by a number of RNA viruses including lymphocytic choriomeningitis virus (LCMV), vesicular stomatitis virus (VSV), influenza A virus and the human foamy retrovirus, without affecting infection by encephalomyocarditis virus (EMCV), a virus known to be IFN resistant.^{37,125,126} Furthermore, PML^{-/-} cells are more readily infected by LCMV than PML^{+/+} cells¹²⁵ and IFN α -pretreated PML^{-/-} cells are more readily infected by

the human foamy retrovirus than IFN α -pretreated PML^{+/+} cells.¹²⁷

If PML actively participates in antiviral defenses, then it would be advantageous to the virus to disable these mechanisms. A large amount of essentially correlative data has been generated showing that both DNA and RNA viruses may benefit by localizing with PML-NBs and directly affecting PML-NB morphology.^{1,36,37} Herpes viruses including herpes simplex virus type 1 (HSV-1) 'unwind' bodies, whereas arenaviruses, including LCMV move PML to the cytoplasm.^{1,19,116} Since PML protein is proapoptotic, the translocation of PML bodies to the cytoplasm during infection may be involved in the antiapoptotic effect of this virus.¹¹⁶ Retroviruses, including human immunodeficiency virus type 1 (HIV-1), may also move PML bodies to the cytoplasm.¹¹⁷ However, other researchers have contradicted this specific observation, demonstrating no apparent modification of PML-NBs following early or late times of HIV-1 infection.¹²⁸ LCMV encodes five proteins, including the 90-residue RING protein, Z.¹²⁹ Z associates with PML nuclear bodies, binds directly to PML, and translocates bodies to the cytoplasm.¹¹⁶ PML and Z interact with the ribosomal P proteins (P0, P1, P2) in the nucleus of uninfected and infected cells, respectively.⁴⁸ The P proteins form part of the large ribosomal subunit and are required for protein synthesis.^{130,131} In addition, like PML, Z protein binds eIF4E and selectively represses cyclin D1 protein production in a RING-dependent manner.¹³² The acquisition of translation machinery such as eIF4E and the P proteins by the virus, and the potential use by the virus of negative regulators of translation (PML), may partially explain how the virus regulates synthesis of its own proteins and selectively decreases the translation of cellular mRNA.^{48,132}

The proteins and genomes of a variety of DNA viruses, including HSV-1 and human cytomegalovirus (HCMV) associate with the periphery of PML-NBs.³⁶ In the case of HSV-1, the viral immediate-early regulatory protein ICP0 disrupts PML-NBs.¹³³ ICP0 protein mediates the proteasome-dependent degradation of both PML and another PML-NB component, Sp100.¹³⁴⁻¹³⁶ Like PML, Sp100 gene expression is also induced by IFN.³⁷ Further studies reveal that ICP0 induces the formation of co-localizing conjugated ubiquitin in PML-NBs, but the direct ubiquitination of PML has yet to be observed.^{123,137} It should be noted that in addition to PML, there are multiple, non-PML-NB-associated, targets for ICP0-mediated decay, including the catalytic subunit of DNA-PK and the centromeric proteins CENP-C, CENP-A. The degradation of these centromeric proteins disrupts centromere structure resulting in mitotic delay and failure.^{36,135} It is currently unclear as to why ICP0 should migrate to and affect both PML-NBs and centromeres, but these observations provide a good example of how complicated it is to assign a discrete biochemical role for PML in antiviral defenses.

Trying to understand how PML contributes to APL pathogenesis

In APL cells, PML-NBs are disrupted as a result of the PMLRAR α translocation.^{1,2} This disruption is correlated with a block in myeloid development and a loss of growth control that may lead to the initiation of APL. It is possible that the characteristic phenotype of APL cells is caused by PML-NB disruption, but alternatively, these APL-related, phenotypic changes may subsequently effect PML-NB morphology. Although many PML and APL reviews state that the loss of

PML's ability to negatively regulate growth contributes to APL pathogenesis, in reality the evidence is not as conclusive.^{1,2,6,18,138} However, given knowledge of direct PML partner proteins, certain potentially disrupted, PML-specific, molecular mechanisms can be postulated to contribute to the uncontrolled growth associated with APL pathogenesis. For instance, under normal circumstances eIF4E, which unlike PML is evolutionarily conserved, directly interacts with PML in mammalian cells.^{14,31} This interaction appears to be essential for PML to negatively regulate eIF4E-dependent activities.^{14,56} In APL cells, while PML-NBs are disrupted, eIF4E nuclear bodies remain intact.¹⁴ Consequently, eIF4E-dependent functions, including the nuclear export of specific mRNAs encoding growth stimulatory proteins,^{14,92,98} are likely to continue at a potentially elevated rate. The increase in mitogenic signals could eventually lead to uncontrolled proliferation and the initiation of APL. Deregulated eIF4E activities have been previously proposed to contribute to the pathogenesis of human tumors derived from a variety of tissues including breast, head and neck, colon, bladder and B-lymphocytes.^{97,139,140} A positive correlation was observed between eIF4E protein levels and disease severity.^{139,141–144} In addition to eIF4E, the functions of other evolutionarily conserved, PML partner proteins including PRH, a protein involved in myeloid differentiation, may be disrupted in APL cells.⁵² PML-NBs, reformed following the ATRA treatment of APL cells, once again colocalize with eIF4E and PRH (Ref. 14, Cohen and Borden, unpublished observations). Therefore, ATRA treatment potentially restores valuable growth control mechanisms that may release the block on differentiation and cause clinical remission. However, these mechanisms are undoubtedly very complex and may be regulated by a variety of PML-NB proteins, not forgetting proteins whose expression is controlled by RAR α . So in addition to disrupting PML functions, PMLRAR α may potentially disrupt the growth regulatory activities of a large number of PML-NB components. New proteomics techniques may be key to understanding how the composition of the PML-NB changes after ATRA treatment and will likely provide useful insight into the role of the body in APL.

Controversy has arisen as to the extent to which the disrupted activities of PML or RAR α influence the development of APL. Although other chromosomal translocations have been identified in rare cases of APL, all reported APL translocations result in fusion proteins containing RAR α .¹ All the fusion partners of RAR α , including PML, PLZF, NPM, Stats and NuMA, are predominantly localized in the nucleus but otherwise share limited commonality.^{1,6} Therefore, various studies have proposed that these RAR α fusion partners play an important, but accessory role in the pathogenesis of APL and that abnormal RAR α activity is primarily responsible for disease progression.⁹ For example, PMLRAR α , in addition to forming complexes with RAR α and PML, is able to form homo-oligomers using its PML-derived, coiled coil domain. Consequently, abnormal, PMLRAR α -containing promyelocytes may be unable to differentiate in response to physiological concentrations of RA due to the higher level of HDAC activity associated with PMLRAR α homo-oligomers.⁹ However, intriguing mouse model studies by Kogan *et al*, have shown that the RA-responsiveness of PMLRAR α , including transcriptional activation by ATRA, may be dispensable for leukemogenesis.^{6,145} A leucine to proline mutation at amino acid 398 of RAR α markedly impairs the ability of RAR α and PMLRAR α to bind ATRA. Both RAR α and PMLRAR α are converted into ATRA-insensitive, transcriptional repressors with dominant negative activity potentially restricted to genes containing retinoic acid

response elements (RAREs), any potential ATRA-independent effects of RAR α would probably be unaffected. As expected, transgenic mice expressing the mutated PMLRAR α exhibit a phenotype similar to that observed in transgenic mice expressing wild-type PMLRAR α . However, the mutated RAR α does not cause these phenotypic changes and does not significantly impair neutrophilic differentiation.¹⁴⁵ While these results suggest that the PML portion of PMLRAR α is essential for the initiation of APL, the possibility exists that the mutated RAR α , though ATRA-insensitive, has lost its ability to form specific, APL-causing oligomers. As outlined above, the coiled coil domain of PML, present in both wild-type and mutated PMLRAR α and required for oligomerization,¹⁵¹ is absent from the mutated RAR α . Alternatively, it is possible that the RAR α portion may still play an important role in the pathogenesis of APL since the mutated PMLRAR α does not fully reconstitute all the APL-like symptoms observed in the mouse models.^{145,146} Unfortunately, it is important to recognize that the low frequency and relatively late age of onset of APL-like symptoms in mouse models of APL makes defining the precise roles of PML and RAR α in development of APL that much more difficult. The large changes observed in PML-NB morphology during APL suggest an important role for PML in APL pathogenesis. However, it is readily apparent that PML's molecular and biochemical roles in APL pathogenesis are far from clear.

The search for a definitive, PML-mediated, molecular/biochemical function continues

The PML protein is physiologically important with established roles in growth arrest, apoptosis, transformation suppression and senescence.¹ Such a wide variety of effects bring to mind similarities with the classical tumor suppressor protein p53.¹⁴⁷ However, unlike p53, there is a tremendous lack of biochemical and molecular data associated with PML. PML protein and/or the PML-NB may potentially be one of a select class of regulatory factors implicated in functions at multiple levels of gene expression including DNA repair, transcription, mRNA transport and as a nuclear storage facility.^{14,19,30,31,62–65,114} In this review, we have discussed some criteria to consider when investigating PML-NB function. PML-NB function has generally been determined through the identification of partner proteins (Table 1). It is important to establish the physiological relevance of an interaction between the PML-NB and a potential partner protein, especially since several elegant studies demonstrate that PML-NBs 'sense' and accumulate around overexpressed or foreign proteins.^{30,49} In addition, a number of potential PML partners may only associate with PML-NBs following the introduction of oncogenic Ras or during specific parts of the cell cycle,^{33,68,71} making their role in normal, PML-mediated processes harder to define.

In an attempt to fill the void left by the lack of information regarding PML-mediated biochemical and molecular processes, many groups have shown that PML protein can modulate CAT and Luc expression in reporter assays.¹ However, the potential for PML to regulate post-transcriptional processes may undermine the tendency to associate PML with transcription based solely on reporter assay data. Indeed, several proteosomal components were originally misidentified as transcription factors due to their ability to modulate reporter assays,¹⁴⁸ highlighting the danger of using these assays alone to establish direct transcriptional activity. The argument for

PML's involvement in transcriptional processes would be substantially strengthened by the ability to obtain direct evidence using newer techniques such as the chromatin immunoprecipitation. Although PML protein has been implicated in a number of critical molecular processes one must remember that PML may not be evolutionarily conserved and PML^{-/-} mice are viable.^{22,23} However, many years ago researchers were equally puzzled by the viability of p53-null mice.¹⁴⁹ Only time and more extensive and vigorous research procedures will elucidate the biochemical function(s) of both the PML protein and the PML nuclear body.

Only a few discrete biochemical functions have been attributed to PML. One is its ability to modulate the 5'm⁷G cap-binding activity of eIF4E, thereby negatively regulating RNA transport and suppressing eIF4E-mediated transformation.^{14,31,56} Also PML undergoes SUMO-1 modification through its ability to interact with both SUMO-1 and Ubc9.⁵¹ In order to fully understand the physiological roles of both PML protein and the PML-NB, we must have a clearer picture of the biochemical processes in which they are involved. Then we will be in a much better position to understand how the disruption of PML-NBs in human disorders, such as APL and HIV infection leads to derailed cell growth.

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