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Functional Potential of P2P-R: A Role in the Cell Cycle and Cell Differentiation Related to its Interactions With Proteins That Bind to Matrix Associated Regions of DNA?

Robert E. Scott,[1,2]* Thomas Giannakouros,[1,2] Sizhi Gao,[1,2] and Philippos Peidis[1,2]

[1]Department of Pathology, University of Tennessee Health Science Center, Memphis, Tennessee 38163
[2]Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, Greece

Abstract P2P-R is the alternately spliced product of the P2P-R/PACT gene in that P2P-R lacks one exon encoding 34 amino acids. The 250 kDa P2P-R protein is the predominate product expressed in multiple murine cell lines. It is a highly basic protein that contains multiple domains including an N-terminal RING type zinc finger, a proline rich domain, an RS region, and a C-terminal lysine-rich domain. P2P-R binds the p53 and the Rb1 tumor suppressors and is phosphorylated by the cdc2 and SRPK1a protein kinases. P2P-R also interacts with scaffold attachment factor-B (SAF-B), a well characterized MARs (for matrix attachment regions) binding factor, and may interact with nucleolin, another MARs binding factor. In addition, P2P-R binds single strand DNA (ssDNA). The expression of P2P-R is regulated by differentiation and cell cycle events. P2P-R mRNA is markedly repressed during differentiation, whereas immunoreactive P2P-R protein levels are >10-fold higher in mitotic than in G_0 cells. The localization of P2P-R also is modulated during the cell cycle. During interphase, P2P-R is present primarily in nucleoli and nuclear speckles whereas during mitosis, P2P-R associates with the periphery of chromosomes. Overexpression of near full length P2P-R induces mitotic arrest in prometaphase and mitotic apoptosis, and overexpression of selected P2P-R segments also can promote apoptosis. This compendium of data supports the possibility that P2P-R may form complexes with the Rb1 and/or p53 tumor suppressors and MARs-related factors, in a cell cycle and cell differentiation-dependent manner, to influence gene transcription/expression and nuclear organization. J. Cell. Biochem. 90: 6–12, 2003.

Key words: p53; Rb1; SAF-B; SRPK1a; nucleolin; p63

In early 1997, full-length P2P-R cDNA [Witte and Scott, 1997] and full-length PACT cDNA [Simons et al., 1997] were cloned and characterized. The highly homologous P2P-R and PACT cDNAs encode a ~250 kDa protein product. P2P-R has now proven to be the alternately spliced product of the PACT/P2P-R gene; P2P-R lacks one exon containing 102 nucleotides that encodes 34 amino acids (Fig. 1A). Recent PCR studies using primers 5′ and 3′ to the alternately spliced exon demonstrated that P2P-R is the predominant product expressed in multiple murine cell lines [Scott and Cox, unpublished observation]. Whether the PACT protein that contains the exon missing in P2P-R is expressed in specific cell types remains to be determined. It is of interest that the peptide encoded by the exon missing in P2P-R has a coiled-coil structure that can determine protein binding specificities, suggesting that the PACT molecule, that contains such a domain, might have unique binding characteristics [Peidis, Giannakouros, and Scott, unpublished observation].

Biochemical data show that P2P-R is a highly basic nuclear protein with an isoelectric point of 9.6. In addition to containing multiple nuclear localization signals, P2P-R contains a series of important domains. Starting at its N-terminus and proceeding to its C-terminus, there exists a RING type zinc finger, a proline-rich domain, an RS-like region and a lysine-rich domain. The C-terminal third of P2P-R also contains an

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*Correspondence to: Robert E. Scott, Department of Pathology, University of Tennessee Health Science Center, Memphis, TN 38163. E-mail: rescott@utmem.edu

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A

5'-CCACAGTTTTCTCCTCAGTTTCCTCCTGGCCAGCCTCCACCAGCAGGATATAGTGTCCCTCCTCCAGGGTTTCCACC
AGCTCCTGCCAATATATCAACACCTTGGGTATCATCAGGAGTGCAGACTGCCCATTCAAATACCATCCCTACAACACA
AGCACCTCCTTTGTCCAGGGAAGAATTCTATAGAGAGCAACGACGGCTAAAGGAAGAG**GAAAAGAAAAAGTCCAA**
GCTAGATGAGTTTACAAATGATTTTGCTAAGGAATTGATGGAATACAAAAAGATTCAAAAGGAGCGTAGGCGC
TCATTTTCCAGGTCTAAATCTCCCTATAGTGGGTCATCGTATTCAAGAAGTTCATACACTTATTCAAAGTCAAGGTCT
GGCTCAACACGTTCACGCTCTTACTCTCGGTCCTTCAGCCGCTCACATTCTCGCTCCTATTCACGATCACCCCCATACC
CCAGGAGAGGCAGAGGCAAGA– 3'

B

P2P-R Protein Domains	P2P-R Binding Factors					
	p53	Rb1	ssDNA	SAF-B	Nucleolin	SRPK1a
1. Ring-type zinc finger (57-107aa)						
2. Proline rich (362-411aa)						
3. SR-like (460-540aa)				+	+	+
4. Rb1 binding (735-908aa)		+				
5. p53/ssDNA binding (1204-1314aa)	+		+			
6. Lysine-rich (1497-1550aa)						

Fig. 1. DNA and protein characteristics of the P2P-R/PACT cDNAs and its product. The P2P-R cDNA differs from PACT cDNA in the absence of one 102 base pair exon that exists 5′ of the segment that encodes the RS protein domain. **A**: Illustrates the region of the P2P-R/PACT gene containing the alternately spliced domain (bold) that is absent from P2P-R. The 5′ and 3′ sequence to which PCR primers that have been prepared to assay the relative expression of P2P-R versus PACT is underlined. **B**: Tabulation of the protein domains of P2P-R and the factors known to bind to specific P2P-R domains.

hnRNP core epitope because the FA12 monoclonal antibody against core hnRNPs was initially used to identify the P2P-R cDNA from a 3T3 expression library [Witte and Scott, 1997].

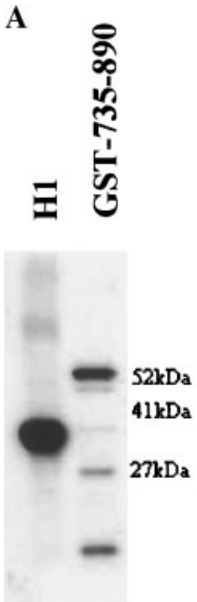
Specific domains of P2P-R interact with important proteins (Fig. 1B). These P2P-R binding proteins include the Rb1 and p53 tumor suppressors. Documentation of the binding of Rb1 and p53 to P2P-R/PACT has been published, showing that aa 735–908 of P2P-R binds to the pocket domain of Rb1, whereas aa 1204–1314 binds to the oligomerization and C-terminal regulatory domain of p53 (aa 347–357) [Simons et al., 1997; Witte and Scott, 1997; Gao and Scott, in press].

Preliminary studies suggest that P2P-R might be a member of a new family of proteins that share a common domain organization with the ability to influence the nuclear matrix. One well-characterized protein of this family is topors [Haluska et al., 1999; Zhou et al., 1999]. It too contains an N-terminal RING type zinc finger, an internal RS region, a p53 binding domain and a lysine rich C-terminal sequence. Topors was initially cloned on the basis of its ability to interact with topoisomerase I (topors, topoisomerase I-binding RS protein), which can affect the nuclear matrix, via its interaction with nucleolin [Haluska et al., 1999].

Two protein kinases can phosphorylate P2P-R. Figure 2A shows that P2P-R can be phosphorylated by the mitotic cdc2 kinase. Such data are relevant to recently published reports showing that the immunoreactivity of P2P-R is markedly modulated during mitosis. More specifically, Western blots using the M56 anti-P2P-R monoclonal antibody detect only low levels of P2P-R in quiescent G_0 cells, whereas mitotic cells show $>10\times$ higher levels of P2P-R [Gao et al., 2002].

In this respect, it should be noted that the localization of P2P-R also changes during the cell cycle. Whereas P2P-R localizes to nucleoli and nuclear speckles in interphase cells, mitotic cells that lack nucleoli and show markedly repressed transcriptional and pre-RNA splicing activities, show most P2P-R at the periphery of chromosomes together with nucleolin and a series of other factors (Fig. 2B) [Gao et al., 2002]. It is possible that such changes in P2P-R localization might be related to an association of P2P-R with the nuclear matrix. The nuclear matrix, as a regulator of nuclear structure and nuclear metabolism, undergoes dynamic changes during the cell cycle. Although

A



B

P2P-R Localization	Interphase Cells	Mitotic Cells
Nucleolar	+	–
Nuclear Speckles	+	–
Perichromosomal	–	+

Fig. 2. Cell cycle-associated changes in P2P-R phosphorylation and intracellular localization. **A**: Evidence that the cdc2 mitotic protein kinase phosphorylates in vitro a GST fusion protein containing 735-890 of P2P-R. The full-length fusion protein migrates with an apparent molecular mass of approximately 52 kDa. The lower bands are thought to represent degradation products. Histone H1 was employed as the positive control substrate for cdc2. **B**: Summary of evidence from confocal microscopy studies documenting that the localization of P2P-R changes during the cell cycle.

traditionally it has been thought that during mitosis, the nuclear matrix just disassembled in prophase and then reassembled in telophase, it now appears that much of the nuclear matrix of interphase cells can be used to form the mitotic apparatus [Mancini et al., 1996].

In addition, SRPK1a has been found to phosphorylate the RS region of P2P-R [Peidis, Giannakouros, Scott, and Gao, unpublished observation]. SRPK1a is encoded by an alternatively processed transcript derived from the *SRPK1* gene and contains an insertion of 171 amino acids at its N-terminal domain [Nikolakaki et al., 2001]. Interestingly, SRPK1a specifically interacts, via its N-terminal domain, with SAF-B, a MARs binding protein [Nikolakaki et al., 2001].

Recent studies have also established that the RS region of P2P-R can bind two factors that associate with MARs. Immunoprecipitation experiments document that P2P-R binds SAF-B [Scott, Peidis, and Giannakouros, unpublished observation, see also Fig. 3A] and preliminary yeast two hybrid results suggest that P2P-R can also bind to nucleolin [Witte and Scott, unpublished observation].

SAF-B was first purified and found to be a novel MARs binding factor in 1996 [Renz and Fackelmayer, 1996]. Subsequently, SAF-B was reported to couple transcription and pre-RNA splicing and to be localized at speckles in the nucleus [Nayler et al., 1998]. Then it was reported that SAF-B is essentially the same protein as HAT that interacts with hnRNPA1 and several other hnRNPs through its RS domain [Weighardt et al., 1999]. Both SAF-B and HAT were also found to be identical to HET, a transcriptional regulator of the heat shock protein 27 gene [Oesterreich et al., 1997]. In this regard, it is intriguing that P2P-R that interacts with SAF-B also contains an hnRNP core epitope recognized by the FA12 monoclonal antibody and a heat shock 90 epitope recognized by the AC88 monoclonal antibody [Witte and Scott, 1997].

The ability of nucleolin to bind to MARs also has been clearly documented [Dickinson and Kohwi-Shigematsu, 1995; Galande, 2002]. Indeed, MARs have been identified in the nucleoli of multiple cell types as diverse as rat cells [Stephanova et al., 1993] and tobacco cells [Fujiwara et al., 2002]. About 30 additional proteins have been reported to bind to MARs. These include: lamins, matrins, SATB1, huRNP-U, topoisomerase II, the Ku autoantigen, histone H1, and poly (ADP-ribose) polymerase [Galande and Kohwi-Shigematsu, 2000; Galande, 2002; Liebich et al., 2002].

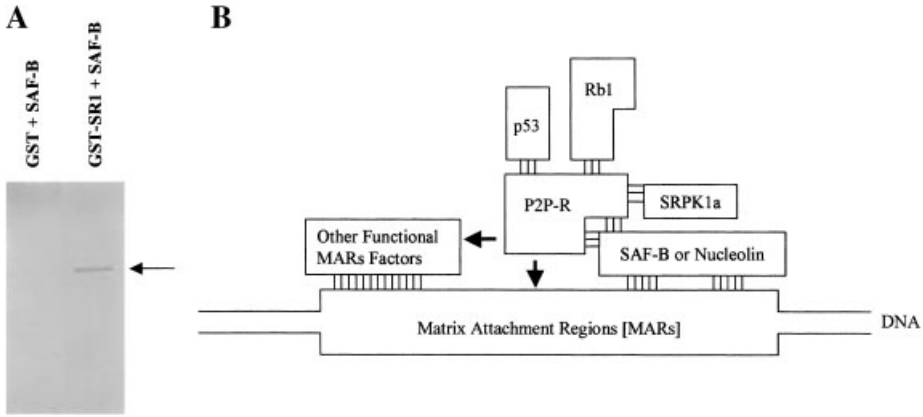


Fig. 3. A complex of P2P-R, SAF-B/nucleolin, p53 and/or RB plus other factors may exist at MARs to influence nuclear organization and DNA transcription. **A**: Evidence that the RS domain of P2P-R [GST-SR1] binds to the MARs binding protein designated SAF-B (←) whereas SAF-B does not bind to control GST alone. **B**: Model based on evidence that P2P-R can interact with the MARs binding proteins SAF-B and nucleolin (preliminary data) in addition to p53, Rb1, and SRPK1a. The model suggests that the complex of P2P-R with SAF-B/nucleolin, p53 and/or Rb1 might directly influence MARs or other functional MARs factors (arrows).

Matrix attachment regions of DNA and associated factors appear to partition chromatin into functional regions [von Kries et al., 1991]. They have been identified primarily in exons but recent data suggest that they may also exist in introns preceding 5′ ends of genes. In both locations, MARs and associated proteins are thought to control transcription and multiple other nuclear activities [Glazko et al., 2003]. Recent reports suggest that a MARs recognition signature sequence may even exist [van Drunen et al., 1999] in contrast to prior studies, which suggested that no consensus sequences exist. MARs tend to be concentrated in nuclear speckles and in nucleoli, which is intriguing since P2P-R also localizes both in the nucleolus and in nuclear speckles in interphase cells [Simons et al., 1997; Gao and Scott, 2002].

Experiments to evaluate the biological outcome of overexpression of near full-length P2P-R or smaller P2P-R segments provide additional insights into the function of P2P-R. The overexpression of near full-length P2P-R induces mitotic arrest at prometaphase and mitotic apoptosis [Gao and Scott, 2002]. In addition, overexpression of selected P2P-R segments that include a 158 aa region contiguous with the p53 and ssDNA binding domains of P2P-R, facilitates apoptosis induced by the topoisomerase I inhibitor camptothecin [Gao and Scott, in press]. In view of the fact that camptothecin-induced apoptosis is linked to its ability to stabilize single strand DNA cleavages induced by topoisomerase I, the finding that specific P2P-R segments have ssDNA binding potential suggests that P2P-R may influence topoisomerase I-camptothecin–DNA interactions [Gao and Scott, in press]. Whether P2P-R interacts directly with topoisomerase I, as does topors, and whether the function of P2P-R in apoptosis relates to its purported association with MARs, remains to be evaluated.

A final set of data, related to the original basis for the cloning of P2P-R, needs to be emphasized. Studies published between 1989 and 1997 [Minoo et al., 1989; Witte and Scott, 1997] established that when 3T3T adipocytes terminally differentiate and when human keratinocytes terminally differentiate or senesce, the expression of a type of hnRNP-like A protein, designated P2P, was markedly repressed. The FA12 anti-core hnRNP antibody that detects P2Ps was therefore used to clone the cDNA designated P2P-R [Witte and Scott, 1997]. Figure 4 shows that the expression of P2P-R mRNA also is markedly repressed by adipocyte differentiation. In contrast, the expression of P2P-R mRNA does not change during the cell cycle in related cell types. This suggests that modulation of P2P-R expression does not result from quiescence but from more complex differentiation-dependent regulatory mechanisms. More specifically, if the level of P2P-R mRNA in G_0 and G_2/M phase cells is set at 100%, Figure 4 shows that the level of P2P-R mRNA in non-terminally and terminally differentiated cells is decreased by 70–95% [Witte and Scott, 1997; Gao et al., 2002].

Differentiation Characteristics	Relative Expression of P2P-R RNA
I. Undifferentiated Specimens	
•Unsynchronized 3T3T cells	100%
•G_2/M 3T3T cells	100%
•Quiescent G_0 3T3T cells	100%
II. Differentiated Specimens	
•Nonterminally differentiated 3T3T adipocytes	30%
•Terminally differentiated 3T3T adipocytes	< 5%

Fig. 4. Differentiation selectively represses P2P-R RNA expression. The relative expression of P2P-R RNA at specific cell cycle and differentiation states was determined by densitometric analysis of Northern blots using either GAPDH levels or ethidium bromide RNA staining as loading controls.

The molecular and biological characteristics of P2P-R therefore have been extensively studied and many insights have been developed. However, the core function of P2P-R has yet to be definitively established.

P2P-R FUNCTIONAL HYPOTHESIS

Our working hypothesis suggests that P2P-R's core function stems from its ability to complex with MARs-associated factors, such as SAF-B and nucleolin, and the tumor suppressors p53 and Rb1 that might be influenced by modification of P2P-R via phosphorylation by SRPK1a or cdc2 protein kinases or other enzymes. This hypothesis also suggests this complex of interactions can be modulated during the cell cycle and cell differentiation. Figure 3B is presented to illustrate such a purported MARs complex that includes P2P-R, SAF-B or nucleolin, with p53, Rb1 and the protein kinase SRPK1a.

It is possible that a complex of P2P-R and SAF-B might localize to specific sites on the nuclear matrix associated with speckles. That complex might also involve p53 and/or Rb1 and it might function to influence the organization of chromatin directly or by modulating the activity of other MARs-associated factors, such as topoisomerases and thereby to influence gene expression.

A complex of P2P-R and nucleolin might also localize to specific sites in the nuclear matrix where rRNA transcription occurs [Ginisty et al., 1998]. That complex might also involve p53 and/or Rb1 to influence MARs directly or the function of other MARs-associated factors.

Both of these possibilities are compatible with the fact that both p53 and Rb can localize to MARs sites under specific biological situations. Hypophosphorylated Rb associates with the nuclear matrix, especially at the nuclear periphery and in nucleolar remnants [Mancini et al., 1994]. In contrast, mutant Rb does not bind to the nuclear matrix [Mancini et al., 1994]. Rb also interacts with specific nuclear matrix proteins, such as p84 [Durfee et al., 1994] and topoisomerase II α [Bhat et al., 1999]. The interaction of Rb with the nuclear matrix may even affect its organization because Rb-deficient mice show a highly relaxed chromatin structure [Herrera et al., 1996]. Rb may thereby influence transcription by its ability to modify the conformation of individual nucleosomes [Morrison et al., 2002].

Although native p53 appears not to bind to MARs sites in DNA, p53 can bind to double and single strand DNA, and at least half of all nuclear p53 appears to bind to specific nuclear structures [Steinmeyer and Deppert, 1988; Zerrahn et al., 1992]. In contrast, mutant p53s have the distinct ability of binding to the nuclear matrix with high affinity [Weissker et al., 1992; Will et al., 1998; Deppert et al., 2000]. Mutant p53s can bind to MARs sites in DNA [Muller et al., 1996; Koga and Deppert, 2000], and the ability of mutant p53 to bind to MARs sites has been proposed to account for the "gain of function" outcome that causes some mutant p53s to act as oncogenes [Deppert, 1996; Deppert et al., 2000].

The model for the proposed role of P2P-R in MARs-related activities (Fig. 3B) includes all the above perspectives and provides a focus for many challenging future experiments. The precise sites within the P2P-R protein that are phosphorylated by SRPK1a and by cdc2 need to be defined and the times that P2P-R phosphorylation is modified during the cell cycle need to be established. The functional impact of P2P-R phosphorylation also needs to be defined, as does the interaction of P2P-R with the nuclear matrix binding factors SAF-B and nucleolin. For example, it needs to be established if P2P-R can be coimmunoprecipitated with SAF-B or nucleolin from cells and if the distribution of P2P-R, SAF-B, and/or nucleolin can be colocalized during the cell cycle using confocal microscopy. The use of cell systems that lack either P2P-R, SAF-B, nucleolin, p53 or Rb1 could also provide important insights into how proposed MARs complexes might function. Ultimately, the functional impact of modulating P2P-R

interactions with MARs complexes also needs to be defined to explain the role of P2P-R in apoptosis [Gao and Scott, 2002; Gao and Scott, in press].

Concerning P2P-R and its potential interaction with MARs in association with differentiation, it has recently been reported that mice devoid of the MARs binding factor SATB1 show major cell lineage specific defects in T-lymphocyte development [Alvarez et al., 2000]. More specifically, SATB1 null mice, which develop with only a small thymus and spleen, die within one month after birth with multiple defects in T-cell function. It therefore is possible that complexes of MARs binding proteins may act as global regulators of cell differentiation [Alvarez et al., 2000]. Ongoing studies to develop a P2P-R knockout mouse model should provide new insights in that regard. It has also been reported that the nuclear matrix is modified by terminal differentiation in a variety of cell types [Davie, 1997; Fischer et al., 1998; Morioka et al., 1998; Cheung et al., 2001; Hawkins et al., 2001]. Finally, the recent discovery that the differentiation-regulatory p63 protein influences P2P-R gene expression [Wu et al., 2003] supports the possibility that linkages exist between differentiation, nuclear matrix characteristics, and P2P-R.

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REFERENCES

Alvarez JD, Yasui DH, Niida H, Joh T, Loh DY, Kohwi-Shigematsu T. 2000. The MAR-binding protein SATB1 orchestrates temporal and spatial expression of multiple genes during T-cell development. Genes Dev 14:521–535.

Bhat UG, Raychaudhuri P, Beck WT. 1999. Functional interaction between human topoisomerase IIalpha and retinoblastoma protein. Proc Natl Acad Sci USA 96: 7859–7864.

Cheung WM, Chu AH, Chu PW, Ip NY. 2001. Cloning and expression of a novel nuclear matrix-associated protein that is regulated during retinoic acid-induced neuronal differentiation. J Biol Chem 276:17083–17091.

Davie JR. 1997. Nuclear matrix, dynamic histone acetylation and transcriptionally active chromatin. Mol Biol Rep 24:197–207.

Deppert W. 1996. Binding of MAR-DNA elements by mutant p53: Possible implications for its oncogenic functions. J Cell Biochem 62:172–180.

Deppert W, Gohler T, Koga H, Kim E. 2000. Mutant p53: "Gain of function" through perturbation of nuclear structure and function? J Cell Biochem Suppl 35:115–122.

Dickinson LA, Kohwi-Shigematsu T. 1995. Nucleolin is a matrix attachment region DNA-binding protein that specifically recognizes a region with high base-unpairing potential. Mol Cell Biol 15:456–465.

Durfee T, Mancini MA, Jones D, Elledge SJ, Lee WH. 1994. The amino-terminal region of the retinoblastoma gene product binds a novel nuclear matrix protein that co-localizes to centers for RNA processing. J Cell Biol 127: 609–622.

Fischer DF, van Drunen CM, Winkler GS, van de Putte P, Backendorf C. 1998. Involvement of a nuclear matrix association region in the regulation of the SPRR2A keratinocyte terminal differentiation marker. Nucleic Acids Res 26:5288–5294.

Fujiwara S, Matsuda N, Sato T, Sonobe S, Maeshima M. 2002. Molecular properties of a matrix attachment region-binding protein located in the nucleoli of tobacco cells. Plant Cell Physiol 43:1558–1567.

Galande S. 2002. Chromatin (dis)organization and cancer: BUR-binding proteins as biomarkers for cancer. Curr Cancer Drug Targets 2:157–190.

Galande S, Kohwi-Shigematsu T. 2000. Caught in the act: Binding of Ku and PARP to MARs reveals novel aspects of their functional interaction. Crit Rev Eukaryot Gene Expr 10:63–72.

Gao S, Scott RE. Stable overexpression of specific segments of the P2P-R protein in human MCF-7 cells promotes camptothecin-induced apoptosis. J Cell Physiol (in press).

Gao S, Scott RE. 2002. P2P-R protein overexpression restricts mitotic progression at prometaphase and promotes mitotic apoptosis. J Cell Physiol 193:199–207.

Gao S, Witte MM, Scott RE. 2002. The P2P-R protein localizes to the nucleolus in interphase cells and the periphery of chromosomes in mitotic cells, which show maximum P2P-R immunoreactivity. J Cell Physiol 191: 145–154.

Ginisty H, Amalric F, Bouvet P. 1998. Nucleolin functions in the first step of ribosomal RNA processing. EMBO J 17:1476–1486.

Glazko GV, Koonin EV, Rogozin IB, Shabalina SA. 2003. A significant fraction of conserved noncoding DNA in human and mouse consists of predicted matrix attachment regions. Trends Genet 19:119–124.

Haluska P, Jr., Saleem A, Rasheed Z, Ahmed F, Su EW, Liu FL, Rubin EH. 1999. Interaction between human topoisomerase I and a novel RING finger/arginine-serine protein. Nucleic Acids Res 27:2538–2544.

Hawkins SM, Kohwi-Shigematsu T, Skalnik DG. 2001. The matrix attachment region-binding protein SATB1 interacts with multiple elements within the gp91phox promoter and is down-regulated during myeloid differentiation. J Biol Chem 276:44472–44480.

Herrera RE, Chen F, Weinberg RA. 1996. Increased histone H1 phosphorylation and relaxed chromatin structure in Rb-deficient fibroblasts. Proc Natl Acad Sci USA 93: 11510–11515.

Koga H, Deppert W. 2000. Identification of genomic DNA sequences bound by mutant p53 protein (Gly245–>Ser) in vivo. Oncogene 19:4178–4183.

Liebich I, Bode J, Frisch M, Wingender E. 2002. S/MARt DB: A database on scaffold/matrix attached regions. Nucleic Acids Res 30:372–374.

Mancini MA, Shan B, Nickerson JA, Penman S, Lee WH. 1994. The retinoblastoma gene product is a cell cycle-dependent, nuclear matrix-associated protein. Proc Natl Acad Sci USA 91:418–422.

Mancini MA, He D, Ouspenski II, Brinkley BR. 1996. Dynamic continuity of nuclear and mitotic matrix proteins in the cell cycle. J Cell Biochem 62:158–164.

Minoo P, Sullivan W, Solomon LR, Martin TE, Toft DO, Scott RE. 1989. Loss of proliferative potential during terminal differentiation coincides with the decreased abundance of a subset of (hnRNP) heterogeneous ribonuclear proteins. J Cell Biol 109:1937–1946.

Morioka K, Tone S, Mukaida M, Takano-Ohmuro H. 1998. The apototic and nonapoptotic nature of the terminal differentiation of erythroid cells. Exp Cell Res 240:206–217.

Morrison AJ, Sardet C, Herrera RE. 2002. Retinoblastoma protein transcriptional repression through histone deacetylation of a single nucleosome. Mol Cell Biol 22:856–865.

Muller BF, Paulsen D, Deppert W. 1996. Specific binding of MAR/SAR DNA-elements by mutant p53. Oncogene 12:1941–1952.

Nayler O, Strätling W, Bourquin J-P, Stagljar I, Lindemann L, Jasper H, Hartmann AM, Fackelmayer FO, Ullrich A, Stamm S. 1998. SAF-B protein couples transcription and pre-mRNA splicing to SAR/MAR elements. Nucleic Acids Res 26:3542–3549.

Nikolakaki E, Kohen R, Hartmann AM, Stamm S, Georgatsou E, Giannakouros T. 2001. Cloning and characterization of an alternatively spliced form of SR protein kinase 1 that interacts specifically with scaffold attachment factor-B. J Biol Chem 276:40175–40182.

Oesterreich S, Lee AV, Sullivan TM, Samuel SK, Davie JR, Fuqua SA. 1997. Novel nuclear matrix protein HET binds to and influences activity of the HSP27 promoter in human breast cancer cells. J Cell Biochem 67: 275–286.

Renz A, Fackelmayer FO. 1996. Purification and molecular cloning of the scaffold attachment factor B (SAF-B), a novel human nuclear protein that specifically binds to S/MAR-DNA. Nucleic Acids Res 24:843–849.

Simons A, Melamed-Bessudo C, Wolkowicz R, Sperling J, Sperling R, Eisenbach L, Rotter V. 1997. PACT: Cloning and characterization of a cellular p53 binding protein that interacts with Rb. Oncogene 14:145–155.

Steinmeyer K, Deppert W. 1988. DNA binding properties of murine p53. Oncogene 3:501–507.

Stephanova E, Stancheva R, Avramova Z. 1993. Binding of sequences from the 5′- and 3′-nontranscribed spacers of the rat rDNA locus to the nucleolar matrix. Chromosoma 102:287–295.

van Drunen CM, Sewalt RG, Oosterling RW, Weisbeek PJ, Smeekens SC, van Driel R. 1999. A bipartite sequence element associated with matrix/scaffold attachment regions. Nucleic Acids Res 27:2924–2930.

von Kries JP, Buhrmester H, Stratling WH. 1991. A matrix/scaffold attachment region binding protein: Identification, purification, and mode of binding. Cell 64:123–135.

Weighardt F, Cobianchi F, Cartegni L, Chiodi I, Villa A, Riva S, Biamonti G. 1999. A novel hnRNP protein (HAP/SAF-B) enters a subset of hnRNP complexes and relocates in nuclear granules in response to heat shock. J Cell Sci 112:1465–1476.

Weissker SN, Muller BF, Homfeld A, Deppert W. 1992. Specific and complex interactions of murine p53 with DNA. Oncogene 7:1921–1932.

Will K, Warnecke G, Wiesmuller L, Deppert W. 1998. Specific interaction of mutant p53 with regions of matrix attachment region DNA elements (MARs) with a high potential for base-unpairing. Proc Natl Acad Sci USA 95: 13681–13686.

Witte MM, Scott RE. 1997. The proliferation potential protein-related (P2P-R) gene with domains encoding heterogeneous nuclear ribonucleoprotein association and Rb1 binding shows repressed expression during terminal differentiation. Proc Natl Acad Sci USA 94: 1212–1217.

Wu G, Nomoto S, Hoque MO, Dracheva T, Osada M, Lee C-CR, Dong SM, Guo Z, Benoit N, Cohen Y, Rechthand P, Califano J, Moon C, Ratovitski E, Jen J, Sidransky D, Trink B. 2003. ΔNp63α and TAp63α regulate transcription of genes with distinct biological functions in cancer and development. Cancer Res 63:2351–2357.

Zerrahn J, Deppert W, Weidemann D, Patschinsky T, Richards F, Milner J. 1992. Correlation between the conformational phenotype of p53 and its subcellular location. Oncogene 7:1371–1381.

Zhou R, Wen H, Ao S-Z. 1999. Identification of a novel gene encoding a p53-associated protein. Gene 235:93–101.