CHAPTER 4

# PML NUCLEAR BODIES AND OTHER TRIM-DEFINED SUBCELLULAR COMPARTMENTS

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**Abstract:** Tripartite motif (TRIM) proteins are defined by their possession of a RING, B-box and predicted coiled coil (RBCC) domain. The coiled-coil region facilitates the oligomerisation of TRIMs and contributes to the formation of high molecular weight complexes that show interesting subcellular compartmentalisations and structures. TRIM protein compartments include both nuclear and cytoplasmic filaments and aggregates (bodies), as well as diffuse subcellular distributions. TRIM19, otherwise known as promyelocytic leukaemia (PML) protein forms nuclear aggregates termed PML nuclear bodies (PML NBs), at which a number of functionally diverse proteins transiently or covalently associate. PML NBs are therefore implicated in a wide variety of cellular functions such as transcriptional regulation, viral response, apoptosis and nuclear protein storage.

## INTRODUCTION

The family of tripartite motif (TRIM) containing proteins are defined by a conserved motif that contains a RING (really interesting new gene) domain, one or two B-box domains and a predicted coiled coil region.[1] TRIM proteins often show interesting subcellular compartmentalisations and structures, which is in part due to the oligomerization abilities of the TRIM motif facilitating both homo- and hetero-oligomerization of TRIM proteins. Whilst some of the TRIM component domains are not always present, their linear sequence order is always preserved (RING domain—B1—B2—coiled coil) and the motif is mostly evolutionarily conserved.[2] Therefore the various sub-domains of the tripartite motif likely

*TRIM/RBCC Proteins*, edited by Germana Meroni.

**Table 1.** Summary of known cellular localizations of TRIM proteins. Most of the cellular localizations are for exogenously expressed protein. Examples of interactions are taken from the Human Protein Reference Database (HPRD)

| Name | Aliases | Domains | Cellular Localization |
|---|---|---|---|
| TRIM1 | FXY2, MID2, RNF60 |  | Filaments (C) |
| TRIM2 | RNF86 |  | Filaments (C) |
| TRIM3 | BERP, RNF22, RNF97, HAC1 |  | Diffuse (C) Speckles (C) Filaments (C) |
| TRIM4 | RNF87 |  | Speckles (C) |
| TRIM5 | RNF88 |  | Speckles (C) |
| TRIM6 | RNF89 |  | Speckles (C) Tracks (N) |
| TRIM7 | GNIP, RNF90 |  | Diffuse (C and N) |
| TRIM8 | GERP, RNF27 |  | Speckles (N) |
| TRIM9 | RNF91 |  | Speckles (C) |
| TRIM10 | RFB30, RNF9, HERF1 |  | Aggregates (C) Speckles (C) |
| TRIM11 | RNF92 |  | Diffuse (C and N) |
| TRIM13 | RNF77, RFP2, LEU5 |  | Speckles (C) |
| TRIM14 | - |  | Speckles (C) |
| TRIM16 | EBBP |  | Diffuse (C) |
| TRIM17 | RBCC, RNF16, TERF |  | Speckles (C) |
| TRIM18 | FXY, MID1, RNF59, XPRF |  | Filaments (C) |
| TRIM19 | PML, MYL, RNF71 |  | Bodies (N) |
| TRIM20 | PYRIN, MEFV |  | Diffuse (C) |

*continued on next page*

function in an integrated manner rather than as separate domains that happen to be adjacent to each other.[2] In terms of biochemical activity, many TRIM proteins that contain the RING domain also have ubiquitin (Ub) E3 ligase activity[3] (which is discussed in Chapter 3 by Ikeda and Inoue). A summary of TRIM proteins with known cellular localizations (mostly of exogenously expressed protein) can be found in Table 1, together with their aliases.

One of the best-characterised TRIM proteins is TRIM19, otherwise known as promyelocytic leukaemia (PML) protein. PML is necessary for the formation of nuclear protein aggregates termed PML nuclear bodies (PML NBs). A number of functionally distinct proteins associate either transiently or covalently with PML NBs and thus PML is implicated in a variety of cellular functions. In this chapter, we will review current knowledge on the tripartite motif with a specific focus on PML NBs.

**Table 1.** Continued

| Name | Aliases | Domains | Cellular Localization |
|---|---|---|---|
| TRIM21 | RNF21, RO52, SSA1 |  | Speckles (C and N) |
| TRIM22 | RNF94, STAF50 |  | Diffuse, Speckles (C) |
| TRIM23 | ARD1, ARFD1, RNF46 |  | Speckles (C and N) |
| TRIM24 | RNF82, TIF1, TIF1A |  | Speckles (C), Bodies (N) |
| TRIM25 | EFP, RNF147, ZNF147 |  | Diffuse and aggregates (C) |
| TRIM26 | AFP, RNF95, ZNF173 |  | Diffuse and aggregates (C) |
| TRIM27 | RFP, RNF76 |  | Speckles (C), Bodies (N) |
| TRIM28 | KAP1, RNF96, TIF1B |  | Chromatin domains |
| TRIM29 | ATDC |  | Filaments (C) |
| TRIM31 | C6orf13, RNF |  | Diffuse (C and N) |
| TRIM32 | HT2A |  | Diffuse and speckled (C and N) |
| TRIM33 | PTC7, RFG7, TIF1G |  | Diffuse and speckled (N) |
| TRIM35 | HLS5 |  | Speckled (C and N) |
| TRIM36 | RBCC728, RNF98, HAPRIN |  | Filaments (C) |
| TRIM37 | MUL, POB1, TEF3 |  | Peroxisome |
| TRIM41 | RINCK |  | Speckles (C and N) |
| TRIM45 | RNF99 |  | Diffuse (C and N) |
| TRIM46 | TRIFIC, GENEY |  | TRIM46 |
| TRIM47 | GOA, RNF100 |  | TRIM47 |
| TRIM54 | MURF, MURF3, RNF30 |  | TRIM54 |
| TRIM55 | MURF2, RNF29 |  | TRIM55 |
| TRIM63 | IRF, MURF1, RNF28, SMRZ |  | TRIM63 |
| TRIM69 | HSD34, RNF36, Trif, Trimless |  | TRIM69 |

Localisation:
N = Nuclear C = Cytoplasmic

Domains:
RING, B-Box 1, B-box 2, Coiled coil, COS box, NHL, ARF, WD40, Fibronectin Type-III, B30.2/SPRY, PHD, Filamin, BROMO, MATH

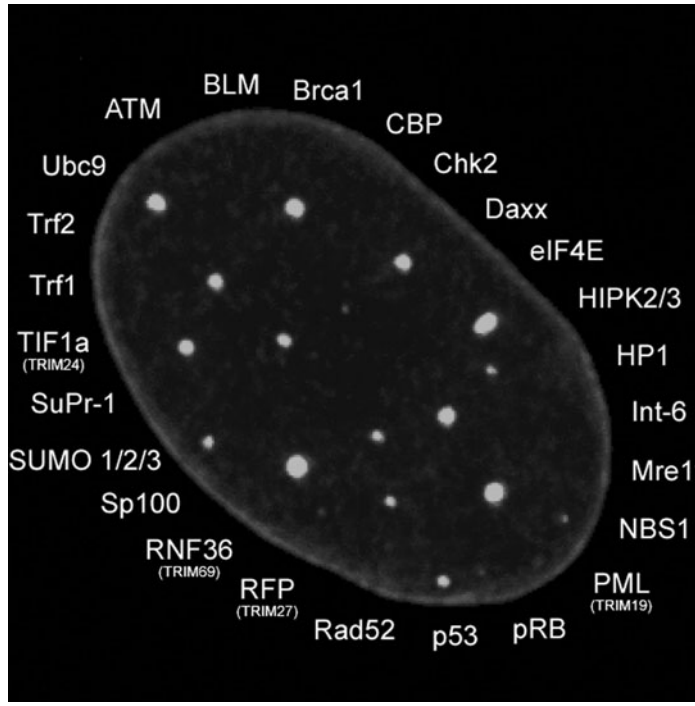


**Figure 1**. Proteins that show associations with PML nuclear bodies. The major constituent proteins of PML NBs are PML and Sp100. All the other proteins show transient localization to the bodies, or are recruited under certain conditions.

## PML NUCLEAR BODIES AND ASSOCIATED PROTEINS

Also known as ND10, PODs and Kremer bodies, PML NBs are spherical bodies typically found within the mammalian cell nucleus. They range from 0.3 to 1.0 μm in diameter and usually number between 10 and 30 per cell, but this varies according to cell line and cell cycle phase.[4] PML and SUMO- (small ubiquitin-like modifier) modified PML are the nucleating components of PML NBs.[5,6] The main constituent protein of the bodies is PML, which is thought to provide a scaffold around which the other protein components accumulate.[6] The aggregation of sumoylated PML protein into PML NBs is thought to prompt the recruitment of other associated proteins to the scaffold. The other known constituent PML NB protein is Sp100.[7] Transiently associated proteins, or proteins recruited to PML NBs under certain conditions include CBP,[8] BLM,[5] Daxx (a transcriptional repressor),[5,9] p53 (an activator of transcription), pRB[10] and SUMO.[11] Since these proteins function in diverse cellular pathways it has been proposed that PML NBs act as storage depots where localised protein concentrations can be regulated.[12] Figure 1 lists some of the known PML NB-associated proteins.

The specific function of PML NBs is unclear, although given the diversity of proteins that are associated with PML, functional roles in tumour suppression,[13] apoptosis,[14] DNA replication and repair,[15] response to viral infection,[16] nuclear protein storage[12] and gene regulation and transcription[17] have been suggested.

## THE TRIPARTITE MOTIF IN PML

The tripartite motif in PML plays an integral role in the formation of PML NBs, PML-homo and hetero interactions as well as PML growth suppressor,[13,18] apoptotic[14,19] and anti-viral activities.[20,21] The influences of each component domain have been investigated thoroughly.

### RING Domain

The RING finger motif is a cysteine-rich zinc-binding module and was first discovered in the human protein RING1.[22] It is found in many cellular and viral proteins and mediates E3 ubiquitin ligase activity.[3,23] Mutations of the conserved zinc-binding cysteines in the RING finger motif of PML disrupt PML NB formation in vivo and results in a nuclear diffuse localization of PML.[24] These mutations correlate with loss of PML growth suppression,[25,26] apoptotic and anti-viral activities.[27,28] Furthermore, mutagenesis studies of surface residues that form part of the PML RING domain resulted in very large nuclear aggregates of PML protein in vivo.[11] The requirement of an intact RING domain for PML NB formation may relate to specific protein-protein interactions mediated by the RING and thereby playing an essential role in supramolecular assembly.[29] There are a number of examples of heterodimer RING-RING interactions (Brca1/Bard), although these are usually associated with the formation of complexes that mediate E3 Ub ligase activity. Although speculative, one cannot discount an indirect role for the PML RING domain in mediating E3 SUMO or Ub ligase activity.

### B Boxes in PML

B boxes are also cysteine/histidine rich protein sequence motifs.[30] The B boxes in PML (B1 and B2) are small (42 and 46 residues respectively) and have been shown to bind zinc.[31] Substitution of conserved zinc ligands in B1 or B2 in PML disrupts PML body formation in vivo but does not prevent multimerization.[31] Together with the RING finger the B-boxes, B1 and B2, exert PML growth suppressor activity.[32] Interestingly B1 SUMO modification has not only been shown to be critical for recruitment of the 11S proteasomal subunit to PML NBs[33] but also necessary for recruitment of Daxx to PML NBs.[34] A recent structure of the human MID1 B-box 1 domain shows that the B-box has a fold similar to the RING domain providing another potential interface for mediating E2 interactions and conferring either E3 or E4 ligase activities.[35]

### Coiled-Coil Domain of PML

PML possesses a weakly predicted $\alpha$-helical coiled-coil domain, which is essential for PML homodimerization and heterodimerization with PML-RAR$\alpha$ without the need for the RING and B-box domains.[26,36-38] The coiled-coil domain is also essential for PML NB formation and full growth suppressor activity in vivo.[32] Removal of the coiled-coil prevents formation of high molecular weight complexes. Recently it has been shown that SIAH-1/2 binds and targets the coiled-coil domain in PML for proteasome-mediated degradation, leading to a loss of PML NBs.[39] These results involve the coiled-coil region in PML as a novel structural determinant for targeted degradation.

## THE FORMATION AND DISSOLUTION OF PML NUCLEAR BODIES

PML protein is found in the nucleoplasm in a soluble fraction that is not modified by SUMO-1.[40] PML NB genesis is thought to be a two-stage process, beginning with the formation of primary aggregates of nonsumoylated PML protein[33] and driven by homodimerization via its coiled-coil domain, part of the tripartite motif.[41] Formation of the mature bodies is mediated by the covalent modification of PML protein by SUMO-1,[33] polymeric SUMO-2[42] and SUMO-3[43] at up to three lysine residues (amino acid positions 65, 160 and 490;[44,45] The structure of the primary and mature bodies differs—mature bodies visualised using electron microscopy possess a shell of PML protein that surrounds other PML NB-associated proteins.[33,46] A PML mutant unable to be SUMO-modified forms aberrant nuclear aggregates and fails to recruit proteins typically associated with PML NBs, such as Daxx and Sp100.[6] Recently the nuclear localization of PML has been shown to be regulated by SUMO-3,[43] one of the least abundant human SUMO isoforms.[47]

PML protein also possesses a SUMO binding motif, known as a SUMO interacting motif or SIM, which is independent of its sumoylation sites and is required for PML NB formation.[48] SUMO is noncovalently bound to PML at the SIM motif and it is proposed that SIM's can confer the ability to aggregate SUMO labelled proteins as well as promote sumoylation of assembled complexes.[49] The proposed model of PML NB formation predicts that PML protein is aggregated via interactions between RBCC motifs. The PML RING domain is in turn required for efficient PML sumoylation.[45] Upon sumoylation in interphase cells, noncovalent binding of PML to SUMO-modified PML through the SIM is the nucleation event for the later recruitment of other sumoylated proteins or proteins containing SIMs to PML NBs.[48] The regulation of PML sumoylation including SUMO polymer chain length is not yet clear, although recent studies now suggest the existence of specific SUMO-targeted E3 ubiquitin ligases linking sumoylation to ubiquitin-mediated degradation.[50] Among these proteins are the RNF4 family of RING proteins that contain SIMs.[51] It will be interesting to see how these newly defined proteins might regulate sumoylated-PML and by inference PML NB formation.

## PML NUCLEAR BODY DYNAMICS—NUMBER, MOVEMENT AND MORPHOLOGY

Levels of endogenous PML expression are variable within a cell-line population,[52] as determined by studying labelling intensity and number of PML NBs and the presence of a nonspeckled diffuse nuclear staining. In HeLa and Hs27 cells, late G1 cells show strong labelling in around 15 bodies. In S phase cells there are many smaller dots in addition to nuclear diffuse staining and in G2 PML NB number decreases until two to three are present in mitotic cells. PML NBs reappear between mitosis and late G1, with cytoplasmic bodies found in addition. Thus it appears that PML nuclear bodies show significant variation within the cell cycle.[52]

PML NB number is also a reflection of cell cycle status, especially at S phase when the number of bodies increases twofold.[4] In general, PML NBs are positionally and structurally stable over extended periods of interphase, with the exception of some smaller bodies that show limited localized or ATP-dependent rapid movement.[53] Stress may also induce the fission of PML microstructures, which also show fast movement, from parental NBs.[54] Also, as cells enter S phase PML NB structural stability is lost which

is exemplified by distortions in shape and by fission and fusion events. The association of fission products with chromatin implies that PML NBs are responding to changes in chromatin organization and topology. A redistribution of PML protein and not de novo synthesis was found to be responsible for the increase in PML NB number with the highest PML protein levels found in G1, dropping by 10 and 30% respectively in S phase and G2.[4]

Perhaps not unexpected, PML NB components like Sp100 and SUMO-1 also undergo changes in a cell cycle-dependent manner. Both are conjugated to SUMO-1 during interphase, but are deconjugated during mitosis.[55] Mitotic PML is stabilised by phosphatase inhibitors; treatment of interphase cells with such inhibitors induces production of PML similar to the mitotic species. Therefore phosphorylation is also important in the differential modification of PML during the cell cycle.[55] Although showing tight colocalization throughout interphase, PML and Sp100 become separated in early prophase of mitosis.

At mitosis PML protein partitions via mitotic accumulations of PML protein (MAPPs), which differ in dynamics, biochemistry and structure to PML NBs.[56] Unlike PML NBs they do not contain Sp100, SUMO-1 or Daxx, their loss occurring prior and concurrent to chromatin condensation and nuclear membrane breakdown respectively.[56] MAPPs contribute to the re-establishment of PML NBs in G1 where some MAPPs remain in association with the mitotic chromosomes, potentially forming nucleation sites for PML NB formation at G1.[56]

## Other Body Morphologies

Up until this point we have discussed typical PML NBs, however, there are some other observed morphologies of PML NBs which are found under conditions of a particular cell cycle phase (giant PML NBs) or senescence (senescence associated nuclear bodies or SANB). There are also cytoplasmic PML bodies, which play a different role in the cell to their nuclear counterparts.

## Giant PML Nuclear Bodies

PML protein may also be involved in heterochromatin remodelling at G2.[57] In immunodeficiency, centromeric instability and facial dysmorphy (ICF) syndrome patients heterochromatin HP1 proteins (HP1$\alpha$, $\beta$ and $\gamma$) accumulate in a giant body (only present in G2) over the 1qh and 16qh juxtacentromeric heterochromatins. PML protein is also present within this body and as such it is proposed to be a giant PML nuclear body. It contains a core of satellite DNA, although controversially, several studies have suggested that PML NBs do not typically contain DNA. By analogy to normal PML NBs , G2 PML NBs may contain PML and Sp100 organized in ordered concentric spherical layers around satellite DNA. These large PML containing structures would function in the re-establishment of a condensed heterochromatic state on late-replicated satellite DNA through an unknown mechanism.[57]

## Senescence-Associated Nuclear Bodies (SANB)

Spontaneous or oncogene retrieval-induced senescence is associated with formation of large PML NBs (SANB) that also contain nucleolar components.[58] SANB appear to associate with the periphery of the nucleolus and perhaps play a role in the ubiquitin-proteasome pathway.

### Cytoplasmic PML Bodies

Cytoplasmic PML bodies have been observed by indirect immunofluorescence in a number of cell lines.[41,59-61] As all PML isoforms comprise a nuclear localisation signal in exon 6,[41] these bodies could represent PML protein that has become trapped outside of the reformed nuclear membrane and lamina. Alternatively, the cytoplasmic bodies could comprise of the PML I isoform since it contains a nuclear export sequence (NES),[62] encoded within exon 9 of the *PML* gene.[63] (The isoforms of PML are discussed further in the following section). The ability of PML to become SUMO-modified and induce poly-PML aggregates leading to PML bodies could be utilised in important cytoplasmic functions. Indeed, it has recently been shown that cytoplasmic PML and PML-I bodies play a key role in the TGF-ß signalling which links cell regulation to a nonnuclear form of PML.[61]

## ISOFORMS OF PML PROTEIN

TRIM family proteins often comprise a number of isoforms and PML protein is the good example of this. The *PML* genomic locus is found on chromosome 15, extends over 35 Kb and consists of nine exons, from which a range of transcripts are produced via alternative splicing. This leads to the production of a number of PML proteins ranging between forty-eight to ninety-seven kDa. There are seven isoforms of PML, PML I to VII, which all share the N-terminal tripartite motif (found within exons one to three), but show C-terminal sequence differences.[2,41] The different isoforms show different cellular localisations; either nuclear, or both nuclear and cytoplasmic.

Since TRIM proteins share a common N-terminal motif, C-terminal differences (such as the presence of other motifs including the BROMO, PHD and SPRY domains) may explain other diverse functionalities. These differences may also result from alternative splicing, such as in the case of PML protein where different PML isoforms have the potential to facilitate isoform-specific cellular activities. The existence of several PML isoforms may also explain the high number of cellular and viral proteins that interact with PML protein.[21] This could also account for the diversity of functionality accredited to PML within the cell.

The expression of specific PML isoforms is not limited to particular primary cells or cell lines.[63] PML III, IV and V were found to be minor isoforms when compared to PML I and II, indicating differential isoform-dependent expression levels. Interestingly stable expression of each of the isoforms (I-V) in a *pml*-null background produces distinct subcellular localizations. PML I has both a nuclear and cytoplasmic distribution (unsurprising since it contains an NES). PML II has a thread-like distribution, PML IV has a large number of small irregular shaped bodies throughout the nucleus and PML V produces large, dense bodies. As in other TRIM proteins, the PML isoforms' C-terminal domains may therefore be involved in interactions with specific cellular compartments.[63] However, co-expression of several isoforms leads to their colocalization, highlighting the ability of TRIM proteins to heterodimerize. Below are examples of proposed isoform-specific functions.

Upon different types of stress (UV-C or γ-irradiation, chemical inhibition of transcription or DNA synthesis and proteasome inhibition) endogenous PML protein forms nucleolar caps that eventually surround nucleolar components.[58] It was observed

that the PML I isoform contains a nucleolar targeting domain within the evolutionarily conserved C-terminus, which contains a predicted exonuclease III fold, both of which are essential for targeting PML-1 to nucleolar fibrillar centres. The function of PML-I at the nucleolus is unclear but the isoform specific localisation implies that PML isoforms can have distinct biochemical functions.

Another PML isoform, PML III, has been demonstrated to localize to the centrosome and the pole of the mitotic spindle through the use of isoform-specific antibodies.[64] PML III is implicated in the control of centrosome duplication as its specific knock-down leads to amplification of centrosomes, (also seen in a significant number of PML -/- MEFs). PML III may regulate this by repression of Aurora A kinase activity, which ultimately leads to the inhibition of Cdk2/cyclin E in its role of regulating centrosome duplication. However this could not be confirmed by others.[63]

It is established that PML protein and NBs play an important role in the regulation of multiple apoptotic pathways.[65,66] To further understand the role of PML, the PML IV isoform was found to specifically bind to the tumour suppressor p53 and regulate its activity.[67] Interestingly, PML IV was shown to recruit p53 to PML NBs implicating PML it in a p53-dependent apoptotic pathway.[68]

## PML NUCLEAR BODIES AND DISEASE

PML protein has shown to be involved in both oncogenesis and response to viral infection. The involvement of TRIM proteins in cancer is explored in Chapter 6 by Cambiaghi et al..

### PML Nuclear Bodies and Acute Promyelocytic Leukaemia

PML protein was first identified as part of the reciprocal chromosomal translocation at t(15;17) q(22;21) found in acute promyelocytic leukaemia (APL). The resulting fusion protein comprises PML fused to retinoic acid receptor $\alpha$ (RAR$\alpha$).[69-72] Interestingly, normal PML protein is unable to form PML NBs, instead showing a microspeckled pattern as a result of the heterodimerization of PML with the PML-RAR$\alpha$ fusion protein.[73-75] APL is characterised by a block in the differentiation of promyelocytes which can be unblocked by treatment with all-trans retinoic acid (ATRA). The restoration of a normal PML NB pattern[76] correlates with the release of the differentiation block in promyelocytes.[77] Patients with APL are often able to go into remission after differentiation therapy.

### Influence of the Coiled-Coil in PML Localization in APL

The PML-RAR$\alpha$ fusion protein has reduced intranuclear mobility and shows mislocalisation when compared with RAR$\alpha$.[78] Using Fluorescence Recovery After Photobleaching (FRAP), PML-RAR$\alpha$ sumoylation site mutants were shown to have the same intracellular localization and reduced mobility as wild type PML-RAR$\alpha$, indicating that sumoylation of PML-RAR$\alpha$ (at each of three different sites) does not contribute to the altered distribution and reduced mobility of PML-RAR$\alpha$. Instead a coiled-coil deletion mutant PML-RAR$\alpha$ indicated that the coiled-coil domain of PML-RAR$\alpha$ is responsible for its immobilization and thus mislocalization within the nucleus.[79]

### PML and the Antiviral Response

Some TRIMs display antiviral properties, such as TRIM1, TRIM5α, TRIM19 and TRIM22,[21] with evidence also for influence over viral replication. TRIMs may act in both the cytoplasm and nucleus, at multiple viral life cycle stages and target various viral proteins.[21] PML protein (with its various antiviral activities) and TRIM5α (possessing Lv1 and Ref1 antiretroviral activities) are the best-characterized TRIM proteins in terms of their involvement in viral infection responses.[20,21]

In early DNA viral infections, PML NBs become associated with the parental genomes and early replication compartments.[80-82] Type I IFNs are known to induce an anti-viral state. Interestingly, interferon induction also induces the expression of PML, producing an increase in the size and number of PML NBs.[83,84] Over-expression of PML also provides resistance to vesicular stomatitis virus and influenza A virus.[85] Infection with adenovirus causes a redistribution of PML nuclear bodies into thread-like structures associated with viral replication centres. The adenovirus Type 5 (Ad5) early region 4 open reading frame (E4 ORF3) product is responsible for this reorganization, colocalizing with the PML threads.[86,87] E4 ORF3 was also found to specifically interact with PML II.[88]

The RBCC containing protein TRIM5α, containing the C-terminal SPRY domain, was found to block HIV-1 infection prior to reverse transcription in the cells of Old World monkeys.[89] Interestingly, TRIM5α also localises to cytoplasmic speckles[21,89,90] which are highly mobile showing a dynamic morphology[91] indicating a functional role for TRIM5α cytoplasmic bodies.

## NUCLEAR COMPARTMENTS SHARING ASSOCIATIONS WITH PML NUCLEAR BODIES

PML nuclear bodies have been shown to specifically associate with a variety of other nuclear compartments. In some cases it is unclear whether these associations are of functional importance or occur through stochastic processes. In the following section, these associations are discussed in the context of PML NB function.

### Transcriptional Compartments

The DNA damage response in cells results in PML sequestration of Mdm2, a p53 ubiquitin-ligase, to the nucleolus thereby potentiating p53 stability.[92] There is little evidence to suggest that PML NBs show association with the nucleoli of normal primary cell lines that have not undergone stress or are not tumour lines. Recently a novel PML compartment in association with nucleolar structures in human mesenchymal stem cells has been observed in growth permitting conditions[93] indicating that PML may be involved in nucleolar functions of normal nontransformed cells, as this compartment is not present in rapidly-growing tumour-derived cells. However, it does lack the morphology of normal PML NBs.

A cell cycle-dependent association of PML NBs with RNA polymerase II active transcription sites has been demonstrated.[94] In this study, 30% of PML NBs in unsynchronized cells associated with active transcription sites containing $RNAPII_O$ (RNA

polymerase II with a hyperphosphorylated CTD, which forms part of the elongation complex). This increased to 70% of PML NBs in G1 cells and 80% upon exposure to IFNγ suggesting a correlation between alteration of PML NB spatial associations with increased transcriptional activity. However the specific functional role of PML NBs in transcription is still unclear.

PML and CREB binding protein (CBP), a transcriptional coactivator and histone acetyl transferase, are found to colocalize at the PML NB.[8,46] PML, CBP and RNA polymerase II have also been found together at a subset of PML NBs, supporting the idea that transcription takes place at PML NBs. This alludes to a possible function for PML in the regulation of transcription.

## Chromatin Associations

There is some evidence for a cell-cycle dependent PML NB-chromatin association, in particular the observed dynamic link between PML NBs and centromeres in G2 that is stabilised upon proteasome-mediated proteolysis inhibition.[95] An observation that Vmw100 (Herpes simplex virus Type 1 regulatory protein) induces proteasome-dependent degradation of both principal PML NB component proteins PML and Sp100 and CENP-C (a centromeric protein) indicated a connection between PML NBs and centromeres in uninfected cells. Proteasome inhibitor treatment revealed an association between the two compartments in a significant number of G2 Hep2 cells and subsequent reassessment of unsynchronised cells revealed rare cells in late G2 that had considerable PML NB-centromere associations.[95]

A subset of PML NBs, known as alternative lengthening of telomeres (ALT) associated PML NBs (APBs), are found to colocalize with telomeres in certain immortalized cell lines. Such cells seek to perpetuate or surpass existing telomeric length but are telomerase (hTERT) negative and thus employ a different method involving homologous recombination.[96,97] APBs contain telomeric DNA and telomere-specific binding proteins TRF1 and TRF2, plus other DNA recombination and replication proteins suggesting a role of PML NBs in regulating telomere length.[98]

The first study of the 3D spatial organization of PML NBs and gene-rich and gene-poor chromosomal regions revealed a highly nonrandom association of the gene-rich major histocompatibility complex (MHC) on chromosome 6 with PML NBs.[99] Interestingly, this association remained when a subsection of this chromosomal region is integrated into another chromosome in a mutant cell line.[99] An extension of this study revealed that the distance between a genomic locus and its nearest PML NB correlates with the transcriptional activity and gene density around that specific locus.[100] Locus-PML NB mean minimum distances for four different loci (6p24, Histone cluster 6p22, TAP/LMP and the centromere of chromosome six were compared between S phase and G1/G0 cells and it was found that only the histone-encoding cluster (transcribed in S phase alone) was more strongly associated with PML NBs in S phase than in G1/G0. These studies provided the first rigorous and statistical meaningful study of PML NB associations with specific genomic regions establishing a direct link between PML NB association with gene-rich and transcriptional active regions. They also alluded to the possibility that PML NBs associations are directly linked to the functionality of the specific gene region such that the insertion of a gene-rich and active region into a different chromosome maintained the PML NB association.

## TRIM PROTEINS THAT LOCALISE TO PML NUCLEAR BODIES

Other members of the TRIM protein family are known to interact with PML protein and thus localise to PML NBs. These are TIF1α (also known as TRIM24), RFP (TRIM27 or RET finger protein) and RNF36 (TRIM69 or Trif). Interestingly like PML, both TIF1 and RFP become oncogenic as the result of chromosomal translocations producing aberrant fusion proteins that contain RFP and TIF1.[101-103]

### TIF1α (TRIM24)

*TIF1α* is fused to *Braf*, resulting in the TIF1α-B-Raf (T18) oncoprotein in mouse hepatocellular carcinomas.[103] PML, TIF1α and RXRα/RARα have been shown to function together in a transcription complex dependent on retinoic acid (RA).[104] PML acts as the ligand-dependent coactivator of RXRα/RARα and interacts with TIF1α and CBP which localise to PML NBs.[104] In *Pml*$^{-/-}$ cells the ability of TIF1α and CBP to act as transcriptional coactivators on RA is impaired. T18 oncoprotein disrupts the RA-dependent activity of the complex in a dominant-negative manner resulting in a growth advantage.[104] TIF1α also fuses to the RET receptor tyrosine kinase in human childhood papillary thyroid carcinomas.[102] Like PML, it was shown that TIF1α is SUMO-1 modified[105] by two SUMO-1 moieties per TIF1α. By analogy to PML and Sp100, TIF1α is also chromatin associated and was shown to interact with HP1 similar to Sp100.[106] Interestingly, PML protein has been implicated as a cofactor in TIF1α-dependent enhancement of RARα-mediated transcriptional activation.[104] Like other TRIM proteins, TIF1α is able to heteromultimerize via the TRIM motifs and can bind TIF1γ.[107]

As mentioned previously, the adenovirus Type 5 E4 ORF3 product is able to cause the reorganization of PML bodies in to tracks within the nucleus.[86,87] Recently, TIF1α has also been identified as a novel E4 ORF3-interacting partner and is also found reorganized into PML-track structures upon E4 ORF3 expression.[108] E4 ORF3 binds TIF1α in vivo and directs this PML NB reorganization via the TRIM domain.

### RFP (TRIM27 or Ret Finger Protein)

Like other TRIM proteins, the subcellular localization of RFP can be nuclear, as detected by a monoclonal antibody (RFP-1) generated against amino acids 148 to 163.[109] In HL-60 cells fractionated into nuclear and cytoplasmic components, the protein reactive with RFP-1 is detected only in the former. Over 90% of nuclei of human spermatogenic cells (but not mature spermatozoon or human testicular tumour cells) were positive for RFP and so were up to 60% of cells in other human adult tissues, which suggests a functional role for RFP in spermatogenesis. In contrast to these cell-specific studies, it has been observed that RFP shows differential nuclear and cytoplasmic distributions in different cell types.[110] RFP is expressed in the nuclei of cells ranging from peripheral and central neurones and hepatocytes to adrenal chromaffin cells and also at high levels in male germ line cells (such as round spermatids, also forming a perinuclear cap in primary spermatocytes). However in some plasma cells, solitary plasmacytoma and multiple myeloma it shows high cytoplasmic expression levels. This cell-specific differential RFP distribution must relate to the different functions of RFP perhaps in the regulation of growth or differentiation of different cell types.

Like PML, RFP, (also known as *Ret* finger protein), is sumoylated and localizes to PML NBs. RFP functions as a transcriptional repressor by interacting with Enhancer of Polycomb 1 (EPC1), (a Polycomb group member that plays a role in heterochromatin formation).[111,112] In this context, RFP shows either a cytoplasmic or nuclear localization depending upon cell type.[113] Within its coiled-coil domain is a NES and in NIH3T3 cells this results in a cytoplasmic localization for RFP, but conversely in HepG2 cells the NES is masked. Treatment with 12-*O*-tetradecanoylphorbol-13-acetate or overexpression of constituently active protein kinase Cα (PKCα) removes this masking and RFP is able to relocate to the cytoplasm. NIH3T3 cells treated with PKC inhibitors show blocked NES function, leading to nuclear localization of RFP. Therefore RFP export appears to be positively regulated by PKC activation, however it is not a direct substrate.[113]

RFP, SUMO-1 and PIASy (protein inhibitor of activated STAT y, an E3 SUMO ligase[114]) were found to localize in a characteristic nuclear structure juxtaposed with the inner nuclear membrane (XY body) of primary spermatocytes in mouse testis.[112]

As previously described, TRIM proteins can homodimerize through their coiled-coil domains.[41] For RFP this interaction requires an intact B box (despite it not being an interacting interface itself), but not the RING finger.[115] The coiled-coil domain of RFP is required for its heterodimerization with PML.[116] Interestingly, RING finger and B box mutations affect RFP subcellular localisation suggesting that homo-oligomerization of RFP and its specific subcellular localisation depends upon an intact and complete TRIM motif.[115] In this context, ectopic expression of RFP in HEK293 cells causes extensive apoptosis with an intact tripartite motif required for this pro-apoptotic function. Interestingly, sole expression of the tripartite motif (without the C-terminal RFP domain) actually results in a further increase in pro-apoptotic activity.[117] Since the RING domain is involved this could suggest that many of the TRIM family proteins play a role in the control of cell survival.

In primary lymphocytes Int-6 (a subunit of the eukaryotic translation initiation factor eIF3), RFP and certain PML bodies colocalise, with RFP shown to trigger translocation of Int-6 into PML NBs in HeLa cells.[118] The interaction of RFP with Int-6 is mediated via its C-terminal Rfp (B30.2) domain (unlike the interaction between RFP and PML, which is coiled-coil dependent).

## RNF36 (TRIM69 or Trif)

The gene for Trif (testis-specific ring finger) was isolated from a mouse testis cDNA library[119] and the protein contains the tripartite motif and C-terminal B30.2/SPRY domain. Later renamed RNF36, the protein is expressed in germ cells at round spermatid stages during spermatogenesis.[120] Expression of full-length GFP-RNF36 in HEK293 cells produces a speckled nuclear localization. However, truncated RNF36 (containing only the RING and B-box domains and retaining a putative NLS) localizes to the nucleus but not in a speckled pattern.[120] From immunofluorescence and coimmunoprecipitation studies full-length RNF36 and PML protein were found to colocalize and interact. Since p38 (a mitogen-activated protein kinase) inhibition leads to its cytoplasmic translocation, RNF36's nuclear localization may be phosphorylation-dependent. Interestingly, over-expression of full-length RNF36 induced about 50% cell death in transfected cells as a result of apoptosis.[120] Given PML's involvement in the regulation of apoptotic pathways and its interaction with RNF36, RNF36's induction of apoptosis may be via a PML-dependent (but possibly p53-independent) pathway.

## OTHER TRIM-DEFINED SUBCELLULAR COMPARTMENTS

The coiled-coil domain of the tripartite motif allows TRIM proteins to oligomerize into large subcellular structures, such as the PML nuclear bodies. Many TRIMs are found to localize to different types of cytoplasmic or nuclear thread-like, filamentous and speckle or body aggregates. These structures can represent endogenous localisation patterns but more commonly are a result of overexpression studies. The ability of TRIM proteins to oligomerize is an interesting biochemical property, that can infer an ability to partition between soluble and insoluble high molecular weight complexes as part of function. An extensive investigation of the subcellular localization of all TRIMs using GFP, found most to localize to discrete cytoplasmic or nuclear structures, occasionally associated with diffuse background staining.[2]

Table 1 lists the currently known cellular localizations of TRIM proteins. Cytoplasmic TRIMs include those that form filaments (TRIM1, 2, 18, 36, 46, 54, 55), speckles (TRIM4, 5, 9, 13, 14, 17) or are diffuse in the cytoplasm (TRIM16 and 20). Other TRIMs show a number of cytoplasmic localizations such as TRIM3 which has cytoplasmic speckles, filaments and a diffuse component, TRIM10 which forms cytoplasmic speckles and aggregates, TRIM22 with its cytoplasmic speckles and diffuse component and TRIMs 25 and 26 with their cytoplasmic aggregates and diffuse component. Nuclear TRIMs can be found in speckles (TRIM8) or nuclear bodies (TRIM19 and 69). TRIM33 localizes to nuclear speckles and shows a diffuse nuclear distribution.

TRIM proteins may also show any other combination of cytoplasmic and nuclear subcellular localizations. TRIM 21, 23, 35 and 41 all form both cytoplasmic and nuclear speckles, whilst TRIM6 and TRIMs 27 and 24 have cytoplasmic speckles, but differ by also localizing to nuclear tracks and nuclear bodies respectively. TRIM63 forms cytoplasmic filaments and nuclear speckles. TRIM7, 11, 31 and 45 all show both cytoplasmic and nuclear diffuse components.

The subcellular localization (and possibly functions) of TRIM proteins can often be correlated with particular domain architecture or C-terminal features. Examples of this include the C-terminal differences in PML isoforms that allow nuclear localization and export and TRIMs containing a PHD or BROMO domain (TRIMs 24, 28 and 33) which can associate with specific chromatin regions.[2,121] Another example of this is that of a subfamily of TRIMs (including TRIM1 (MID2), TRIM9, TRIM18 (MID1), TRIM36 and TRIM46 (TRIFIC)) in which all analyzed members associate with the microtubule cytoskeleton.[122] All subfamily members share an identical domain arrangement and contain a novel motif, the COS box, found adjacent to the coiled-coil. As expected most of the COS box-containing proteins in Table 1 show a cytoplasmic filamentous subcellular localization. Often the presence of the fibronectin Type III (fnIII) domain correlates with the presence of the COS box. 13% of the TRIMs described in Table 1 possess a COS box. Mutations in this motif abolish microtubule-binding ability and if incorporated into a nonmicrotubule-binding TRIM, the protein is redirected to the microtubules.[122] The majority of TRIM proteins (just over 60% of TRIM proteins described in Table 1) contain a SPRY/B30.2 domain. B30.2 is actually a SPRY domain plus an additional PRY domain and is found in TRIMs, having been evolutionarily selected and maintained, speculatively as part of the innate immune recognition of retroviruses.[123] This tallies with current thinking that many TRIM proteins possess antiviral functionality, such as in the example of TRIM5$\alpha$, which in fact does contain a SPRY/B30.2 domain.[21] However this domain is noticeably absent from PML.

As seen with PML protein in APL, delocalization from expected subcellular localizations is often an indicator of disease. A further example of this is TRIM37, which shows a particularly interesting subcellular localization and is mutated in the autosomal recessive genetic disorder Mulibrey (muscle, liver, brain and eye) nanism syndrome[124] to produce a nonfunctional truncated protein. TRIM37 is normally targeted to the peroxisome, however this localization is compromised upon transient expression of *TRIM37* containing the major mutation found in the Finnish population, becoming homogeneously distributed throughout the cytoplasm and the nucleus.[125] TRIM37 has also been shown to be an E3 ubiquitin ligase,[126] perhaps indicating that defective ubiquitin-dependent degradation plays a role in the pathogenesis of this disease.

## CONCLUSION

The tripartite motif is present in a large number of proteins. The RBCC domain of TRIMs is essential for their subcellular compartmentalization, as illustrated by the formation of PML NBs which are the best characterized of TRIM subcellular compartments. The coiled-coil domain of the tripartite motif confers an ability to oligomerize, thus mediating the aggregation of large multi-protein complexes and the partitioning of soluble and insoluble protein fractions. The ability for both homo- and hetero-oligomerisation gives rise to increased functional diversity amongst TRIMs. TRIM subcellular compartments may be found in both nuclear and cytoplasmic locations and are able to form both transient and stable complexes with other proteins in the cell, again potentiating functionality. In addition, the RING finger plays a role in the mediation of E3 ubiquitin ligase activity, suggesting that all TRIMs are potential E3 ubiquitin ligases.

Whilst the tripartite motif allows oligomerisation of TRIM proteins, it is the domain structure and composition of the rest of the protein that tends to influence the functions and subcellular locations of TRIM-defined compartments. This makes unambiguous definition of TRIM functionality difficult. There is redundancy in the C-terminal domain composition of TRIM proteins, the most common domain of which is SPRY/B30.2. Others of interest are the PHD and BROMO domains (involved in localization to chromatin) and the COS domain (often correlating with the presence of the fnIII domain and required for localization to microtubules). Interestingly a number of TRIM proteins including PML are associated with human disease, often resulting in their delocalization from their subcellular compartments.

Undoubtedly as more TRIM proteins are investigated in depth the link between subcellular localization and domain structure will become clearer. Within the field of PML, future research will certainly extend insight at both a biochemical and cellular level into the formation of PML nuclear bodies and the interactions between PML and its associated proteins.

## REFERENCES

1. Reddy BA, Etkin LD, Freemont PS. A novel zinc finger coiled-coil domain in a family of nuclear proteins. Trends Biochem Sci 1992; 17(9):344-345.
2. Reymond A, Meroni G, Fantozzi A et al. The tripartite motif family identifies cell compartments. EMBO J 2001; 20(9):2140-2151.
3. Meroni G, Diez-Roux G. TRIM/RBCC, a novel class of 'single protein RING finger' E3 ubiquitin ligases. Bioessays 2005; 27(11):1147-1157.

4. Dellaire G, Ching RW, Dehghani H et al. The number of PML nuclear bodies increases in early S phase by a fission mechanism. J Cell Sci 2006; 119(Pt 6):1026-1033.
5. Ishov AM, Sotnikov AG, Negorev D et al. PML is critical for ND10 formation and recruits the PML-interacting protein daxx to this nuclear structure when modified by SUMO-1. J Cell Biol 1999; 147(2):221-234.
6. Zhong S, Muller S, Ronchetti S et al. Role of SUMO-1-modified PML in nuclear body formation. Blood 2000; 95(9):2748-2752.
7. Szostecki C, Guldner HH, Netter HJ et al. Isolation and characterization of cDNA encoding a human nuclear antigen predominantly recognized by autoantibodies from patients with primary biliary cirrhosis. J Immunol 1990; 145(12):4338-4347.
8. Boisvert FM, Kruhlak MJ, Box AK et al. The transcription coactivator CBP is a dynamic component of the promyelocytic leukemia nuclear body. J Cell Biol 2001; 152(5):1099-1106.
9. Li H, Leo C, Zhu J et al. Sequestration and inhibition of Daxx-mediated transcriptional repression by PML. Mol Cell Biol 2000; 20(5):1784-1796.
10. Alcalay M, Tomassoni L, Colombo E et al. The promyelocytic leukemia gene product (PML) forms stable complexes with the retinoblastoma protein. Mol Cell Biol 1998; 18(2):1084-1093.
11. Boddy MN, Duprez E, Borden KL et al. Surface residue mutations of the PML RING finger domain alter the formation of nuclear matrix-associated PML bodies. J Cell Sci 1997; 110(Pt 18):2197-2205.
12. Negorev D, Maul GG. Cellular proteins localized at and interacting within ND10/PML nuclear bodies/ PODs suggest functions of a nuclear depot. Oncogene 2001; 20(49):7234-7242.
13. Salomoni P, Pandolfi PP. The role of PML in tumor suppression. Cell 2002; 108(2):165-170.
14. Takahashi Y, Lallemand-Breitenbach V, Zhu J et al. PML nuclear bodies and apoptosis. Oncogene 2004; 23(16):2819-2824.
15. Dellaire G, Bazett-Jones DP. PML nuclear bodies: dynamic sensors of DNA damage and cellular stress. Bioessays 2004; 26(9):963-977.
16. Fabunmi RP, Wigley WC, Thomas PJ et al. Interferon gamma regulates accumulation of the proteasome activator PA28 and immunoproteasomes at nuclear PML bodies. J Cell Sci 2001; 114(Pt 1):29-36.
17. Zhong S, Salomoni P, Pandolfi PP. The transcriptional role of PML and the nuclear body. Nat Cell Biol 2000; 2(5):E85-90.
18. Strudwick S, Borden KL. Finding a role for PML in APL pathogenesis: a critical assessment of potential PML activities. Leukemia 2002; 16(10):1906-1917.
19. Sanchez-Pulido L, Valencia A, Rojas AM. Are promyelocytic leukaemia protein nuclear bodies a scaffold for caspase-2 programmed cell death? Trends Biochem Sci 2007; 32(9):400-406.
20. Everett RD, Chelbi-Alix MK. PML and PML nuclear bodies: implications in antiviral defence. Biochimie 2007;89(6-7):819-830.
21. Nisole S, Stoye JP, Saib A. TRIM family proteins: retroviral restriction and antiviral defence. Nat Rev Microbiol 2005; 3(10):799-808.
22. Freemont PS. The RING finger. A novel protein sequence motif related to the zinc finger. Ann NY Acad Sci 1993; 684:174-192.
23. Joazeiro CA, Weissman AM. RING finger proteins: mediators of ubiquitin ligase activity. Cell 2000; 102(5):549-552.
24. Borden KL, Boddy MN, Lally J et al. The solution structure of the RING finger domain from the acute promyelocytic leukaemia proto-oncoprotein PML. EMBO J 1995; 14(7):1532-1541.
25. Mu ZM, Chin KV, Liu JH et al. PML, a growth suppressor disrupted in acute promyelocytic leukemia. Mol Cell Biol 1994; 14(10):6858-6867.
26. Le XF, Yang P, Chang KS. Analysis of the growth and transformation suppressor domains of promyelocytic leukemia gene, PML. J Biol Chem 1996; 271(1):130-135.
27. Borden KL, CampbellDwyer EJ, Salvato MS. The promyelocytic leukemia protein PML has a pro-apoptotic activity mediated through its RING domain. FEBS Lett 1997; 418(1-2):30-34.
28. Regad T, Chelbi-Alix MK. Role and fate of PML nuclear bodies in response to interferon and viral infections. Oncogene 2001; 20(49):7274-7286.
29. Kentsis A, Gordon RE, Borden KL. Control of biochemical reactions through supramolecular RING domain self-assembly. Proc Natl Acad Sci USA 2002; 99(24):15404-15409.
30. Borden KL. RING fingers and B-boxes: zinc-binding protein-protein interaction domains. Biochem Cell Biol 1998; 76(2-3):351-358.
31. Borden KL, Lally JM, Martin SR et al. In vivo and in vitro characterization of the B1 and B2 zinc-binding domains from the acute promyelocytic leukemia protooncoprotein PML. Proc Natl Acad Sci USA 1996; 93(4):1601-1606.
32. Fagioli M, Alcalay M, Tomassoni L et al. Cooperation between the RING + B1-B2 and coiled-coil domains of PML is necessary for its effects on cell survival. Oncogene 1998; 16(22):2905-2913.

33. Lallemand-Breitenbach V, Zhu J, Puvion F et al. Role of promyelocytic leukemia (PML) sumolation in nuclear body formation, 11S proteasome recruitment and As2O3-induced PML or PML/retinoic acid receptor alpha degradation. J Exp Med 2001; 193(12):1361-1371.
34. Zhu J, Zhou J, Peres L et al. A sumoylation site in PML/RARA is essential for leukemic transformation. Cancer Cell 2005; 7(2):143-153.
35. Massiah MA, Simmons BN, Short KM et al. Solution structure of the RBCC/TRIM B-box1 domain of human MID1: B-box with a RING. J Mol Biol 2006; 358(2):532-545.
36. Grignani F, Testa U, Rogaia D et al. Effects on differentiation by the promyelocytic leukemia PML/RARalpha protein depend on the fusion of the PML protein dimerization and RARalpha DNA binding domains. EMBO J 1996; 15(18):4949-4958.
37. Kastner P, Perez A, Lutz Y et al. Structure, localization and transcriptional properties of two classes of retinoic acid receptor alpha fusion proteins in acute promyelocytic leukemia (APL): structural similarities with a new family of oncoproteins. EMBO J 1992; 11(2):629-642.
38. Perez A, Kastner P, Sethi S et al. PMLRAR homodimers: distinct DNA binding properties and heteromeric interactions with RXR. EMBO J 1993; 12(8):3171-3182.
39. Fanelli M, Fantozzi A, De Luca P et al. The coiled-coil domain is the structural determinant for mammalian homologues of Drosophila Sina-mediated degradation of promyelocytic leukemia protein and other tripartite motif proteins by the proteasome. J Biol Chem 2004; 279(7):5374-5379.
40. Muller S, Matunis MJ, Dejean A. Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of PML within the nucleus. EMBO J 1998; 17(1):61-70.
41. Jensen K, Shiels C, Freemont PS. PML protein isoforms and the RBCC/TRIM motif. Oncogene 2001; 20(49):7223-7233.
42. Tatham MH, Jaffray E, Vaughan OA et al. Polymeric chains of SUMO-2 and SUMO-3 are conjugated to protein substrates by SAE1/SAE2 and Ubc9. J Biol Chem 2001; 276(38):35368-35374.
43. Fu C, Ahmed K, Ding H et al. Stabilization of PML nuclear localization by conjugation and oligomerization of SUMO-3. Oncogene 2005; 24(35):5401-5413.
44. Kamitani T, Kito K, Nguyen HP et al. Identification of three major sentrinization sites in PML. J Biol Chem 1998; 273(41):26675-26682.
45. Duprez E, Saurin AJ, Desterro JM et al. SUMO-1 modification of the acute promyelocytic leukaemia protein PML: implications for nuclear localisation. J Cell Sci 1999; 112(Pt 3):381-393.
46. LaMorte VJ, Dyck JA, Ochs RL et al. Localization of nascent RNA and CREB binding protein with the PML-containing nuclear body. Proc Natl Acad Sci USA 1998; 95(9):4991-4996.
47. Desterro JM, Rodriguez MS, Kemp GD et al. Identification of the enzyme required for activation of the small ubiquitin-like protein SUMO-1. J Biol Chem 1999; 274(15):10618-10624.
48. Shen TH, Lin HK, Scaglioni PP et al. The mechanisms of PML-nuclear body formation. Mol Cell 2006; 24(3):331-339.
49. Matunis MJ, Zhang XD, Ellis NA. SUMO: the glue that binds. Dev Cell 2006; 11(5):596-597.
50. Prudden J, Pebernard S, Raffa G et al. SUMO-targeted ubiquitin ligases in genome stability. EMBO J 2007; 26(18):4089-4101.
51. Sun H, Leverson JD, Hunter T. Conserved function of RNF4 family proteins in eukaryotes: targeting a ubiquitin ligase to SUMOylated proteins. EMBO J 2007; 26(18):4102-4112.
52. Koken MH, Linares-Cruz G, Quignon F et al. The PML growth-suppressor has an altered expression in human oncogenesis. Oncogene 1995; 10(7):1315-1324.
53. Muratani M, Gerlich D, Janicki SM et al. Metabolic-energy-dependent movement of PML bodies within the mammalian cell nucleus. Nat Cell Biol 2002; 4(2):106-110.
54. Eskiw CH, Dellaire G, Mymryk JS et al. Size, position and dynamic behavior of PML nuclear bodies following cell stress as a paradigm for supramolecular trafficking and assembly. J Cell Sci 2003; 116(Pt 21):4455-4466.
55. Everett RD, Lomonte P, Sternsdorf T et al. Cell cycle regulation of PML modification and ND10 composition. J Cell Sci 1999; 112(Pt 24):4581-4588.
56. Dellaire G, Eskiw CH, Dehghani H et al. Mitotic accumulations of PML protein contribute to the re-establishment of PML nuclear bodies in G1. J Cell Sci 2006; 119(Pt 6):1034-1042.
57. Luciani JJ, Depetris D, Usson Y et al. PML nuclear bodies are highly organised DNA-protein structures with a function in heterochromatin remodelling at the G2 phase. J Cell Sci 2006; 119(Pt 12):2518-2531.
58. Condemine W, Takahashi Y, Le Bras M et al. A nucleolar targeting signal in PML-I addresses PML to nucleolar caps in stressed or senescent cells. J Cell Sci 2007; 120(Pt 18):3219-3227.
59. Fagioli M, Alcalay M, Pandolfi PP et al. Alternative splicing of PML transcripts predicts coexpression of several carboxy-terminally different protein isoforms. Oncogene 1992; 7(6):1083-1091.
60. Lin HK, Bergmann S, Pandolfi PP. Cytoplasmic PML function in TGF-beta signalling. Nature 2004; 431(7005):205-211.

61. Salomoni P, Bellodi C. New insights into the cytoplasmic function of PML. Histol Histopathol 2007; 22(8):937-946.
62. Henderson BR, Eleftheriou A. A comparison of the activity, sequence specificity and CRM1-dependence of different nuclear export signals. Exp Cell Res 2000; 256(1):213-224.
63. Condemine W, Takahashi Y, Zhu J et al. Characterization of endogenous human promyelocytic leukemia isoforms. Cancer Res 2006; 66(12):6192-6198.
64. Xu ZX, Zou WX, Lin P et al. A role for PML3 in centrosome duplication and genome stability. Mol Cell 2005; 17(5):721-732.
65. Wang ZG, Ruggero D, Ronchetti S et al. PML is essential for multiple apoptotic pathways. Nat Genet 1998; 20(3):266-272.
66. Hofmann TG, Will H. Body language: the function of PML nuclear bodies in apoptosis regulation. Cell Death Differ 2003; 10(12):1290-1299.
67. Fogal V, Gostissa M, Sandy P et al. Regulation of p53 activity in nuclear bodies by a specific PML isoform. EMBO J 2000; 19(22):6185-6195.
68. Guo A, Salomoni P, Luo J et al. The function of PML in p53-dependent apoptosis. Nat Cell Biol 2000; 2(10):730-736.
69. de The H, Lavau C, Marchio A et al. The PML-RAR alpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. Cell 1991; 66(4):675-684.
70. Kakizuka A, Miller WH Jr, Umesono K et al. Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor, PML. Cell 1991; 66(4):663-674.
71. Pandolfi PP, Grignani F, Alcalay M et al. Structure and origin of the acute promyelocytic leukemia myl/RAR alpha cDNA and characterization of its retinoid-binding and transactivation properties. Oncogene 1991; 6(7):1285-1292.
72. Goddard AD, Borrow J, Freemont PS et al. Characterization of a zinc finger gene disrupted by the t(15;17) in acute promyelocytic leukemia. Science 1991; 254(5036):1371-1374.
73. Dyck JA, Maul GG, Miller WH Jr et al. A novel macromolecular structure is a target of the promyelocyte-retinoic acid receptor oncoprotein. Cell 1994; 76(2):333-343.
74. Koken MH, Puvion-Dutilleul F, Guillemin MC et al. The t(15;17) translocation alters a nuclear body in a retinoic acid-reversible fashion. EMBO J 1994; 13(5):1073-1083.
75. Weis K, Rambaud S, Lavau C et al. Retinoic acid regulates aberrant nuclear localization of PML-RAR alpha in acute promyelocytic leukemia cells. Cell 1994; 76(2):345-356.
76. Daniel MT, Koken M, Romagne O et al. PML protein expression in hematopoietic and acute promyelocytic leukemia cells. Blood 1993; 82(6):1858-1867.
77. Huang ME, Ye YC, Chen SR et al. Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. Blood 1988; 72(2):567-572.
78. Dong S, Stenoien DL, Qiu J et al. Reduced intranuclear mobility of APL fusion proteins accompanies their mislocalization and results in sequestration and decreased mobility of retinoid X receptor alpha. Mol Cell Biol 2004; 24(10):4465-4475.
79. Huang Y, Qiu J, Chen G et al. Coiled-coil domain of PML is essential for the aberrant dynamics of PML-RARalpha, resulting in sequestration and decreased mobility of SMRT. Biochem Biophys Res Commun 2008; 365(2):258-265.
80. Everett RD. Interactions between DNA viruses, ND10 and the DNA damage response. Cell Microbiol 2006; 8(3):365-374.
81. Ishov AM, Maul GG. The periphery of nuclear domain 10 (ND10) as site of DNA virus deposition. J Cell Biol 1996; 134(4):815-826.
82. Maul GG, Ishov AM, Everett RD. Nuclear domain 10 as preexisting potential replication start sites of herpes simplex virus type-1. Virology 1996; 217(1):67-75.
83. Chelbi-Alix MK, Pelicano L, Quignon F et al. Induction of the PML protein by interferons in normal and APL cells. Leukemia 1995; 9(12):2027-2033.
84. Lavau C, Marchio A, Fagioli M et al. The acute promyelocytic leukaemia-associated PML gene is induced by interferon. Oncogene 1995; 11(5):871-876.
85. Chelbi-Alix MK, Quignon F, Pelicano L et al. Resistance to virus infection conferred by the interferon-induced promyelocytic leukemia protein. J Virol 1998; 72(2):1043-1051.
86. Carvalho T, Seeler JS, Ohman K et al. Targeting of adenovirus E1A and E4-ORF3 proteins to nuclear matrix-associated PML bodies. J Cell Biol 1995; 131(1):45-56.
87. Doucas V, Ishov AM, Romo A et al. Adenovirus replication is coupled with the dynamic properties of the PML nuclear structure. Genes Dev 1996; 10(2):196-207.
88. Hoppe A, Beech SJ, Dimmock J et al. Interaction of the adenovirus type 5 E4 Orf3 protein with promyelocytic leukemia protein isoform II is required for ND10 disruption. J Virol 2006; 80(6):3042-3049.

89. Stremlau M, Owens CM, Perron MJ et al. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. Nature 2004; 427(6977):848-853.
90. Perez-Caballero D, Hatziioannou T, Yang A et al. Human tripartite motif 5alpha domains responsible for retrovirus restriction activity and specificity. J Virol 2005; 79(14):8969-8978.
91. Campbell EM, Dodding MP, Yap MW et al. TRIM5 alpha cytoplasmic bodies are highly dynamic structures. Mol Biol Cell 2007; 18(6):2102-2111.
92. Bernardi R, Scaglioni PP, Bergmann S et al. PML regulates p53 stability by sequestering Mdm2 to the nucleolus. Nat Cell Biol 2004; 6(7):665-672.
93. Janderova-Rossmeislova L, Novakova Z, Vlasakova J et al. PML protein association with specific nucleolar structures differs in normal, tumor and senescent human cells. J Struct Biol 2007; 159(1):56-70.
94. Kiesslich A, von Mikecz A, Hemmerich P. Cell cycle-dependent association of PML bodies with sites of active transcription in nuclei of mammalian cells. J Struct Biol 2002;140(1-3):167-179.
95. Everett RD, Earnshaw WC, Pluta AF et al. A dynamic connection between centromeres and ND10 proteins. J Cell Sci 1999; 112(Pt 20):3443-3454.
96. Bryan TM, Englezou A, Dalla-Pozza L et al. Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. Nat Med 1997; 3(11):1271-1274.
97. Dunham MA, Neumann AA, Fasching CL et al. Telomere maintenance by recombination in human cells. Nat Genet 2000; 26(4):447-450.
98. Yeager TR, Neumann AA, Englezou A et al. Telomerase-negative immortalized human cells contain a novel type of promyelocytic leukemia (PML) body. Cancer Res 1999; 59(17):4175-4179.
99. Shiels C, Islam SA, Vatcheva R et al. PML bodies associate specifically with the MHC gene cluster in interphase nuclei. J Cell Sci 2001; 114(Pt 20):3705-3716.
100. Wang J, Shiels C, Sasieni P et al. Promyelocytic leukemia nuclear bodies associate with transcriptionally active genomic regions. J Cell Biol 2004; 164(4):515-526.
101. Hasegawa N, Iwashita T, Asai N et al. A RING finger motif regulates transforming activity of the rfp/ret fusion gene. Biochem Biophys Res Commun 1996; 225(2):627-631.
102. Klugbauer S, Rabes HM. The transcription coactivator HTIF1 and a related protein are fused to the RET receptor tyrosine kinase in childhood papillary thyroid carcinomas. Oncogene 1999; 18(30):4388-4393.
103. Le Douarin B, Zechel C, Garnier JM et al. The N-terminal part of TIF1, a putative mediator of the ligand-dependent activation function (AF-2) of nuclear receptors, is fused to B-raf in the oncogenic protein T18. EMBO J 1995; 14(9):2020-2033.
104. Zhong S, Delva L, Rachez C et al. A RA-dependent, tumour-growth suppressive transcription complex is the target of the PML-RARalpha and T18 oncoproteins. Nat Genet 1999; 23(3):287-295.
105. Seeler JS, Marchio A, Losson R et al. Common properties of nuclear body protein SP100 and TIF1alpha chromatin factor: role of SUMO modification. Mol Cell Biol 2001; 21(10):3314-3324.
106. Le Douarin B, Nielsen AL, Garnier JM et al. A possible involvement of TIF1 alpha and TIF1 beta in the epigenetic control of transcription by nuclear receptors. EMBO J 1996; 15(23):6701-6715.
107. Peng H, Feldman I, Rauscher FJ 3rd. Hetero-oligomerization among the TIF family of RBCC/TRIM domain-containing nuclear cofactors: a potential mechanism for regulating the switch between coactivation and corepression. J Mol Biol 2002; 320(3):629-644.
108. Yondola MA, Hearing P. The adenovirus E4 ORF3 protein binds and reorganizes the TRIM family member transcriptional intermediary factor 1 alpha. J Virol 2007; 81(8):4264-4271.
109. Kikuchi T, Takahashi M, Ueda R et al. Nuclear localization of antigens detected by a monoclonal antibody against a synthetic peptide of rfp finger protein. Hybridoma 1990; 9(2):189-200.
110. Tezel G, Nagasaka T, Iwahashi N et al. Different nuclear/cytoplasmic distributions of RET finger protein in different cell types. Pathol Int 1999; 49(10):881-886.
111. Shimono Y, Murakami H, Hasegawa Y et al. RET finger protein is a transcriptional repressor and interacts with enhancer of polycomb that has dual transcriptional functions. J Biol Chem 2000; 275(50):39411-39419.
112. Matsuura T, Shimono Y, Kawai K et al. PIAS proteins are involved in the SUMO-1 modification, intracellular translocation and transcriptional repressive activity of RET finger protein. Exp Cell Res 2005; 308(1):65-77.
113. Harbers M, Nomura T, Ohno S et al. Intracellular localization of the Ret finger protein depends on a functional nuclear export signal and protein kinase C activation. J Biol Chem 2001; 276(51):48596-48607.
114. Schmidt D, Muller S. PIAS/SUMO: new partners in transcriptional regulation. Cell Mol Life Sci 2003; 60(12):2561-2574.
115. Cao T, Borden KL, Freemont PS et al. Involvement of the rfp tripartite motif in protein-protein interactions and subcellular distribution. J Cell Sci 1997; 110(Pt 14):1563-1571.
116. Cao T, Duprez E, Borden KL et al. Ret finger protein is a normal component of PML nuclear bodies and interacts directly with PML. J Cell Sci 1998; 111(Pt 10):1319-1329.
117. Dho SH, Kwon KS. The Ret finger protein induces apoptosis via its RING finger-B box-coiled-coil motif. J Biol Chem 2003; 278(34):31902-31908.

118. Morris-Desbois C, Bochard V, Reynaud C et al. Interaction between the Ret finger protein and the Int-6 gene product and colocalisation into nuclear bodies. J Cell Sci 1999; 112(Pt 19):3331-3342.
119. Shyu HW, Hsu SH, Hsieh-Li HM et al. A novel member of the RBCC family, Trif, expressed specifically in the spermatids of mouse testis. Mech Dev 2001; 108(1-2):213-216.
120. Shyu HW, Hsu SH, Hsieh-Li HM et al. Forced expression of RNF36 induces cell apoptosis. Exp Cell Res 2003; 287(2):301-313.
121. Bienz M. The PHD finger, a nuclear protein-interaction domain. Trends Biochem Sci 2006; 31(1):35-40.
122. Short KM, Cox TC. Subclassification of the RBCC/TRIM superfamily reveals a novel motif necessary for microtubule binding. J Biol Chem 2006; 281(13):8970-8980.
123. Rhodes DA, de Bono B, Trowsdale J. Relationship between SPRY and B30.2 protein domains. Evolution of a component of immune defence? Immunology 2005; 116(4):411-417.
124. Avela K, Lipsanen-Nyman M, Idanheimo N et al. Gene encoding a new RING-B-box-Coiled-coil protein is mutated in mulibrey nanism. Nat Genet 2000; 25(3):298-301.
125. Kallijarvi J, Avela K, Lipsanen-Nyman M et al. The TRIM37 gene encodes a peroxisomal RING-B-box-coiled-coil protein: classification of mulibrey nanism as a new peroxisomal disorder. Am J Hum Genet 2002; 70(5):1215-1228.
126. Kallijarvi J, Lahtinen U, Hamalainen R et al. TRIM37 defective in mulibrey nanism is a novel RING finger ubiquitin E3 ligase. Exp Cell Res 2005; 308(1):146-155.