Review article

The promyelocytic leukemia protein, PML, and its biological function

Sadeq Vallian

Division of Genetics, Department of Biology, Faculty of Science, University of Isfahan, Isfahan, I.R. Iran.

Abstract
The PML gene was first identified at the breakpoint region of t(15;17) chromosomal translocation in acute promyelocytic leukemia (APL). There has been a large attention toward the elucidation of the biological function of PML in the cell. Our studies and those of others during the last decade resulted in elucidation of several fundamental biological functions for PML. These include: i) Regulation of transcription in a promoter dependent manner; ii) Suppression of growth and tumorigenisity of cells transformed by oncogenes; iii) Involvement in cell cycle progression through interaction with several key proteins involved in cell cycle regulation and apoptosis, e.g. p53 and pRb; iv) Association and mediation of the effects of a number of viral regulatory protein via localization in the nucleus. These findings have introduced not only PML as a multifunction protein, but also opened new windows for analysis of mechanisms involved in leukemogenesis of the APL disease. The present article focused on the studies involving the elucidation of different biological properties of PML.
Keywords: *Promyelocytic Leukemia Protein; Acute Promyelocytic Leukemia; RARα; Transcription; Cellular Growth; Cell Cycle.*

Table of contents:

Correspondence to: **Sadeq Vallian, PhD**
Telefax: +98 0311 793 2456
E-mail: svallian@sci.ui.ac.ir

INTRODUCTION

The t(15;17) chromosomal translocation is the main chromosomal abnormality in acute promyelocytic leukemia (APL). As a consequence of this translocation the retinoic acid alpha (RARα) gene located on chromosome 17 is translocated to a previously unknown gene called *PML* (for promyelocytic leukemia) located on chromosome 15 (Alcaly *et al.*, 1991; De The *et al.*, 1991; Goddard *et al.*, 1991 and Kastner *et al.*, 1992). As this is a reciprocal translocation, two fusion genes are formed, the PMLRARα gene on the recombinant 15q+ chromosome and its reciprocal RARαPML on the recombinant 17q- chromosome, (Fig. 1). PML localizes to the nucleus in a novel speckled pattern, but PMLRARα is mainly remained in the cytoplasm. In APL cells the normal pattern of PML in the nucleus is disrupted and PML as well as the PMLRARα protein are detected in the cytoplasm. Treatment of APL cells with *all*-trans retinoic acid induces degradation of PMLRARα and relocalization of PML to the nucleus (Goddard *et al.*, 1991; Grignani *et al.*, 1993; Koken *et al.*, 1994; McKenzie, 2005 and Jing, 2004). Therefore, it was hypothesized that cytopalsmic sequestration of the PML protein as well as its disruption by the t(15;17) translocation may contribute to the leukemogenesis of the APL phenotype (Grignani *et al.*, 1994). Investigations towards the elucidation of the role of PML during the last 10 years have resulted in several key functions for the protein in cellular growth, oncogenesis and transcription regulation of which some of the main aspects are reviewed in this article.

The genomic structure of the PML gene: Cloning and characterisation of the human PML gene revealed

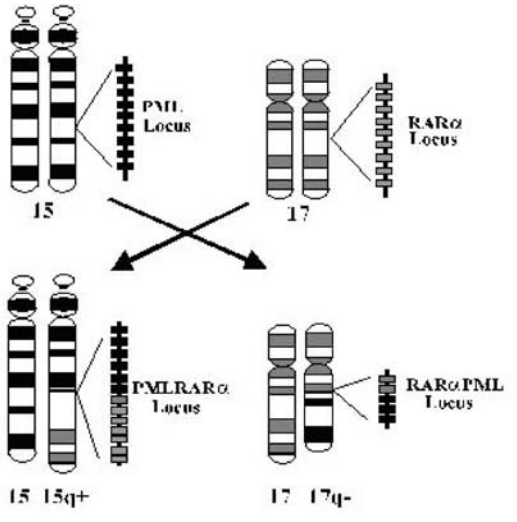

Figure 1: Schematic representation of t(15;17) chromosomal translocation. The translocation derived chromosomes are represented as 15q+ and 17q-, on which the PMLRARα and RARαPML loci are show.

that, it contains nine exons which span a minimum of 35 kb. However, the initiation codon (ATG) is found in exon 1 and three alternative TGA termination codons are present in exons 7, 8 and 9 (Fagioli *et al.*, 1992 and Pandolfi *et al.*, 1992). Analysis of the expression pattern of PML indicated the presence of several (at least four) mRNA isoforms, with differences in their 3 termini (Fagioli *et al*., 1992). These observations suggest that all PML transcripts may originate from a long primary transcript during the splicing process rather than genomic heterogeneity. Results from several studies demonstrate that the PML coding region is contained within exons 1-7 (Chang *et al.*, 1992).

Cloning and mapping of the fusion point of the t(15;17) from different APL patients has revealed that the chromosome 15 breakpoint is variably located in three regions of the PML locus (Alcaly *et al.*, 1991; De The *et al.*, 1991 and Goddard *et al.*, 1991). In most cases, the breakpoint is located within intron 6 which results in the longest isoform of PMLRARα. It is noted that the translocation breakpoint on chromosome 17 always occurs within intron two of the RARα gene (Fagioli *et al.*, 1992 and Pandolfi *et al.*, 1992). Therefore it would be expected that all isoforms of PMLRARα, retain the RARα exon 3, and the 3´ sequences which code for domains B-F. Further analysis of fusion points of PMLRARα from a large number of APL patients, resulted in the indication of two more breakpoint cluster regions (bcrs). One of these regions spans exon 5, intron 5 and exon 6 (bcr2), and the other is between the 3´ end of intron 2 to the 5´ end of intron 4 (bcr3) (Pandolfi *et al.*, 1992). Western blot analysis of APL samples using antibody against the F domain of RARα, resulted in the identification of different isoforms of PMLRARα ranging from 83-89 and 96-110 kDa, corresponding to samples from APLs with bcr1/bcr2 and bcr3, respectively (Fig. 2) (Fagioli *et al.*, 1992 and Pandolfi *et al.*, 1992).

Furthermore, northern and western blot analysis of samples from different APLs has revealed that the RARαPML gene is also transcriptionally active, and therefore both RARαPML transcripts and proteins can be detected (Kastner *et al.*, 1992). Further analysis of samples with bcr3 resulted in the identification of relatively short PMLRARα molecules ranging from 22 to 48 kDa, corresponding to exons 1-3 of PML joined to exon 3 of RARα. These PMLRARα transcripts are expected to encode aberrant PML proteins, as a termination codon is present a few nucleotides downstream of the fusion point (Kastner *et al.*, 1992; Pandolfi *et al*, 1992). These truncated PML proteins are encoded by alternatively spliced PMLRARα transcripts that are spliced in such a way that the longest open reading frames (ORFs) of PML and RARα are not aligned, and a stop codon is found a few base pairs 3´ to the PMLRARα junction resulting in the production of a C-terminal truncated PML protein. These proteins, which can be detected in all APL cases, retain the PML RING finger domain. The RARαPML protein which contains only the A domain of RARα and part of the coiled-coil (α-helix) region of PML can also be detected in about 70-80% of APL cases (Chang *et al.*, 1992).

Structure of the PML protein: Analysis of the primary structure of the PML protein, and a comparison with other proteins, has resulted in the identification of several interesting regions. As depicted in figure 3, at the N-terminal end of the protein there is a proline-rich region which is followed by a region containing three clusters of cysteine residues. These two regions are retained in all PML and PMLRARα isoforms (Kastner *et al*., 1992 and Borden *et al*., 1995). Although database search for other proteins with homology to the proline-rich region has not found any similarity, proline-rich regions have bee found in association with transcription activation domains (Freemont *et al.*, 1991). Furthermore, stretches of proline, when linked to the DNA binding domain of GAL4, have been shown to activate transcription from GAL4-responsive promoters both *in vitro* and *in vivo* (Gerber *et al*, 1994 and Reddy *et al*., 1992). This suggests the presence of a possible transcriptional activity for this region of

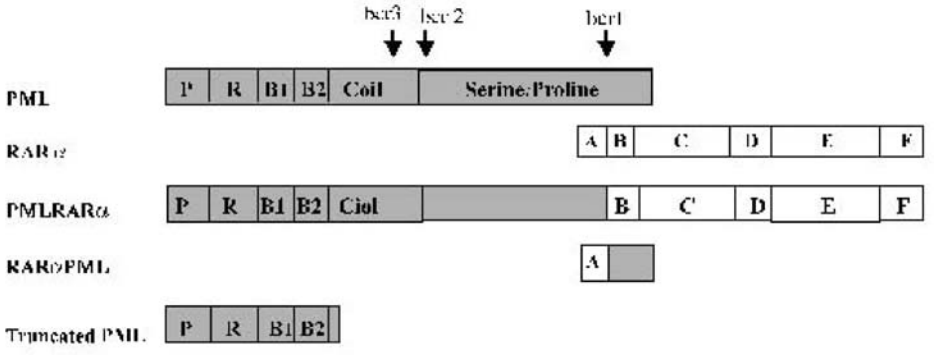

Figure 2: Schematic structure of PML, RARα, PMLRARα and RARαPML. Diagram of PML, RAR?, PMLRARα and RARαPML (translocation fusion products) as well as the aberrant PML are illustrated. Arrows indicate the position of translocation breakpoints which result in different isoforms of PMLRARα. P, proline-rich; R, RING finger; B1 and B2, B-boxes; coil, coiled-coil, and serine/proline-rich regions are shown. The A-F domains of RARα also indicated. For more details see the text.

PML, a question not yet directly addressed. The cysteine-rich region of PML is intriguing, as its first cluster of cysteines, shows that it belongs to a new class of proteins called RING finger or B-box proteins (Kakizuka *et al.*, 1991; Borden *et al.*, 1995 and Gerber *et al.*, 1994). The RING finger domain which is conserved in all members of the family can be summarised as C3HC4, where C and H indicate the cysteine and histidine residues. Examples of the members of this family which are involved in transcriptional regulatory activities include, Rpt-1, a negative regulator of the interleukin 2 receptor gene; RAG-1, a human recombination-activating gene product; *Xenopus* XNF-7, a developmental regulatory protein; Rad 18, a protein involved in repair of DNA damaged by UV. Two members of this family, T18 and Rfp, have been found implicated in oncogenesis (Kakizuka *et al.*, 1991). Moreover, some members of the RING finger family share one (e.g. XNF-7, RPT-1) or two (e.g. T18) extra cysteine-rich domains with PML. These regions are referred to as B-boxes, B1 and B2. Analysis of the crystal structure of the RING finger domain of PML has revealed that this region contains two zinc atoms (Zn^{2+}) which are required for its autonomous folding (Fig. 3).

In addition to the RING finger and B-box like motifs, all the members of the RING family share a region which is predicted to form an α-helical structure. This region in PML contains four clusters of hydrophobic amino acids, in which every forth and seventh position is an amphipatic residue. Similar repeats have been reported in the DNA-binding domain of thyroid hormone, retinoic acid and vitamin D3 receptors and are believed to form the protein's dimerisation interface (Forman and Samuels, 1990; Lazar, 1993). Also, in this region, are three repeats of lysine, in which this amino acid has been repeated at every seventh position. This heptad repeat orientation for lysine residues is reminiscent of the classical leucine zipper domains (Kouzarides and Ziff, 1988). Furthermore, a segment in this region shows homology to the leucine zipper domain of the c-Fos family of proteins, including Fra-1, c-Fos and Fos-B (Kouzarides and Ziff, 1989).

PML encodes a nuclear protein localized to PODs: The results from extensive immunostaining experiments for PML have demonstrated that PML localizes to the nucleus in a speckled pattern, and is associated with nuclear bodies named POD (PML oncogenic domain) or PML NB (PML nuclear body) (Koken, *et al.*, 1994 and Dyck *et al.*, 1994). It seems that PML is the major component of POD. Many proteins co-localized with POD have been identified, including SP100, the ubiquitin-like protein modifier SUMU-1/PIC-1/sentrin, the interferon induced protein ISG20, immediate early viral proteins IE1 and IE4, and the HTLV-1 encoded Tax associated protein int-6 (Ahn and Hayward, 1997; Ahn *et al.*, 1998; Desbois *et al.*, 1996; Everet and Maul, 1994; Guccione *et al.*, 2004).

The SUMO1/PIC1/sentrin conjugated PML are exclusively localized to the POD, indicating that linking of the SUMO1 modifier is important for assembling the PML NB(Kamitani *et al.*, 1998a, 1998b and Lee *et al.*, 2004). PML NBs are frequent target of viral oncoproteins such as the HSV-1 gene product Vmw110, the adenovirus proteins E1A and E4-ORF3, the Epstein-Barr virus-encoded nuclear antigen

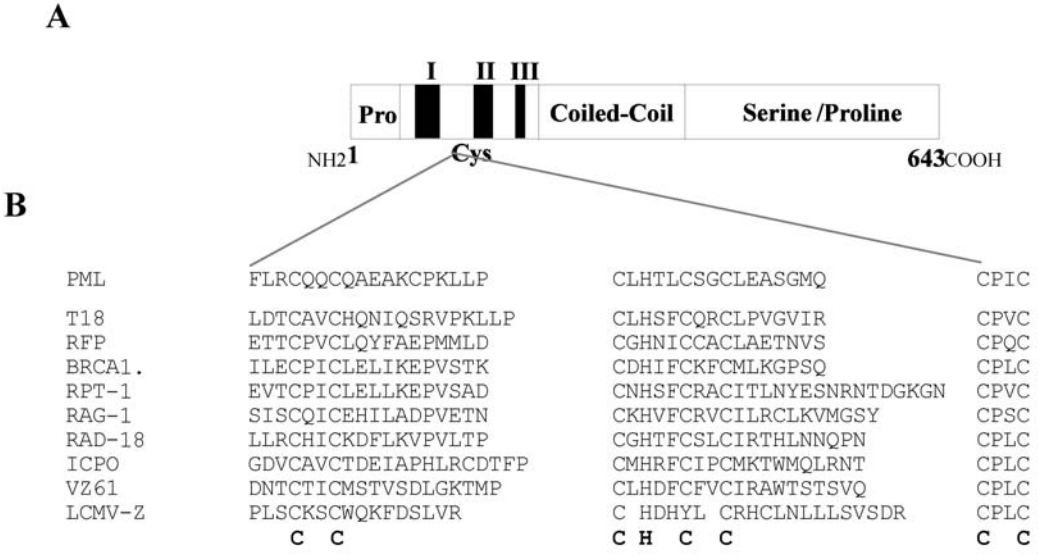

Figure 3: Homology between the cysteine-rich domain of PML and the RING finger proteins. A) Schematic diagram of PML. Different domains of the PML protein (pro, proline-rich; cys, cysteine-rich, coiled-coil and serine/proline) are shown. The solid boxes represent three clusters of cysteine (I, II, and III). B) Sequence alignment of the cluster I of the cysteine-rich domain of PML, and RING finger proteins.

EBNA-5, and the CMV major immediate-early protein IE1 and IE2 (Everet and Maul, 1994). After adenovirus infection, the viral protein e.g. E4-ORF targeted to PML NB, disrupted its organization and recruited its component (SP100 and NDP55) to the viral replication domain. The CMV immediate early gene product IE-1 physically interacts with PML (Doucas *et al.*, 1996).

PML has been reported to mediate the effects of interferon (IFN) and viral mutants lacking ICP0 ($\Delta\alpha0$ mutants) (Crowder *et al.*, 2005). It has been shown that HSV-1 disaggregates POD structures and disperses PML to preclude IFN-mediated antiviral effects. Analysis of the accumulation of viral proteins and virus yields from murine $PML^{+/+}$ and $PML^{-/-}$ cells mock treated or exposed to IFN-α, IFN-γ, or both and infected with the wild-type or $\Delta\alpha0$ mutant virus showed that the anti-HSV state induced by exogenous IFN is mediated by PML (Crowder *et al.*, 2005). Moreover, the virus targets the POD structures and disseminates PML in order to preclude the establishment of the antiviral state induced by IFNs.

It has been shown that the RING finger domain of PML is responsible for its speckled distribution in the nucleus (PODs), as deletion mutants in this region are able to localize to the nucleus but with an altered pattern of distribution, ranging from uniform to microspeckled patterns (Kastner *et al.*, 1992 and Koken *et al.*, 1994). Therefore, the nuclear localisation of PML and the involvement of Zn^{2+} in its RING finger domain suggest that PML's RING finger may have a DNA target.

At the C-terminal region of PML there is a region rich in serine and proline residues. This region contains several X-S-P-X type repeats that have been identified as the minimum sequence of a serine/threonine kinase and casein kinase II (CKII) phosphorylation site (Fagioli *et al.*, 1992). Taking advantage of the presence of possible phosphorylation sites in PML, (Chang *et al.* 1995) have investigated this possibility using ^{32}P-orthophosphate incorporation assays and found that PML is indeed a phosphoprotein and that serine and tyrosine residues are phosphorylated. It has been recently shown that phosphorylation affects the role of PML in the cell. As an instance, Arsenic trioxide (As_2O_3) treatment induces apoptosis in acute promyelocytic leukemia cells through an incompletely understood mechanism (Shen *et al.*, 1997). It has been recently reported that As_2O_3 treatment of APL cells induces phosphorylation of the PML protein through a mitogen-activated protein (MAP) kinase pathway. Increased PML phosphorylation is associated with increased sumoylation of PML and increased PML-mediated apoptosis. Conversely, MAP kinase cascade inhibitors, or the introduction of phosphorylation or sumoylation-defective mutations of PML, impair As_2O_3-mediated apoptosis by PML. These data show that phosphorylation by MAP kinase cascades potentiates the antiproliferative functions of PML and helps mediate the proapoptotic effects of As_2O_3 (Hayakawa and Privalsky, 2004).

PML is a ubiquitsly expressed protein: Investigations using mono- and polyclonal antibodies revealed that PML is expressed in a variety of cell lines and tissues examined. The highest levels of protein were present in post-mitotic, differentiated cell types, such as endothelial cells, epithelial cells and tissue macrophages, especially activated ones (Flenghi *et al*., 1995). The cell lines tested include myeloid cell lines (e.g. HL-60, U937), T cell line (MOLT), neuroblastoma cell line (NB 100) (Goddard *et al*., 1991), and human breast carcinoma cell lines (MCF-7, ZR-75, T47D, BT474, MDA-MB-231, MDA-MB-468, Hs578T) (Li *et al*., 1994). The PML protein was also detected in several tissues including, adult brain, gut, liver, lung, muscle, placenta and testes (Goddard *et al*., 1991).

It has been reported that PML displays an altered expression during tumorigenesis. Analysis of PML expression in different stages of tumor progression indicated that the level of PML decreases during the progression from benign to malignant, and from malignant to invasive tumors (Koken *et al.*, 1994; Gurrieri *et al.*, 2004a). These findings suggest an inverse relation between PML expression and tumor growth.

The status and expression of the *PML* gene in solid tumors of multiple histologic origins was recently analyzed using a tumor tissue microarrays (TTMs) created with samples from patients with different tumors. The data showed that PML protein expression is frequently lost in human cancers of various histologic origins, and its loss associates with tumor grade and progression in some tumor histotypes was reduced or abolished (Gurrieri *et al*., 2004a and 2004b).

The ubiquitous expression of the *PML* gene could classify it among the housekeeping genes in the cell with putative function as a fundamental regulator involved in different aspects of the cellular processes. Therefore, lack of PML expression could impair different cellular pathways.

Involvement of PML in transcription regulation: The localization of PML to the nucleus, the presence of proline-rich (at the N-terminal), and serine/proline-rich (at the C-terminal) regions, which are associated with activation domains (Gerber *et al*., 1994), and the presence of a cysteine-rich domain, suggest that PML may function as a regulator of transcription (Freemont *et al.*, 1991) Moreover, PML NB was found to be the site of viral DNA replication and transcription. Nascent RNA polymerase II transcripts were found within the PML NB and that PML colocalized with the cAMP enhancer binding protein (CREB)- binding protein (CBP) (Doucas *et al.*, 1999). Indeed, it was shown that CBP associated with PML *in vitro* and is recruited to the PODs *in vivo*. Through its association with CBP, wild-type PML dramatically stimulates nuclear receptor transcriptional activity. These results demonstrate that a fraction of CBP is compartmentalized to the POD through its association with PML and thus suggest that PML and other POD-associated proteins may play an unexpectedly broad role in aspects of transcriptional regulation and human disease (Doucas *et al.*, 1999).

It has been demonstrated that transcription activity associated with PODs is selectively repressed by the recruitment of Bach2 around these nuclear bodies (Tashiro *et al.*, 2004). Bach2 is a member of the BTB-basic region leucine zipper factor family and represses transcription activity directed by the 12-O-tetradecanoylphorbol-13-acetate response element, the Maf recognition element, and the antioxidant-responsive element. Bach2 forms nuclear foci associated with PODs upon oxidative stress. The Bach2 N-terminal region including the BTB domain is essential for the focus formation. Sumoylation of Bach2 is required for the recruitment of the protein around PODs (Tashiro *et al.*, 2004). These observations represent that transcription activity associated with PODs which is modulated by a sequence-specific transcription factor upon oxidative stress.

Our study and others showed that PML was directly involved in regulation of transcription; it activates transcription of steroid hormone receptor and transcription mediated by Fos/AP-1 (Guiochon-Mantel *et al.*, 1995 and Vallian *et al*., 1997; 1998). When fused to the GAL4 DNA-binding domain, PML acted as a transcription suppressor to inhibit transcription from the GAL4-responsive promoter and that specific domain of the PML protein was involved in the transcription repressing events (Vallian *et al.*, 1997).

In a search for PML's target genes, several promoters were found to be regulated by PML including thymidine kinase (TK), epidermal growth factor receptor (EGFR) and dehydrofolate reuductase (DHFR) (Mu *et al.*, 1994). Among these promoters, the repressive effects of PML on EGFR and DHFR were investigated in detail (Vallian and Chang, 2004a and 2004b; Vallian *et al.*, 1988a). It was shown that that PML's repression of EGFR transcription is caused by inhibition of EGFR's Sp1-dependent activity. On functional analysis, the repressive effect of PML was mapped to a 150-bp element (the sequences between -150 and -16, relative to the ATG initiation site) of the promoter. Transient transfection assays showed that the transcription of this region was regulated by Sp1 and that

the Sp1-dependent activity of the promoter was suppressed by PML in a dose-dependent manner. Coimmunoprecipitation and mammalian two-hybrid assays demonstrated that PML and Sp1 were associated in vivo. Analysis of the effects of PML on Sp1 DNA binding by electrophoretic mobility shift assay (EMSA) showed that PML could specifically disrupt the binding of Sp1 to DNA (Vallian *et al.*, 1998b). Furthermore, cotransfection of PML specifically repressed Sp1, but not the E2F1-mediated activity of the dihydrofolate reductase promoter (Vallian and Chang, 2004a). Together, these data suggest that the association of PML and Sp1 represents a novel mechanism for negative regulation of EGFR and other Sp1 target promoters.

Interestingly, it was also found that the effects of PML on the activity of dihydrofolate reuductase (DHFR) promoter, mainly regulated by Sp1 (Vallian and Chang, 2004b). On functional analysis, transient transfection of PML into mammalian cells resulted in a significant repression of the DHFR promoter. Moreover, electrophoretic mobility shift assay (EMSA) using Sp1-containing oligonucleotide probes showed significant reduction in Sp1 binding in the presence of PML, confirming the necessity of the Sp1 DNA-binding sites for PML's repressive effects (Vallian and Chang, 2004b). Analysis of DNA synthesis using [3H]thymidine incorporation assay showed a significant reduction in DNA synthesis in HeLa cells overexpressing PML (Mu *et al.*, 1997). Together, the data demonstrated that PML could function as a negative regulator of the DHFR promoter, which may represent a novel mechanism for the known repressive effects of PML on cellular growth.

Because DHFR and EGFR promoters function as key regulators in DNA synthesis and cellular growth, their repression by PML represent a novel mechanism for the well documented growth inhibitory effects of PML (Le *et al.*, 1998 and Gurrieri *et al.*, 2004 b). Also, these data could provide a link between growth suppression and transcription regulatory function of the PML protein.

Investigations toward the mechanisms underlying the transcriptional repression property of PML by our group showed that PML is functionally and physically associated with all three isoforms of histone deacetylase complex (HDAC1-3) through specific domains *in vivo* and silent transcription by deacetylation of the target promoter (Wu *et al.*, 2001). Both the C-terminal and the N-terminal of the PML protein are necessary for efficient binding to HDAC. The retinoblastoma sudceptibility protein (pRb) interacts with PML and HDAC (Wu *et al.*, 2001; Wang *et al.*, 1998 and Alcaly *et al.*, 1998). This study showed that the pattern of PML interaction with HDACs is different from Rb and other HDAC interacting proteins in a sense that they bind only HDAC1 and HDAC2. Analysis of the HDAC domain that interacts with PML, we found that the N-terminal domain (amino acids 1-180) is responsible for the interaction. This region is different from the Rb binding domain that was localized to the C-terminal of HDAC. The Rb binding domain is highly conserved between HDAC1 and HDAC2 but not in HDAC3, this explains why Rb does not bind HDAC3(Wu *et al.*, 2001). Moreover, the data showed that that PML de-represses transcriptional repression of E2F target gene by Rb, which may affect the Rb/HDAC/E2F transcriptional repression complex. These results provided evidence for the first time that the *in vivo* function of PML is directly linked to Rb/E2F complex, the major G1/S checkpoint regulator (Wang *et al.*, 1998; Wu *et al.*, 2001 and Mallette *et al.*, 2004).

Interestingly the study showed that the PML-RARα mutant encoded from the breakpoint of t(15;17) in APL was unable to bind HDAC. That PML-RARα unable to interact with HDAC may have important implication that a loss of function of a yet unknown event normally controls by PML by recruiting HDAC to its target genes may have occurred in APL cells. This event, as a result of t(15;17) translocation may have contributed to the leukemogenesis of the APL disease.

PML functions as a suppressor of cellular growth: The fact that that PML interacts with transcription factor Sp1 and inhibits its binding to the target sites (see above) (Vallian *et al.*, 1998a), and the interaction of Sp1 with E2F and synergistically activation of transcription of G1/S checkpoint genes (Karlseder *et al.*, 1996), suggest that PML could play a role in regulating cell cycle progression by its association with the Rb/E2F/Sp1 complex. The PML's role in cell cycle progression is also supported by several reports documented the finding that PML nuclear body is the target of several viral oncoproteins similar to other tumor suppressors involved in the control of G1/S checkpoint (Le *et al.*, 1998).

Expression of PML in U937 cells can be up-regulated following the exposure of these cells to interferon gamma (an inducer of monocyte/ macrophage differentiation and activation), vitamin D3 and transforming growth factor β1 (TGF β1)(Lin *et al.*, 2004). These results show that PML has a regulatory function in cellular proliferation and/or differentiation, or act as a target for factors involved in these processes. Consistent

with these findings, a number of reports indicated that PML is involved in oncogenesis and can function as a growth suppressor. Expression of PML in the APL cell line, NB4, has resulted in repression of anchorage-independent growth of these cells in soft agar and also their transformation ability in nude mice (Mu *et al.*, 1997 and Shin *et al.*, 2004). In addition, the expression of PML in mouse fibroblasts, NIH3T3, could inhibit transformation of these cells by an activated *neu* oncogene. Results from the same study have indicated that transformation of rat embryo fibroblasts, REF, transfected with Ha-*ras* and either c-*myc* or mutant *p53* can be significantly inhibited in the presence of PML.

Insights into the mechanism of the role of PML in cell growth and transformation suppression come from several findings; i) PML NB is regulated during progression of the cell cycle and the highest number was found in the G1; ii) PML induced a G1 arrest and apoptosis in MCF-7 and normal human lung fibroblasts; iii) In HeLa, PML NB induced growth inhibition by lengthening G1 phase of the cell cycle (Wang *et al*., 1998 and Shin *et al.*, 2004). Moreover, the *PML* gene knock-out study strongly support a crucial role for PML in the control of cell growth. These studies demonstrated that PML is essential for multiple pathways of program cell death by using $PML^{-/-}$ mice and in cells over expressing PML (Wang *et al.*, 1998 and Shin *et al.*, 2004).

Regulation of cell cycle progression and apoptosis by PML: It has been reported that overexpression of PML induced a G1 cell cycle arrest both in cancer cell line and in normal human lung fibroblasts. Stable overexpression of PML in HeLa cell caused an alteration of cell cycle progression by lengthening G1 (Mu *et al.*, 1997). Based on these findings it was hypothesize that PML normally plays a role in regulating cell cycle progression. Expression of PML has been reported to be cell-cycle dependent. Immunofluorescence staining and confocal microscopic analysis of HeLa cells, which were synchronised at different phases of the cell cycle, indicated that PML was expressed at a lower level in S, G2, and M phases and at a significantly higher level in G1 phase (Chang *et al.*, 1995).

Moreover, it seems that PML affects cell cycle progression by modulating the expression of several key proteins involved in G1/S checkpoint. PML interacts with multiple factors involved in cell cycle and growth regulation including p53, Rb, Sp1, PLZF, CMV immediate early protein IE1 (Alcaly *et al.*, 1998; Fogal *et al.*, 2000; Bernardi *et al.*, 2004 and Vallian *et al.*, 1998a). It is well documented that Rb plays a central role in controlling cell-cycle progression by modulating transcriptional activity of E2F. Transcription of many genes involved in the G1 to S transition is controlled by E2F. At G1, the hypophosphorylated form of Rb recruits HDAC, interacts with E2F and inactivates its transactivation function. During G1/S cell cycle transition, Rb becomes phosphorylated by cyclin-dependent kinase, cyclin D/Cdk4 or cyclin E/Cdk2. The phosphorylated form of Rb releases E2F and HDAC and reactivates E2F target genes, e.g. DNA polymerase, thymidine kinase, DHFR, and cyclin A etc, this enable cell cycle progression from G1 to S. Rb also acts as a transcription regulator by association with several other transcription factors. For example Rb regulates transcription of genes containing the Rb controlled element (RCE). The biological significance of interaction of PML with Rb is not clear. It is speculated that PML normally regulate cell cycle progression by modulating the functional activity of the Rb/E2F complex. In this sense, disruption of PML function by t(15;17) in APL should contribute to the development of acute leukemia.

The association of PML and p53 was examined in several studies (Hongyan *et al.*, 2003; Insinga *et al.*, 2004; Pearson and Pelicci, 2001 and Stanchina *et al.*, 2004). The data show that p53 is recruited into PODs by a specific PML isoform (PML3) or by co-expression of SUMO-1 and hUbc9. POD targeting depends on the direct association of p53, through its core domain, with a C-terminal region of PML3. The relocalization of p53 into NBs enhances p53 transactivation in a promoter-specific manner and affects cell survival. Current models suggest that recruitment to PML-NBs activate p53 by bringing it in close proximity with CBP/p300 (Torchia *et al.*, 1997). Acetylation of p53 by CBP/p300 then increases p53 DNA binding affinity, leading to an activation of p53-responsive genes. Theses results indicate the existence of a cross-talk between PML- and p53-dependent growth suppression pathways, implying an important role for PODs and their resident proteins as modulators of p53 functions. This activation of p53 likely contributes to the tumor suppressor function of PML.

It has been reported that that SIRT1, the human Sir2 homolog, is recruited to the PML-NBs upon over expression of either PML or oncogenic Ras (HarasV12) (Langley, 2002). SIRT1 binds and deacetylates p53, a component of PML-NBs (see above), and it can repress p53-mediated transactivation. Moreover, this study showes that SIRT1 and p53 co-localize in PML-NBs upon PML upregulation. When overexpressed in primary mouse embryo fibroblasts (MEFs), SIRT1 antagonizes PML-induced acetylation of p53 and rescues PML-mediated premature cellular senes-

cence (Langley, 2002). The data together establish the SIRT1 deacetylase as a novel negative regulator of p53 function capable of modulating cellular senescence.

The fact that PML interacts functionally and physically with MDM2 independent of p53 (Hongyan *et al.*, 2003), may indicate that p53, mdm2, and PML can colocalize in PML-NBs with one another, and this can occur in the absence of p53:mdm2 binding (Hongyan *et al.*, 2003 and Haupt *et al.*, 2003).

References

Ahn JH, Brignole EJ, Hayward GS (1998). Disruption of PML subnuclear domains by the acidic IE1 protein of human cytomegalovirus is mediated through interaction with PML and may modulate a RING finger-dependent cryptic transactivator function of PML. *Mol Cell Biol.* 18:4899-4913.

Ahn JH, GS Hayward (1997). The major immediate-early proteins IE1 and IE2 of human cytomegalovirus colocalize with and disrupt PML-associated nuclear bodies at very early times in infected permissive cells. *J Virol.* 71:4599-4613

Alcalay ML, Tomassoni E, Colombo S, Stoldt F, Grignani M, Fagioli L, Szekely K, Helin, P. G. Pelicci. (1998). The promyelocytic leukemia gene product (PML) forms stable complexes with the retinoblastoma protein. *Mol Cell Biol.* 18:1084-1093

Alcalay, M, Zangrilli D, Pandolfi PP, Longo L, Mencarelli A, Giacomucci A, Rocchi M, Biondi A, Rambaldi A, Lo Coco F, Diverio D, Donti E, Grignani F, Pelicci PG (1991). Translocation breakpoint of acute promyelocytic leukemia lies within the retinoic acid receptor alpha locus. *Proc Natl Acad Sci USA.* 88: 1977-1981.

Bernardi R, Scaglioni PP , Bergmann S, Horn HF, Vousden KH, Pandolfi PP (2004). PML regulates p53 stability by sequestering MDM2 to the nucleolus. *Nat Cell Biol.* 6: 7: 665-72.

Borden KLB, Boddy MN, Lally J, O'Reilly J, Martin S, Howe K, Solomon E, Freemont PS (1995). The solution structure of the RING finger domain from the acute promyelocytic leukemia proto-oncoprotein, PML. *EMBO J.* 14: 1532-1541.

Chang KS, Fan YH, Andreeff M, Liu JX, Mu ZM (1995). The pml gene encodes a phosphoprotein associated with the nuclear matrix. *Blood,* 85: 3646-3653.

Chang KS, Stass SA, Chu DT, Deaven LL, Trujillo JM, Freireich EJ (1992). Characterization of a fusion cDNA (RARa/myl) transcribed from the t(15;17) translocation breakpoint in acute promyelocytic leukemia. *Mol Cell Biol.* 12: 800-810.

Crowder C, Dahle O, Davis RE, Gabrielsen OS, Rudikoff S (2005) PML mediates IFN-alpha-induced apoptosis in myeloma by regulating TRAIL induction. *Blood.* 105:1280-7.

De The H, Lavau H, Marchio C, Chomienne A, Degos L, Dejean A (1991). The PML-RARα fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell,* 66: 675-684.

Desbois C, Rousset R, Bantignies F, Jalinot P (1996). Exclusion of Int-6 from PML nuclear bodies by binding to the HTLV-I Tax oncoprotein. *Science,* 273:951-953.

Doucas V, Tini M, Egan DA, Evans RM (1999). Modulation of CREB binding protein function by the promyelocytic (PML) oncoprotein suggests a role for nuclear bodies in hormone signaling. *Proc Natl Acad Sci USA.* 96: 2627–2632.

Doucas V, Ishov AM, Romo A, Juguilon H, Weitzman MD, Evans RM, Maul GG (1996). Adenovirus replication is coupled with the dynamic properties of the PML nuclear structure. *Genes Dev.* 10:196-207.

Dyck JA, Maul GG, Miller J, WH, Chen JD, Kakizuka A, Evans RM (1994). A novel macromolecular structure is a target of the promyelocyte-retinoic acid receptor oncoprotein. *Cell,* 76: 333-343.

Everett RD, GG Maul (1994). HSV-1 IE protein Vmw110 causes redistribution of PML. *EMBO J.* 13:5062-5069.

Fagioli M, Alcalay M, Pandolfi PP, Venturini L, Mencarelli A, Simeone A, Acampora D, Grignani F, Pelicci PG (1992). Alternative splicing of PML transcripts predicts coexpression of several carboxy-terminally different protein isoforms. *Oncogene,* 7: 1083-1091.

Flenghi L, Fagioli M, Tomassoni L, Pileri S, Gambacorta M, Pacini R, Grignani F, Casini T, Ferrucci PF, Martelli MF, Pelicci PG, Falini B (1995). Characterization of a new monoclonal antibody (PG-M3) directed against the aminoterminal portion of the PML gene product: Immunocytochemical evidence for high expression of PML proteins on activated macrophages, endothelial cells, and epithelia**.** *Blood,* 85: 1871-1880.

Fogal V, Gostissa M, Sandy P, Zacchi P, Sternsdorf T, Jensen K, Pandolfi PP, Will H, Schneider C, Del Sal G (2000). Regulation of p53 activity in nuclear bodies by a specific PML isoform. *EMBO J.* 19**:** 6185-6195.

Forman BM, Samuels HH (1990). Interactions among a subfamily of nuclear hormone receptors -the regulatory zipper model. *Mol Endocrinol.* 44:1293-1301.

Freemont PS, Hanson IM, Trowsdale J (1991). A novel cystein-rich sequence motif. *Cell,* 64: 483-484.

Gerber HP, Seipel K, Georgiev O, Hofferer M, Hug M, Rusconi S, Schaffner W (1994). Transcriptional activation modulated by homopolymeric glutamine and proline stretches. *Science,* 263: 808-811.

Goddard AD, Borrow J, Freemont PS, Solomon E (1991) Characterization of a zinc finger gene disrupted by the t(15;17) in acute promyelocytic leukemia. *Science,* 254: 1371-1374

Grignani F, Fagioli M, Alcalay M, Longo L, Pandolfi PP, Donti E, Biondi A, Lo Coco F, Pelicci PG (1994). Acute promyelocytic leukemia: From genetics to treatment. *Blood,* 83: 10-25.

Grignani F, Ferrucci PF, Testa U, Talamo G, Fagioli M,

Alcalay M, Mencarelli A, Peschle C, Nicoletti I, Pelicci PG (1993). The acute promyelocytic leukemia-specific PML-RARalpha fusion protein inhibits differentiation and promotes survival of myeloid precursor cells. *Cell*, 74: 423-431.

Guccione E, Lethbridge KJ, Killick N, Leppard KN, Banks L (2004). HPV E6 proteins interact with specific PML isoforms and allow distinctions to be made between different POD structures. *Oncogene*, 23: 4662-72.

Guiochon-Mantel A, Savouret FJ, Quignon F, Delaber K, Milgrom E, de The H (1995). Effect of PML and PML-RAR on the transactivation properties and subcellular distribution of steroid hormone receptors. *Mol Endocrinol.* 9:1791-1803

Gurrieri C, Nafa K, Merghoub T, Bernardi R, Capodieci P, Biondi A, Nimer S, Douer D, Cordon-Cardo C, Gallagher R, Pandolfi PP (2004a). Mutations of the PML tumor suppressor gene in acute promyelocytic leukemia. *Blood*, 103: 2358-2362.

Gurrieri C, Capodieci P, Bernardi R, Scaglioni PP, Nafa K, Rush LJ, Verbel DA, Cordon-Cardo C, Pandolfi PP. (2004b). Loss of the tumor suppressor PML in human cancers of multiple histologic origins. *J Natl Cancer Inst*. 96: 269 - 279.

Haupt Y, Maya R, Kazaz A, Oren M (2003). MDM2 promotes the rapid degradation of p53. *Nature*, 387: 296-299.

Hayakawa F, ML Privalsky (2004). Phosphorylation of PML by mitogen-activated protein kinases plays a key role in arsenic trioxide-mediated apoptosis. *Cancer Cell*, 5: 389-401.

Hongyan Z, Wu L, Maki CG (2003). MDM2 and promyelocytic leukemia antagonize each other through their direct interaction with p53. *J Biol Chem.* 278: 49286-49292.

Insinga A, Monestiroli S, Ronzoni S, Carbone R, Pearson M, Pruneri G, Viale G, Appella E, Pelicci PG, Minucci S (2004). Impairment of p53 acetylation, stability and function by an oncogenic transcription factor. *EMBO J*, 23: 1144-1154.

Jing Y (2004). The PML-RARalpha fusion protein and targeted therapy for acute promyelocytic leukemia. *Leuk Lymphoma.* 45: 639-48.

Kakizuka A, Miller JWH, Umesono K, Warrel J, Frankle SR, Murty VS, Dmitrovsky E, Evans RM (1991). Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RARα with a novel putative transcription factor. *Cell*, 66: 663-674.

Kamitani T, Nguyen HP Kito K, Fukuda-Kamitani T, Yeh ET (1998a). Covalent modification of PML by the sentrin family of ubiquitin-like proteins. *J Biol Chem.* 273:3117-3120.

Kamitani T, Kito K, Nguyen KP, Wada H, Fukuda-Kamitani T, Yeh ET (1998b). Identification of three major sentrinization sites in PML. *J Biol Chem.* 273:26675-26682.

Karlseder J, Rotheneder H, Wintersberger E (1996). Interaction of Sp1 with the growth-and cell cycle-regulated transcription factor E2F. *Mol Cell Biol.* 16:1659-1667.

Kastner P, Perez A, Lutz Y , Egly CR, Gaube MP, Durand B, Lanotte M, Berger R, Chambon P (1992). Structure, localization and transcriptional properties of two classes of retinoic acid receptor α fusion proteins in acute promyelocytic leukemia (APL): structural similarities with a new family of oncoproteins. *EMBO J.* 11: 629-642.

Koken MHM, Dutilleul FP, Guillemin CM, Viron A, Cruz GL, Stuurman N, de Jong L, Szostecki C, Calvo F, Chomienne C, Degos L, Puvion E, De The H (1994). The t(15;17) translocation alters a nuclear body in a retinoic acid-reversible fashion. *EMBO J.* 13: 1073-1083.

Kouzarides T, Ziff E (1988). The role of the leucine zipper in the fos-jun interaction. *Nature*, 336: 646-651.

Kouzarides T, Ziff E (1989). Leucine zippers of fos, jun and gcn4 dictate dimerization specificity and thereby control DNA binding. *Nature*, 340: 568-571.

Lazar MA (1993). Thyroid hormone receptors: multiple forms, multiple possibilities. *Endocr Rev. 14*, 184-193.

Langley E, Pearson M, Faretta M, Bauer UM, Frye RA, Minucci S, Pelicci PG, Kouzarides T (2002). Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence. *EMBO J.* 21:2383-96.

Le XF, Vallian S, Mu ZM, Hung MC, Chang KS (1998). Recombinant PML adenovirus suppresses growth and tumorigenisity of human breast cancer cells by inducing G1 cell cycle arrest and apoptosis. *Oncogene*, 16: 1839-1849.

Lee HR, Kim DJ, Lee JM, Choi CY, Ahn BY, Hayward GS, Ahn JH (2004). Ability of the human cytomegalovirus IE1 protein to modulate sumoylation of PML correlates with its functional activities in transcriptional regulation and infectivity in cultured fibroblast cells. *J Virol.* 78: 6527-6542.

Li XS, Sheikh MS, Shao ZM, Lanzkron S, Chen JC, Fontana JA (1994). Regulation of PML mRNA expression in human breast carcinoma cells. *Int J Oncol. 5*:1177-1181.

Lin HK, Bergmann S, Pandolfi PP (2004). Cytoplasmic PML function in TGF-beta signaling. *Nature*, 431: 7005: 205-11.

Mallette FA, Goumard S, Gaumont-Leclerc MF, Moiseeva O, Ferbeyre G (2004). Human fibroblasts require the Rb family of tumor suppressors, but not p53, for PML-induced senescence. *Oncogene*, 23: 91-9.

McKenzie SB (2005). Advances in understanding the biology and genetics of acute myelocytic leukemia. *Clin Lab Sci*. 18:28-37.

Mu ZM, Chin KV, Liu JH, Lozano G, Chang KS (1994). PML, a growth suppressor disrupted in acute promyelocytic leukemia. *Mol Cell Biol.* 14: 6858-6867.

Mu, ZM, Le XF, Vallian S, Glassman AB, Chang HP (1997). Stable overexpression of PML alters regulation of cell cycle progression in HeLa cells. *Carcinogenesis*, 18:2063-2069.

Pandolfi PP, Alcalay M, Fagioli M, Zangrilli D, Mencarelli A, Diverio D, Biondi A, Lo Coco F, Rambaldi A, Grignani F, Rochette-Egly C, Gaube MP, Chambon P, Pelicci PG (1992). Genomic variability and alternative splicing generate multiple PMLRARα transcripts that encode aberrant PML proteins and PMLRARα isoforms in acute promyelocytic leukemia. *EMBO J.* 11:1397-1407.

Pearson M, Pelicci PG (2001). PML interaction with p53 and its role in apoptosis and replicative senescence. *Oncogene*, 49: 7250-7256.

Reddy BA, Etkin LD, Freemont PS (1992). A novel zinc finger coiled-coil domain in a family of nuclear proteins. *TIBS.* 17: 344-345.

Shen ZX, Chen GQ, Ni JH, Li XS, Xiong SM, Qiu QY, Tang W, Sun GL, Yang KQ, Chen Y, Zhou L, Fang ZW, . Wang YT, Ma J, Zhang P, Zhang TD, Chen SJ, Chen Z, Wang ZY (1997). Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL). II. Clinical efficacy and pharmacokinetics in relapsed patients. *Blood*, 89: 3354-3360.

Shin J, Park B, Cho S, Lee S, Kim Y, Lee SO, Cho K, Lee S, Jin BS, Ahn JH, Choi EJ, Ahn K (2004). Promyelocytic Leukemia is a direct inhibitor of SAPK2/p38 mitogen-activated protein kinase. *J Biol Chem.* 279: 40994- 41003.

Stanchina E de, Querido E, Narita M, Davuluri RV, Pandolfi PP, Ferbeyre G, Lowe SW. (2004). PML is a direct p53 target that modulates p53 effector functions. *Mol Cell*, 13: 523-35.

Tashiro S, Muto A, Tanimoto K, Tsuchiya H, Suzuki H, Hoshino H, Yoshida M, Walter J, Igarashi K (2004). Repression of PML nuclear body-associated transcription by oxidative stress-activated Bach2. *Mol Cell Biol.* 24:3473-3484.

Torchia J, Rose DW, Inostroza J, Kamei Y, Westin S, Glass CK, Rosenfeld MG. (1997). The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. *Nature,* 387:677-684

Vallian S, Chang KS (2004a). Down regulation of DHFR promoter activity by PML transcription repressor. *J. Sci.* I.R Iran. 15:233-239.

Vallian S, Chang KS (2004b). Expression of PML tumor suppressor in A431 cells reduces cellular growth by inhibiting the EGFR expression. *Iranian J Sci Technol, Transaction A*, 28: A1, 57-64.

Vallian S, Chin KV, Chang KS (1998a). The promyelocytic leukemia protein interacts with Sp1 and inhibits its transactivation of the epidermal growth factor receptor promoter. *Mol Cell Biol.* 18:7147-7156.

Vallian S, Gaken J, Gingold EB, Kouzarides T, Chang KS, Farzaneh F (1998b). Regulation of Fos-mediated AP1 transcription by the promyelocytic leukemia protein. *Oncogene*, 16:2843-2853.

Vallian S, Gaken JA, Trayner ID, Gingold EB, Kouzarides T, Chang KS, Farzaneh F (1997). Transcriptional repression by the promyelocytic leukemia protein, PML. *Exp Cell Res.* 237:371-382.

Wang ZG, Ruggero D, Ronchetti S, Zhong S, Gaboli M, Rivi R, Pandolfi PP (1998). PML is essential for multiple apoptotic pathways. *Nat Genet.* 20:266-272.

Wu WS, Vallian S, Seto E, Yang WM, Edmondson D, Roth S, Chang KS (2001). The growth suppressor PML represses transcription by functionally and physically interacting with histone deacetylases. *Mol Cell Biol.* 21:2259-2268.