Journal of Cellular Biochemistry 76:559–566 (2000)

Differential Nuclear Localization and Nuclear Matrix Association of the Splicing Factors PSF and PTB

Michael Meissner,[1] **Thomas Dechat,**[2] **Christopher Gerner,**[1] **Rudolf Grimm,**[3] **Roland Foisner,**[2] **and Georg Sauermann**[1]*

[1]Institute of Tumor Biology–Cancer Research, University of Vienna, A-1090 Vienna, Austria
[2]Institute of Biochemistry and Molecular Cell Biology, University of Vienna, A-1030 Vienna, Austria
[3]Hewlett-Packard, Chemical Analytical Division, D-76337 Waldbronn, Germany

Abstract A monoclonal antibody raised against nuclear matrix proteins detected a protein of basic pI in human nuclear matrix protein samples of various cellular origin. The ubiquitously occurring (common) nuclear matrix protein was identified as splicing factor PSF (PTB associated splicing factor). The interaction between the splicing factors PSF and PTB/hnRNP I was confirmed by co-immunoprecipitation from nuclear salt extracts. However, the nuclear localization of PSF and PTB and their distribution in subnuclear fractions differed markedly. Isolated nuclear matrices contained the bulk of PSF, but only minor amounts of PTB. In confocal microscopy both proteins appeared in speckles, the majority of which did not co-localize. Removing a large fraction of the soluble PTB structures by salt extraction revealed some colocalization of the more stable PTB fraction with PSF. These PTB/PSF complexes as well as the observed PSF-PTB interaction may reflect the previously reported presence of PTB and PSF in spliceosomal complexes during RNA processing. The present data, however, point to different cellular distribution and nuclear matrix association of the majority of PSF and PTB. J. Cell. Biochem. 76:559–566, 2000.

Key words: nuclear matrix; human splicing factors; polypyrimidine tract-binding; intranuclear localization

The nuclear matrix protein fraction has been defined as the insoluble material resisting sequential treatment of isolated nuclei with detergents, nucleases, and high-ionic-strength buffers [Berezney and Coffey, 1974]. The proteins have been considered to represent components of the fibrillar network identified in various structural studies of the interphase nucleus. Furthermore, the nuclear matrix has been described as being involved in essential nuclear processes, such as DNA replication, DNA transcription, RNA processing, RNA transport, or steroid hormone action. Accordingly, common nuclear matrix proteins that are shared among various cell types have been differentiated from cell type-specific and cell state-specific nuclear matrix proteins [for reviews, see Stuurman et al., 1992; Berezney and Jeon, 1995; Stein et al., 1996].

In the present study the identification of PSF, the PTB-associated splicing factor, as a human common nuclear matrix protein is described. In addition, the association of PTB to PSF and their respective intranuclear localization were investigated.

In the pre-mRNA splicing process non-coding intervening sequences (introns) are removed from primary DNA transcripts. The reaction takes place in nuclear multicomponent complexes termed spliceosomes. In step I (spliceosome assembly) several intermediate complexes, termed E, A, B, and C, are engaged [for reviews, see Lamm and Lamond, 1993; Kramer, 1996]. PSF and PTB, both binding to the polypyrimidine tract at the 3′ end of introns, are constituting proteins of complexes B and C [Gozani et al., 1994]. PSF has been described to be essential for the following catalytic step II of the splicing reaction [Gozani et al., 1994]. In steps II and III introns are released and exons ligated. During these steps PTB, also described as an isoform of the hnRNP I protein [Ghetti et

Abbreviations used: hnRNP, heterogeneous nuclear ribonucleoprotein; PSF, PTB associated splicing factor; PTB, polypyrimidine tract-binding protein.

*Correspondence to: Georg Sauermann, Institute of Tumor Biology–Cancer Research, Borschkegasse 8a, A-1090 Vienna, Austria. E-mail: Georg.Sauermann@univie.ac.at

Received 30 April 1999; Accepted 16 August 1999

This article published online in Wiley InterScience, January 2000.

al., 1992], regulates alternative splice site selection [Gil et al., 1991; Singh et al., 1995; Gooding et al., 1998].

Several findings have indicated a specific interaction between PSF and PTB. The two proteins have been found copurifying during biochemical fractionation procedures [Patton et al., 1991; Gozani et al., 1994]. In addition, selective association of PSF and PTB has been observed in in vitro protein binding assays [Patton et al., 1993]. In contrast, the present data shows the different nuclear distribution and nuclear matrix association of the majority of PSF and PTB molecules.

MATERIALS AND METHODS

Cells

HeLa S3 and HL-60 (ATCC CRL-1964) cells were cultured in RPMI medium, supplemented with 10% fetal calf serum, at 37°C, in a humidified atmosphere containing 5% CO_2. Leukocytes of healthy human donors were purified as described previously [Holzmann et al., 1997]. Human tissue and tumor samples were kindly provided by Dr. P. Obrist and Dr. C. Ensinger, University of Innsbruck, Austria.

Preparation of Monoclonal Antibody mAb CTS32

Six-week-old Balb/c mice were immunized with a rat liver nuclear protein fraction [Foisner and Gerace, 1993]. Hybridoma cells were obtained by fusion of spleen cells with myelomas (P3-X63-Ag.8.853). Fusion, cloning, and hybridoma growth were performed according to standard protocols [Harlow and Lane, 1988]. Other conditions were as detailed in Gotzmann et al. [1997]. For immunodetection and immunoprecipitation hybridoma supernatants were used.

Preparation of Nuclear Matrices and Subcellular Fractions

Nuclear matrices were isolated as described elsewhere [Gerner et al., 1998]. In brief, isolated nuclei were exposed to vanadyl ribonucleoside complexes, detergents, DNAse I, and high-ionic strength buffer, finally yielding the insoluble nuclear matrix protein pellet. For preparation of subcellular fractions, cells were homogenized in 10 mM HEPES/NaOH, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$. After adjusting to 0.25 M sucrose and 0.35% Triton X-100 nuclei were pelleted through a 10% sucrose cushion, and the supernatant referred to as cytosol. For further fractionation, pelleted nuclei were suspended in PBS containing either 0.7 M NaCl, or 0.2% sodium deoxycholate and 0.4% Tween 40 (detergents; Sigma, St. Louis, MO), or 40 µg/ml DNAse I and/or 50 µg/ml RNAse A. Centrifugation at 700*g* for 5 min yielded the supernatant and pellet fractions.

Immunoprecipitations

For microsequencing, isolated HeLa cell nuclei were extracted with 1% Triton X-100, 1% SDS in 500 mM Tris/HCl pH 8.0, 1.5 M NaCl, 50 mM EDTA. After pelleting insoluble material, the supernatant was diluted 10-fold and incubated with CTS32 hybridoma supernatant at 4°C for 14 h. Following additional 3 h incubation with anti-mouse IgM agarose beads (Sigma) the beads were collected by centrifugation, washed, and proteins recovered in sample buffer. For co-immunoprecipitation experiments the 0.7 M salt extract of HeLa cell nuclei was used directly, or after adjusting to 0.4 M salt by addition of 50 mM HEPES pH 8.0.

Electrophoresis

High resolution two-dimensional electrophoresis was performed according to Hochstrasser et al. [1988] in the Mini-Protean system (Bio-Rad, Richmond, CA). Proteins were dissolved in 10 M urea, 4% CHAPS, 0.5% SDS, 100 mM DTT supplemented with 2% (v/v) ampholytes pH 7–9. NEPHGE (Non-equilibrium pH-gradient electrophoresis) was performed at 540 Vh in 70 × 1 mm polyacrylamide (4%) rod gels containing 8.6 M urea, 0.03% Nonidet P-40, 0.08% CHAPS, and 2% carrier ampholytes (1.2% pH 3.5–10, 0.4% pH 7–9, and 0.4% pH 9–11, E. Merck, Germany). IEF and SDS-PAGE (10% polyacrylamide), silver staining, blotting, and immunostaining were performed as previously described [Gotzmann et al., 1997]. Monoclonal antibody 7G12 against PTB/hnRNP I was a kind gift of Dr. G. Dreyfuss, University of Pennsylvania, Philadelphia, PA.

Microsequencing of Proteins

Immunoprecipitated proteins were separated by SDS-PAGE and electroblotted. The antigen was hydrolyzed with trypsin (Promega, Madison, WI) according to Aebersold et al. [1987]. Peptides were separated by reversed-phase-HPLC on a 1.0 × 250 mm Vydac C-18 column

(Hewlett-Packard, Waldbronn, Germany) at 40°C with a flow rate of 50 µl/min, using the Hewlett-Packard 1090 HPLC Series II system (Hewlett-Packard) and analyzed by means of the Hewlett-Packard G1005A protein sequencing system (Palo Alto, CA). Database searches were conducted, using the GCG software analysis package at http://www.at.embnet.org/.

Immunofluorescence Microscopy

HeLa cells grown on coverslips were permeabilized with 0.3% Triton X-100 in PBS. Cells were fixed with 2% formaldehyde either immediately (Fig. 4A) or after 5 min treatment with 250 mM NaCl (Fig. 4B) or 5 min treatment with detergents and 15 min with nucleases (Fig. 4C). Slides were first incubated with mAb CTS32 (undiluted hybridoma supernatant) and a rabbit polyclonal anti-PTB antibody (diluted 1:1,000 in PBS, a kind gift of Dr. M. Garcia-Blanco, Duke University Medical Center, Durham, NC), followed by incubation with the respective Texas-red and FITC labeled secondary antibodies (Jackson, West Grove, PA; diluted 1:200 in PBS). Samples were viewed in a Zeiss Axiophot microscope, equipped with the MRC 600 laser scanning device (Bio-Rad).

RESULTS

Detection of a Human Common Nuclear Matrix Protein

For the characterization and identification of nuclear matrix proteins, monoclonal antibodies were prepared against a rat liver nuclear protein fraction [Foisner and Gerace, 1993]. One of the resulting hybridomas produced an IgM-class antibody, termed mAb CTS32, that recognized in immunoblotting experiments a protein with an apparent molecular weight of 100 kDa in total cell lysates of cultured human cells (Fig. 1A). Furthermore, the antibody efficiently precipitated the protein from SDS-solubilized cell fractions. Subcellular fractionation analysis revealed that the antigen was exclusively found in nuclei or nuclear matrices (Fig. 1A).

Further analysis of nuclear matrices isolated from various human cell lines, hematopoietic cells, and normal and tumor tissues showed that the antigen was expressed at significant levels in all samples examined (Fig. 1B). Therefore, the antigen was classified as a common nuclear matrix protein and in accordance with designations used in other studies [Korosec et al., 1997; Holzmann et al., 1997, 1998; Gerner et al., 1998, 1999] termed human nuclear matrix protein 613 (hNMP 613).

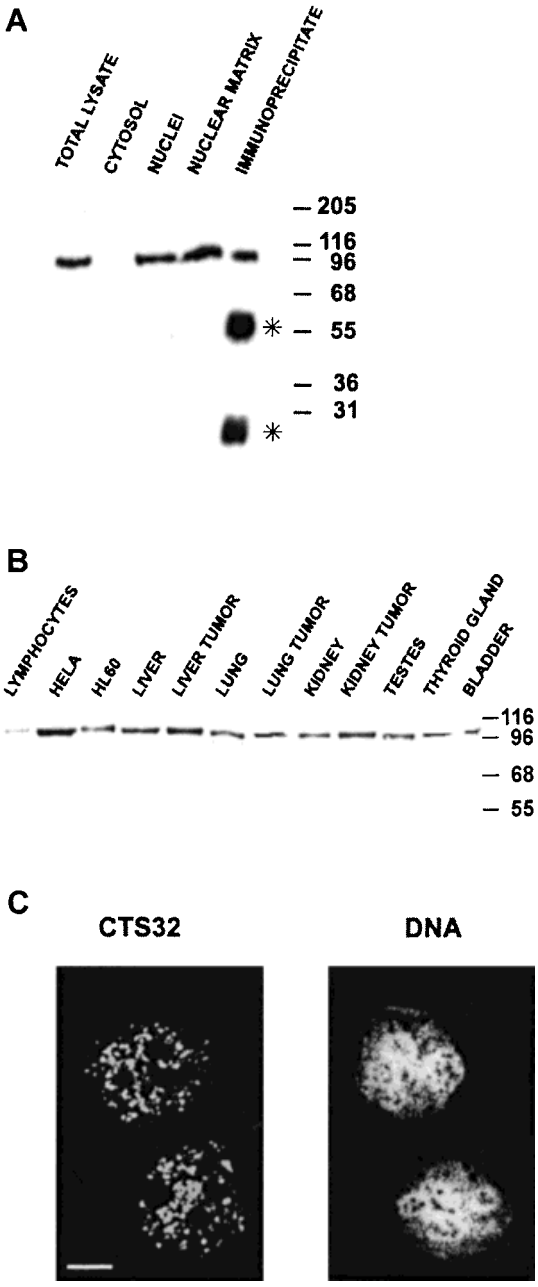

Fig. 1. Detection of a human common nuclear matrix protein by mAb CTS32. **A**: Cell fractions and an immunoprecipitate from SDS-soluble nuclear fractions of HeLa cells as indicated (asterisks mark IgM chains). **B**: Nuclear matrices prepared from various cells and tissues. **C**: Subcellular localization of the antigen in intact cells. Confocal microscopy of interphase HeLa cells stained with mAb CTS32 **(left)** and for DNA with propidiumiodide dye **(right)**. Scale bar = 5 µm.

In order to investigate the intracellular distribution of the antigen we performed confocal

immunofluorescence microscopic analysis of HeLa cells using mAb CTS32. The protein appeared in speckles randomly scattered throughout the nucleus, being excluded from nucleolar regions (Fig. 1C). Thus, the staining pattern was reminiscent of non-nucleolar nuclear matrix proteins.

Identification of the Antigen

For sequence analysis, the antigen was immuno-precipitated from a nuclear lysate under stringent conditions. By microsequencing two internal amino acid sequences, MGYMDPR and FGQGGAGPVG, were obtained that were identical to published sequences of the human splicing factor PSF. According to Swiss-Prot database entry Nr. P23246, the above sequences extend from aa positions 600–606 and 667–677 of the human PSF polypeptide, consisting of 707 amino acids [Patton et al., 1993].

After high resolution 2-D IEF of HeLa cell nuclear matrix proteins, none of the blotted proteins, ranging from pI 4 to 7.5, were recognized by mAb CTS32 (not shown). Following separation of basic proteins (pI 7.5–12.0) by 2-D NEPHGE, a protein spot was detected at 100 kDa, pI 9.5 (Fig. 2). This corresponded to the calculated pI value and the reported electrophoretic mobility of PSF [Gozani et al., 1994] and confirmed the identity of the antigen as human PSF.

Differential Distribution of PSF and PTB in Subnuclear Fractions

In protein binding assays PSF has been found to specifically interact with PTB, a feature determining the name of the protein [Patton et al., 1993]. In addition, the two proteins have been described to copurify in spliceosomes [Patton et al., 1993] and to be components of the same subgroup of nuclear splicing complexes [Gozani et al., 1994]. Although these observations would suggest that similar amounts of PSF and PTB are present in nuclear matrices, we only detected comparably faint signals for PTB in 2D immunoblots of nuclear matrix protein fractions (Fig. 2B). Therefore, the distribu-

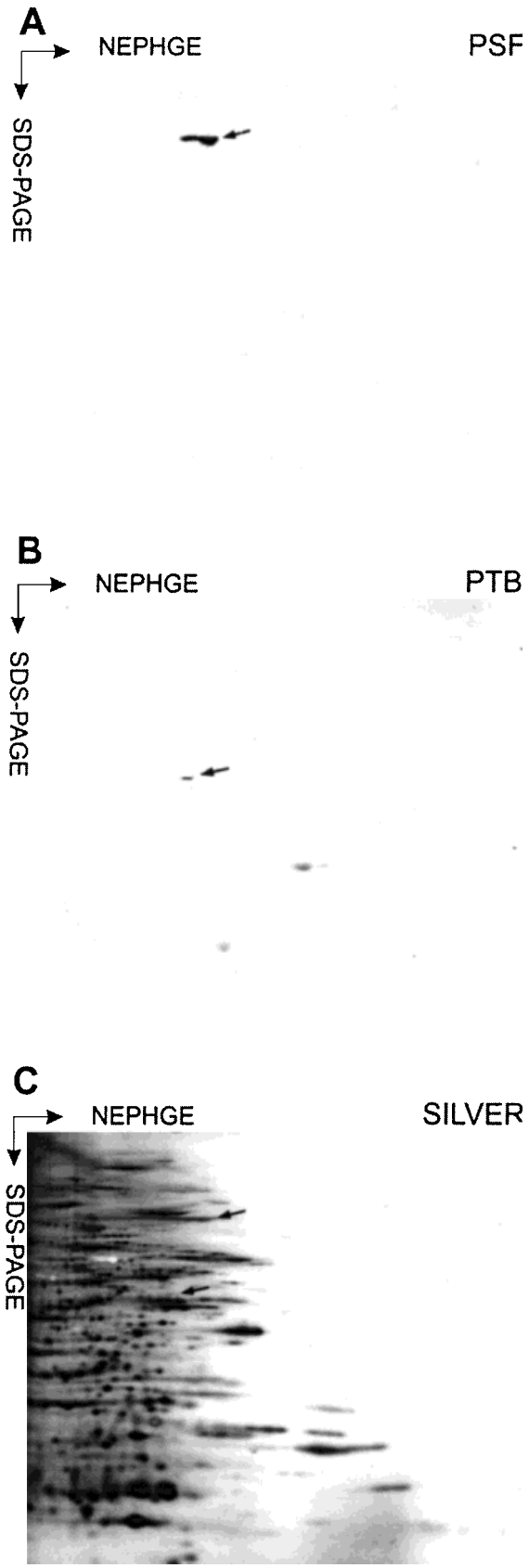

Fig. 2. Position of PSF and PTB in the 2D NEPHGE pattern of nuclear matrix proteins. Immunodetection by (**A**) mAb CTS32, (**B**) mAb 7G12. Only minor amounts of PTB were detectable. Additional spots are due to nonspecific cross-reactions of secondary antibody in long-time exposures (B). Positions of the antigens are indicated by arrows. **C**: Silver stain.

tion of the two splicing factors in subnuclear fractions was investigated in more detail. Isolated HeLa cell nuclei were treated with varying combinations of detergents, DNAse-I, RNAse, high ionic strength salt buffers, and the resulting soluble and insoluble residual nuclear fractions were tested by immunoblotting for the presence of PSF and PTB.

When nuclei were extracted in buffers containing 0.7 M NaCl without prior DNAse treatment, which apparently disrupted nuclear structures, both PSF and PTB were completely solubilized (Fig. 3A, lane 2). However, under any other conditions examined, including those used for the isolation of the nuclear matrices (lane 7), PSF was entirely retained in the residual nuclear pellet fraction. In contrast, the majority of PTB was released even under mild extraction with detergents or after treatment of nuclei with DNAse-I or RNAse.

Stability of the PSF-PTB Complex

The antibody mAb CTS32 was capable of co-precipitating PSF and PTB, thus allowing analysis of the stability of the complex. We immunoprecipitated PSF from 0.7 M salt extracts of HeLa nuclei (compare to Fig. 3A, lane 2) using mAb CTS32. Upon analysis of the PSF immunoprecipitate by 2D NEPHGE and immunoblotting using antibodies to PSF and PTB we only identified PSF, whereas no signal was detectable at the position of PTB. This clearly showed that PSF and PTB were not found in the same complex under high salt conditions and confirmed our solubility studies. However, upon lowering to 0.4 M salt concentration prior to immunoprecipitation both PSF and PTB were identified in the precipitates. Thus, under less stringent conditions PSF and PTB form a stable complex, supporting previous reports on the association of the proteins.

To exclude that the interaction of PSF and PTB was indirectly mediated by DNA or RNA we immunoprecipitated PSF from DNAse and RNAse treated nuclear extracts at 0.4 M salt. As both PSF and PTB were found in the immunoprecipitate (Fig. 3B) it may be concluded that they form complexes also in the absence of intact DNA and RNA structures.

Intranuclear Localization of PSF and PTB

In order to test whether PSF-PTB complexes existed also in the nucleus under more physiological conditions we performed double immu-

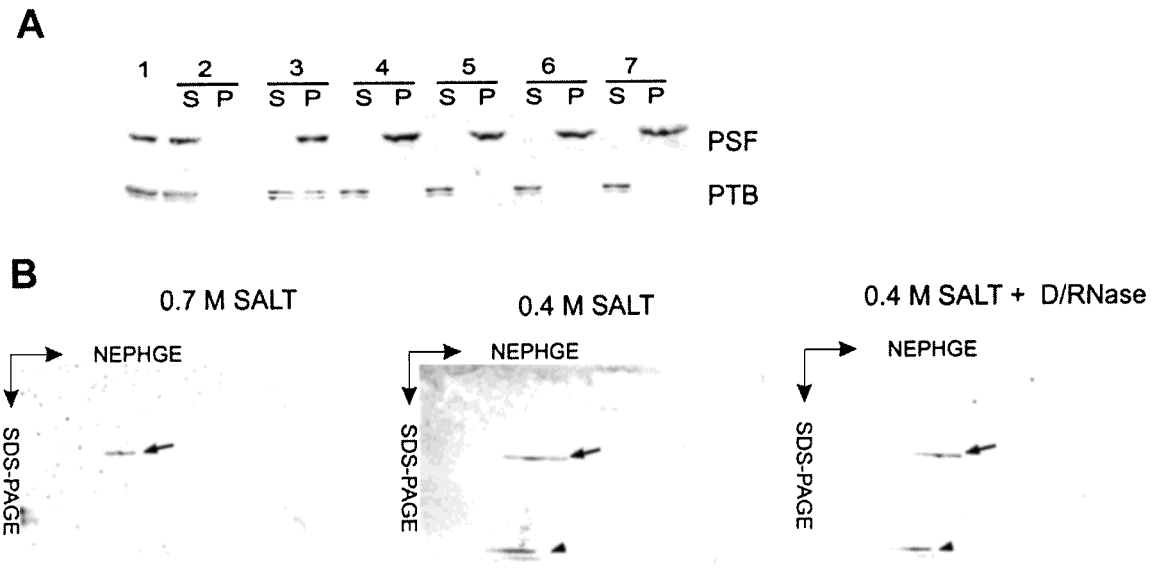

Fig. 3. Subnuclear fractionation and co-immunoprecipitation of PSF and PTB. **A**: HeLa nuclei were incubated with various combinations of detergents, salt, and nucleases, and resulting soluble (S) and residual nuclear pellet (P) fractions analyzed by immunoblotting using mAb CTS32 and mAb 7G12. Control nuclei **(lane 1)** and fractions upon treatment with 0.7 M salt **(lane 2)**, detergents **(lane 3)**, DNAse I **(lane 4)**, RNAse **(lane 5)**, DNAse + RNAse **(lane 6)**, DNAse and RNAse followed by 0.7 M NaCl **(lane 7)**. **B**: Triton-washed nuclei were extracted with 0.7 M salt and soluble fractions were used either directly for immunoprecipitation with mAb CTS32 or after reducing salt concentration to 0.4 M or after salt dilution and DNAse and RNAse treatment. The precipitates were analyzed by 2D-NEPHGE immunoblotting using mAb CTS32 and mAb 7G12. Positions of PSF are indicated by arrows, those of PTB by arrowheads.

nofluorescence microscopy. Confocal images of cells, fixed immediately after removal of culture medium, visualized both proteins in speckles throughout the nucleus (Fig. 4A). However, merged images of PSF and PTB revealed that only minor fractions of PSF and PTP co-localized, as indicated in yellow. Thus, the majority of PSF and PTB was not found in the same complexes in proliferating cells.

Next, cells were extracted with 250 mM salt, a concentration not dissociating PSF-PTB complexes (see Fig. 3B). Apparently, staining of PTB structures was markedly reduced, suggesting the release of PSF-independent PTB. On the other hand, resistant speckles of PTB colocalizing with PSF were evident (Fig. 4B, right panel).

If cells were extracted in buffers containing detergents and nucleases, conditions which solubilize most of PTB and retain PSF in the pellet (see Fig. 3A), we detected only a faint staining of PTB in the nucleus, whereas the speckle type staining pattern of PSF was preserved (Fig. 4C). Unlike PSF staining, PTB staining was

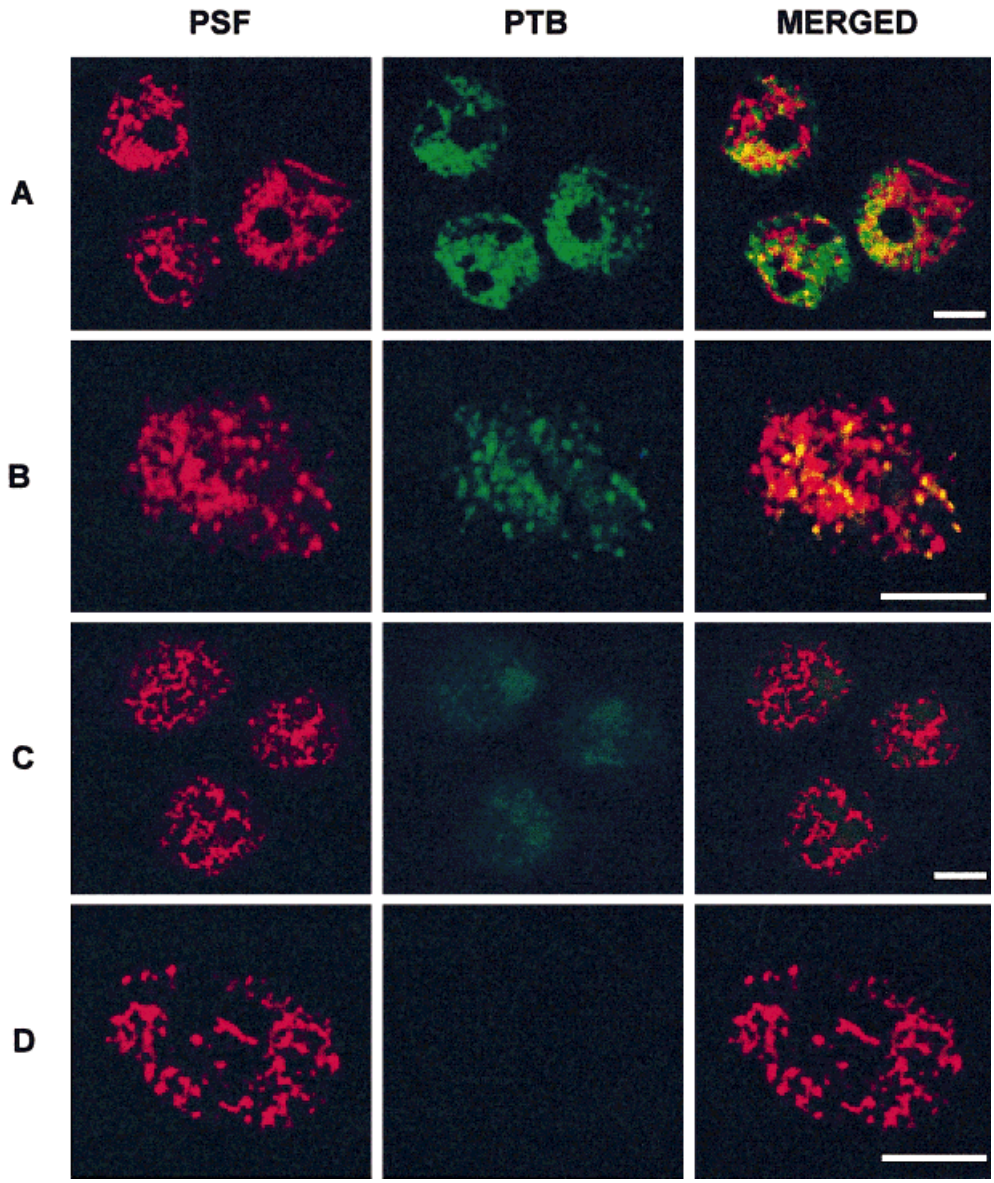

Fig. 4. Differential localization of PSF and PTB in HeLa cells. Double immunofluorescence microscopy using mAb CTS32 and a polyclonal antibody to PTB. Single and superimposed (merged) confocal images of stained PSF and PTB, with colocalizing PSF and PTB in yellow. **A**: Permeabilized cells. **B**: After extraction with 250 mM salt. **C**: After treatment with detergent and nucleases. **D**: Isolated nuclear matrices. Scale bars = 5 μm.

almost completely lost after extraction under more stringent conditions applied during the preparation of the nuclear matrix (Fig. 4D).

In conclusion, our data indicates that PSF forms stable nuclear structures, highly resistant to detergent/high salt, while the majority of PTB is found in PSF independent structures, easily extractable. Nevertheless, a fraction of PTB seems to be more firmly associated with PSF structures, which might be important for spliceosome formation.

DISCUSSION

The finding that metabolically active complexes or constituents are preferentially bound to the nuclear matrix has been taken as indicative for a role of the nuclear matrix in diverse nuclear processes [Stuurman et al., 1992; Berezney and Jeon, 1995]. The cell-state and cell-type dependent association of distinct components, as e.g., PCNA or hnRNPs [Gerner and Sauermann, 1999], to the nuclear matrix has also shown the dynamic nature of the conceived nuclear framework. A function of the nuclear matrix in DNA transcription and RNA processing has been indicated by reported association of transcription factors, newly synthesized RNA, splicing factors, hnRNPs, and snRNPs to the nuclear matrix [Stuurman et al., 1992; Berezney and Jeon, 1995]. The present identification of PSF, a major factor of active spliceosomal complexes, as common nuclear matrix protein gives further evidence for a role of the nuclear matrix in RNA processing.

Another aspect is the observed difference in the subnuclear localization of two splicing factors known for their specific interaction [Patton et al., 1993; Gozani et al., 1994]. By microscopic examination and nuclear fractionation studies, PSF was exclusively detected in the nuclear matrix. In contrast, only low amounts of PTB were found in the nuclear matrix. The bulk of PTB was localized in PSF independent nuclear structures, which were readily solubilized by treatment of cells or nuclei with detergent, salt, and nucleases.

The non-matching patterns of punctuate PSF and PTB seen in the confocal microscope would agree with currently discussed interpretations of such phenomena [for review, see Singer and Green, 1997]. As the functional requirement of both, PSF and PTB, in splicing reactions has been established [Patton et al., 1991] it may be concluded that only the small amount of PSF and PTB structures which were found to colocalize in the nucleus are involved in splicing reactions. It is unclear at present, which function the individual structures of PTB and PSF may fulfill. There might be a highly dynamic exchange of PTB between the PSF containing complexes and the PSF independent structures, leading to a steady state equilibrium with the majority of cellular PTB not being associated with spliceosomal complexes. Alternatively, those complexes containing either PSF or PTB might represent different types of spliceosomal complexes at various intermediates of splicing assembly. The speckled appearance of PSF may be taken as indicative for its association with the nuclear matrix, as observed in case of other nuclear matrix proteins [Blencowe et al., 1994; Chabot et al., 1995]. Therefore, a potential function of PSF might be to link PTB to the nuclear matrix at the sites where spliceosomes are to be assembled. The punctuate appearance of PTB, however, may be due to the presence of bulk PTB in interchromatin granule clusters, reassembly and storage sites, reported for other metabolically non-engaged splicing components [Huang and Spector, 1996; Mattern et al., 1999].

It may, therefore, be concluded that depending on the biological state of the cells, PTB molecules may be recruited from nuclear storage sites to nuclear matrix associated metabolically active splicing sites. Putatively, this population might be anchored to nuclear matrix bound PSF.

ACKNOWLEDGMENTS

We wish to thank Christine Stadler for help in the production of mAb CTS32 and Josef Gotzmann for technical advice and for critical reading of the manuscript. This study was supported by grants from the Austrian Science Research Fund (FWF) (to R.F.) and from the Österreichische Nationalbank Jubiläumsfond (to G.S.).

REFERENCES

Aebersold RH, Leavitt J, Saavedra R, Hood LE, Kent SBH. 1987. Internal amino acid sequence analysis of proteins separated by one-or two-dimensional gel electrophoresis after in situ protease digestion on nitrocellulose. Proc Natl Acad Sci USA 84:6970–6974.

Berezney R, Coffey DS. 1974. Identification of a nuclear protein matrix. Biochem Biophys Res Commun 60:1410–1417.

Berezney R, Jeon KW, editors. 1995. Structural and functional organization of the nuclear matrix. Int Rev Cytol 162A, 162B. San Diego: Academic Press.

Blencowe BJ, Nickerson JA, Issner R, Penman S, Sharp PA. 1994. Association of nuclear matrix antigens with exon-containing splicing complexes. J Cell Biol 127:593–607.

Chabot B, Bisotto S, Vincent M. 1995. The nuclear matrix phosphoprotein p255 associates with splicing complexes as part of the [U4/U6.U5] tri-snRNP particle. Nucleic Acids Res 23:3206–3213.

Foisner R, Gerace L. 1993. Integral membrane proteins of the nuclear envelope interact with lamins and chromosomes, and binding is modulated by mitotic phosphorylation. Cell 73:1267–1279.

Gerner C, Holzmann K, Grimm R, Sauermann G. 1998. Similarity between nuclear matrix proteins of various cells revealed by an improved isolation method. J Cell Biochem 71:363–374.

Gerner C, Sauermann G. 1999. Nuclear matrix proteins specific for subtypes of human hematopoietic cells. J Cell Biochem 72:470–482.

Ghetti A, Pinol-Roma S, Michael WM, Morandi C, Dreyfuss G. 1992. HnRNP I, the polypyrimidine tract-binding protein: distinct nuclear localization and association with hnRNAs. Nucleic Acids Res 20:3671–3678.

Gil A, Sharp PA, Jamison SF, Garcia-Blanco MA. 1991. Characterization of cDNAs encoding the polypyrimidine tract-binding protein. Genes Dev 5:1224–1236.

Gooding C, Roberts GC, Smith CWJ. 1998. Role of an inhibitory pyrimidine element and polypyrimidine tract binding protein in repression of a regulated α-tropomyosin exon. RNA 4:85–100.

Gotzmann J, Eger A, Meissner M, Grimm R, Gerner C, Sauermann G, Foisner R. 1997. Two-dimensional electrophoresis reveals a nuclear matrix-associated nucleolin complex of basic isoelectric point. Electrophoresis 18: 2645–2653.

Gozani O, Patton JG, Reed R. 1994. A novel set of spliceosome-associated proteins and the essential splicing factor PSF bind stably to pre-mRNA prior to catalytic step II of the splicing reaction. EMBO J 13:3356–3367.

Harlow E, Lane D, editors. 1988. Antibodies— a laboratory manual. Cold Spring, NY: Cold Spring Harbor Laboratory Press.

Hochstrasser DF, Harrington MG, Hochstrasser AC, Miller MJ, Merill CR. 1988. Methods for increasing the resolution of two-dimensional protein electrophoresis. Anal Biochem 173:424–435.

Holzmann K, Korosec T, Gerner C, Grimm R, Sauermann G. 1997. Identification of human common nuclear matrix proteins as heterogeneous nuclear ribonucleoproteins H and H′ by sequencing and mass spectrometry. Eur J Biochem 244:479–486.

Holzmann K, Gerner C, Korosec T, Pöltl A, Grimm R, Sauermann G. 1998. Identification and characterization of the ubiquitously occurring nuclear matrix protein NMP 238. Biochem Biophys Res Comm 252:39–45.

Huang S, and Spector DL. 1996. Dynamic organization of pre-mRNA splicing factors. J Cell Biochem 62:191–197.

Korosec T, Gerner C, Sauermann G. 1997. Common nuclear matrix proteins in rat tissues. Electrophoresis 18:2109–2115.

Kramer A. 1996. The structure and function of proteins involved in mammalian splicing. Annu Rev Biochem 65: 367–409.

Lamm GM, Lamond AI. 1993. Non-snRNP protein splicing factors. Biochim Biophys Acta 1173:247–265.

Mattern KA, Van der Kraan I, Schul W, De Jong L, Van Driel R. 1999. Spatial organization of four hnRNP proteins in relation to sites of transcription, to nuclear speckles, and to each other in interphase nuclei and nuclear matrices of HeLa cells. Exp Cell Res 246:461–470.

Patton JG, Mayer SA, Tempst P, Nadal-Ginard B. 1991. Characterization and molecular cloning of polypyrimidine tract-binding protein: a component of a complex necessary for pre-mRNA splicing. Genes Dev 5:1237–1251.

Patton JG, Porro EB, Galceran J, Tempst P, Nadal-Ginard B. 1993. Cloning and characterization of PSF, a novel pre-mRNA splicing factor. Genes Dev 7:393–406.

Singer RH, Green MR. 1997. Compartmentalization of eucaryotic gene expression: causes and effects. Cell 91:291–294.

Singh R, Valcárel J, Green MR. 1995. Distinct binding specificities and functions of higher eucaryotic polypyrimidine tract-binding proteins. Science 268:1173–1176.

Stein GS, Stein JL, Lian JB, van Wijnen AJ, Montecino M. 1996. Functional interrelationships between nuclear structure and transcriptional control: contributions to regulation of cell cycle- and tissue-specific gene expression. J Cell Biochem 62:198–209.

Stuurman N, DeJong L, Van Driel R. 1992. Nuclear frameworks: concepts and operational definitions. Cell Biol Int Reports 16:837–852.