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REVIEW

Proteins Tightly Bound to DNA: New Data and Old Problems

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Abstract—Proteins tightly bound to DNA (TBP) comprise a group of proteins that remain bound to DNA after usual deproteinization procedures such as salting out and treatment with phenol or chloroform. TBP bind to DNA by covalent phosphotriester and noncovalent ionic and hydrogen bonds. Some TBP are conservative, and they are usually covalently bound to DNA. However, the TBP composition is very diverse and significantly different in different tissues and in different organisms. TBP include transcription factors, enzymes of the ubiquitin–proteasome system, phosphatases, protein kinases, serpins, and proteins of retrotransposons. Their distribution within the genome is nonrandom. However, the DNA primary structure or DNA curvatures do not define the affinity of TBP to DNA. But there are repetitive DNA sequences with which TBP interact more often. The TBP distribution within genes and chromosomes depends on a cell's physiological state, differentiation type, and stage of organism development. TBP do not interact with DNA in the sites of its association with nuclear matrix and most likely they are not components of the latter.

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Key words: proteins tightly bound to DNA, nuclear matrix, repetitive DNA sequences, serpins, phosphatases, transcription factors, differentiation

Protein binding to specific DNA sequences and their release from complexes with DNA is a key event in chromatin packaging and gene activity regulation. Complexes formed upon the interaction of DNA with tightly bound proteins, are resistant to salts and detergents and are probably very important for genome functioning. Tight bonds provide for chromatin loop joining to nuclear matrix via special AT-rich DNA regions (MAR). These complexes are necessary not only for DNA structuring and packaging, they also play an important role in replication, transcription, repair, and recombination [1-7]. In addition to interactions of DNA with nuclear matrix, DNA also forms more stable, sometimes covalent complexes with so-called tightly bound proteins (TBP) that remain bound to DNA after usual deproteinization procedures like salting out and treatment with phenol or chloroform. In the 1980-1990s TBP were intensely studied in laboratories of Georgiev and Razin [8-11], Werner [12-14], and Tsanev [15]. Chemical aspects of the problem were studied by Juodka et al. [16-18]. Despite interesting results and numerous unsolved problems, these investigations almost ceased, in many respects for subjective reasons. In recent years TBP investigations have been resumed using modern techniques [19-21]. In this review we generalize the earlier and new data about TBP, consider functional role of TBP, and formulate unsolved problems in this field. Among main questions connected with TBP composition and functions, the following were chosen:

– are TBP evolutionarily conservative and homologous in different organisms or are they species- and tissue-specific?

Abbreviations: DNP, deoxyribonucleoprotein(s); MAR, AT-rich DNA regions; TBP, proteins tightly bound to DNA.

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– do TBP bind to definite DNA sequences or are they randomly distributed within the genome?

– are TBP a part of nuclear matrix?

METHODS OF TBP PREPARATION

There are two main methods for purification of DNA complexes with TBP: DNA and DNP fractionation on nitrocellulose [13] and production of residual DNA–protein complexes after enzymatic hydrolysis of DNA [22]. In the first case DNA is first fragmented by enzymatic or mechanical treatment in high ionic strength solution and then filtered through nitrocellulose. DNA–protein complexes bind to nitrocellulose, while "pure" DNA passes through the filter. This fraction is called F (filtered) fraction. Then tight DNP are released from nitrocellulose by successive washings with the low ionic strength solution (R1, retained) and weak alkali solution (R2). In the second method DNA undergoes complete hydrolysis with DNase or benzonase. For efficiency of enzyme action the released nucleotides are dialyzed and residual complexes are precipitated by ethanol. It should be noted that TBP obtained by different methods may have different composition. For example, TBP from barley seedling leaves obtained by chromatography on nitrocellulose [19] and by DNase treatment [22] comprise different polypeptides. The method of DNA isolation may also influence the TBP composition. Thus, replacement of phenol deproteinization by DNA salting out significantly increases TBP yield and variability; supramolecular complexes are detected [23]. However, replacement of phenol deproteinization by equilibrium centrifugation in a cesium chloride density gradient with sarcosyl does not influence TBP composition [9].

CHEMICAL BONDS BETWEEN TBP AND DNA

The existence in a DNA molecule of protein linkers covalently bound by phosphodiester bonds between a tyrosine residue in the protein and the 5′ end of the DNA strand (Fig. 1a, according to [15]) was suggested previously [24, 25]. It was shown later that covalently bound TBP are retained by the alkali-labile phosphotriester bond between tyrosine residue and internucleotide phosphate group (Fig. 1b, [26]). However, the concept of protein linkers was not completely rejected. A group of Hungarian researchers showed that in DNA of various cells there is a single protein-masked single-stranded break per each 50 kb [27, 28]. The complex also contains RNA [29]. By these parameters protein linkers very much resemble "classical" DNA–TBP complexes [9-11]. However, far from all TBP are covalently bound to DNA. If nitrocellulose filter with adsorbed TBP–DNA complexes is washed with a high concentration lithium chloride and urea solution (0-4 M LiCl, 8 M urea), a portion of the DNA, retained in a complex with protein by hydrogen and ionic bonds, can be released from the filter. Another DNA portion is washed off the filter by the heated to 90°C solution of 4 M LiCl, 8 M urea; it is probably bound to protein by steric interactions (the protein pivot is introduced through a partially unwound DNA region). Covalently bound protein–DNA complexes remain on the filter [30].

Polypeptide composition of TBP and its tissue- and species-specificity. It was noted in the first report on TBP composition in various cell types that DNA treatment by phenol, proteases, and alkali does not remove certain polypeptides with molecular mass between 54 and 68 kDa [24]. Later the TBP composition was refined: main fragments were 62, 52, and 40 kDa polypeptides that formed supramolecular structures in the form of globules 12.8 nm in diameter. Minor proteins were also characterized [31].

DNA and TBP complexes localized at the points of chromatin loop attachment to nuclear matrix were studied in detail [9-11]. It was shown that these complexes consisted of 7-8 polypeptides, DNA, and RNA.

Also, the uniformity of TBP composition in different rat tissues such as spermatozoa, hepatocytes, and hepatoma was shown [32]. Peptide maps of TBP obtained from Drosophila embryos, carp liver, ram sperm, chicken erythrocytes, frog liver, and maize seedlings appeared to be very similar [15]. Sets of polypeptides tightly bound to DNA, purified by salting out, were almost identical in mouse and human cells [23]. In yeast cells the TBP content is lower as a whole; therefore the variability of these proteins and homology with mammalian TBP are revealed only upon isolation from large amounts of DNA, otherwise only one polypeptide absent from mammalian TBP preparations is detected [23, 33]. No differences were registered in TBP composition in undifferentiated and differentiated cells of Friend erythroleukemia as well as upon comparison of these proteins in Ehrlich ascites carcinoma and Friend erythroleukemia cells [33, 34]. However, the spectrum of TBP obtained from salted out DNA of *Tetrahymena* cells and from pike milt and eggs significantly differed from that in mouse tumors. However, additional treatment by stronger deproteinization agents (sodium dodecyl sulfate, sarcosyl at high temperature, protease, guanidine chloride, urea, phenol) leveled interspecies differences between TBP. Proteins of 62, 52, and 40 kDa were recognized as common for eukaryotes. Recognition of proteins from different organisms by TBP-specific antibodies from Ehrlich ascites cells supported this conclusion [33]. Recently, to obtain TBP we have used deproteinization with chloroform without protease treatment because it seems that the latter cleaves TBP and makes difficult their recognition. The use of RNase and restriction endonucleases was also excluded from the protocol of TBP preparation because purified

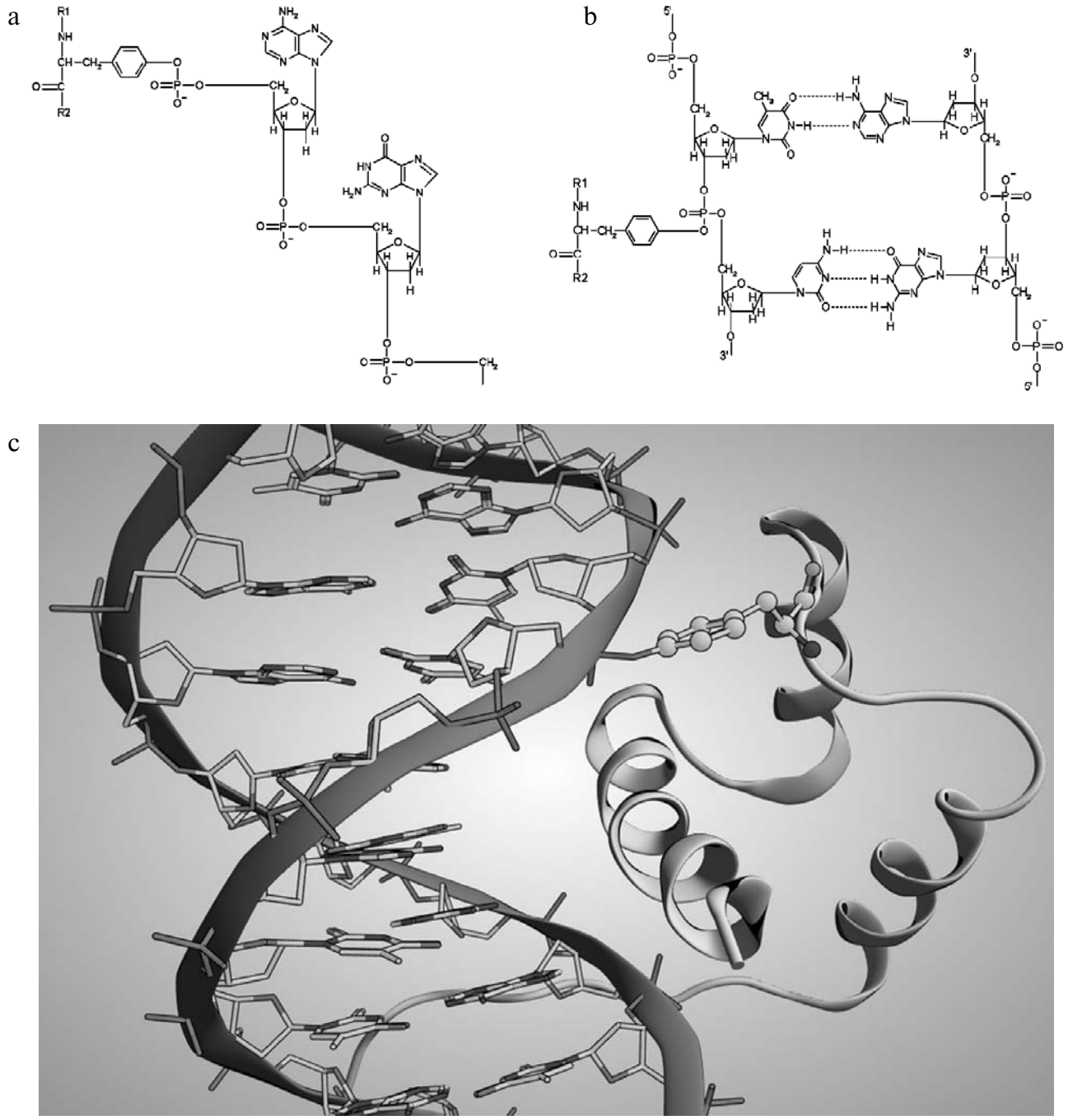

Fig. 1. Scheme of covalent bond formation between TBP and DNA. a) Protein binding to the oligodeoxyribonucleotide 5′ end. b) Protein bonding at internucleotide phosphate group. c) Spatial model of bonding the tyrosine residue in the hypothetical protein homeodomain to internucleotide phosphate group. The image was obtained using the MOE (Molecular Operating Environment) program version 2009.10, software (Chemical Computing Group Inc., Montreal, Canada).

enzymes added in high concentrations to isolated DNA may form stable artifact DNA–protein complexes [35, 36]. It appeared that the TBP set is different in barley seedling leaves, roots, and coleoptiles [19-21, 37]. In some cases it is also possible to reveal changes in the TBP spectrum during seedling development [20]. Differences were also found between TBP isolated from rat organs, chicken liver and erythrocytes, as well as between TBP from normal rat liver, ascites hepatoma Zajdela, and solid hepatoma G-27 (Fig. 2).

Enzymic and accompanying activities of TBP. One of the minor components of TBP of Ehrlich ascites carcinoma cells exhibited phosphatase activity. Active enzyme was formed by subunits of 56 and 59 kDa [31]. Later in

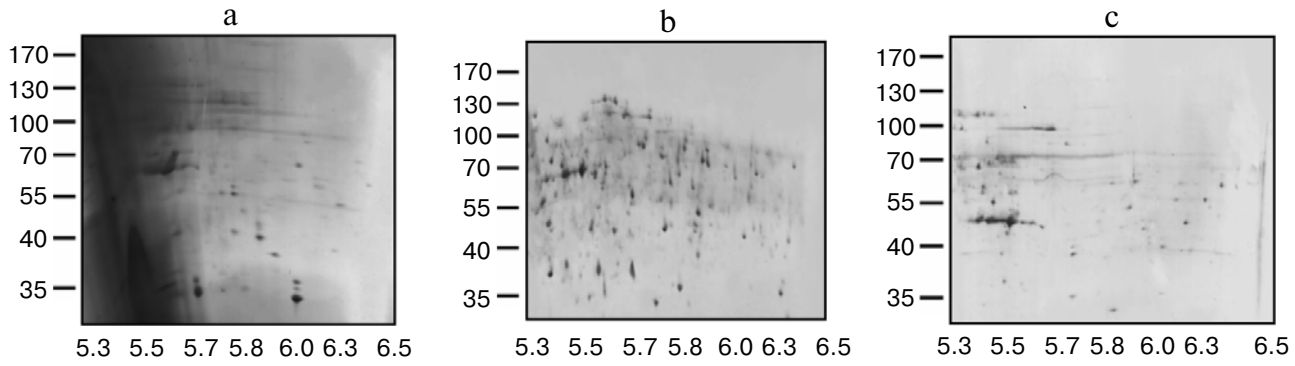

Fig. 2. Two-dimensional electrophoregrams of tightly bound to DNA proteins from rat liver (a), Zajdela ascites hepatoma (b), and hepatoma G-27 (c) (silver staining). Isoelectrofocusing in narrow pH gradient 5.3-6.5 was carried out in the first dimension using Immobiline dry strips (Amersham Biosciences). The sample was separated in the second dimension in 8-18% polyacrylamide gel gradient on a Multiphor II device (Amersham Biosciences). Positions of marker proteins (kDa) are shown on the left; corresponding pH values are shown at the bottom.

TBP of these cells protein kinase activity was also found. Both these activities are also present in TBP from Friend erythroleukemia cells [38].

A small tightly bound to DNA 16 kDa protein C1D exhibiting signal of nuclear localization and able to activate protein kinases appeared to be a proapoptotic factor whose level is regulated by the ubiquitin–proteasome system, while excessive expression results in cell apoptosis [39-41]. The 52 kDa glycoprotein incorporated in TBP of Ehrlich ascites carcinoma cells was homologous to alpha-1 serum inhibitor of proteases [42]. Later three proteins from the group of serum protease inhibitors (serpins) known as Spi-1, Spi-2, and Spi-3 were found among TBP. Serpins Spi-1 and Spi-2 are encoded by the same gene, while signal sequence at the polypeptide *N*-terminus provides for nuclear localization of these proteins. It is supposed that in addition to the long known function of serum protease inhibitors these proteins also fulfill certain intranuclear functions [43], in particular, they are involved in DNA repair [44].

Identified TBP. Combination of two- or one-dimensional protein electrophoresis in denaturing conditions with mass spectrometry (MALDI TOF-MS) allowed us to characterize a number of TBP from plant and animal tissues. Among TBP isolated from barley leaves, some chromatin and nuclear matrix proteins were found (NMCP1, histone acetyl transferase HAC12, RNA helicase, the DEMETER group enzyme carrying out DNA demethylation, a homolog of DNA repair enzyme RAD51), as well as numerous transcription factors belonging to different groups. Among found tightly bound to DNA transcription factors there were factors WRKY and Squamosa interacting with DNA via zinc fingers, factors AGAMOUS and MADS-box, whose specific DNA-binding domains are formed by 56 amino acids (MADS box), and factor TGA4 interacting with DNA via a leucine zipper. The TEOSINTE BRANCHED 1 factor binding to DNA via a noncanonical helix–loop–helix motif also was resistant to deproteinization [37, 45]. Thus, our results show that many transcription factors containing different DNA-binding domains are resistant to a deproteinization procedure. Numerous transcription factors are also found within nuclear matrix. Hydrogen bonds joining DNA with corresponding domains of transcription factors appeared to be resistant to salts and detergents [46]. It is quite possible that these bonds in many cases are resistant to organic solvents, owing to which transcription factors remain on DNA after deproteinization and are detected among TBP. Besides, hydroxyl groups of tyrosine residues in DNA-binding domains of transcription factors, localized near phosphodiester bond in DNA, may spontaneously form phosphotriester bonds like in the example shown in Fig. 1c. Both "classical" serpins and protein kinases were found among TBP of barley leaves. The protein encoded by retrotransposon *Ty3-gypsy* appeared to be specific for plant TBP. Heat shock proteins and immunophilins are also found among barley leave TBP [37, 45].

TBP obtained from yeast cells comprised a number of DNA-binding proteins, many of which are involved in DNA repair (RAD7, RHC31) and chromatin rearrangement (CAF-1, BAF-1). The TBP-specific kinases and phosphatases as well as components of the ubiquitin–proteasome system of protein degradation were also found [47].

Mass spectrometry of individual TBP peptides of rat liver identified 43 different proteins, the affiliation of most of which to TBP does not seem obvious. Nuclear enzymes such as DNA-methyl transferase were among the hepatocyte TBP. Another enzyme tightly bound to DNA, ribonuclease UK114, is mainly expressed in liver and kidney cells. Enzyme expression decreases upon tumor transformation of hepatocytes, and transfer of the protein into the cell nucleus occurs in response to stress

[48]. Several proteins involved in transcription regulation were identified. The latter include β-catenin, which binds chromatin and launches transcription of genes regulated via the Wnt signal pathway [49, 50]. One TBP turned out to be the translin-associated protein X that is also involved in transcription regulation of some translin genes [51]. The BAF factor (barrier-to-autointegration factor) tight binding to DNA is quite predictable because this DNA-binding protein interacts with lamin A and emerin; it is incorporated in nuclear matrix, a very tight DNA–protein complex [52]. The same protein was also found among yeast TBP. Another TBP, parafibromin, interacting with RNA polymerase within PAF1 regulatory complex, rather tightly binds to DNA [53]. Among TBP that remain complexed with DNA after chloroform treatment, there are proteins binding to SH3 domain (like SH3-domain-binding protein 5) and involved in signal transduction in Ras cascades and NFκB transcription factor, in function of the ubiquitin–proteasome system and RNA processing [54]. Localization of these proteins in the cell nucleus is especially pronounced in malignant tumors [55].

GTP-binding protein Mx3 induced by interferon and involved in antiviral protection is also belongs to the TBP class [56]. The leucine zipper of this protein retains it on DNA during deproteinization.

Detection of the above-mentioned proteins among TBP can be explained and even sometimes predicted, but identification in this group of inositol-3,4,5-triphosphate receptor and protein kinase C (known as signal system components) and hormones, binding to plasma membranes, is not so obvious. However, if the work of Russian [57] and Italian [58-60] researchers revealing this signal system in the cell nucleus are remembered, then everything is found in appropriate positions. Our data show that in addition to intranuclear localization of receptors and enzymes, the latter tightly interact with DNA. Phosphodiesterase, a component of nuclear cAMP-dependent signal system was found among TBP [61, 62]. It should be noted that this enzyme can also be involved in hydrolysis of covalent phosphotriester bonds between TBP and DNA [15, 26].

The insulin-dependent signal system localized mainly on the nuclear matrix also exists in the cell nucleus [63-67]. Some of its components (precursor of protein 2, binding to insulin-like growth factor, protein kinase beta, the Ras family protein Rab-18) were found among hepatocyte TBP. It is possible that TBP homologous to interleukin 18, neurotrophic brain factor, and to hepatocyte growth factor were formed due to intranuclear transport of growth factors [68, 69].

Detection among TBP of E3 ubiquitin-ligase NEDD4 and cullin, components of the ubiquitin–proteasome system of protein degradation, was quite predictable. According to personal communication of D. Werner, proteasome-resembling particles were found on electron microphotographs of TBP preparations. It is assumed that proteasomes are actively involved in transcription regulation [70]. They carry out degradation of numerous nuclear proteins including transcription factors, repair enzymes [71], and the TBP C1D [72]. Proteasomal proteins are well-characterized components of nuclear matrix [73-75].

Seemingly, there is no place for choline-ethanolamine-kinase and the fatty acid binding protein among TBP, but data on intranuclear lipid biosynthesis [76] explain this fact to some extent.

Unlike the above-mentioned proteins, the presence among rat liver TBP of a number of membrane, microsomal, and mitochondrial proteins is difficult to explain in any way except their artifactual binding to DNA during cell lysis. DNase [36] and RNase [35] are known to form tight artificial complexes with DNA. Owing to this, we do not use exogenous enzymes in TBP isolation. For some time there existed suspicion that TBP is keratin, contaminating DNA preparations [15]. Therefore, possible artifacts should be treated very carefully. On the other side, the presence of serpins among TBP first seemed strange [42], but presently it is an unquestionable fact.

DNA sequences interacting with TBP. Attempts to solve the question whether TBP bind DNA in definite sequences or randomly were made long ago. It was shown in early works that the ovalbumin gene sequence was involved both in complexes with TBP and with "pure" DNA [77]. At the same time, proteins covalently bound to the chicken β-globin gene enhancer were found [78]. These complexes were detected in reticulocytes, but they are absent from thymocytes [79], i.e. their formation depends on the level of gene transcription.

Several works were carried out on cloning and sequencing the TBP-bound DNA. It was shown that in human cells TBP binds to satellite DNA sequences [12]. Several repetitive sequences of mouse genome also form complexes with TBP [14, 22]. It was also shown that TBP binds to a specific oligonucleotide repeat (AGAGG/TCTCC) in chicken cells (here and further these are oligodeoxyribonucleotides) [13]. It is interesting that the same pentanucleotide sequence was found in the centromere DNA of gramineous plants [80, 81]. However a consensus sequence common for all organisms was not detected. Also, no homologies were found between TBP-binding DNA fragments in a different organism. The data indicate that very different sequences are able to bind TBP or that the DNA sequence is absolutely not important for complex formation with TBP [12]. It should be noted that these conclusions were based on analysis of very few clones.

T. G. Sjakste and M. Roder cloned 600 inserts of TBP-associated DNA (manuscript in preparation). Protein–nucleic acid complexes were obtained from different organs of barley seedlings by two methods: fractionation on nitrocellulose and DNase treatment. The CT

motif, most often represented by the $CC(TCTCCC)_2TC$ sequence, was identified in many DNA fragments (18.9% of all inserts). It is interesting that the "core" of this repeat is identical to the TCTCC repeat that binds TBP with DNA in chicken cells [13] and is characteristic of centromere DNA of gramineous plants [80, 81]. A different 49-bp GC-rich sequence was found in 6.9% of all inserts. Interestingly, frequency of these repeats depended on the procedure of TBP isolation and investigated seedling organ.

Thus, undoubtedly there are DNA sequences to which TBP exhibit increased affinity. However, it can be supposed that TBP do not obligatorily interact with just these sequences, i.e. the DNA sequence does not define the possibility of TBP–DNA complex formation. Such conclusion can be drawn after analysis of new data concerning TBP distribution at the gene and chromosome levels obtained by hybridization with microarrays of genomic probes and by PCR [19-21].

The TBP distribution in the chicken α-globin gene domain has been studied for a long time [8, 10]. It was shown that TBP bind to globin genes in erythroid cells and do not bind in non-erythroid cells [8]. The study of TBP distribution on 40 kb of the domain has shown the prevalent TBP binding to fragments exhibiting enhancer activity and bound to nuclear matrix [82]. Recent investigation of TBP distribution on 100 kb of this gene domain, using hybridization with microarrays of genomic probes revealed rearrangements of these proteins along the domain depending on transcription, differentiation, and apoptosis (Fig. 3 [21]). The microarrays were a set of oligonucleotides complementary to regions of the DNA domain remote from each other by 2000 bp. Oligonucleotides were fixed on the membrane using a device for blot hybridization. The microseries hybridization was carried out with radiolabeled DNA enriched in the fraction TBP (fraction R) or with DNA "free" of these proteins (fraction F, see above). The ratio of hybridization intensities of R and F fractions characterizes TBP binding with this domain region. It should be noted that this domain does not contain the above-mentioned repeat (AGAGG/TCTCC), enriching chicken DNA that interacts with TBP [13], while TBP still bind DNA in this domain, especially because their binding is of doubtless functional importance. Changes in TBP distribution depending on stage of barley grain ripeness were detected in α-amylase *Amy32b* and β-amylase *Bmy1* genes. In the *Amy32b* gene transition from watery to milk ripeness is accompanied by decrease in TBP binding along the whole gene, especially in the promoter and intron 2 region. The *Bmy1* gene expression associated with ripening was accompanied by release of exon 3 and intron 3 sequences from complexes with TBP [20]. Thus, TBP are differently distributed on the same sequence in grains of different ripeness. TBP rearrangements depending on barley seedling organ and development stage were also described at the chromosome level [19, 20].

TBP binding to bent DNA. We have also analyzed the possible involvement of bent DNA in formation of tight DNA–protein complexes. DNA curvatures are considered as a characteristic feature of the nuclear matrix binding sites [83-85]. Curvatures are also described in TBP-bound DNA [86, 87]. Experiments on hybridization with microarrays allowed us to clarify this question as well. In the chicken α-globin domain the bend.it program on the DNA tools site (http://www.icgeb.trieste.it/dna/) identifies curvatures in the region of 36,390 and 58,450 nucleotides from the beginning of published domain sequence (accession number AY016020). The existence of curvatures was checked experimentally by retardation of DNA fragment migration in polyacrylamide gel (Fig. 4). The curvature localized in position 36,390 of the domain 5′ untranslated region caused retardation of a corresponding fragment in polyacrylamide gel, a weaker (according to the program) curvature in the domain 3′ untranslated region did not influence the rate of fragment migration. The first sequence containing a stronger curvature (fraction 18, Fig. 4, b and c) was insignificantly enriched in TBP in erythroblasts (Fig. 4b). The second sequence whose position corresponds to oligonucleotide 60 in Fig. 4a and oligonucleotide 30 in Fig. 4 (b and c) was shown in the erythroblast cell culture DNA associated with nuclear matrix (Fig. 4a) and in the TBP-enriched erythrocyte DNA (Fig. 4c), although the existence of a curvature here was not confirmed experimentally. However, this sequence in erythroblasts is not bound to TBP (Fig. 4b). Thus, DNA curvatures do not define the TBP binding to DNA.

RELATIONSHIPS OF TBP WITH NUCLEAR MATRIX

The question whether TBP are components of nuclear matrix and points of DNA and TBP interaction correspond to sites of DNA binding to nuclear matrix is still discussed from the time when both these structures were described. Some researchers assume that they are identical structures [88, 89], while others believe that DNA–TBP complexes are not associated with sites of nuclear matrix interaction with DNA [15, 32].

Comparison of distribution of TBP [21] and sites of DNA binding to nuclear matrix [90] in the α-globin gene domain of HD3 cells (Fig. 4, a and b), determined by microarray hybridization clearly illustrates differences in the two distributions. Sites of binding with nuclear matrix and TBP coincide only for a single fraction (34 for Fig. 4a, 17 for Fig. 4b).

Sites of TBP binding with nuclear matrix on the chromosomal level was similarly compared on barley chromosomes 1H and 7H using a set of primers for

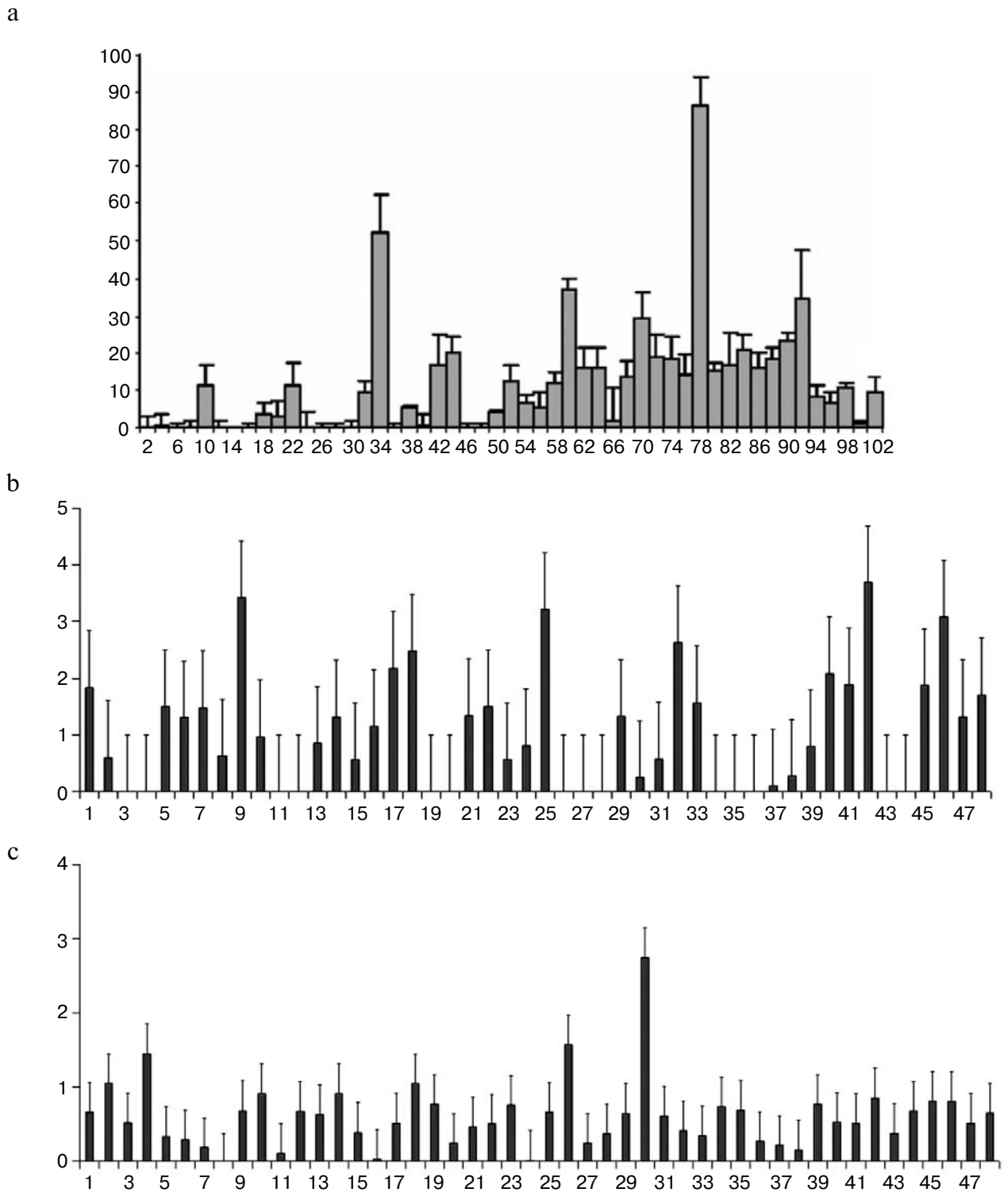

Fig. 3. Intensity of hybridization with genomic probe microarrays for chicken α-globin domain. Compilation of data from [21, 90]. Probes correspond to domain sequences arranged at the interval of 1 kb (a) or 2 kb (b, c). a) Distribution of sites of DNA interaction with nuclear matrix in α-globin domain in chicken erythroleukosis culture cells HD3. Data are given as the ratio of intensity of nuclear matrix DNA hybridization signal to that of total DNA. b) Distribution of sites of DNA interaction with TBP in α-globin domain in chicken erythroleukosis culture cells HD3. Data are given as the ratio of the fraction R DNA hybridization signal intensity to that of fraction F DNA (see text). c) The same with chicken erythrocytes.

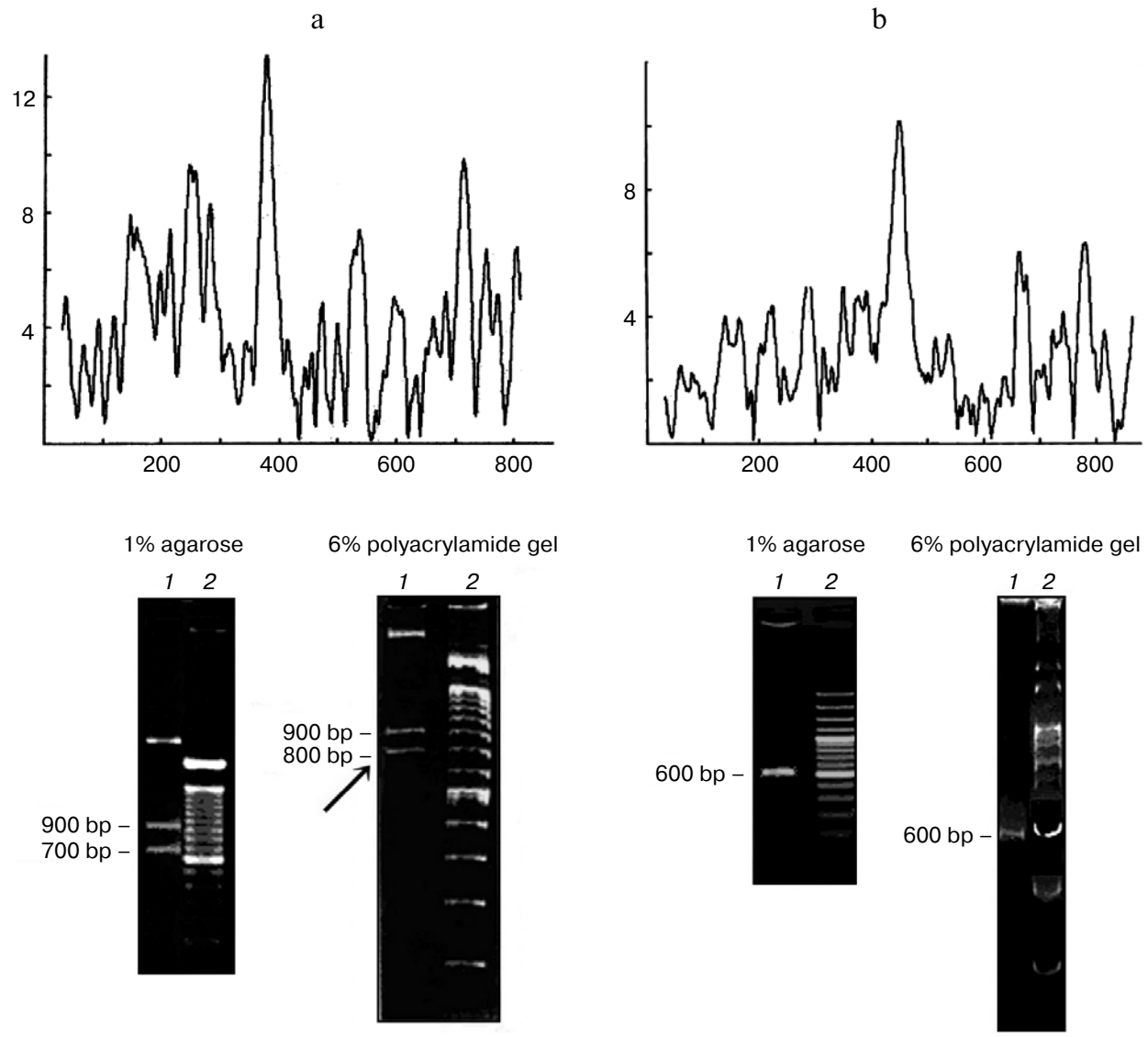

Fig. 4. Prediction and checking the existence of DNA curvatures in chicken α-globin domain. Upper row, theoretically predicted curvatures. Bottom row, electrophoresis of DNA fragments in agarose and polyacrylamide gels. a) Upper row: analysis of possible DNA curvatures using the bend.it program between 36,000 and 36,800 bp of the AY016020 sequence. Base pair numbers are shown on the abscissa axis, the predicted bending (degrees per 10.5 bp) is shown on the ordinate axis. Bottom row: electrophoresis of *Hind*III-*Bgl*II (36,329-37,059 bp) and *Bgl*II-*Hind*III (37,059-37,972 bp) fragments in agarose (1%) and polyacrylamide (6%) gels. Fragments were obtained from cloned H3 fragment of *Hind*III-*Hind*III. *1*) Restriction fragments; *2*) molecular mass markers. The band with decreased migration rate is marked by an arrow. b) Upper row: analysis of possible DNA bends using the bend.it program between 58,000-58,800 bp of the AY016020 sequence. Designations as for panel (a). Bottom row: electrophoresis of amplified fragment (600 bp), containing the supposed bending, in agarose and polyacrylamide gels. *1*) Amplified DNA fragment; *2*) molecular mass markers.

microsatellite genetic markers of these chromosomes. DNA complexes with nuclear matrix and TBP from organs of barley seedlings of different age were obtained. The presence of any marker sequence in DNA fraction was determined by amplification product formation in PCR with a given pair of primers. It is seen in Fig. 5 that the distribution of microsatellite markers in DNA bound to nuclear matrix and TBP does not coincide [20].

It should also be noted that no typical MAR sequences were revealed in clones of TBP-bound DNA [91]. Thus, it can be concluded that the TBP–DNA and nuclear matrix–DNA complexes are different structures. Taking into account TBP heterogeneity (see above) and complexity of nuclear matrix composition [46, 92], it is not surprising that some proteins of nuclear matrix can also be found in TBP.

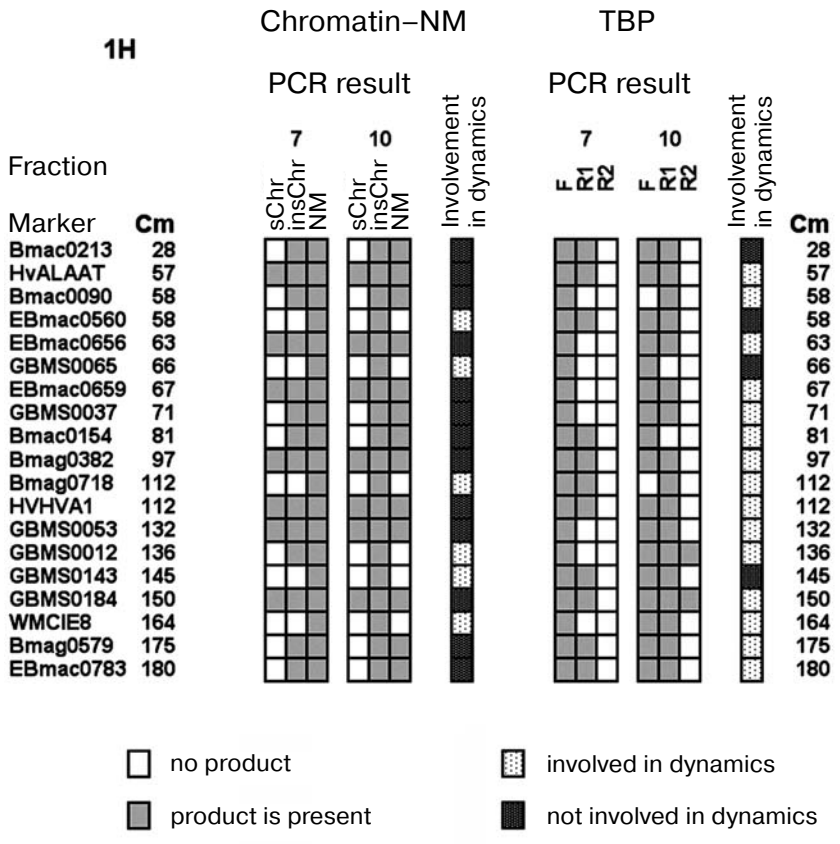

Fig. 5. Distribution of microsatellite markers of barley chromosome 1H in DNA bound with nuclear matrix and TBP. Compilation according to [20]. sChr, soluble chromatin; insChr, insoluble chromatin; NM, nuclear matrix; F, DNA fraction free of TBP; R1 and R2, fractions of tight protein–nucleic acid complexes released from nitrocellulose by successive washings with low ionic strength solution (R1) and weak alkali solution (R2).

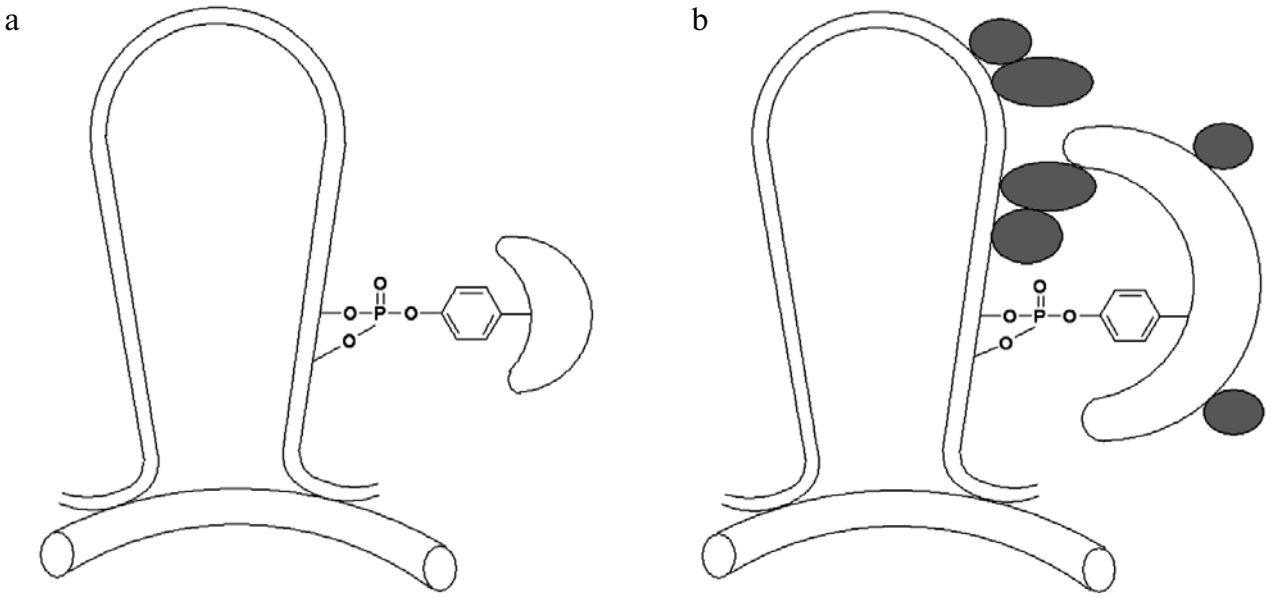

Fig. 6. Generalized scheme. a) Transcription-inactive domain. Outside the site of binding to nuclear matrix, DNA is covalently bound to "conservative" TBP. b) Domain activation is accompanied by association of additional proteins with DNA by noncovalent bonding. Complex includes protein components not bound to DNA but interacting with TBP due to protein–protein interactions.

In conclusion, we shall answer questions formulated in the introduction.

1. Are proteins tightly bound to DNA homologous in many organisms and evolutionarily conservative or are they species- and tissue-specific? It appears that some TBP are really conservative, most likely these are proteins covalently bound to DNA. However, the TBP composition is very diverse, owing to which the spectrum of these proteins in different tissues and in different organisms differs significantly. Investigations of TBP composition should be continued; it is necessary to exclude proteins artificially interacting with DNA, i.e. molecules of proteins tightly binding to DNA in cell lysate during nucleic acid isolation, and to reveal proteins that characterize the group as such, i.e. those tightly bound to DNA in a living cell. For the present it can be said that TBP from different sources are transcription factors, other proteins interacting with DNA and chromatin, enzymes of the ubiquitin–proteasome system, phosphatases, protein kinases, serpins, and retrotransposon proteins. Further investigations will show which of these proteins are "real" TBP and which are occasional fellow travelers.

2. Do TBP bind to definite or random DNA sequences in the genome? Certainly, TBP are not accidentally distributed along the genome. However, the DNA primary structure or curvatures do not define the affinity of TBP to it. However, sequences are revealed with which TBP interact more often than with others. The TBP distribution in genes and chromosomes depends on the cell physiological state, differentiation type, and stage of organism development.

3. Are TBP a part of nuclear matrix? Most likely not; these proteins interact with DNA not in the sites of its association with nuclear matrix. The hypothesis of possible TBP localization on the chromatin loop is shown in Fig. 6. In the absence of transcription in the chromatin domain outside the site of binding to nuclear matrix, DNA is covalently bound to "conservative" TBP. Domain activation is accompanied by noncovalent association of additional proteins with DNA. The complex is also replenished by protein components not bound to DNA but interacting with TBP due to protein–protein interactions.

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