PML bodies associate specifically with the MHC gene cluster in interphase nuclei

Carol Shiels[1,5], Suhail A. Islam[2], Radost Vatcheva[1], Peter Sasieni[3], Michael J. E. Sternberg[2], Paul S. Freemont[4,5,*] and Denise Sheer[1,*]

[1]Human Cytogenetics Laboratory, Imperial Cancer Research Fund, London, WC2A 3PX, UK
[2]Biomolecular Modelling Laboratory, Imperial Cancer Research Fund, London, WC2A 3PX, UK
[3]Mathematics and Statistics Group, Imperial Cancer Research Fund, London, WC2A 3PX, UK
[4]Molecular Structure Laboratory, Imperial Cancer Research Fund, London, WC2A 3PX, UK
[5]Centre for Structural Biology, Imperial College of Science, Technology and Medicine, South Kensington, London, SW7 2AZ, UK
*Authors for correspondence (e-mail: p.freemont@ic.ac.uk; sheer@icrf.icnet.uk)

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SUMMARY

Promyelocytic leukemia (PML) bodies are nuclear multi-protein domains. The observations that viruses transcribe their genomes adjacent to PML bodies and that nascent RNA accumulates at their periphery suggest that PML bodies function in transcription. We have used immuno-FISH in primary human fibroblasts to determine the 3D spatial organisation of gene-rich and gene-poor chromosomal regions relative to PML bodies. We find a highly non-random association of the gene-rich major histocompatibilty complex (MHC) on chromosome 6 with PML bodies. This association is specific for the centromeric end of the MHC and extends over a genomic region of at least 1.6 megabases. We also show that PML association is maintained when a subsection of this region is integrated into another chromosomal location. This is the first demonstration that PML bodies have specific chromosomal associations and supports a model for PML bodies as part of a functional nuclear compartment.

Key words: Immuno-FISH, Major histocompatibility complex, Nuclear organisation, PML nuclear body, Transcription

INTRODUCTION

The mammalian interphase nucleus is responsible for tasks such as DNA replication, control of gene expression, transcription and RNA processing. Despite the absence of intranuclear membranes, many of the proteins involved in these processes have been found to accumulate in discrete nuclear domains or foci, leading to the concept of a functionally compartmentalised nucleus (Spector, 1993). One such nuclear domain, originally recognised by autoantibodies against the Sp100 protein (Szostecki et al., 1990) and named ND10 (nuclear domain 10) or Kremer bodies (Kr), was subsequently found to contain the promyelocytic leukemia protein PML (Dyck et al., 1994; Koken et al., 1994; Weis et al., 1994). PML is expressed as a fusion protein with RARα in patients with acute promyelocytic leukemia, resulting in a block in promyelocyte differentiation and concomitant redistribution of PML-containing foci (de The et al., 1991; Goddard et al., 1991; Kakizuka et al., 1991). This lead to these foci being commonly known as PML bodies or PML oncogenic domains (PODs) (Hodges et al., 1998; Maul et al., 2000; Seeler and Dejean, 1999; Sternsdorf et al., 1997).

In addition to the defining Sp100 and PML proteins, PML bodies also contain a heterogeneous mix of functionally important proteins (Seeler and Dejean, 1999). The PML protein plays a pivotal role in accumulating components at PML bodies, since proteins such as Blooms (BLMs), CREB binding protein (CBP), DAXX and Sp100 do not localise to PML bodies in the absence of PML (Ishov et al., 1999; Zhong et al., 1999; Zhong et al., 2000b; Zhong et al., 2000a). Cells lacking BLM or Sp100 have otherwise normal PML body components (Ishov et al., 1999). There is also an essential role for the small ubiquitin-related protein SUMO-1 in PML body formation, since a PML mutant that cannot be SUMO-modified does not form PML bodies (Muller et al., 1998; Zhong et al., 2000b). The heterogeneity of proteins localising to PML bodies has made it difficult to propose a single function for this class of nuclear domain. The observation that proteins, such as the tumour suppressor BRCA1, only localise to PML bodies when overexpressed (Maul et al., 1998) suggests that PML bodies are a type of nuclear storage site, perhaps regulating the levels of active proteins within the nucleus (Maul, 1998). The pRB tumour suppressor protein for example, only localises to PML bodies in an inactive non-phosphorylated form (Alcalay et al., 1998). In support of this idea is the recent finding that misfolded proteins can accumulate in PML bodies prior to degradation by ubiquitin-mediated proteolysis via the proteosome (Anton et al., 1999).

PML is also implicated in regulating transcription. The recent demonstration that nascent RNA accumulates at the periphery of PML bodies suggests that PML bodies could act to concentrate regulatory factors for transcriptional events at the surface of PML bodies (Boisvert et al., 2000). The observation that highly acetylated chromatin can also be found associated with PML bodies is of particular interest (Boisvert et al., 2000), since PML bodies have been observed to contain the histone acetyltransferase CBP (LaMorte et al., 1998; Zhong et al., 2000b). Additional evidence for an association with

transcriptionally active chromatin comes from the finding that PML bodies are often embedded in the SC35 enriched nuclear domains, where expressed genes are often localised (Ishov et al., 1997; Smith et al., 1999). The PML protein is also implicated in the regulation of certain genes, including the upregulation of genes involved in MHC class I processing (Zheng et al., 1998), and the inhibition of Sp1-mediated activation at the EGFR promoter (Vallian et al., 1998). Viral genomes also localise to PML bodies in order to transcribe their DNA, again indicating that PML bodies are transcription permissible domains (Ishov et al., 1997).

Given the accumulating evidence that PML regulates the expression of specific target genes, we set out to determine the 3D spatial relationship between PML bodies and gene dense/gene poor genomic regions in primary human fibroblast nuclei. We examined two gene-rich regions, namely, the major histocompatibility complex (MHC) on chromosome 6 and the epidermal differentiation complex (EDC) on chromosome 1; and the gene-poor 6p24 region on chromosome 6. We found that there is a significantly higher association between PML bodies and the MHC region in comparison to the other genomic regions examined. We have mapped the association locus to a region of at least 1.6 Mb that encompasses the centromeric end of the MHC. We also show that in a B-lymphoblastoid cell line, PML association occurs with a sub-region of the 1.6 Mb MHC locus when integrated into chromosome 18. These data show for the first time that PML bodies have specific genomic associations that are independent of transcription and support a model for PML bodies as functional domains.

MATERIALS AND METHODS

Cell culture

MRC5 human primary fibroblasts were obtained from the ATCC. AHB human B-lymphoblastoid cells were obtained from Lady J. Bodmer, ICRF, Oxford, UK. The B-lymphoblastoid cell line 721.174 containing a large homozygous deletion of the MHC class II region (DeMars et al., 1984) and the 4D1D10.2 cell line containing the MHC class II YAC integrated into chromosome 18 (Fabb et al., 1997) were the kind gifts of J. Trowsdale, University of Cambridge, UK and J. Ragoussis, King's College, London, UK, respectively. All cells were cultured in RPMI media supplemented with 10% FCS and 2 mM glutamine. Recombinant human interferon γ (IFN-γ, R&D Systems), was used at a concentration of 200U/ml for 24 hours to upregulate transcription of MHC genes (Boehm et al., 1997; Girdlestone, 1996; Rohn et al., 1996). Global transcription by RNA polymerase II was inhibited by incubation with α-amanitin (5 μg/ml) or DRB (50 μg/ml) for 5 hours before fixation. Cells in S phase were labelled by adding BrdU (Sigma) to the culture medium at a final concentration of 100 μM for 1hour immediately before fixation.

Antibodies and DNA probes

The following antibodies were used in this study: Rabbit polyclonal anti-PML (Borden et al., 1995) to detect PML nuclear bodies, mouse anti-BrdUTP (Boehringer Mannheim) to detect BrdUTP incorporated into newly replicating DNA and mouse anti-p80 coilin to detect Cajal bodies (Almeida et al., 1998). Secondary antibodies used were anti-rabbit Alexa-488, anti-mouse Alexa-488, anti-mouse Alexa-546 (all from Molecular Probes) and anti-rabbit cy5 (Amersham Pharmacia). Genomic loci were detected using the following DNA probes: the MHC (LMP/TAP) region was detected using cosmids HA14, U15, U10 and M4 containing the LMP and TAP genes (Beck et al., 1996); the EDC region was detected using cosmids K0695, D01101, K1632, F0823, J1113 and F0969 containing the SPRR genes (South et al., 1999); a subsection of the 6p24 region was detected using cosmids A9.5, B5.10, B10.10, B12.10, E11.2, F1.6 (Davies et al., 1998; Olavesen et al., 1997); the DAXX region was detected using PACs 229D22, 36A2 and cosmids I0332, B2046, P0717 (Herberg et al., 1998a); the BAK region was detected using cosmid A094 (Herberg et al., 1998b); the TCP11 region was detected using cosmids 3N2, 3A2, 3B21 and 11H10 (Tripodis et al., 1998); the MHC class I region was detected using cosmids C0247, C0426, I1421 and A0622 adjacent to the classical class I gene HLA-A (Goldsworthy et al., 1996); the centromere of chromosome 6 was detected using a commercial biotinylated alpha satellite (Appligene Oncor); and band 18q11 was delineated using a partial chromosome paint specific for this chromosomal band (www.biologia.uniba.it/rmc).

Combined immunofluorescence and FISH (immuno-FISH)

For immuno-FISH of adherent cell lines, cells grown on chamber-slides were pre-extracted in CSK (0.1 M $NaCl_2$, 0.3 M sucrose, 3 mM $MgCl_2$, 10 mM Pipes, pH 6.8) (Carter et al., 1991) containing 0.5% Triton X-100 for 5 minutes on ice before fixation in 4% formaldehyde for 10 minutes. To facilitate detection of DNA sequences by FISH, we also performed additional nuclear permeabilisation steps consisting of repeated freeze-thaw in liquid nitrogen and treatment with 0.1 M HCl for 10 minutes (Kurz et al., 1996). Suspension cells were subjected to the same permeabilisation steps, but were not attached to slides by cytocentrifugation until after the fixation step in order to prevent distortion of the cells during centrifugation (Ferguson and Ward, 1992). These permeabilisation steps have previously been shown to be optimal for preservation of nuclear structure and detection of DNA by FISH (Carter et al., 1991; Kurz et al., 1996; Verschure et al., 1999). For visualisation of genomic loci by FISH, cells were denatured in 70% deionised formamide, 2× SSC at 72°C for 2 minutes and washed for 1 minute in cold 2× SSC before addition of denatured DNA probe. Hybridisation was at 37°C overnight, followed by washes at 42°C in 50% formamide, 2× SSC for 3× 5 minutes and in 2× SSC for 3× 5 minutes. Biotin-labelled DNA probes were detected using streptavidin Alexa-546 (Molecular Probes) and digoxigenin-labelled DNA probes were detected using anti digoxigenin FITC (Boehringer Mannheim). Detection of nuclear proteins by immunofluorescence was performed simultaneously with the FISH detection steps.

Microscopy and statistical analysis

Images were captured as optical sections using a Zeiss LSM 510 confocal laser scanning microscope equipped with a Plan Apo 63×/NA 1.4 objective. Sections were collected at 0.4 μm intervals through each nuclei and processed using the newly developed Image3D program. This produces 3D co-ordinates of centroid positions for PML bodies and each genomic locus as shown in Fig. 3. The Euclidean distance between each pair of fluorescent foci was calculated from their co-ordinates.

For each genomic locus, we considered the set of fluorescent foci consisting of the locus and all PML bodies. For each focus in each set we calculated the distance to its nearest neighbour. For a genomic locus this is the distance to the nearest PML body and is referred to as the minimum genomic locus-PML distance. For a PML body this is either the distance to the nearest PML body or the distance to the genomic locus in question, whichever is closest. For each cell we then calculated the mean minimum genomic locus-PML distance and the mean minimum PML-PML distance (the latter being the average of the minimum PML-PML distances for all PML bodies in the nucleus). For statistical analysis, let the mean minimum PML-PML distance in cell i be denoted d0i and let the corresponding mean minimum genomic locus 1-PML distance be denoted d1i and similarly for genomic locus 2. In order to explore whether a particular gene tends to be close to at least one PML body, we performed a paired *t*-test using the pairs d1i, d0i. The *t*-statistic was calculated in the usual way

and the two-sided *p*-values quoted in this paper are for the null hypothesis of equal means. Large negative values indicate that the gene tends to be closer to a PML body than PML bodies are to other PML bodies. Three-colour experiments also provide a direct comparison between the minimum genomic locus-PML distances for the two genomic loci. This was explored using a paired *t*-test of the values d1i and d2i. The third type of comparison is between minimum genomic locus-PML distances in a set of untreated cells and in a set of treated cells, or between two different cell populations. The effect of treatment is then investigated using an unpaired *t*-test on the two sets of d1i distances under the assumption of equal variance. Where the mean minimal PML-PML distances are quite different between the two sets of cells, the comparison is made between the differences d1i–d0i.

RESULTS

PML bodies associate specifically with the MHC genomic region

To investigate the potential role of PML bodies in transcription, the spatial distribution of PML bodies was examined in primary human fibroblasts in relation to genomic regions of varying gene density and transcriptional activity. These regions included the MHC, the EDC and the 6p24 region (genomic locations illustrated in Fig. 1A). The MHC on chromosome 6 is one of the most gene-rich regions of the human genome (average gene density of 1 gene per 16 kb); (The MHC sequencing Consortium, 1999) and contains a number of genes that are constitutively expressed in fibroblasts (Milner and Campbell, 1992; Trowsdale, 1995). The EDC locus is also extremely gene-rich (27 genes within a 2.5 megabase region) (South et al., 1999), but has only three genes known to be expressed in fibroblasts (Okada et al., 1998; Schafer and Heizmann, 1996). The third region we investigated is a 500 kb subregion of band 6p24 on chromosome 6 (Fig. 1A) containing no known genes and flanked by two genes, (Olavesen et al., 1997), neither of which are expressed in mammalian fibroblasts (Ebisawa et al., 1999; Huang and Domann, 1999). The constitutively expressed LMP2, LMP7, TAP1 and TAP2 genes located within the class II region of the MHC (Fig. 1B) are of particular interest, since they have been found to be transcriptionally upregulated by PML (Zheng et al., 1998).

In order to visualise genomic regions and PML bodies simultaneously, we used immuno-FISH on three-dimensionally preserved MRC5 mammalian fibroblasts. The MHC region was detected using probes hybridising to the LMP/TAP genes, the EDC region was detected using probes hybridising to the SPRR genes and the 6p24 region was detected using probes hybridising to a 500 kb subregion of band 6p24 (see Materials and Methods) (Fig. 1A). Three-dimensional images of nuclei were collected by confocal microscopy and the relative genomic locus-PML associations analysed (see Materials and Methods). As shown in Fig. 2, PML bodies are either in close association or overlapping with both the MHC and the EDC genomic regions. A visual examination of confocal images demonstrates that PML bodies are associating with at least one MHC homologue in ~42% of nuclei (n=55). By contrast, PML bodies associate with the EDC and 6p24 regions at a much lower frequency, with only ~24% (n=55) and ~20% (n=30) of nuclei, respectively, having at least one allele associating with a PML body. These results demonstrate that PML bodies can associate with specific chromosomal regions and appear more frequently associated with the MHC region than with the EDC and 6p24 regions. Given that each nucleus contains a relatively large number of PML bodies (10-15 per nucleus), we therefore tested the statistical significance of the observed associations

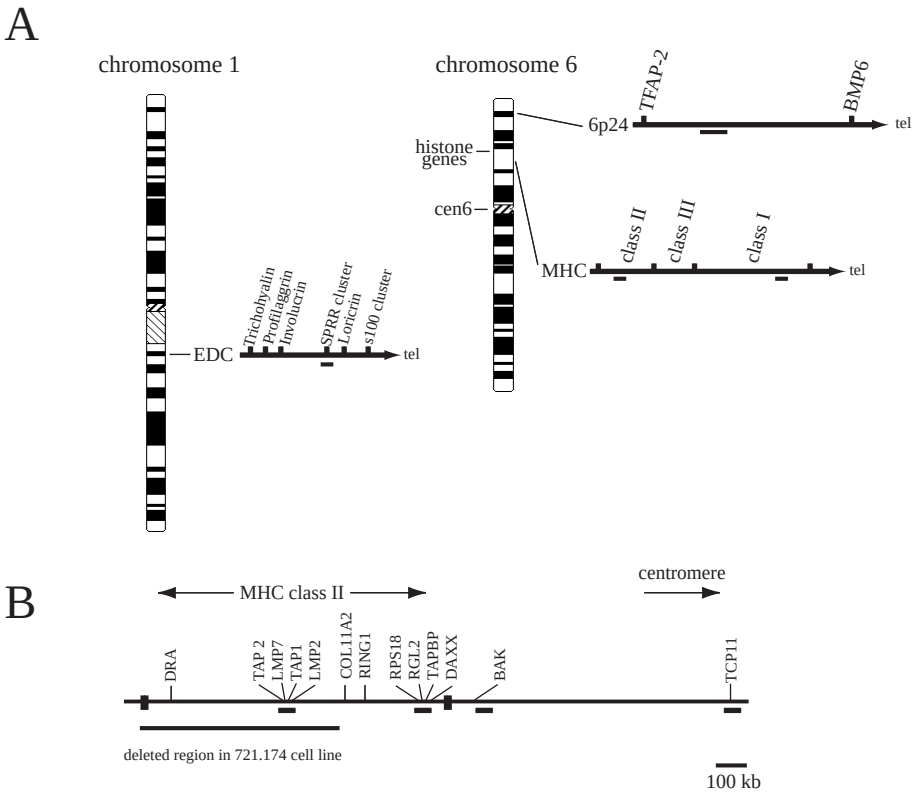

Fig. 1. Schematic maps indicating the organisation of the MHC, EDC and 6p24 genomic regions and the location of the genomic probes used for FISH analysis. (A) Ideograms show the positions of the EDC on chromosome 1 and the MHC and 6p24 regions on chromosome 6. The EDC contains 27 genes and spans a genomic region of 2.5 Mb, it is mainly composed of genes involved in keratinocyte differentiation and can be visualised using genomic probes to the small proline rich region (SPRR). The 6p24 region is relatively gene-poor, with the 500 kb subsection studied flanked by only two known genes. The MHC is divided into three classes and contains many genes involved in the immune response. It is one of the most gene-rich regions of the genome, with 224 gene loci identified over a genomic region of 3.9 Mb. A more detailed map of the MHC class II and adjacent centromeric region is shown in (B), with the relative positions of the LMP/TAP, DAXX, BAK and TCP11 regions indicated. A number of genes mapping to this region are shown, for a more comprehensive list refer to the Sanger Centre website (www.sanger.ac.uk). The region deleted in the 721.174 cell line is shown as a solid bar and covers a large proportion of the MHC class II region from the DRA gene up to the LMP/TAP region.

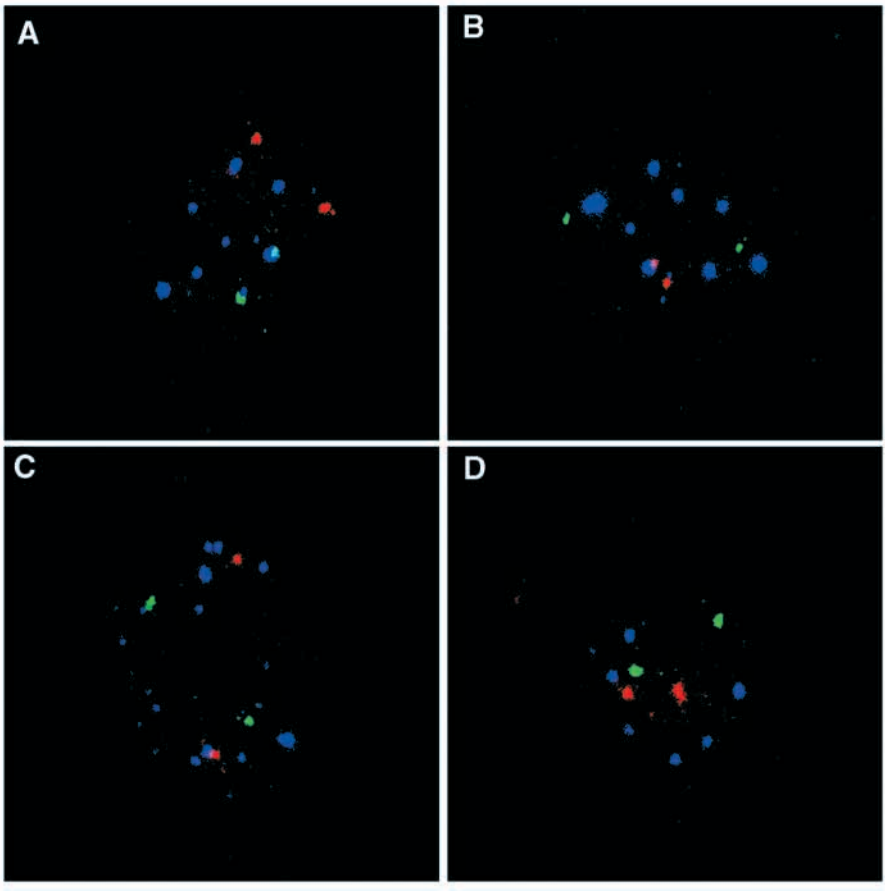

Fig. 2. Spatial distribution of PML bodies relative to the MHC and EDC genomic regions. Immuno-FISH was performed on three-dimensionally preserved fibroblast nuclei, detecting PML bodies by immunofluorescence (blue) and simultaneously visualising the MHC using genomic probes recognising the LMP/TAP region (green) and the EDC using genomic probes recognising the SPRR region (red). Projections of serial optical sections through each nucleus are shown. PML bodies can be observed either closely associated or overlapping with both the MHC and the EDC in a percentage of the nuclei investigated. A-D demonstrates the variability in PML body association, with PML bodies found in association with both MHC regions (A), both EDC regions (B), associating with one MHC region and one EDC region (C) and associating with neither genomic region (D).

using a newly devised minimal distance method. This method involves a 3D reconstruction from serial confocal images of fluorescent signals above a certain threshold to obtain centroid xyz co-ordinates for each PML body and genomic locus (see Materials and Methods). From these centroid co-ordinates, the average minimal distance between PML bodies in each nucleus can be calculated and compared with values between PML and each genomic locus (Fig. 3). This allows a direct estimate of

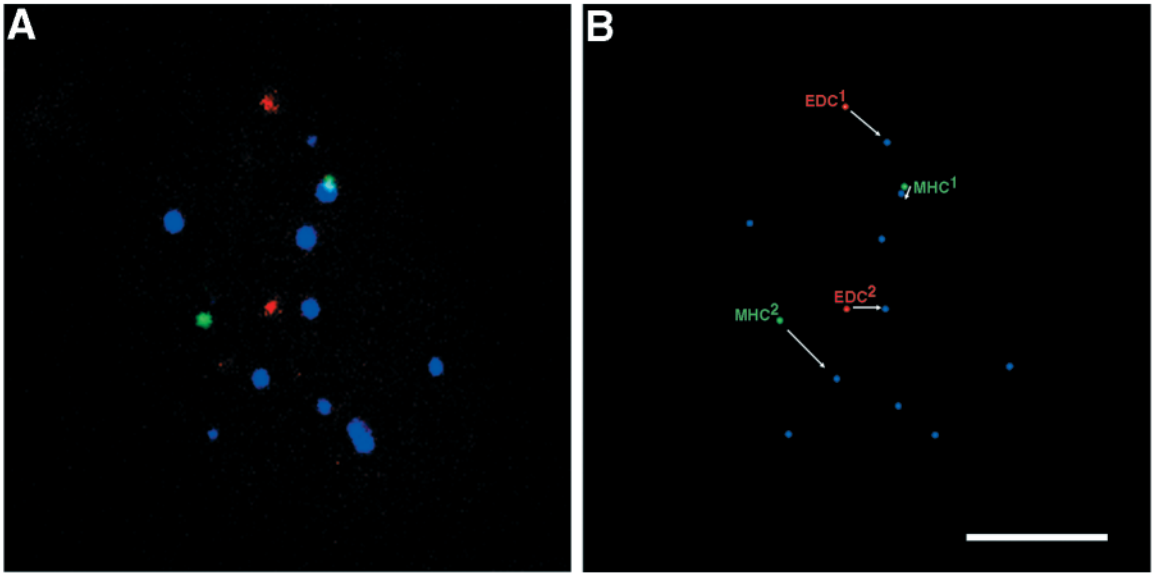

Fig. 3. Determining minimal intranuclear genomic locus-PML body distances. Images of three-dimensionally preserved fibroblast nuclei collected by confocal microscopy (A) were processed using the image3D program to determine the x,y,z centroid co-ordinates of each PML body, MHC and EDC region in the nucleus (B). Using these coordinates it was possible to calculate the distance between each genomic locus and its closest PML body: MHC^1-PML=1.3 μm; MHC^2-PML=3 μm; EDC^1-PML=2.3 μm; EDC^2-PML=1.7 μm. Note that these images are 2D representations of 3D images. Bar, 5 μm.

Table 1. Statistical analysis of genomic locus-PML associations

Cell	Genomic locus	N	$\overline{X}_{PML}$	$\overline{X}_{MHC(LMP/TAP)}$	*t*(MHC)*	$\overline{X}_{genomic\ locus}$	*t*(genomic locus)*	t(MHC: genomic locus)‡
MRC5	EDC(SPRR)	55	2.15	1.51	–9.38	1.88	–3.19	3.98 (*P*=0.0002)
“	6p24	30	2.27	1.57	–8.13	2.12	–1.79	6.17 (*P*<0.0001)
MRC5	MHC class I	52	2.41	1.68	–9.42	1.92	–6.17	3.99 (*P*=0.0002)
“	DAXX	30	2.14	1.52	–7.55	1.42	–9.51	–2.66 (*P*=0.01)
“	BAK	30	2.17	1.46	–8.90	1.47	–7.79	0.03 (*P*=0.97)
“	TCP11	36	2.15	1.56	–7.77	1.56	–7.66	–0.06 (*P*=0.95)
“	cen6	36	2.14	1.55	–9.74	2.11	–0.61	6.23 (*P*<0.0001)
4D1D10.2§	DAXX	31	2.10	1.62	–3.41	1.55	–6.27	–0.38 (*P*=0.7)
4D1D10.2¶	18q11 (-YAC)	70	2.13	1.83	–3.98	2.02	–1.50	1.45 (*P*=0.15)

*The significance of MHC-PML and genomic locus-PML associations was calculated using a one sample *t*-test, as described in Materials and Methods. Large negative values ($t \leq -2$) indicate that the MHC or genomic locus tend to be closer to a PML body than PML bodies are to themselves.

‡A paired *t*-test was used to directly compare MHC-PML distances with genomic locus-PML distances. A positive *t*(MHC:genomic locus) of ≥2 indicates that the MHC locus is more closely associated with a PML body than is the test genomic locus. The degrees of freedom for each *t*-statistic is one less than the number of cells.

§The MHC locus in this experiment is contained within the YAC on one copy of chromosome 18.

¶The MHC locus in this experiment is represented by the 18q11 locus adjacent to the MHC YAC integration site; see Fig. 8.

Abbreviations: $\overline{X}_{PML}$, mean minimal PML-PML distance (μm); $\overline{X}_{MHC}$, mean minimal MHC-PML distance (μm); $\overline{X}_{genomic\ locus}$, mean minimal genomic locus-PML distance (μm); N, number of cells.

the statistical significance of specific genomic locus-PML associations within the same nucleus, where a *t*-statistic can be calculated (see Materials and Methods). A large negative *t*-statistic ($t \leq -2$) indicates a significant genomic locus-PML body association. More importantly, by examining PML associations with two different genomic loci within the same nucleus, a paired *t*-test can be performed to allow direct statistical comparisons of specific genomic locus-PML associations.

By applying this statistical approach to our immuno-FISH observations, we find striking differences for the association of PML bodies with each of the three genomic loci studied. In a set of 55 nuclei, the MHC-PML mean minimal distance ($\overline{X}_{MHC}$=1.51μm) is less than the mean minimal PML-PML distance ($\overline{X}_{PML}$=2.15μm; *t*=–9.38; Table 1), indicating that the MHC region tends to be more closely associated with a PML body than PML bodies are to themselves. In the same set of nuclei, the mean minimal EDC-PML distance ($\overline{X}_{EDC}$=1.88 μm) is intermediate between distances for $\overline{X}_{MHC}$ and $\overline{X}_{PML}$ and has a less negative, although still significant, *t*-statistic (*t*=–3.19; Table 1). Direct comparison, however, of MHC-PML and EDC-PML associations clearly shows that the MHC region is more closely associated with a PML body than is the EDC region (*P*=0.0002; Table 1). A similar comparison of PML body associations with the MHC and 6p24 regions again confirms that the MHC-PML association is statistically significant, whereas the 6p24 region is less closely associated (*P*<0.0001; Table 1). A summary of the distributions of minimal genomic locus-PML distances is given in Fig. 4, with the smaller MHC-PML mean minimal distances (compared with EDC and 6p24) clearly demonstrated.

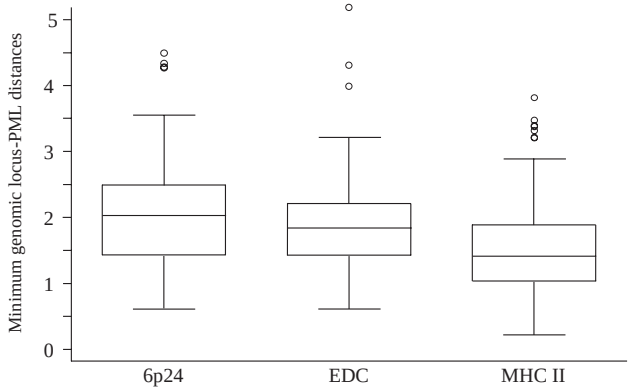

Fig. 4. Comparison and distribution of minimal genomic locus-PML body distances. The distribution of minimum distances between each genomic locus and its nearest PML body is shown as a box and whiskers plot, where the box extends from the lower quartile (where 25% of observed genomic locus-PML body distances are smaller), to the upper quartile (where 25% of observed genomic locus-PML body distances are larger) and the horizontal line in the middle of the box is the median. The small circles show outlying points and the ‘whiskers’ extending from the box show the range of minimum genomic locus-PML body distances. Note that the distribution of minimal MHC-PML distances is clearly shorter in comparison to the minimal EDC-PML and 6p24-PML distances.

Alterations in transcriptional activity do not affect the MHC-PML association

The increased association of PML bodies with the MHC region could suggest an association that is dependent upon the transcriptional status of the region. We therefore tested the effects of both positive and negative regulators of global transcription on the observed MHC-PML association. The overall transcriptional activity of the MHC region is increased using IFNγ (Boehm et al., 1997; Girdlestone, 1996; Rohn et al., 1996), including the specific upregulation of the LMP and TAP genes (Seliger et al., 1997) (data not shown). IFNγ also induces PML gene expression as part of an anti-viral response pathway (Lavau et al., 1995), although the concentration of IFNγ used here causes an increase in PML body size only and does not increase PML body number (data not shown). In IFNγ-treated fibroblasts, we find no statistically significant difference in the MHC-PML association compared with untreated fibroblasts, despite upregulation of the LMP and TAP genes (compare $\overline{X}_{MHC}$ values in untreated and treated cells;

Table 2; Fig. 5A,B). This shows that, within the sample of fibroblasts studied, any IFNγ-induced increase in MHC transcriptional activity is unlikely to result in an increased MHC-PML association.

We next tested the effects of the global transcription inhibitors α-amanitin (Nguyen et al., 1996) and DRB (Haaf and Ward, 1996) on MHC-PML association. Mammalian fibroblasts were treated with either α-amanitin or DRB, with both treatments resulting in complete inhibition of LMP2 and TAP1 transcription (data not shown). Immuno-FISH of treated and untreated cells allows us to test for changes in observed MHC-PML associations. We find that although fibroblasts treated with α-amanitin have a larger mean minimal MHC-PML distance ($\overline{X}_{MHC}$=1.75 μm) compared with untreated cells ($\overline{X}_{MHC}$=1.49 μm; Table 2), this difference is not statistically significant (*P*=0.06). These results show that alterations in global transcription do not effect the association of PML bodies with the MHC region. The spatial distribution of PML bodies also appears unaffected by α-amanitin treatment in the majority of nuclei (Table 2), although in ~10% of cells we observe a clustering of PML bodies not present in untreated fibroblasts (compare Fig. 5A and C). These cells were subsequently excluded from our analysis. The effect on PML body distribution was more pronounced in DRB-treated cells, where in ~36% of cells an aberrant PML clustering was observed (an example of the severe PML clustering observed is shown in Fig. 5D). This clustering and fusing of PML bodies prevents both an accurate estimate of PML body centroid positions and any subsequent statistical analysis.

MHC-PML association can occur throughout the cell cycle

From our studies it is clear that the MHC region does not associate with PML bodies in all nuclei examined (Fig. 2). This could suggest that the observed MHC-PML association is either a transient event, occurring during a certain stage of the cell cycle, or a dynamic event, occurring throughout the cell cycle. To investigate this further, fibroblasts at two different stages of the cell cycle were examined and the MHC-PML association at each stage compared. To differentiate between G_1 and S-phase nuclei, bromodeoxyuridine (BrdU) was incorporated into unsynchronised mammalian fibroblasts, labelling only those cells in S-phase with actively replicating DNA. Immuno-FISH was used to visualise PML bodies, MHC regions and the newly replicating DNA simultaneously. Cells in G_1 were identified by both the absence of BrdU incorporation and the presence of two MHC regions (Fig. 5E). Cells in S-phase were visualised by the presence of BrdU incorporation (Fig. 5F).

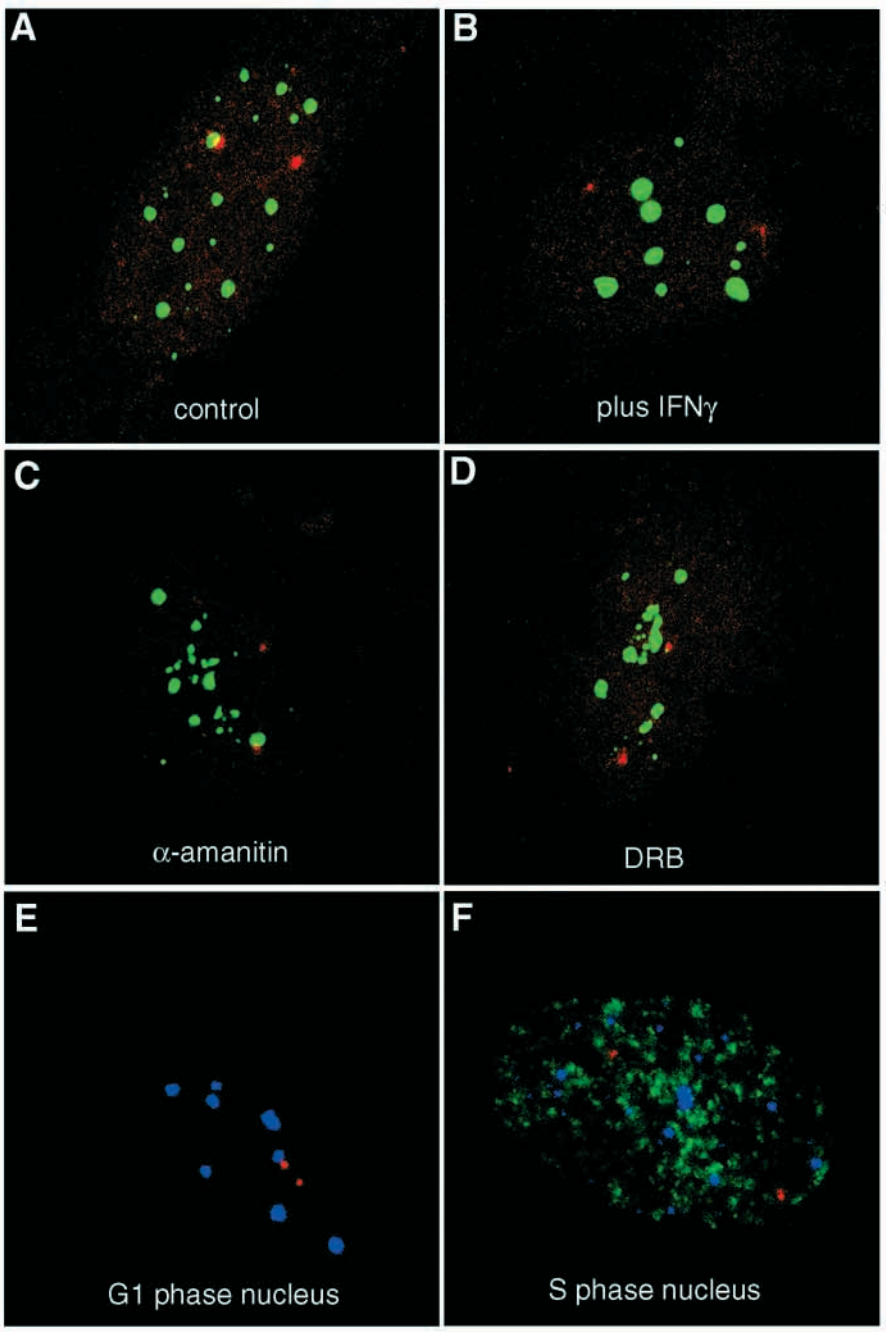

Fig. 5. Further analysis of MHC-PML association. (A-D) Immuno-FISH was used to examine the MHC-PML association in untreated control fibroblasts (A) and compared with the association found in fibroblasts after treatment with interferon-γ (B), α-amanitin (C) and DRB (D). PML bodies were detected by immunofluorescence (green) and the MHC region was visualised simultaneously using genomic probes recognising the LMP/TAP region (red). Note in C and D the clustering of PML bodies observed in a percentage of cells after transcriptional inhibition. (E-F) The MHC-PML association was also examined in fibroblast nuclei during G_1 phase (E) and S phase (F) of the cell cycle, detecting PML bodies (blue) and BrdU (green) by immunofluorescence, and visualising the MHC region by FISH using genomic probes recognising the LMP/TAP region (red). Projections of optical sections through each nucleus are shown.

Table 2. Statistical analysis of MHC-PML association in cells under different experimental conditions and in different cell types

Cells	Genomic locus	N	$\overline{X}_{PML}$	$\overline{X}_{genomic\ locus}$	$\overline{X}_{PML}$-$\overline{X}_{genomic\ locus}$	*t*(genomic locus)*	*t*(cells)‡
MRC5 untreated	MHC(LMP/TAP)	47	2.24	1.65	–	–8.06	
MRC5+IFNγ	"	46	2.28	1.63	–	–6.48	
Untreated vs treated							0.14 (*P*=0.89)
MRC5 untreated	MHC(LMP/TAP)	32	2.08	1.49	–	–5.42	
MRC5+α-amanitin	"	28	2.16	1.75	–	–3.85	
Untreated vs treated							–1.90 (*P*=0.06)
G_1 phase MRC5	MHC(LMP/TAP)	30	2.12	1.66	0.46	–4.97	
S phase MRC5	"	31	1.79	1.37	0.42	–6.04	
G_1 vs S phase							0.31 (*P*=0.76)§
721.174 B cells	DAXX	37	1.87	1.49	–	–4.40	
Control B cells	"	30	1.81	1.27	–	–8.08	
721.174 vs control							1.69 (*P*=0.09)

*The significance of genomic locus-PML associations was calculated using a one sample *t*-test, as described in Materials and Methods. Large negative values ($t \leq -2$) indicate that the MHC or genomic locus tend to be closer to a PML body than PML bodies are to themselves.
‡An unpaired *t*-test was used to compare genomic locus-PML distances between different sets of cells as indicated, *t*(cells) values ±2 indicate that there is unlikely to be any significant difference in the association between the two data sets. The degrees of freedom for each *t*-statistic is two less than the number of cells.
§The *t*(cells) for this experiment was calculated based on X_{PML} - $X_{genomic\ locus}$.
Abbreviations: $\overline{X}_{PML}$, mean minimal PML-PML distance (μm); $\overline{X}_{MHC}$, mean minimal MHC-PML distance (μm); $\overline{X}_{genomic\ locus}$, mean minimal genomic locus-PML distance (μm); N, number of cells.

We find that the association of PML bodies with the MHC region is statistically significant at both stages of the cell cycle (*t*=–4.97 in G_1, *t*=–6.04 in S-phase; Table 2). However, a comparison of MHC-PML associations between both cell populations (based on $\overline{X}_{MHC}$) is complicated by the increased number of PML bodies in S-phase cells compared with G_1 [also noted previously (Terris et al., 1995)], resulting in a significant decrease in $\overline{X}_{PML}$ and $\overline{X}_{MHC}$ distances in S-phase cells (Table 2). Instead, we compared the MHC-PML association in S and G_1-phase cells based on the difference between $\overline{X}_{PML}$ and $\overline{X}_{MHC}$ distances within each cell population ($\overline{X}_{PML}$–$\overline{X}_{MHC}$=0.46 μm in G_1 compared with $\overline{X}_{PML}$–$\overline{X}_{MHC}$=0.42 μm in S-phase; Table 2). We find that these distances are not significantly different (*P*=0.76) and conclude that the observed MHC-PML association, within the sample of fibroblasts studied, occurs throughout the cell cycle and that there is no significant difference in MHC-PML association between S and G_1-phase cells.

Mapping the PML association along the short arm of chromosome 6

We next examined PML body association with other genomic regions along the short arm of chromosome 6 (Fig. 6). Adjacent regions were examined in combination with the LMP/TAP locus and their relative PML associations compared. The adjacent DAXX, BAK and TCP11 loci, (~500 kb, 700 kb and 1600 kb centromeric of the LMP/TAP region respectively; see Fig. 1B), have remarkably similar mean PML distances compared to those for the LMP/TAP region (Table 1). Each of the genomic regions are as close to a PML body as is the LMP/TAP region, with the DAXX locus marginally closer (*P*=0.01; Table 1). In contrast, more distant genomic regions, such as the MHC class I region located ~3 Mb telomeric of the LMP/TAP region, show an increase in their mean minimal genomic locus-PML distance (Fig. 6), and are not as closely associated with a PML body as is the LMP/TAP region (*P*=0.0002, Table 1). Similarly, genomic regions several tens of megabases distant of the MHC region such as the 6p24 region (as discussed previously) and the centromere region of chromosome 6, are not as closely associated with PML bodies when compared with the LMP/TAP region (Table 1; Fig. 6).

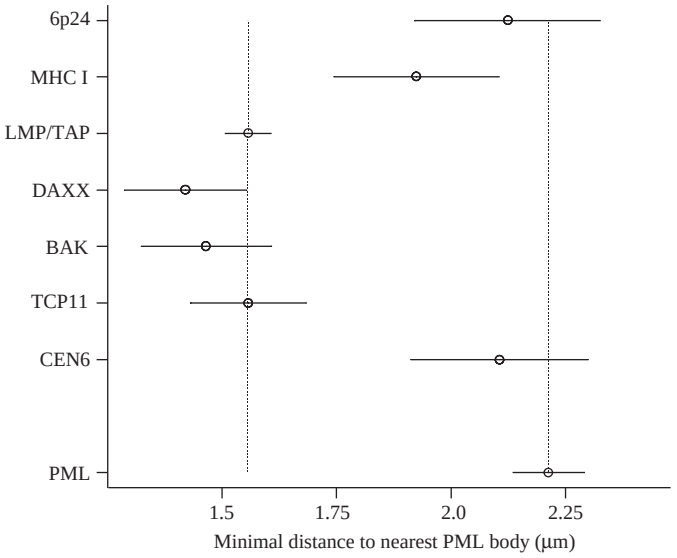

Fig. 6. Graphical representation of minimal distances to nearest PML body for each genomic locus along the short arm of chromosome 6. A small circle marks the mean distance and the horizontal line extends 2 standard errors either side of the mean. The standard errors are calculated based on a single mean minimum distance from each nucleus. Note that the error bars for PML-PML and LMP/TAP-PML distances are shorter than the others, because these distances were measured in all the experiments. The dashed vertical lines are at the mean LMP/TAP-PML and mean PML-PML distances.

MHC-PML association in relation to other nuclear domains

PML bodies have previously been shown to associate with other nuclear foci or domains including Cajal bodies (Grande et al., 1996) and cleavage bodies (Schul et al., 1996). Since Cajal bodies are well characterised in terms of their nuclear distribution and protein components, we examined the spatial arrangement of Cajal bodies in relation to the observed MHC-PML associations. We used HeLa cells, where Cajal bodies can be clearly visualised with an anti-p80-coilin antibody (Almeida et al., 1998), and their association with the histone gene cluster on chromosome 6 (Fig. 1A) has previously been reported (Frey and Matera, 1995). Fig. 7 shows an MHC-PML body association (top inset) and although a number of coiled bodies are found in this nucleus, they are clearly not involved in the observed association, suggesting that the MHC-PML association is independent of Cajal bodies. Further examination of this nucleus also reveals a PML-Cajal body association (middle inset) as previously observed (Grande et al., 1996). Interestingly, an association between a Cajal body and the MHC region can also be observed (lower inset). We attribute this to the known Cajal body-histone gene cluster association (Frey et al., 1999), given the close proximity of the MHC to this region on chromosome 6 (Fig. 1A). Together, these data show that the associations between PML and Cajal bodies with gene-rich regions of chromosome 6 are independent of one another.

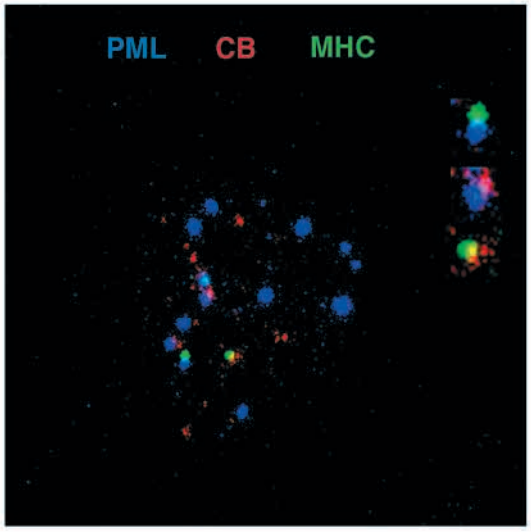

Fig. 7. The spatial distribution of PML bodies and coiled bodies with respect to the MHC region. The relative associations of PML bodies (blue), coiled bodies (CB) (red) and MHC regions (green) were examined in three dimensionally preserved HeLa cell nuclei. Insets on the right (from top to bottom) show examples of associations between PML bodies and the MHC region, PML bodies and coiled bodies, and coiled bodies and the MHC region. A projection of optical sections through the nucleus is shown.

Specificity of the MHC-PML association

To investigate the specificity of the observed MHC-PML body association, a B-lymphoblastoid cell line (721.174) monosomic for the short arm of chromosome 6 and containing a large deletion of the MHC class II region was used (Fig. 1B). This cell line allows us to determine whether the observed MHC-PML association is dependent upon the presence of MHC sequences from DRA to LMP/TAP. The mean minimal DAXX-PML distance in this cell line is significantly smaller (1.49 μm; t=–4.40) than the mean minimal PML-PML distance (1.87 μm; Table 2), indicating that removal of the DRA-LMP/TAP region does not disrupt PML body associations with the remaining MHC class II region. In fact, the DAXX-PML association in 721.174 cells is not significantly different to that found in a control B-lymphoblastoid cell line (AHB) containing two intact copies of the MHC region (Fig. 8A,B; Table 2). These results suggested that genomic sequences from DRA to LMP/TAP may not be involved in specific PML body association, however it is possible that more than one region within the MHC gene cluster associates independently with PML bodies. To investigate this further, we next examined a derivative of the 721.174 B-cell line (4D1D10.2) that resulted from stable transfections of YACs containing a large proportion of the deleted MHC class II region, including the LMP and TAP genes (Fabb et al., 1997) (Fig. 1). In this cell line, several copies of the YAC have been integrated into the long arm of chromosome 18 close to the centromere and the MHC genes encoded by the YAC are functional, although their gene products are expressed at reduced levels (Fabb et al., 1997). Using the 4D1D10.2 cell line, PML bodies were visualised together with the MHC class II YAC region on chromosome 18 and the DAXX region on chromosome 6 (Fig. 8C). The results show PML bodies to have a statistically significant association with both the DAXX region on chromosome 6 (t=–6.27) and the MHC class II YAC region integrated into chromosome 18 (t=–3.41). Furthermore, the mean minimal YAC-PML distance (1.62 μm) is similar to the mean minimal DAXX-PML distance (1.55 μm) as are their respective PML associations (P=0.7; Table 1).

To investigate whether the observed association between PML bodies and the MHC class II YAC DNA is due to the presence of class II genomic sequences, we investigated the association of normal chromosome 18 with PML bodies in the 4D1D10.2 cell line. We used as a probe a partial chromosome paint that hybridises close to the YAC integration site on 18q (www.biologia.uniba.it/rmc) to delineate the integration site. By visualising the 18q11 and MHC class II YAC regions together, we are able to differentiate between the copy of chromosome 18 that contains the class II YAC and the normal copy of chromosome 18 that contains no exogenous sequences (Fig. 8D). When mean minimal 18q11-PML distances for each copy are compared (Table 1), it is striking that only the copy of chromosome 18 containing the MHC class II YAC significantly associates with PML bodies (t=–3.98; Table 1). In the absence of the class II YAC, PML bodies have no statistically significantly association with this region of chromosome 18 (t=–1.50; Table 1).

DISCUSSION

There has been much speculation as to the functional role of PML bodies within the nucleus (Ruggero et al., 2000). The finding that many proteins localising to PML bodies are involved in the control of transcription, coupled with the observation that viruses transcribe their genomes at PML

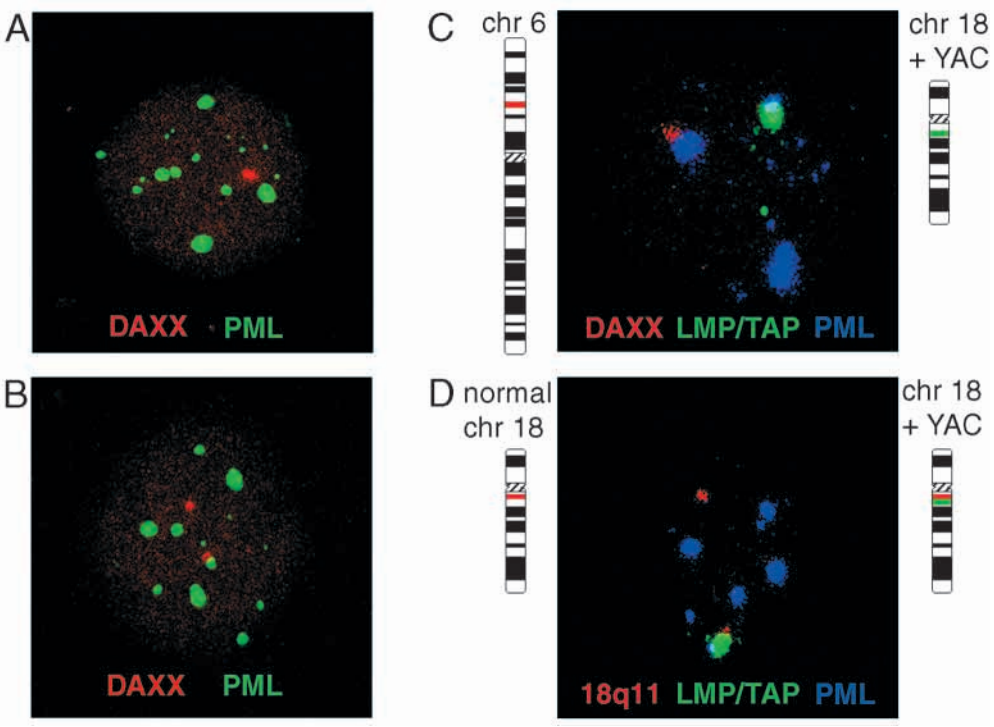

Fig. 8. Specificity of the MHC-PML association. (A-B) MHC-PML association was investigated in the 721.174 B lymphoblastoid cell line (A) and the control B-lymphoblastoid cell line AHB (B) by immuno-FISH on three-dimensionally preserved nuclei. 721.174 cells have only one copy of the short arm of chromosome 6 containing a large deletion of the MHC class II region (from DRA to LMP/TAP). The MHC region was visualised using genomic probes recognising the adjacent DAXX region (red) and PML bodies were detected simultaneously by immunofluorescence (green). (C-D) MHC-PML association was investigated in a derivative of the 721.174 cell line (4D1D10.2), resulting from stable integration of an MHC YAC into chromosome 18. (C) The MHC class II YAC DNA on chromosome 18 was detected using genomic probes recognising the LMP/TAP region (green) and the residual MHC region on chromosome 6 using genomic probes recognising the DAXX region (red). (D) The relative association of PML bodies with each copy of chromosome 18 was determined using a partial chromosome paint (red) to delineate the YAC integration site on band 18q11 and the MHC class II YAC DNA was detected using genomic probes recognising the LMP/TAP region (green). The approximate chromosomal position of these regions is illustrated on the ideograms.

bodies, has suggested that these domains may play a direct role in transcriptional regulation (Zhong et al., 2000c). This view was recently supported by the finding that acetylated chromatin and nascent RNA accumulate at the surface of PML bodies, suggesting that PML bodies are directly associated with transcriptionally active genes (Boisvert et al., 2000). Furthermore, PML has been shown to both repress and activate gene transcription (Ruggero et al., 2000), possibly through direct physical interactions with transcription factors such as Sp1 (Vallian et al., 1998), co-activators such as CBP (LaMorte et al., 1998; von Mikecz et al., 2000; Zhong et al., 2000b) and co-repressors such as pRb (Alcalay et al., 1998). Given these data, the spatial location and association of PML bodies with other nuclear components is clearly of functional importance. It is well established that PML bodies are nuclear matrix associated (Stuurman et al., 1992) and recent data now indicates that they are static structures within the nuclear volume (Plehn-Dujowich et al., 2000). The data reported here provides the first evidence for a PML body-chromosomal association and in particular a specific association with the MHC gene cluster on chromosome 6.

For this study, we chose three genomic regions, the MHC, the EDC and the 6p24 region, on the basis that they differ in gene density and transcriptional activity in primary fibroblasts. We used a combination of immunofluorescence and FISH (immuno-FISH) to co-label PML bodies and specific chromosome regions, and devised a statistical method based on minimal distances to provide a quantitative basis for our analyses. The method we have developed treats fluorescent foci as single points in cartesian space and thereby overcomes any difficulties associated with differential or inefficient fluorescence of labelling fixed cells. By analysing these centroid co-ordinates, we were able to directly compare the distances between specific genomic regions and PML bodies and carry out a number of statistical tests.

From this statistical analysis, we find that the MHC gene cluster is more strongly associated with PML bodies than is the EDC region on chromosome 1 or the transcriptionally silent region 6p24. This finding is of particular interest, since it has previously been shown that PML can upregulate the transcription of the MHC class II TAP and LMP genes (Zheng et al., 1998). One possible explanation for our observations could be the position of gene-rich regions (compared with gene-poor regions) within chromosomal territories. Both individual genes and gene-rich regions have been shown to be preferentially localised at the periphery of chromosomal territories (Kurz et al., 1996; Volpi et al., 2000), similarly, PML bodies tend to be positioned either at the border of (or are excluded from) chromosomal territories (Bridger et al., 1998). Thus PML bodies localised to the inter-chromosomal space are by inference in close proximity to gene-coding DNA. However, this does not explain the clear preferential association of the MHC region for PML bodies both in normal primary fibroblasts and in B-cells where MHC class II sequences have been artificially integrated into chromosome 18. These latter data show that the observed MHC-PML associations appear sequence specific and can be mediated by particular genomic sequences, irrespective of their chromosomal location. Theoretical matrix attachment region (MAR) sequences (van Drunen et al., 1999) have recently been identified in the MHC class II region and have been shown to bind to the nuclear matrix (R. Horton and R. Donev, personal communication).

Since PML bodies are also tightly associated with the nuclear matrix (Stuurman et al., 1992), it is possible that the MHC region may associate with PML bodies through its MAR sequences. The association of genes and transcription factors with the nuclear matrix has been suggested to lead to the formation of nuclear domains that facilitate transcriptional control (Stein et al., 1998). Our data now provide support to the intriguing possibility that PML bodies associate with certain gene-rich clusters as part of a functional compartment involved in gene regulation. We are currently investigating the minimum sequence requirements for PML body association.

Previous studies have suggested that PML can upregulate the transcription of the MHC class II TAP and LMP genes (Zheng et al., 1998). One could speculate that any MHC-PML body association is related to these previous observations, where PML protein is required to actively promote transcription of specific MHC genes (via unknown mechanisms). However, some doubt has arisen about the role of PML in regulating LMP and TAP transcription. APL cells that lack functional PML express normal levels of MHC class I molecules (Larghero et al., 1999), as do PML$^{-/-}$ cells derived from knockout mice (Ruggero et al., 2000). Our studies also show that the observed MHC-PML association is not directly dependent upon the presence of LMP and TAP sequences, since the association is maintained even when a substantial portion of the class II region containing these genes is deleted. Interestingly, in the primary fibroblasts studied, the number of MHC genes that are transcriptionally active is considerably higher compared with the EDC region. So it is possible that the transcriptional activity of a particular genomic locus influences the frequency of PML body association. Our experiments using positive and negative regulators of transcription would argue against this, as changes in transcriptional status did not significantly alter the observed MHC-PML association. We note that both IFNγ and global transcriptional inhibitors have pleiotropic effects and do not specifically target MHC genes. Also, it is well established that PML is upregulated by IFNγ (Lavau et al., 1995), but we see little change in the MHC-PML body association after IFNγ treatment. This suggests that the observed PML body associations appear independent of the concentration of PML protein. The static nature of both chromatin (Shelby et al., 1996) and PML bodies (Plehn-Dujowich et al., 2000) would suggest that once associations have been formed, they are maintained irrespective of the status of the cell. This is further highlighted by our results during different stages of the cell cycle, where little change in MHC-PML association is observed.

What is the functional significance of a MHC-PML body association? If PML bodies play a role in the transcriptional regulation of the MHC, it is difficult to explain why other functionally unrelated proteins, with no obvious involvement in transcription, localise to PML bodies (Borden et al., 1998). Although there may be functional heterogeneity between different PML bodies, another possibility could be that PML bodies coordinate and regulate the concentrations of active proteins (Maul, 1998). Evidence for this includes the observation that misfolded viral proteins, ubiquitin and proteosomal components can localise to PML bodies, suggesting that PML bodies serve as sites for protein degradation (Anton et al., 1999). Of particular interest are recent observations that transcriptional activators can be targeted in vivo for rapid ubiquitin-mediated degradation at sites of active transcription (Molinari et al., 1999; Salghetti et al., 2000). Combining these observations with our own data leads to the following plausible hypothesis. PML bodies associate with certain gene-rich genomic regions and in particular the MHC, to form part of a functional compartment involved in regulating the transcription of that particular region. We note that PML bodies are often associated with other nuclear domains including Cajal bodies (Grande et al., 1996) supporting the notion of discrete functional compartments comprising several multi-protein complexes or bodies juxtaposed to specific chromosomal regions. Specific proteins including transcription factors, co-activators and co-repressors could be targeted for degradation at PML bodies via the proteasome-mediated pathway or stored for subsequent use via the SUMO-1 modification pathway. The observed specific MHC-PML association could thus be necessary for transcriptional regulation of certain genes within the MHC region. The demonstration that PML interacts with the transcription factor Sp1, inhibiting its transactivation properties (Vallian et al., 1998), and the fact that Sp1 is involved in the constitutive expression of a number of genes within the MHC region, including LMP and TAP (Wright et al., 1995), would support this theory.

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REFERENCES

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

Almeida, F., Saffrich, R., Ansorge, W. and Carmo-Fonseca, M. (1998). Microinjection of anti-coilin antibodies affects the structure of coiled bodies. *J. Cell Biol.* **142**, 899-912.

Anton, L. C., Schubert, U., Bacik, I., Princiotta, M. F., Wearsch, P. A., Gibbs, J., Day, P. M., Realini, C., Rechsteiner, M. C., Bennink, J. R. et al. (1999). Intracellular localization of proteasomal degradation of a viral antigen. *J. Cell Biol.* **146**, 113-124.

Beck, S., Abdulla, S., Alderton, R. P., Glynne, R. J., Gut, I. G., Hosking, L. K., Jackson, A., Kelly, A., Newell, W. R., Sanseau, P. et al. (1996). Evolutionary dynamics of non-coding sequences within the class II region of the human MHC. *J. Mol. Biol.* **255**, 1-13.

Boehm, U., Klamp, T., Groot, M. and Howard, J. C. (1997). Cellular responses to interferon-gamma. *Annu. Rev. Immunol.* **15**, 749-795.

Boisvert, F. M., Hendzel, M. J. and Bazett-Jones, D. P. (2000). Promyelocytic leukemia (PML) nuclear bodies are protein structures that do not accumulate RNA. *J. Cell Biol.* **148**, 283-292.

Borden, K. L., Boddy, M. N., Lally, J., O'Reilly, N. J., Martin, S., Howe, K., Solomon, E. and Freemont, P. S. (1995). The solution structure of the RING finger domain from the acute promyelocytic leukaemia proto-oncoprotein PML. *EMBO J.* **14**, 1532-1541.

Borden, K. L., Campbelldwyer, E. J., Carlile, G. W., Djavani, M. and Salvato, M. S. (1998). Two RING finger proteins, the oncoprotein PML and

the arenavirus Z protein, colocalize with the nuclear fraction of the ribosomal P proteins. *J. Virol.* **72**, 3819-3826.

Bridger, J. M., Herrmann, H., Munkel, C. and Lichter, P. (1998). Identification of an interchromosomal compartment by polymerization of nuclear-targeted vimentin. *J. Cell Sci.* **111**, 1241-1253.

Carter, K. C., Taneja, K. L. and Lawrence, J. B. (1991). Discrete nuclear domains of poly(A) RNA and their relationship to the functional organization of the nucleus. *J. Cell Biol.* **115**, 1191-1202.

Davies, A. F., Imaizumi, K., Mirza, G., Stephens, R. S., Kuroki, Y., Matsuno, M. and Ragoussis, J. (1998). Further evidence for the involvement of human chromosome 6p24 in the aetiology of orofacial clefting. *J. Med. Genet.* **35**, 857-861.

DeMars, R., Chang, C. C., Shaw, S., Reitnauer, P. J. and Sondel, P. M. (1984). Homozygous deletions that simultaneously eliminate expressions of class I and class II antigens of EBV-transformed B-lymphoblastoid cells. I. Reduced proliferative responses of autologous and allogeneic T cells to mutant cells that have decreased expression of class II antigens. *Hum. Immunol.* **11**, 77-97.

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

Dyck, J. A., Maul, G. G., Miller, W. H., Jr., Chen, J. D., Kakizuka, A. and Evans, R. M. (1994). A novel macromolecular structure is a target of the promyelocyte- retinoic acid receptor oncoprotein. *Cell* **76**, 333-343.

Ebisawa, T., Tada, K., Kitajima, I., Tojo, K., Sampath, T. K., Kawabata, M., Miyazono, K. and Imamura, T. (1999). Characterization of bone morphogenetic protein-6 signaling pathways in osteoblast differentiation. *J. Cell Sci.* **112**, 3519-3527.

Fabb, S. A., Davies, A. F., Correa, I., Kelly, A. P., Mackie, C., Trowsdale, J. and Ragoussis, J. (1997). Generation of novel human MHC class II mutant B-cell lines by integrating YAC DNA into a cell line homozygously deleted for the MHC class II region. *Hum. Mol. Genet.* **6**, 1295-1304.

Ferguson, M. and Ward, D. C. (1992). Cell cycle dependent chromosomal movement in pre-mitotic human T-lymphocyte nuclei. *Chromosoma* **101**, 557-565.

Frey, M. R. and Matera, A. G. (1995). Coiled bodies contain U7 small nuclear RNA and associate with specific DNA sequences in interphase human cells. *Proc. Natl. Acad. Sci. USA* **92**, 5915-5919.

Frey, M. R., Bailey, A. D., Weiner, A. M. and Matera, A. G. (1999). Association of snRNA genes with coiled bodies is mediated by nascent snRNA transcripts. *Curr. Biol.* **9**, 126-135.

Girdlestone, J. (1996). Transcriptional regulation of MHC class I genes. *Eur J. Immunogenet.* **23**, 395-413.

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

Goldsworthy, M., Sampath, A. and Powis, S. H. (1996). Isolation and ordering of HLA class I region cosmids. *DNA Seq.* **7**, 27-30.

Grande, M. A., van der Kraan, I., van Steensel, B., Schul, W., de The, H., van der Voort, H. T., de Jong, L. and van Driel, R. (1996). PML-containing nuclear bodies: their spatial distribution in relation to other nuclear components. *J. Cell Biochem.* **63**, 280-291.

Haaf, T. and Ward, D. C. (1996). Inhibition of RNA polymerase II transcription causes chromatin decondensation, loss of nucleolar structure, and dispersion of chromosomal domains. *Exp. Cell Res.* **224**, 163-173.

Herberg, J. A., Beck, S. and Trowsdale, J. (1998a). TAPASIN, DAXX, RGL2, HKE2 and four new genes (BING 1, 3 to 5) form a dense cluster at the centromeric end of the MHC. *J. Mol. Biol.* **277**, 839-857.

Herberg, J. A., Phillips, S., Beck, S., Jones, T., Sheer, D., Wu, J. J., Prochazka, V., Barr, P. J., Kiefer, M. C. and Trowsdale, J. (1998b). Genomic structure and domain organisation of the human Bak gene. *Gene* **211**, 87-94.

Hodges, M., Tissot, C., Howe, K., Grimwade, D. and Freemont, P. S. (1998). Structure, organization, and dynamics of promyelocytic leukemia protein nuclear bodies. *Am. J. Hum. Genet.* **63**, 297-304.

Huang, Y. and Domann, F. E. (1999). Transcription factor AP-2 mRNA and DNA binding activity are constitutively expressed in SV40-immortalized but not normal human lung fibroblasts. *Arch. Biochem. Biophys.* **364**, 241-246.

Ishov, A. M., Stenberg, R. M. and Maul, G. G. (1997). Human cytomegalovirus immediate early interaction with host nuclear structures: definition of an immediate transcript environment. *J. Cell Biol.* **138**, 5-16.

Ishov, A. M., Sotnikov, A. G., Negorev, D., Vladimirova, O. V., Neff, N., Kamitani, T., Yeh, E. T., Strauss, J. F., 3rd and Maul, G. G. (1999). PML is critical for ND10 formation and recruits the PML-interacting protein Daxx to this nuclear structure when modified by SUMO-1 [In Process Citation]. *J. Cell Biol.* **147**, 221-234.

Kakizuka, A., Miller, W. H., Jr., Umesono, K., Warrell, R. P., Jr., Frankel, S. R., Murty, V. V., Dmitrovsky, E. and Evans, R. M. (1991). Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor, PML. *Cell* **66**, 663-674.

Koken, M. H., Puvion-Dutilleul, F., Guillemin, M. C., Viron, A., Linares-Cruz, G., Stuurman, N., de Jong, L., Szostecki, C., Calvo, F., Chomienne, C. et al. (1994). The t(15;17) translocation alters a nuclear body in a retinoic acid- reversible fashion. *EMBO J.* **13**, 1073-1083.

Kurz, A., Lampel, S., Nickolenko, J. E., Bradl, J., Benner, A., Zirbel, R. M., Cremer, T. and Lichter, P. (1996). Active and inactive genes localize preferentially in the periphery of chromosome territories. *J. Cell Biol.* **135**, 1195-1205.

LaMorte, V. J., Dyck, J. A., Ochs, R. L. and Evans, R. M. (1998). Localization of nascent RNA and CREB binding protein with the PML-containing nuclear body. *Proc. Natl. Acad. Sci. USA* **95**, 4991-4996.

Larghero, J., Zassadowski, F., Rousselot, P., Padua, R. A., Degos, L. and Chomienne, C. (1999). Alteration of the PML proto-oncogene in leukemic cells does not abrogate expression of MHC class I antigens. *Leukemia* **13**, 1295-1296.

Lavau, C., Marchio, A., Fagioli, M., Jansen, J., Falini, B., Lebon, P., Grosveld, F., Pandolfi, P. P., Pelicci, P. G. and Dejean, A. (1995). The acute promyelocytic leukaemia-associated PML gene is induced by interferon. *Oncogene* **11**, 871-876.

Maul, G. G. (1998). Nuclear domain 10, the site of DNA virus transcription and replication. *Bioessays* **20**, 660-667.

Maul, G. G., Jensen, D. E., Ishov, A. M., Herlyn, M. and Rauscher, F. J., 3rd. (1998). Nuclear redistribution of BRCA1 during viral infection. *Cell Growth Differ.* **9**, 743-755.

Maul, G. G., Negorev, D., Bell, P. and Ishov, A. M. (2000). Review: properties and assembly mechanisms of ND10, PML bodies, or PODs. *J. Struct. Biol.* **129**, 278-287.

Milner, C. M. and Campbell, R. D. (1992). Genes, genes and more genes in the human major histocompatibility complex. *Bioessays* **14**, 565-571.

Molinari, E., Gilman, M. and Natesan, S. (1999). Proteasome-mediated degradation of transcriptional activators correlates with activation domain potency in vivo. *EMBO J.* **18**, 6439-6447.

Muller, S., Matunis, M. J. and Dejean, A. (1998). Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of PML within the nucleus. *EMBO J.* **17**, 61-70.

Nguyen, V. T., Giannoni, F., Dubois, M. F., Seo, S. J., Vigneron, M., Kedinger, C. and Bensaude, O. (1996). In vivo degradation of RNA polymerase II largest subunit triggered by alpha-amanitin. *Nucleic Acids Res.* **24**, 2924-2929.

Okada, H., Danoff, T. M., Fischer, A., Lopez-Guisa, J. M., Strutz, F. and Neilson, E. G. (1998). Identification of a novel cis-acting element for fibroblast-specific transcription of the FSP1 gene. *Am. J. Physiol.* **275**, F306-F314.

Olavesen, M. G., Bentley, E., Mason, R. V., Stephens, R. J. and Ragoussis, J. (1997). Fine mapping of 39 ESTs on human chromosome 6p23-p25. *Genomics* **46**, 303-306.

Plehn-Dujowich, D., Bell, P., Ishov, A. M., Baumann, C. and Maul, G. G. (2000). Non-apoptotic chromosome condensation induced by stress: delineation of interchromosomal spaces. *Chromosoma* **109**, 266-279.

Rohn, W. M., Lee, Y. J. and Benveniste, E. N. (1996). Regulation of class II MHC expression. *Crit. Rev. Immunol.* **16**, 311-330.

Ruggero, D., Wang, Z. G. and Pandolfi, P. P. (2000). The puzzling multiple lives of PML and its role in the genesis of cancer. *Bioessays* **22**, 827-835.

Salghetti, S. E., Muratani, M., Wijnen, H., Futcher, B. and Tansey, W. P. (2000). Functional overlap of sequences that activate transcription and signal ubiquitin-mediated proteolysis. *Proc. Natl. Acad. Sci. USA* **97**, 3118-3123.

Schafer, B. W. and Heizmann, C. W. (1996). The S100 family of EF-hand calcium-binding proteins: functions and pathology. *Trends Biochem. Sci.* **21**, 134-140.

Schul, W., Groenhout, B., Koberna, K., Takagaki, Y., Jenny, A., Manders, E. M., Raska, I., van Driel, R. and de Jong, L. (1996). The RNA 3′ cleavage factors CstF 64 kDa and CPSF 100 kDa are concentrated in nuclear domains closely associated with coiled bodies and newly synthesized RNA. *EMBO J.* **15**, 2883-2892.

Seeler, J. S. and Dejean, A. (1999). The PML nuclear bodies: actors or extras? *Curr. Opin. Genet. Dev.* **9**, 362-367.

Seliger, B., Hammers, S., Hohne, A., Zeidler, R., Knuth, A., Gerharz, C. D. and Huber, C. (1997). IFN-gamma-mediated coordinated transcriptional regulation of the human TAP-1 and LMP-2 genes in human renal cell carcinoma. *Clin. Cancer Res.* **3**, 573-578.

Shelby, R. D., Hahn, K. M. and Sullivan, K. F. (1996). Dynamic elastic behavior of alpha-satellite DNA domains visualized in situ in living human cells. *J. Cell Biol.* **135**, 545-557.

Smith, K. P., Moen, P. T., Wydner, K. L., Coleman, J. R. and Lawrence, J. B. (1999). Processing of endogenous pre-mRNAs in association with SC-35 domains is gene specific. *J. Cell Biol.* **144**, 617-629.

South, A. P., Cabral, A., Ives, J. H., James, C. H., Mirza, G., Marenholz, I., Mischke, D., Backendorf, C., Ragoussis, J. and Nizetic, D. (1999). Human epidermal differentiation complex in a single 2.5 Mbp long continuum of overlapping DNA cloned in bacteria integrating physical and transcript maps. *J. Invest. Dermatol.* **112**, 910-918.

Spector, D. L. (1993). Macromolecular domains within the cell nucleus. *Annu. Rev. Cell Biol.* **9**, 265-315.

Stein, G. S., van Wijnen, A. J., Stein, J. L., Lian, J. B., Pockwinse, S. and McNeil, S. (1998). Interrelationships of nuclear structure and transcriptional control: functional consequences of being in the right place at the right time. *J. Cell Biochem.* **70**, 200-212.

Sternsdorf, T., Grotzinger, T., Jensen, K. and Will, H. (1997). Nuclear dots: actors on many stages. *Immunobiology* **198**, 307-331.

Stuurman, N., de Graaf, A., Floore, A., Josso, A., Humbel, B., de Jong, L. and van Driel, R. (1992). A monoclonal antibody recognizing nuclear matrix-associated nuclear bodies. *J. Cell Sci.* **101**, 773-784.

Szostecki, C., Guldner, H. H., Netter, H. J. and Will, H. (1990). Isolation and characterization of cDNA encoding a human nuclear antigen predominantly recognized by autoantibodies from patients with primary biliary cirrhosis. *J. Immunol.* **145**, 4338-4347.

Terris, B., Baldin, V., Dubois, S., Degott, C., Flejou, J. F., Henin, D. and Dejean, A. (1995). PML nuclear bodies are general targets for inflammation and cell proliferation. *Cancer Res* **55**, 1590-1597.

The MHC sequencing Consortium (1999). Complete sequence and gene map of a human major histocompatibility complex. *Nature* **401**, 921-923.

Tripodis, N., Mason, R., Humphray, S. J., Davies, A. F., Herberg, J. A., Trowsdale, J., Nizetic, D., Senger, G. and Ragoussis, J. (1998). Physical map of human 6p21.2-6p21.3: region flanking the centromeric end of the major histocompatibility complex. *Genome Res.* **8**, 631-643.

Trowsdale, J. (1995). 'Both man & bird & beast': comparative organization of MHC genes. *Immunogenetics* **41**, 1-17.

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

van Drunen, C. M., Sewalt, R. G., Oosterling, R. W., Weisbeek, P. J., Smeekens, S. C. and van Driel, R. (1999). A bipartite sequence element associated with matrix/scaffold attachment regions. *Nucleic Acids Res.* **27**, 2924-2930.

Verschure, P. J., van Der Kraan, I., Manders, E. M. and van Driel, R. (1999). Spatial relationship between transcription sites and chromosome territories. *J. Cell Biol.* **147**, 13-24.

Volpi, E. V., Chevret, E., Jones, T., Vatcheva, R., Williamson, J., Beck, S., Campbell, R. D., Goldsworthy, M., Powis, S. H., Ragoussis, J. et al. (2000). Large-scale chromatin organization of the major histocompatibility complex and other regions of human chromosome 6 and its response to interferon in interphase nuclei. *J. Cell Sci.* **113**, 1565-1576.

von Mikecz, A., Zhang, S., Montminy, M., Tan, E. M. and Hemmerich, P. (2000). CREB-binding protein (CBP)/p300 and RNA polymerase II colocalize in transcriptionally active domains in the nucleus. *J. Cell Biol.* **150**, 265-273.

Weis, K., Rambaud, S., Lavau, C., Jansen, J., Carvalho, T., Carmo-Fonseca, M., Lamond, A. and Dejean, A. (1994). Retinoic acid regulates aberrant nuclear localization of PML-RAR alpha in acute promyelocytic leukemia cells. *Cell* **76**, 345-356.

Wright, K. L., White, L. C., Kelly, A., Beck, S., Trowsdale, J. and Ting, J. P. (1995). Coordinate regulation of the human TAP1 and LMP2 genes from a shared bidirectional promoter. *J. Exp. Med.* **181**, 1459-1471.

Zheng, P., Guo, Y., Niu, Q., Levy, D. E., Dyck, J. A., Lu, S., Sheiman, L. A. and Liu, Y. (1998). Proto-oncogene PML controls genes devoted to MHC class I antigen presentation. *Nature* **396**, 373-376.

Zhong, S., Hu, P., Ye, T. Z., Stan, R., Ellis, N. A. and Pandolfi, P. P. (1999). A role for PML and the nuclear body in genomic stability. *Oncogene* **18**, 7941-7947.

Zhong, S., Salomoni, P., Ronchetti, S., Guo, A., Ruggero, D. and Pandolfi, P. P. (2000a). Promyelocytic leukemia protein (PML) and Daxx participate in a novel nuclear pathway for apoptosis. *J. Exp. Med.* **191**, 631-640.

Zhong, S., Muller, S., Ronchetti, S., Freemont, P. S., Dejean, A. and Pandolfi, P. P. (2000b). Role of SUMO-1-modified PML in nuclear body formation. *Blood* **95**, 2748-2752.

Zhong, S., Salomoni, P. and Pandolfi, P. P. (2000c). The transcriptional role of PML and the nuclear body. *Nat. Cell. Biol.* **2**, E85-E90.