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Structure, Organization, and Dynamics of Promyelocytic Leukemia Protein Nuclear Bodies

Maria Hodges,[1] Catherine Tissot,[1] Kathy Howe,[2] David Grimwade,[2] and Paul S. Freemont[1]

[1]Molecular Structure and Function Laboratory, Imperial Cancer Research Fund, and [2]Division of Medical and Molecular Genetics, Guys and St. Thomas's Medical and Dental School, Guys Hospital, London

The interphase nucleus compartmentalizes its components to give rise to a highly organized and tightly controlled environment. Individual chromosomes occupy discrete areas, termed "chromosome territories," that are separated from each other by a channel called the "interchromosomal domain" (reviewed in Lamond and Earnshaw 1998). Actively transcribed genes tend to be at the periphery of chromosomal territories, whereas newly made RNA transcripts localize into the interchromosomal domain, where they can undergo further processing and transport. Movement within the nucleus (Ferreira et al. 1997) may permit chromosomes to enter "factories" that contain all the necessary enzymatic machinery for replication (reviewed in Jackson 1995).

Of the many discrete domains identified throughout the nucleus, the largest are nucleoli, sites of ribosomal RNA synthesis and processing, and sites of preribosomal particle assembly (reviewed in Scheer and Weisenberger 1994). Other subnuclear bodies that appear as punctate structures under immunofluorescence (IF) microscopy include various dynamic structures involved in the maintenance and replication of DNA and RNA synthesis, processing, and transport (reviewed in Nickerson et al. 1995): replication foci, transcript foci, speckled domains, coiled bodies, gems, and promyelocytic leukemia protein (PML) nuclear bodies. Spliceosomal small nuclear (sn) ribonucleoprotein (RNP) components and a subset of non-snRNP splicing factors can be found concentrated in discrete subnuclear domains called "coiled bodies" (Matera and Frey 1998 [in this issue]). It is becoming increasingly apparent that the nucleus has an organization and contains a number of discrete macromolecular domains that coordinate a variety of nuclear processes.

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Address for correspondence and reprints: Dr. Paul Freemont, Molecular Structure and Function Laboratory, 44 Lincoln's Inn Fields, London WC2A 3PX, United Kingdom. E-mail: freemont@icrf.icnet.uk

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Among these domains is a class of nuclear bodies that were originally described as an autoantigenic target in patients with primary biliary cirrhosis and were termed "multiple nuclear dots" (reviewed in Sternsdorf et al. 1997). Several subsequent IF studies showed that the human acute promyelocytic leukemia (APL) proto-oncoprotein PML localizes to these distinctive nuclear dots. They are known by a variety of names, including "PML NBs" (PML nuclear bodies), "ND10" (nuclear domain 10), "Kr bodies," "PODs" (PML oncogenic domains), and "PML bodies" (reviewed in Doucas and Evans 1996). PML nuclear bodies vary in number between 10 and 30 per nucleus, and they typically have a diameter of between 0.2 and 1μm, although their morphology and size alters during the cell cycle. Analysis with immunoelectron microscopy has shown PML nuclear bodies to be doughnut-like in shape, with a dense fibrillar ring containing PML surrounding a central core that does not contain PML (reviewed in Sternsdorf et al. 1997). PML nuclear bodies appear to be nuclear matrix–associated, because they are resistant to RNase and DNase (Ascoli and Maul 1991) and they are detectable in the residual insoluble fraction, although a soluble nuclear fraction of PML has also been observed (Muller et al. 1998; M. N. Boddy and P. S. Freemont, unpublished observations).

Components of PML Nuclear Bodies

At least 15 components have been identified as part of the PML bodies (see fig. 1); some will be discussed in this review.

PML and RFP

PML, one of the defining components of PML nuclear bodies, acts as a cell-growth suppressor (Mu et al. 1994; Ahn et al. 1995; Koken et al. 1995; Le et al. 1996). This protein exists in many different isoforms, which vary in size from 47 to 160 kD and are generated by alternative splicing and variable C-terminal lengths (reviewed in Grimwade and Solomon 1997), but all isoforms contain several conserved cysteine-rich zinc-binding motifs,

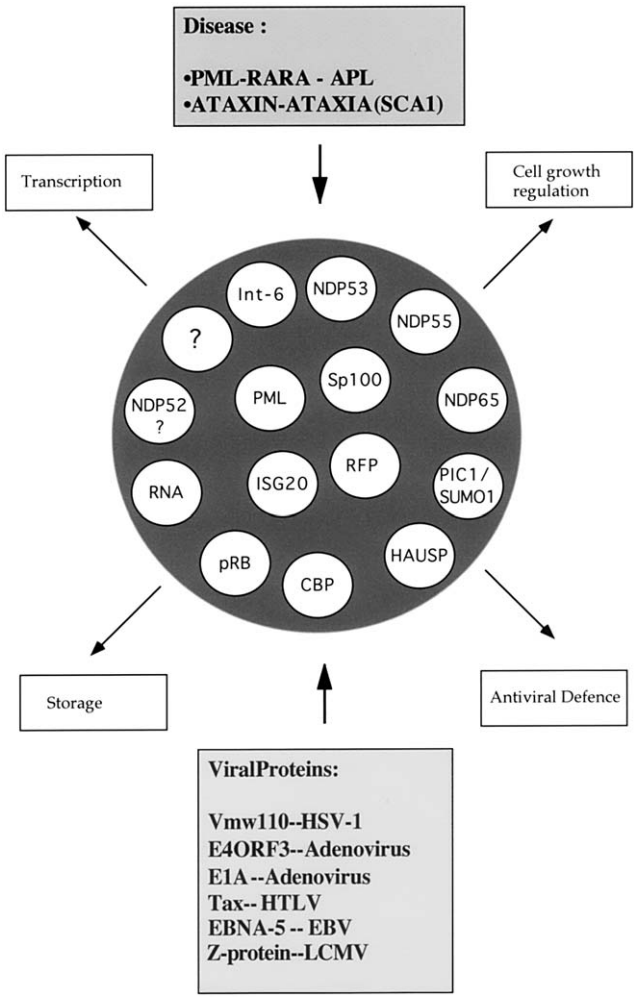

Figure 1 PML nuclear-body components and potential cellular functions. PML nuclear bodies (*large circle*) comprise at least 15 components (*small circles*; reviewed in Sternsdorf et al. 1997). They are targets for viral infection and are disrupted in acute APL and in SCA1. PML nuclear bodies may also play a role in transcription, cell growth and regulation, antiviral defense, or storage of proteins.

known as the RING finger, B1, and B2 B-boxes (reviewed in Saurin et al. 1996). These domains are followed by a predicted α-helical coiled-coil region, all of which together form a tripartite motif known as the RBCC motif, which is thought to mediate assembly of PML nuclear bodies. Recently, it has been shown that the coiled-coil domain can form a hetero-oligomeric interaction with another RBCC protein called ret finger protein (RFP), another component of PML nuclear bodies (Cao et al. 1998). RFP becomes oncogenic when fused to the *RET* proto-oncogene (Takahashi et al. 1988). RFP associates with only a subset of PML nuclear bodies, possibly reflecting the dynamic nature of these structures.

ISG20 and INT6

ISG20 colocalizes with PML nuclear bodies and has homology with the *Xenopus* protein XPMC2, a negative regulator of cell division (Gongora et al. 1997). INT-6, a putative dominant negative oncoprotein, also colocalizes with PML nuclear bodies (Desbois et al. 1996), which are delocalized as a result of viral infection (see below).

PIC1/SUMO-1 and HAUSP

PIC1/SUMO-1 is a ubiquitin-like protein that covalently modifies PML (Boddy et al. 1996; Muller et al. 1998). This modification is reversible and phosphorylation-dependent, with the unmodified PML found in the soluble nucleoplasmic fraction, whereas the modified form apparently partitions into the nuclear matrix (Muller et al. 1998). PIC1/SUMO-1 has 73% homology at the protein level with the SMT3 protein from *Saccharomyces cerevisiae* (Boddy et al. 1996). *SMT3* is an essential yeast gene and was described as a high copy suppressor of mutations in *MIF2,* whose product is required for mitotic spindle integrity at anaphase. The mechanisms by which PIC1/SUMO-1 is conjugated to and removed from PML are beginning to be understood. One member of the ubiquitin-specific protease family, HAUSP, is found in PML nuclear bodies (Everett et al. 1997), but it fails to cleave PIC1/SUMO-1 modified proteins (Everett et al. 1998).

CBP and pRB

Cyclic AMP response element–binding factor (CREB)-binding protein (CBP) is a coactivator for a variety of transcription factors, including nuclear hormone receptors, and it possesses histone acetyl transferase activity (reviewed in Shikama et al. 1997). In interphase nuclei, CBP has a microparticulate distribution with a few larger dots, which colocalize with PML nuclear bodies, suggesting a transient or indirect association (LaMorte et al. 1998). Retinoblastoma protein (pRB), which regulates cell proliferation by controlling a set of transcription factors, has been localized to PML nuclear bodies and forms a stable complex with PML when pRB is hypophosphorylated (Alcalay et al. 1998). Nascent RNA has also been localized to the interior, electron-dense part of some PML nuclear bodies, suggesting that these structures act as a transcriptional regulator (LaMorte et al. 1998).

Dynamic Organization

Two dramatic examples of the dynamic nature of PML bodies are the disruption of these structures in APL (fig. 2) and their enlargement in virally infected cells. The

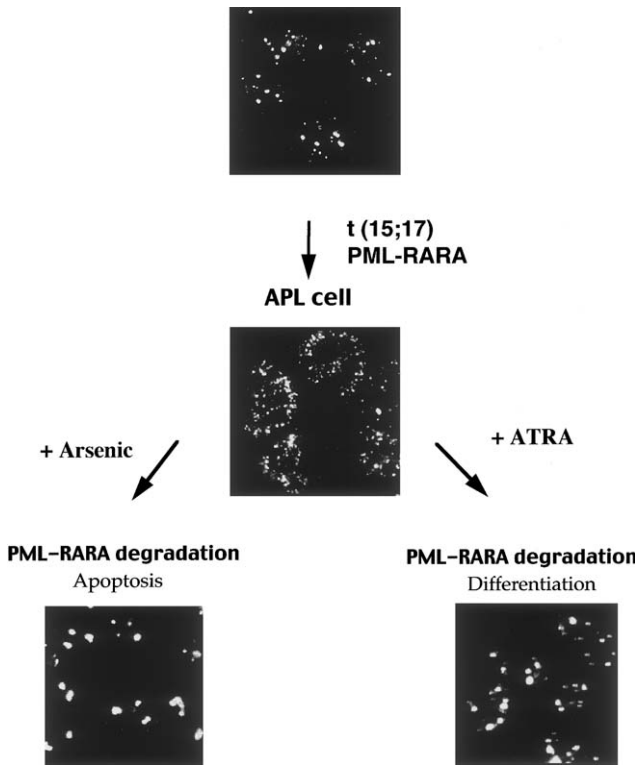

Figure 2 PML nuclear bodies disrupted in APL cells. PML nuclear bodies in non-APL leukemia blasts are shown, *top,* as detected by indirect IF using a specific PML antibody. In APL-derived NB4 cells, which express PML/RARα, a microparticulate pattern of PML staining is observed. In cells cultured with arsenic, there is a degradation of the PML/RARα fusion protein, which leads to a reformation of PML nuclear bodies and subsequent apoptosis (Zhu et al. 1997). NB4 cells cultured in the presence of ATRA are able to differentiate. The PML/RARα fusion protein is degraded, and this is accompanied by reformation of PML nuclear bodies (Dyck et al. 1994; Weis et al. 1994).

PML protein occurs in similar-sized structures in both the nucleus and the cytoplasm (Stuurman et al. 1997), and the continual incorporation and release of PML from nuclear bodies has led to the suggestion that PML (and possibly other components of the PML nuclear bodies) shuttles between nuclear and cytoplasmic structures (Stuurman et al. 1997).

The number and size of PML nuclear bodies varies throughout the cell cycle. The smallest average number is seen in G_0, slowly increasing during progression to G_1, with the highest number observed in S phase (Terris et al. 1995; Koken et al. 1995). A weak, nuclear-diffuse form of PML is visible by IF at G_0, intensifying as the cell progresses to S phase (Koken et al. 1995; Terris et al. 1995). Sp100 and PML colocalize at all stages during the cell cycle except during mitosis, when PML forms large aggregates at the periphery of the cell and Sp100 is distributed more diffusely (reviewed in Sternsdorf et al. 1997). The localization of all the components throughout the cell cycle is not known, but many of the transitory interactions among components of PML nuclear bodies are likely to be sensitive to the cell cycle. Several components, including pRB, are themselves implicated in cell cycle regulation. PML, too, regulates cell cycle progression by modulating the expression of cell cycle regulatory proteins, including Cdk2 and cyclin E (Mu et al. 1997). It will be interesting to determine in more detail the role of PML nuclear bodies and their components in cell cycle regulation.

A number of chemical reagents or alterations in cell growth conditions can significantly affect the morphology and number of PML nuclear bodies. Cystine-deficient media, for example, cause a reversible increase in the size of the nuclear bodies, whereas histidine- or methionine-deficient media have no such effect (Kamei 1997). PML nuclear bodies are also affected by stress factors such as heavy metals and heat shock proteins. Heat shock changes the PML nuclear-body distribution into a microspeckled pattern, and, as the cell recovers, tracklike structures are observed, which finally form normal nuclear bodies (Maul et al. 1995).

Mutagenesis based on the three-dimensional structure of the PML RING finger revealed that a number of domains of PML, in particular the RBCC motif, can influence the formation of PML nuclear bodies (Borden et al. 1995; Boddy et al. 1997). Mutations of the RING finger cysteines result in a nuclear-diffuse but matrix-associated PML when overexpressed, whereas specific surface residue mutations result in abnormally large PML nuclear bodies (Boddy et al. 1997). Other studies have shown that the B-box and coiled-coil domains also influence nuclear-body formation (Kastner et al. 1992; Borden et al. 1996). PML can homo-oligomerize through the coiled-coil domain, although this is not sufficient for the formation of PML nuclear bodies in vivo (Borden et al. 1996). Loss of PML nuclear-body formation has also been linked to a loss of growth and transformation activity of PML (Le et al. 1996), although recent contradictory studies suggest that localization of PML to PML nuclear bodies is not necessary for it to suppress cell growth (Fagioli et al. 1998).

The Effect of Interferon and Viral Infection on PML Nuclear Bodies

Interferons are a family of secreted cytokines with antiviral, antiproliferative, and immunomodulatory activities (reviewed in Darnell et al. 1994). IFN treatment leads to an enlargement in both the size and the number of PML nuclear bodies. The expression of PML, Sp100, and ISG20 (reviewed in Sternsdorf et al. 1997) are all

inducible by both type I (α/β) and type II (γ) IFNs. PML has been shown to contribute to the IFN antiviral action (Doucas et al. 1996; Chelbi-Alix et al. 1998).

Altered localization of PML and structural changes within PML nuclear bodies have been shown to occur during DNA and RNA viral infections (reviewed in Doucas and Evans 1996; Sternsdorf et al. 1997). Some examples are as follows: the T-cell leukemia virus type 1 (HTLV-1) tax oncoprotein, which induces the specific redistribution of INT-6 (Desbois et al. 1996), and the adenovirus, which targets the viral protein E4-ORF3 to PML nuclear bodies and causes their reorganization from spherical to fibrous structures (termed "nuclear tracks"; reviewed in Doucas and Evans 1996). Nuclear bodies are also reorganized after human cytomegalovirus (CMV) infection (Kelly et al. 1995; Korioth et al. 1996)

The herpes simplex virus type 1 (HSV-1) immediate-early protein Vmw110 (also called "ICP0") transiently colocalizes with and subsequently disrupts PML nuclear bodies (Everett and Maul 1994). Recently, it has been shown that Vmw110 binds specifically to HAUSP (see above), which, at early stages of viral infection, increases the proportion of PML nuclear bodies that contain HAUSP (Everett et al. 1997). The cytoplasmic RNA virus lymphocytic choriomeningitis (LCMV) also affects the distribution of PML nuclear bodies, with the Z protein of LCMV interacting with PML to form large cytoplasmic bodies (Borden et al. 1998). Nuclear bodies are sites for the early stages of transcription and replication of DNA and RNA viruses and are also sites for the subsequent cellular antiviral defense mechanisms using IFNs.

Functions of PML Nuclear Bodies

Despite the indications that the proper organization of PML-body components is essential for normal cell proliferation, the functions of these bodies remains elusive after numerous and wide-ranging studies. Recent results, with the ablation of PML by homologous recombination, show that mice are viable but more susceptible to tumorigenesis and infections (Wang et al. 1998). The phenotype of these $PML^{-/-}$ mice supports the role of PML as a negative growth regulator and tumor suppressor and as a specific regulator of hematopoietic differentiation (Wang et al. 1998). The presence of nascent RNA and the transcriptional coactivator CBP within the internal core of PML nuclear bodies suggests that these structures act as transcriptional regulators (LaMorte et al. 1998), and, indeed, Alcalay et al. (1998) have found that PML can abolish activation of glucocorticoid receptor–regulated transcription by pRB. PML nuclear bodies have also been observed to be closely associated with replication domains in middle to late S phase (Grande et al. 1996). Also, viral transcripts and replication factories produced during viral infections have been localized to areas adjacent to PML bodies, suggesting that viral transcription requires some PML nuclear-body components (Ishov et al. 1997).

The exact molecular mechanism by which PML and PML nuclear bodies carry out any of these functions, however, remains to be elucidated. One possibility is that PML nuclear bodies act as "nuclear dumps" or storage "PODS," where different proteins can be deposited or stored and subsequently distributed where necessary. An alternative theory is that PML bodies localize PML NB components to their functionally active site either within the nuclear body or adjacent to it.

PML Nuclear Bodies and Human Disease

In addition to being targeted by a wide range of viral infections, PML nuclear bodies, particularly PML, are involved in other human disease processes. Indeed, it was the characterization of genetic changes underlying APL that actually led to the discovery of PML and its localization within nuclear-body structures (reviewed in Grimwade and Solomon 1997). APL, one of the commonest subtypes of acute myeloid leukemia, is characterized by a block in differentiation leading to replacement of the bone marrow with abnormal promyelocytes. This differentiation block can be overcome by retinoids such as all-trans-retinoic acid (ATRA), which induces complete remission in the majority of patients and has transformed clinical practice (Grimwade and Solomon 1997, and references therein). A reciprocal translocation, t(15;17) (q22;q21), which leads to a rearrangement between genes encoding PML and RARα, is almost invariably found in APL tumor cells (Grimwade and Solomon 1997). RARα is a member of the steroid hormone nuclear receptor family that mediates the effect of retinoic acid at specific DNA response elements. When RARα is complexed with its ligand, the corepressor complex, which includes SMRT (silencing mediator of retinoid and thyroid receptor) or N-CoR and histone deacetylase (HDAC), dissociates from the receptor, thus allowing transcriptional activation (Chambon 1996). In addition, retinoid responses are dependent on members of the distinct retinoid–X-receptor family, which heterodimerize with RARα and mediate high-affinity binding to specific DNA response elements. The t(15; 17) typically leads to the formation of two fusion gene products, PML/RARα and RARα/PML. Most studies have focused on PML/RARα, since it retains the RBCC motif of PML as well as key functional domains of RARα (reviewed in Grimwade and Solomon 1997). The critical role played by PML/RARα in APL is also supported by transgenic mice studies (Brown et al. 1997; Grisolano et al. 1997; He et al. 1998) and by the characterization of

APL cases in which PML/RARα is the sole fusion gene formed (Grimwade et al. 1997).

RARα has been implicated in normal hemopoiesis (Tsai and Collins 1993), suggesting that the PML/RARα fusion might cause APL by inhibiting the wild-type receptor. However, recent studies have demonstrated that expression of mutant RARα (Tsai and Collins 1993) or overexpression of normal receptor (Onodera et al. 1995) block myeloid differentiation but are insufficient to induce APL, suggesting that the PML-derived portion of the fusion protein is essential for leukemic transformation. It has also become apparent that expression of the PML/RARα fusion protein disrupts PML nuclear bodies (fig. 2; Dyck et al. 1994; Weis et al. 1994). In cells derived from normal and neoplastic tissues or from hematological malignancies other than APL, <30 such nuclear bodies are typically observed, whereas, in APL cases with the t(15;17) translocation, a characteristic, microspeckled PML nuclear-staining pattern is detected (fig. 2; Dyck et al. 1994; Weis et al. 1994). Treatment of APL cells with ATRA causes the fusion protein to be degraded and restores the normal PML nuclear-staining pattern; parallel with these events, cellular differentiation resumes (fig. 2). Similarly, reversion to a wild-type pattern of PML staining has been observed when APL blasts are exposed to arsenic compounds (fig. 2; Zhu et al. 1997). However, it has been noted that some cases of APL with *PML/RARα* rearrangements are resistant to retinoids and maintain a microparticulate PML nuclear-staining pattern (Dyck et al. 1994). This suggests that disruption of PML bodies could play a fundamental role in the pathogenesis of APL and that reversal of this process is essential to overcoming the block in differentiation that characterizes APL (Dyck et al. 1994). Furthermore, PML appears to function as a growth suppressor (Mu et al. 1994; Wang et al. 1998; Le et al. 1998), raising the possibility that disruption of PML nuclear bodies in APL could also be important in promoting leukemic transformation.

Further insights into the mechanisms underlying the differentiation block in APL have been gained by the characterization of a series of rare cases of APL lacking the t(15;17) translocation, in which *RARα* was found to be fused to novel partner genes. These include the Krüppel-like zinc finger gene *PLZF* (reviewed in Pandolfi 1996), the gene encoding the nuclear mitotic apparatus protein (*NuMA;* Wells et al. 1997), and the nucleophosmin gene (*NPM*; Pandolfi 1996), whose gene product is involved in RNA processing. These fusion proteins form as a result of t(11;17)(q23;q21), t(11;17)(q13;q21), and t(5;17)(q32;q21) rearrangements, respectively. Of these rare APL-associated translocations, the best studied is the *PLZF/RARα* rearrangement. Although PLZF and PML bear little structural similarity, both suppress cell growth in a transformation assay (Pandolfi 1996), and both are localized to discrete, possibly related nuclear domains. However, in contrast to t(15;17) APL patients, those with a *PLZF/RARα* rearrangement do not resume differentiation when treated with ATRA alone. It is now clear that this reflects differential binding of the PML/RARα and PLZF/RARα fusion proteins to nuclear corepressors such as SMRT or N-COR (He et al. 1997; Grignani et al. 1998; Guidez et al. 1998; Lin et al. 1998). At physiological levels of RA, both fusion proteins bind the corepressor complex, leading to repression of retinoid response elements. In the presence of pharmacological levels of ATRA, binding of ligand to the fusion proteins is associated with displacement of N-COR/SMRT and HDAC from the PML/RARα receptor complex. In the case of PML/RARα, this leads to transcriptional activation at retinoid response elements. However, because PLZF/RARα binds the complex through a second, ATRA-insensitive site, ATRA fails to activate transcription in t(11;17) APL cells.

Recent studies have suggested that PLZF can interact directly with PML in vivo (Koken et al. 1997), suggesting that disruption of PML nuclear bodies could be a common feature in the pathogenesis of APL. However, in APL cases associated with PLZF/RARα (Grimwade et al. 1997), NuMA/RARα (Wells et al. 1997), and NPM/RARα (Redner et al. 1997), a wild-type pattern of PML-staining has been observed, suggesting that PML nuclear bodies are intact in these cases. Therefore, disruption of PML nuclear bodies may not be an essential component of the differentiation block in APL. This is in agreement with in vitro studies showing that the differentiation block and ATRA response are independent of delocalization of PML from nuclear bodies (Grignani et al. 1996).

Whether the growth-suppressor effects attributed to PML—or, indeed, to PLZF—are mediated by the proteins themselves or require other nuclear-body constituents remains to be established. Disruption of PML nuclear bodies may act specifically in the etiology of t(15;17)-associated APL, possibly by causing putative growth suppressors such as PML, pRB, or PLZF to be mislocalized. Interestingly, recent cotransfection studies involving PML/RARα and PLZF/RARα show that both fusion proteins colocalize to microspeckles (Koken et al. 1997), implying that some common mislocalization is involved in all forms of APL. Further characterization of the components of PML nuclear bodies and their intranuclear dynamics in APL may provide further insights into the process of leukemogenesis and the response to retinoids and arsenic compounds.

APL is not the only human disease that is linked with an alteration in the localization of PML nuclear bodies. Disruption of these bodies is also observed in spinocerebellar ataxia type I (SCA1), a neurogenerative disorder. Normal ataxin-1 protein is associated with the nuclear

matrix, whereas expression of a mutant ataxin-1 associated with SCA1 causes the specific redistribution of PML from the nuclear bodies; however, there does not seem to be a general loss of nuclear structure integrity, because three other nuclear components were unaffected by the expression of mutant ataxin-1 (Skinner et al. 1997). Whether this contributes to SCA1 development or is a result of SCA1 is not known, but it is interesting that these bodies appear to be disrupted.

Recent progress in understanding nuclear organization has shown it to be highly organized and dynamic. Future studies will increase our understanding of how the nuclear compartments are assembled in a precise temporal and spatial manner and how disruption of this organization contributes to human disease.

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