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# The nuclear bodies inside out: PML conquers the cytoplasm

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## Abstract

The promyelocytic leukemia (PML) protein is the core component of nuclear substructures that host more than 70 proteins, termed nuclear domains 10 or PML-nuclear bodies. PML was first identified as the gene participating in the translocation responsible for the pathogenesis of acute promyelocytic leukemia (APL). The notion that PML is a tumor suppressor gene was soon extrapolated from leukemia to solid tumors. The last decade has radically changed the view of how this tumor suppressor is regulated, how it can be therapeutically targeted, and how it functions. Notably, one of the most recent and striking features uncovered is how PML regulates cellular homeostasis outside its original niche in the nucleus. These new findings open an exciting new area of research in extra-nuclear PML functions.

## The identification of PML and the APL saga

The 90s started with a breakthrough discovery from several groups that would change the research of the deadly acute promyelocytic leukemia (APL): the mapping of the breakpoint of the reciprocal translocation with chromosome 17 [1]. Soon after, the promyelocytic leukemia gene (PML, MYL, RNF71, PP8675, and TRIM19) was identified as the most frequent translocation partner of the retinoic acid receptor alpha (RARα in APL) [2–5] (Figure 1).

Since its discovery and for the next five years, the tumor suppressive activity of PML was restricted to leukemia, but was soon extrapolated to solid tumors [6,7]. This led to the current notion that PML is a tumor suppressor lost in cancers from multiple origins [8].

One of the most relevant breakthroughs in PML research was the discovery of two drugs which would target PML and/or the PML–RARα fusion oncoprotein. On the one hand, All-Trans Retinoic Acid (ATRA), produced in Shangai and used to treat patients with APL [9], was found to promote the rapid degradation of the fusion protein [10]. On the other hand, two groups described that same year the potential mechanism of arsenic trioxide (ATO), an ancient Chinese medicine used for the treatment of APL [11–13], inducing apoptosis with the concomitant regulation of PML localization and stability [14,15]. This phenomenon would be, a decade later, shown to depend on the binding of the arsenic molecule to PML to promote its SUMOylation-dependent ubiquitin- mediated degradation by RNF4 [16••,17••, 18••].



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The notion that the PML–RARα fusion oncoprotein is sufficient to drive APL was recapitulated in the mouse by several groups [19–21], in turn generating preclinical tools which would later become key in the discovery of an effective therapy for APL. These preclinical efforts in faithful mouse models of APL proved definitive game changer in the treatment of this deadly disease. Genetic mouse models of APL were critical for the formal demonstration of the combined efficacy of ATRA and ATO in APL [22,23]. In turn, these seminal studies would transform a devastating disease in a curable one.

## PML nuclear functions

PML is the essential component of a macromolecular nuclear substructure, the PML-nuclear bodies (PML-NB [24]). Indeed, PML functions as the scaffold of this structure allowing other proteins to shuttle in and out, a process which is regulated by SUMO-mediated modifications and interactions [25,26•]. PML has multiple splicing variants, giving rise to a wide variety of isoforms, whose differential expression and function is yet poorly understood [27,28]. These PML isoforms move dynamically between NBs at a different rate, thus suggesting that the PML-NB composition might be heterogeneous and functionally different [29].

Among the recently discovered nuclear functions of PML, we will focus on the control of gene expression and protein modification, which have become of great interest.

### PML and transcription

PML-NBs host a wide variety of transcriptional regulators, including transcriptional activators, repressors, and histone modifiers. Moreover, PML-NBs have been shown to localize adjacent to transcriptionally active chromatin regions, to Major Histocompatibility Class 1 and p53 loci [30–33]. Furthermore, PML colocalizes with the histone acetyltransferase Creb Binding Protein (CBP) and with RNA Pol II in a cell cycle-dependent manner [34]. On the other hand, PML can repress transcription through the interaction with histone deacetylases and heterochromatin protein 1 (HP1) [35,36] and through the regulation of heterochromatin recondensation in satellite DNA [37]. The transcriptional regulation and heterochromatin remodeling induced by PML have highlighted the importance of this tumor suppressor in the induction of cellular senescence [38,39], with recent evidence pointing at a role for PML in regulating this process through the Rb-E2F pathway [40].

### PML and post-translational modifications

PML-NBs are a factory for protein modifications. It has been shown that PML regulates protein acetylation, phosphorylation, ubiquitination, and SUMOylation among others, through the correct formation of the PML-NBs. These structures host all kinds of protein modifiers, from acetyltransferases to deacetylases, E3 ligases, deubiquitinases, phosphatases, kinases, and more. These regulatory processes have been shown to be of critical importance for cell homeostasis, and in turn, loss of PML results in deregulated modulation of protein function.

Firstly, PML is found to modulate the activity of both protein phosphatases and kinases. On the one hand, PML tunes the function of the protein phosphatases 1A and 2A (PP1A and PP2A). Loss of *Pml* results in increased proliferation and reduced differentiation of neural progenitors [41•], an event that arises from the delocalization of PP1A and reduced activity towards pRb. On the other hand, PML positively regulates the activity of PP2A towards AKT in the PML-NBs, and, as a result, *Pml*-loss exacerbates the phenotype of *Pten* heterozygous mice and leads to more aggressive forms of cancer [42]. Furthermore, PML regulates the activity of several kinases to promote protein phosphorylation. As an example,

PML promotes the phosphorylation of p53 by HIPK2, which increases the rate of acetylation of p53 and its transcriptional activation [43].

Secondly, PML regulates protein stability and function by affecting the activity of E3-ligases and deubiquitinases (DUBs). PML inhibits KLHL2 through its recruitment to the PML-NBs and the physical separation from its target, DAPK, which results in apoptosis and autophagy [44]. Also, PML has been shown to recruitMDM2 to a distinct subnuclear structure, the nucleolus, hence preventing p53 ubiquitination and proteosomal degradation [45]. Conversely, PML negatively regulates the DUB HAUSP/USP7, thus opposing PTEN deubiquitination and cytoplasmic translocation. This molecular framework is found altered in APL, where PML–RARα disrupts the PML-NBs, increases PTEN deubiquitination by HAUSP, and leads to PTEN nuclear exclusion [46].

Lastly, the PML nuclear bodies have a predominant role in the regulation of protein acetylation. PML activates p53-dependent gene expression during oncogene-induced senescence by promoting its acetylation by CBP in the PML-NBs [47]. The regulation of p53 by PML is a balance between the acetylation by CBP and the deacetylation by SIRT1, which also resides in the PML-NBs. Indeed, SIRT1 overexpression tilts the equilibrium towards p53 deacetylation and transcriptional repression, which results in the impairment of the cellular senescence program [48]. Interestingly, PML–RARα exerts the opposite activity on p53, by promoting its deacetylation by a different family of deacetylases, HDACs [49].

The numerous functions described for PML in the control of post-translational modifications raise the question of whether the nuclear bodies serve the main function of providing a microenvironment where these reactions can occur. It remains to be clarified whether other activities of PML attributed to a scaffolding function (e.g., regulation of mTOR by PML [50,51•]) are also the result of the modulation of post-translational modification pathways.

## PML cytoplasmic functions

As we mentioned above, a nuclear localization signal (NLS) located in exon 6 of PML isoforms restricts the localization of the majority of PML protein to the nucleus. However, nucleo-cytoplasmic fractionation reveals that a fraction of PML resides in the cytoplasm [28,52,53••], a 10% of total PML in asynchronously growing primary mouse cells, and probably a higher percentage in other cell types (A.C. and P.P.P., unpublished observations). Of note, a PML isoform, namely PML-VII, lacks the fraction encoding for the NLS and it is therefore purely cytoplasmic (reviewed in [54]).

### Functions of PML-VII in the cytoplasm

The first report to unveil a cytoplasmic function of PML came upon the study of the activity of PML-VII [52]. PML-VII or PMLc (cytoplasmic) was found to be essential for the proper activation of the cytokine transforming growth factor beta (TGFβ) signaling. In the absence of PML (using *Pml* knockout mouse embryonic fibroblasts) the intracellular signaling elicited by TGFβ receptor activation was diminished, and in line with this notion, these cells were refractory to the induction of growth arrest, senescence, and apoptosis by the cytokine. This effect was shown to be mediated by the interaction of PMLc with SMAD2/3 and Smad anchor for receptor activation (SARA), and the regulation of their cytoplasmic compartmentalization. In addition, the activation of TGFβ signaling by PMLc is negatively regulated by the homeodomain protein TGIF (TG-interacting factor), which disrupts its association with SARA in concert with c-Jun [55]. Further research will be needed in order to determine whether this activity of PMLc is also exerted by other PML isoforms in the cytoplasm (Figure 2).

Although most of the research related to cytoplasmic PML has been linked to the use of PML-VII, in the recent years, more data have been brought up to light regarding the function of other PML isoforms and mutants in this compartment.

### Tumor suppressive activities of cytoplasmic PML

As we mentioned before, all PML isoforms can be found in the cytoplasm upon subcellular fractionation analysis. One of the most exciting new roles of this cytosolic PML pool was unveiled when carefully evaluating the precise localization of cytoplasmic PML fraction [53••]. Surprisingly, PML was found to localize to specific membrane structures termed mitochondria-associated membranes, or MAMs for short, which connect the endoplasmic reticulum (ER) and the mitochondria. The MAMs play a central role in apoptosis through the regulation of calcium influx from the ER to the mitochondria. Indeed, *Pml*-deficient cells exhibited impaired calcium influx to the mitochondria and resistance to ER-stress-induced cell death. In line with the notion that PML in the cytoplasm could form structures that host proteins also found in the PML-NB [56], PML was found to exert this proapoptotic activity through the regulation of a large complex involving PP2A, AKT, and the inositol triphosphate receptor (IP3R), which ultimately controls calcium flux to the mitochondria. To formally demonstrate the relevance of cytoplasmic PML fraction in this activity, the resistance to apoptosis of *Pml*-deficient cells was reverted upon expression of an ER-targeted PML construct. These results provide an exciting new potential mechanism whereby cytoplasmic PML could regulate apoptosis from MAM in parallel with the modulation of proapoptotic factors by nuclear PML [57].

### Oncogenic activities of cytoplasmic PML mutants

The localization of PML to the nucleus requires a functional NLS located within exon 6. In this sense, genetic mutations affecting this domain might result in dysfunctional PML. Indeed, two independent studies described that mutations in PML leading to NLS-less PML mutants have oncogenic consequences. On the one hand, in a plasmacytoma cell linePML was found to be mutated in exon 3, which resulted in the expression of a truncated protein confined to the cytoplasm [58]. Surprisingly, this cytoplasmic PML functions as a dominant negative, oncogenic form of the tumor suppressor. On the other hand, in two aggressive forms of PML–RARα APL, the nontranslocated moiety of PML was found mutated [59]. Both mutations led to the expression of a cytoplasmic PML mutant. Interestingly, these mutants form cytoplasmic PML bodies (PML-CB) and host some of the components found in the PML-NB. Indeed, PML-CB was able to bind PML–RARα, sequester it in the cytoplasm, further inhibit RA-dependent transcription, and potentiate the block in differentiation in APL [56]. These reports raise the interesting possibility that an aberrant PML in the cytoplasm could function as an oncogenic cue. However, it remains to be determined whether an aberrant cytoplasmic PML is oncogenic through its activity on nuclear or cytoplasmic PML physiological functions.

## Cytoplasmic PML: what next?

The view of how this multitasking protein operates has dramatically changed with the most recent developments, attributing to PML extra-nuclear oncogenic and tumor suppressive activities. However, the field is just in its infancy. As an example, a recent interesting study analyzed systematically the sites of PML cytoplasmic localization by expressing all PML isoforms harboring mutations in the NLS. Surprisingly, many of PML mutant isoforms localized to endosomes and lysosomes [60••]. This study, along with the various mentioned in this review raises new and exciting questions in need of answers: *Where else does PML localize in the cytoplasm and what does it do there?* The notion that PML could reside or shuttle between these compartments opens the possibility that, as in the MAMs and the

nucleus, this protein could be regulating the localization, post-translational modification, and function of residents of 'PML-related bodies'. *How is PML nucleocytoplasmic shuttling regulated?* PML presents multiple phosphorylation and acetylation sites surrounding the NLS [61,62] and it is therefore plausible that these modifications alter the accessibility to the NLS and in turn the compartmentalization of PML. Two decades of PML research has therefore identified multiple new unexpected lines of research to be pursued. On the basis of this premise, the next decade of research will certainly change further our view of this mysterious and exciting protein.

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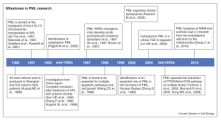

**Figure 1.**
Milestones in PML research from its discovery in the beginning of the 90s.

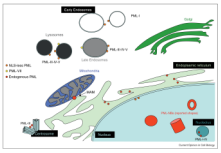

**Figure 2.**
Current view of PML compartmentalization in the cell. Nuclear PML in PML-NBs (with suggested shapes and forms) and cytoplasmic PML localization.