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REVIEW

Y-Box-Binding Protein 1 (YB-1) and Its Functions

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Abstract—This review describes the structure and functions of Y-box binding protein 1 (YB-1) and its homologs. Interactions of YB-1 with DNA, mRNAs, and proteins are considered. Data on the participation of YB-1 in DNA reparation and transcription, mRNA splicing and translation are systematized. Results on interactions of YB-1 with cytoskeleton components and its possible role in mRNA localization are discussed. Data on intracellular distribution of YB-1, its redistribution between the nucleus and the cytoplasm, and its secretion and extracellular functions are summarized. The effect of YB-1 on cell differentiation, its involvement in extra- and intracellular signaling pathways, and its role in early embryogenesis are described. The mechanisms of regulation of *YB-1* expression in the cell are presented. Special attention is paid to the involvement of YB-1 in oncogenic cell transformation, multiple drug resistance, and dissemination of tumors. Both the oncogenic and antioncogenic activities of YB-1 are reviewed. The potential use of YB-1 in diagnostics and therapy as an early cancer marker and a molecular target is discussed.

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Key words: YB-1, Y-box binding proteins, regulation of gene expression, DNA repair, transcription, translation, oncogenesis, oncomarker, multidrug resistance, metastasis

The multifunctional vertebrate Y-box-binding protein 1 (YB-1) is a member of a large family of proteins with an evolutionally ancient cold shock domain. This protein is involved in a number of cellular processes including proliferation, differentiation, and stress response. The *YB-1* gene knockout in mice results in serious distortions of embryonic development and in early (prenatal) death. A high YB-1 content is typical for all mouse organs both at prenatal and early postnatal stages of development. However, the amount of this protein gradually decreases with aging, and in old age it is virtually not found in any organ except the liver.

The YB-1 protein performs its functions both in the cytoplasm and in the cell nucleus. It can also be secreted from cells and by binding to the receptors on their surface it can activate intracellular signaling.

YB-1 is a DNA- and RNA-binding protein that has properties of a nucleic acid chaperone, and it interacts with a great variety of other proteins. By binding to nucleic acids, YB-1 is involved in almost all DNA- and mRNA-dependent processes including DNA replication and repair, transcription, pre-mRNA splicing, and mRNA translation. It packs and stabilizes mRNAs as well as realizes global and specific regulation of gene expression at different levels.

Inasmuch as the content of YB-1 drastically increases in tumor cells, this protein is considered to be one of the most indicative markers of malignant tumors.

By passing from the cytoplasm to the cell nucleus, YB-1 activates transcription of genes of several protective proteins, including proteins that provide multidrug resistance of cells. When involved in DNA repair in the nucleus, YB-1 also enhances resistance of cells to xenobiotics and ionizing radiation. That is why YB-1 nuclear localization is an early marker of multidrug resistance of malignant cells.

An increase of YB-1 level in the cytoplasm prevents oncogenic cell transformation by the PI3K/Akt signaling pathway, and simultaneously it can promote transforma-

Abbreviations: A/P, alanine/proline-rich domain; CRS, cytoplasmic retention site; CSD, cold shock domain; CTD, C-terminal domain; dbp A and B, DNA-binding proteins A and B; EMT, epithelial mesenchymal transition; FRGY1 and 2, frog Y-box proteins 1 and 2; IFNγ, interferon γ; mRNP, messenger ribonucleoproteins; NLS, nuclear localization signal; PABP, poly(A) binding protein; TGFβ, transforming growth factor β; TNFα, tumor necrosis factor α; UTR, untranslated region; YB-1, Y-box binding protein 1.

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tion of differentiated epithelial cells into mesenchymal ones with higher migration activity. This is favorable for cell spreading in the organism and metastasis. Thus, YB-1 can be a marker of metastasis in remote organs.

In this review, we have tried to summarize and describe in detail the most significant of the available results on the YB-1 protein, a most multifunctional and intriguing object of the current molecular biology.

HISTORY OF DISCOVERY AND NOMENCLATURE OF Y-BOX BINDING PROTEINS

Y-Box binding proteins were first described in the 1970s as universal major components of cytoplasmic mRNP from bird (duck) [1, 2] and mammal (rabbit) reticulocytes [3]. Later they were found in mRNPs of other mammalian cells: KB [4], HeLa [5], ascitic Ehrlich carcinoma [6], kidney cells [7], etc. [8] as well as in oocytes of amphibians (*Xenopus laevis*) [9]. On SDS polyacrylamide gel electrophoresis the proteins possessed motility corresponding to that of proteins with molecular mass of about 50 kDa, and thereby they were for a long time designated as p50, p54/p56, etc. One of these proteins was discovered and for the first time cDNA-sequenced in 1988 as a DNA-binding protein interacting with the so-called Y-box motif in the promoter of the major histocompatibility complex class II genes [10]. It was named "Y-box binding protein 1 (YB-1)". After that the determination of the primary structure of *X. laevis* p54/p56 [11, 12] and rabbit p50 [13] permitted their identification as Y-box binding proteins.

In 1988 it was demonstrated that two proteins, named dbpB and dbpA, bind specifically to the enhancer region of the gene encoding the receptor of the human epidermal growth factor [14]. It appeared that the amino acid sequence of dbpB is completely identical to that of YB-1, and dbpA is homologous to it by about 46%. The YB-1 and dbpA proteins had an evolutionary conserved sequence near the N-terminus, which was 44% identical to the sequence of protein CspA (Cold shock protein A) from *Escherichia coli* [15]. This part of the molecule of YB-1 and its homologs was called a "cold shock domain" (CSD), since the CspA protein belongs to the group of the so-called major bacterial cold shock proteins. The presence of a CSD is a specific feature of Y-box binding proteins and allows attributing them to a wider group of proteins containing a cold shock domain. Other representatives of this group can consist of one (bacterial proteins CspA, CspB, CspE, and CspD) or of several (mammalian protein UNR, Upstream of N-Ras) cold shock domains and can have, apart from CSD, additional domains and sequences such as zinc finger domains (protein lin-28 from *Caenorhabditis elegans*) or glycine-rich sequences (protein lin-28 from *C. elegans*, protein RBP16 from *Trypanosoma brucei*, and protein GRP2 from *Arabidopsis thaliana*).

At present amino acid sequences of about two dozens of Y-box binding proteins of vertebrates have been determined, and some of their functions have been clarified. Y-Box binding proteins can fall into three subfamilies (Table 1).

The first subfamily (YB-1) includes the human YB-1 *per se* (see other names in Table 1), rabbit p50, chicken chk-YB-1, bovine EFI-A, FRGY1 from *X. laevis*, mouse MSY-1, and some others. As a rule, these proteins are characteristic of somatic cells and perform various functions. They are the most studied ones, and the greater part of this review will be devoted to them.

The second subfamily (YB-2) involves proteins FRGY2 (p54/56) from *X. laevis* and mouse MSY-2. The human cell proteins from this subfamily are named YB-2, dbpC, and contrin. They are specific for germ cells.

And finally, the YB-3 subfamily contains human dbpA (csdA) protein, mouse MSY-3, and YB-3 from *X. laevis*. It is supposed that these proteins are synthesized during embryonic development and disappear by the moment of birth [16]. However *YB-3* mRNA can be detected in some tissues of an adult organism [17]. It is also noted that *YB-3* mRNA has two isoforms, a short and a long one, that are produced by an alternative splicing of pre-mRNA [17].

It is difficult to assign proteins Ct-p50/p40 from *Chironomus tentans* and Yps from *Drosophila melanogaster* to any of the subfamilies of vertebrate Y-box binding proteins because their similarity with the proteins of subfamilies YB-1, YB-2 and YB-3 is restricted to only high homology of cold shock domains, very similar amino acid composition, and characteristic cluster allocation of charged amino acid residues in the C-terminal domain (CTD).

PROPERTIES AND STRUCTURAL AND FUNCTIONAL ORGANIZATION OF Y-BOX BINDING PROTEINS

General properties of Y-box binding proteins. The basic peculiarities of all members of the three subfamilies of vertebrate Y-box binding proteins are as follows:

– high content of alanine and proline in the N-terminal domain (hence its other name – the A/P domain);

– the presence of a cold shock domain;

– the presence of an elongated C-terminal domain containing alternating clusters of positively and negatively charged amino acid residues (Fig. 1a).

A comparison of amino acid sequences of proteins from various subfamilies has demonstrated that their cold shock domains are identical by more than 90% (Fig. 1b), while in the other parts of the molecules no significant homology is observed.

Within a subfamily the homology is rather high. For example, human YB-1 protein is 96% identical to mouse

Table 1. Y-Box binding proteins

	YB-1	YB-2	YB-3
Homo sapiens (human)	YB-1 (Y-box binding protein 1); NSEBP 1 (Nuclease sensitive element binding protein 1); EFI-A (Enhancer factor I subunit A); dbpB (DNA-binding protein B)	YB-2 (Y-box binding protein 2, Germ cell-specific Y-box binding protein); Contrin; dbpC (DNA-binding protein C)	dbpA (DNA-binding protein A); csdA (Cold shock domain protein A); Single-strand DNA binding protein NF-GMB
Mus musculus (mouse)	MSY-1 (Mouse Y-box binding protein 1); dbpB	MSY-2 (Mouse Y-box binding protein 2)	MSY-3/4 (Mouse Y-box binding protein 3/4); dbpA; csdA
Rattus norvegicus (rat)	RYB-a (Rat Y-box binding protein-a); YB-1		
Oryctolagus cuniculus (rabbit)	p50; YB-1		
Bos taurus (bovine)	EFI-A; YB-1		
Gallus gallus (chicken)	Chk-YB-1 (Chicken Y-box protein 1)	Chk-YB-2 (Chicken Y-box protein 2)	
Xenopus laevis (African clawed frog)	FRGY1 (Frog Y-box protein 1)	FRGY2 (Frog Y-box protein 2); p54/p56; mRNP4	YB-3
Danio rerio (carp family fish)	YB-1		
Chironomus tentans (mosquito)	Ct-p50/p40		
Drosophila melanogaster (fruit fly)	Yps; Y-box binding protein		

MSY-1, 80% identical to FRGY1 from *X. laevis*, and 67% identical to YB-1 protein from *Danio rerio*.

In line with a structure prediction, N- and C-terminal domains are disordered (Fig. 2). Probably this is the reason why there has been no success in determining the three-dimensional structure of full-size Y-box binding proteins. There is a hypothesis according to which the conformation of these domains is fixed only upon binding to ligands and may vary in complexes with different ligands. That is why it would be expedient to study three-dimensional structures of Y-box binding proteins in a complex with various partners.

Based on the data of circular dichroism (CD) spectroscopy, it was assumed that protein FRGY2 contains the structure of poly(L-proline) helix type II [18].

The three-dimensional structure of protein CspA that is 44% identical to the YB-1 CSD was determined by X-ray analysis and nuclear magnetic resonance (NMR) long ago [19, 20]. However, the structure of the human YB-1 CSD was determined using NMR just about 10 years later [21]. Three-dimensional structures of the YB-1 CSD and CspA turned out to be very close to each other, which could be expected from the high homology of these proteins. The YB-1 CSD and CspA consist of five β-strands packed antiparallel in a β-barrel at the ends of which there are loops that connect the β-strands. CSD has the so-called RNP-1 and RNP-2 consensus sequences (K/N-G-F/Y-G-F-I/V and V-F-V-H-F, respectively) [22] involved in specific and nonspecific interaction with DNA [23] and RNA [24, 25].

In accord with the NMR data, the YB-1 CSD has a low stability: at 25°C in solution only about 70% of CSD molecules are in the native state [26] whereas, judging by the microcalorimetry data, the melting temperature of protein CspA from *E. coli* is 56°C [27]. This difference in the stability of CSD of pro- and eukaryotes is associated with the presence of a long flexible loop in the YB-1 CSD, which is absent in prokaryotic proteins [21].

YB-1 and its homologs form oligomers with a mass up to 800 kDa [13, 23]. Using electron and atomic force microscopy, it was found that oligomers adsorbed on a substrate have a rounded shape, their diameter being 35-40 nm and the height 9-10 nm [28]. Oligomerization may occur because of the interaction of oppositely charged amino acid clusters of the C-terminal domains from different protein molecules [23]. An isolated CSD is a monomer [26].

It has been established recently that at high ionic strength YB-1 forms elongated fibrils of 15-20 nm in diameter and several micrometers long. The fibrils look like helices with a period of 50-52 nm [29]. It may be that

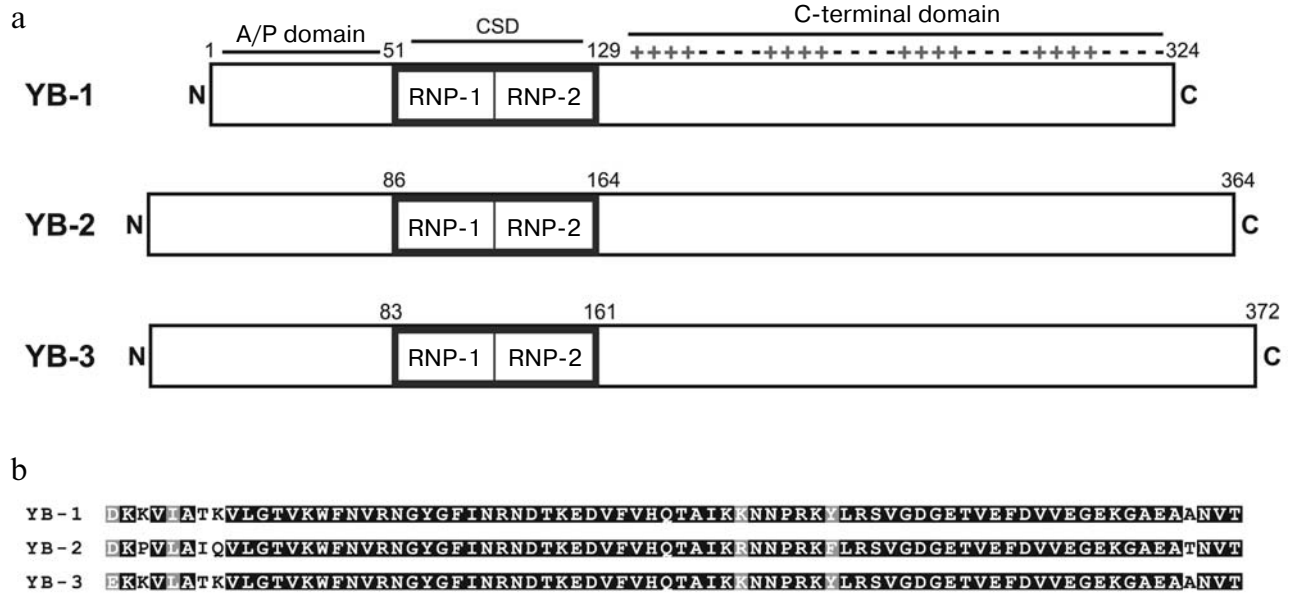

Fig. 1. Y-Box binding proteins domain organization. a) Human Y-box binding proteins. b) Comparison of amino acid sequences of human Y-box binding proteins cold shock domains.

CSD is involved in the formation of fibrils in a partly unfolded state, which is specific of its existence in solution in equilibrium with CSD in the native state.

Properties of Y-box binding protein 1 (YB-1). Human YB-1 consists of 324 amino acid residues, the predominating ones being Arg (11.7%), Gly (12%), Pro (11%), and Glu (8.3%). Its molecular mass calculated by the amino acid sequence is about 35.9 kDa, but as mentioned above, during SDS gel electrophoresis YB-1 migrates as a protein with a mass of about 50 kDa, i.e. it shows anomalous behavior. A specific feature of YB-1 is an extremely high isoelectric point of about 9.5 [30].

Peculiarities of interaction of YB-1 with DNA and RNA. YB-1 was discovered as a DNA-binding protein specifically interacting with the Y-box (5′-CTG**ATTG-G**$^{C}/_{T}{}^{C}/_{T}$AA-3′); however, later it was clarified that it can bind to a great variety of sequences in DNA [31-33]. When analyzing the interaction with oligodeoxyribonucleotides immobilized onto a microchip, it was found that YB-1 has the greatest preference to the single-stranded motif GGGG, then to single- and double-stranded motifs CACC and CATC, and a lower affinity to the sequences occurring in Y-boxes [33]. By binding to DNA, YB-1 significantly decreases the melting temperature of double helices, by three orders of magnitude accelerates the formation of DNA double helices from mutually complementary strands in physiological conditions, and also catalyzes the exchange of complementary strands in imperfect duplexes to generate the most elongated and fully-matched double helices, i.e. reveals features of a DNA-chaperone [28, 33, 34]. It was shown by many authors that YB-1 has a far higher affinity to a single-stranded DNA than to a double-stranded one. It is suggested that the binding of YB-1 to the CT-rich strand results in the generation of nuclease-sensitive regions in the DNA and the formation of the DNA H-structure in the second strand [31, 35-37]. Besides, YB-1 has a higher affinity to cisplatin-modified DNA or DNA containing abasic sites or mismatches [31, 36, 38-40]. By binding to such DNA, YB-1 causes local melting of duplexes, which may promote its efficient repair [36, 40]. The discovered peculiarities of the interaction of YB-1 with DNA define its functional activity in such processes as transcription and DNA repair.

The functioning of YB-1 in pre-mRNA splicing, translation, stabilization, and packing is dependent on its

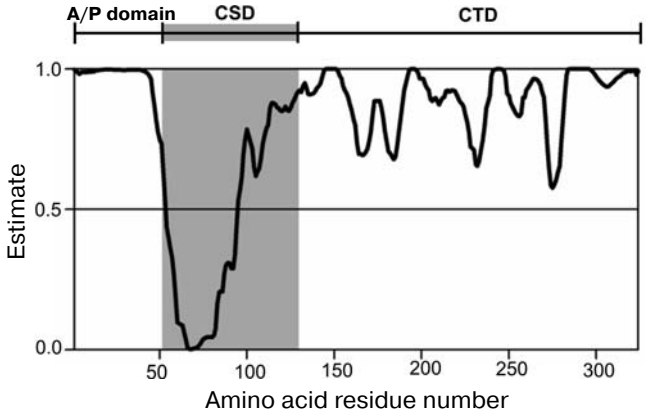

Fig. 2. Disorder in YB-1 structure. Each residue is compared to a disorder estimate by the PONDR VL-XT (www.pondr.com) algorithm. Value ≥0.5 means disorder.

ability to bind RNA. It was demonstrated that YB-1 has a high nonspecific affinity to a wide variety of sequences, though showing preferences to some of them. When binding to homopolyribonucleotides, YB-1 has the highest affinity to poly(G) and a gradually decreasing affinity to poly(U), poly(A), and poly(C). The dissociation constant of YB-1 complexes with globin mRNA and 16S rRNA is $4 \cdot 10^{-9}$ M [30, 41]. The specific sequence with which YB-1 homologs from *X. laevis* (FGRY1 and FRGY2) preferably interact was determined by the SELEX method. It is the hexanucleotide sequence 5′-AA**C**A**U****C**-3′ called YRS (FRGY recognized sequence) [25]. Then similar sequences, to which YB-1 from different organisms specifically binds, were found by footprinting in the *YB-1* mRNA (5′-UC**C**A$^{A}/_{G}$**C**A-3′) [42], *protamine* mRNA (5′-UC**C**A**U****C**A-3′) [43], *VEGF* mRNA (Vascular endothelial growth factor) (5′-AAC$^{C}/_{U}$UCU-3′) [44], Rous sarcoma virus RNA (5′-GUAC**C**A**C****C**-3′) [45], and Dengue virus (+)RNA (5′-UC**C**A**G**GCA-3′) [46]. All of them are rich in A and C, and in addition, as shown by point mutagenesis, C in the third position, A in the fourth, and C in the sixth (bold typed) are nucleotides determining a higher affinity of YB-1 to these sequences [25, 43].

It should be mentioned that YB-1 has a higher affinity to RNA containing an oxidized base 8-oxoguanine. Such modifications may occur under oxidative stress conditions and can cause different errors during protein biosynthesis. It is assumed that by recognizing the modified mRNA, YB-1 is capable of blocking its translation [47].

Binding to RNA, YB-1 melts its secondary structure, yet the melting is incomplete (during interaction with YB-1 up to 60% of the initial secondary structure in α-globin mRNA is melted) [13]. Under physiological conditions, YB-1 accelerates annealing and catalyzes the exchange of complementary RNA strands to generate the most elongated and perfect duplexes, i.e. acts as an RNA chaperone [34]. It is important that the ratio of RNA-melting and RNA-annealing activities of YB-1 is dependent on the YB-1/RNA ratio within the complex: RNA-annealing activity is prevailing in complexes not saturated with the protein, whereas RNA-melting activity is prevailing in protein-saturated complexes [13]. It is probable that at a low YB-1/mRNA ratio, YB-1 helps mRNA to assume the conformation appropriate for correct recognition of mRNA by mRNA-binding factors.

CTD is thought to be responsible for nonspecific binding to RNA, though according to some data it prefers pyrimidine-rich sequences [24]. It is CTD that provides a high affinity of YB-1 to nucleic acids. The cold shock domain accounts for the specific binding with RNA, while CTD and perhaps A/P enhance and stabilize this interaction [18, 25, 48].

Interaction of YB-1 with proteins. All the three YB-1 domains are involved in the interaction with proteins (Fig. 3). The **A/P domain** contains binding sites for actin [49], splicing factor SRp30c [50], transcription factor p53 [51], and cyclin D1 [52].

CSD can interact with Akt kinase [53] and E3 ubiquitin ligase FBX33 [54].

CTD supports protein homomultimerization [23, 25, 55]. This domain has binding regions for some important regulatory proteins such as hnRNP K [56], hnRNP D

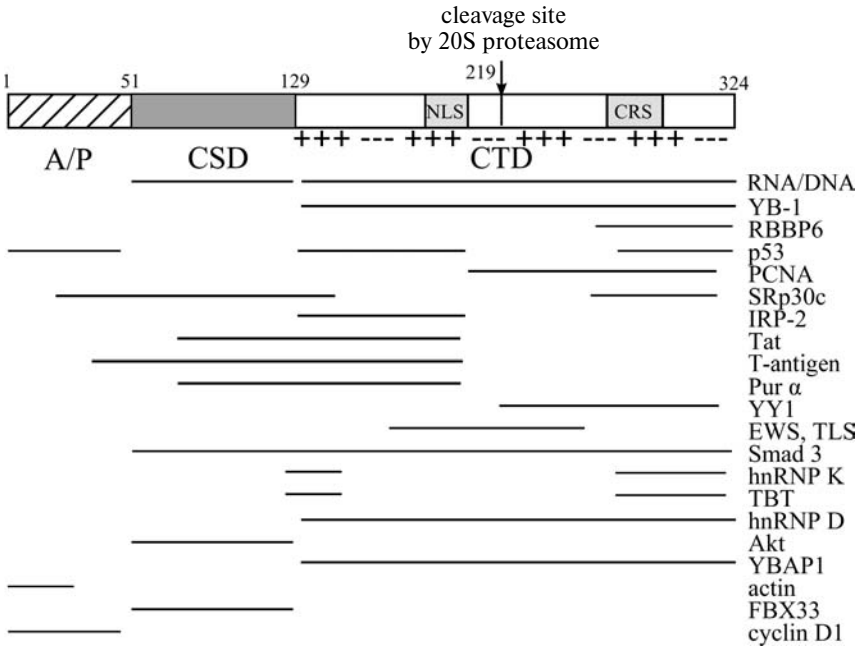

Fig. 3. Scheme of binding sites for YB-1 partners. Pluses and minuses indicate positions of clusters of positively and negatively charged amino acids.

[57], TATA-binding protein TBP [56], transcription factor p53 [51], YBAP1 (Y-box protein-associated acidic protein 1) [58], PCNA (Proliferating cell nuclear antigen) [39], IRP-2 (Iron regulatory protein 2) [59], E3 ubiquitin ligase RBBP6 [60], transcription factor YY1 [61], TLS (Translocated in liposarcoma protein), and EWS (Ewing's sarcoma breakpoint region) [62].

CTD and CSD jointly form binding regions for transcription factor Purα [63], HIV-1 transcription factor Tat [64], large T-antigen of JCV polyomavirus [65], transcription factor Smad3 [66], and splicing factor SRp30c [50].

YB-1 is also known to interact with transcription factor Sox1 [67], CARP (Cardiac ankyrin repeat protein) [68], tubulin [69], Ankrd2 (Ankyrin repeat domain-containing protein 2) [70], heat shock protein HSP60 [71], transcription factor RelA [72], transcription factor CTCF [73], Purβ (Purine-rich element binding proteins β) [74], transcription factor AP-2 [75], histone acetyltransferase p300 [66], tumor suppressor IRF-1 [76], and DEAD-like RNA-helicase [77]. However regions of the YB-1 molecule involved in these interactions have not been clearly determined.

Posttranslational modifications of YB-1. YB-1 is known to be subject to phosphorylation, limited proteolysis with 20S proteasome, ubiquitination, and probably acetylation.

The total mass-spectrometric studies of the phosphoproteome show that YB-1 is phosphorylated at the following amino acid residues: Ser165 and/or Ser167, Ser174 and/or Ser176, Ser313 and/or Ser314, and Tyr162 [78-81]. YB-1 can be phosphorylated by kinases ERK2 and GSK3β, such phosphorylation enhancing the YB-1 binding to the *VEGF* gene promoter [82].

YB-1 is phosphorylated at Ser102 *in vitro* and *in vivo* by kinase Akt [53, 83] as well as kinase RSK [84].

YB-1 can be completely degraded by the 26S proteasome after ubiquitination [54]. On the other hand, YB-1 can undergo limited proteolysis (processing) by the 20S proteasome. In the latter case, the cleavage of YB-1 into two fragments after Glu219 is ATP- and ubiquitin-independent [85]. Complete and limited proteolysis of the protein is triggered in different physiological conditions: complete proteasomal destruction may be associated with the beginning of apoptosis, because protein FBX33 (F-box protein 33), which is a YB-1-recognizing component of the multimeric E3 ubiquitin ligase, is activated just at this moment [54]. Recently one more E3 ligase has been discovered that is capable of ubiquitinating YB-1 – RBBP6 (Retinoblastoma binding protein 6) [60]. The 20S proteasome-mediated processing occurs upon treatment of cells with DNA-damaging xenobiotics [85] and possibly upon treatment of endothelial cells with thrombin [86].

It is supposed that YB-1 can be acetylated at residues Lys301 and Lys304. This modification may be crucial for secretion of YB-1 from the cell [87].

FUNCTIONS OF YB-1 IN THE NUCLEUS

When in the nucleus, YB-1 is involved in transcription of various genes, in DNA repair and replication, and in pre-mRNA splicing.

YB-1 in transcription. YB-1 can influence transcription of many genes including virus ones [88]. In particular, YB-1 regulates the activity of genes involved in cell division, apoptosis, immune response, multidrug resistance, stress response, and tumor growth (Table 2).

YB-1 can both stimulate and inhibit transcription. It is supposed that YB-1 regulates transcription through its direct interaction with the specific Y-box-containing regions in gene promoters as well as with DNA single-stranded regions that can have no Y-box sequence. Having formed a complex with DNA, YB-1 may attract other proteins in this complex. Moreover, it can interact with DNA only when being associated with other proteins, or be involved in complexes with DNA via other proteins that have already bound to DNA. A particular mechanism of the action of YB-1 on transcription has not been yet established in any single case, although transcriptional regulation of certain genes has been studied quite thoroughly. Let us analyze some of such examples.

Long ago it was demonstrated that YB-1 can stimulate transcription of *MDR1* gene (Multidrug resistance) encoding P-glycoprotein. It was suggested that this occurs as a result of YB-1 binding to the Y-box sequence in the promoter of this gene [98, 99, 133]. However, some researchers do not find YB-1 within DNA/protein complexes assembled in nuclear lysates on double-stranded oligonucleotides corresponding to the *MDR1* gene promoter regions [134, 135]. This challenge can be explained by different experimental conditions or by different ways of detecting YB-1 within these complexes – the knockdown of *YB-1* in the first case and the use of antibodies in the second. It can be assumed that YB-1 is involved in the activation of *MDR1* transcription only under strictly specific conditions and interacts with the gene promoter only when in complex with other proteins. Indeed, it has been shown recently that the interaction of YB-1 with the *MDR1* promoter is dependent on APE1 protein and proceeds in complex with the latter and histone acetyltransferase p300. Histone acetyltransferase p300 is established to attract RNA polymerase II, facilitate transition of chromatin into a relaxed state, as well as recognize and bind acetylated residues in regulatory proteins. The APE1–p300–YB-1 complex is formed upon acetylation of APE1. The maximal binding of YB-1 and p300 to APE1 is observed during acetylation of APE1 at Lys6 and Lys7. It is remarkable that APE1 is a cofactor of a number of transcription factors and interacts with them under stress conditions, when it undergoes various modifications. For example, APE1 is acetylated under DNA-damaging stress caused by treatment with cisplatin or etoposide [100].

Table 2. Some cellular and virus genes regulated by YB-1

Regulated gene	References
Activation	
CCL5 gene (chemokine)	[89, 90]
CD44 gene (surface glycoprotein binding hyaluronic acid)	[91]
CD49f gene (integrin α6)	[91]
Gelatinase A/matrix metalloproteinase 2 gene	[75, 92-94]
DNA polymerase α gene	[95]
EGFR (epidermal growth factor receptor) and *HER-2* (human epidermal growth factor receptor 2) genes	[90, 96]
MET gene (hepatocyte growth factor receptor)	[97]
MDR1 gene (P-glycoprotein responsible for development of multidrug resistance)	[98-100]
MLC-2v gene (myosin light chain 2v)	[68, 101]
LRP/MVP gene (protein involved in protection of cells from xenobiotics and in development of multidrug resistance)	[102]
PDGFB gene (platelet-derived growth factor B-chain)	[86]
PI3KCA gene (phosphoinosytol-3-kinase catalytic α subunit)	[103]
PTP1B gene (tyrosine phosphatase)	[104]
Smad7 gene (adapter protein involved in TGFβ 1 receptor degradation)	[105]
Cyclin A and B1 genes	[106]
Adenovirus late genes under control of promoter E2	[107]
Genes under control of HIV-1 TAR-promoter	[64, 108]
Polyomavirus JCV promoters (late)	[63, 65, 72, 109, 110]
Repression	
α-Actin gene	[74, 111-113]
COL1A1 gene (type α1(I) collagen)	[114, 115]
COL1A2 gene (type α2(I) collagen)	[66, 105, 114, 116, 117]
CPS-1 gene (carbamoyl phosphate synthetase 1)	[118]
Fas gene (*CD95/Apo-I*) (TNF/NGF family receptor involved in apoptosis)	[119]
GM-CSF gene (granulocyte and macrophage colony-stimulating factor)	[120-122]
GRP78/BiP gene (protein chaperon, cold shock protein 5)	[61]
Matrix metalloproteinase 12 gene	[123]
Matrix metalloproteinase 13 gene	[124]
MHC I genes (proteins of the major histocompatibility I complex)	[125]
MHC II (*HLA DRα*, *I-Aβ*) genes (proteins of the major histocompatibility II complex)	[126-128]
Mrp2/Abcc2 gene (proteins involved in formation of multidrug resistance)	[129]
Transcription factor c-*myc* gene	[56, 73]
LRP/MVP (protein involved in protection of cells from xenobiotics and in formation of multiple drug resistance) gene	[130]
p21 gene (cyclin-dependent kinase inhibitor 1A)	[51]
p53 gene	[131]
Thyrotropin receptor gene	[132]
Polyomavirus JCV (early) promoters	[63, 65, 72, 109, 110]
VEGF gene (vascular endothelial growth factor)	[37, 82]

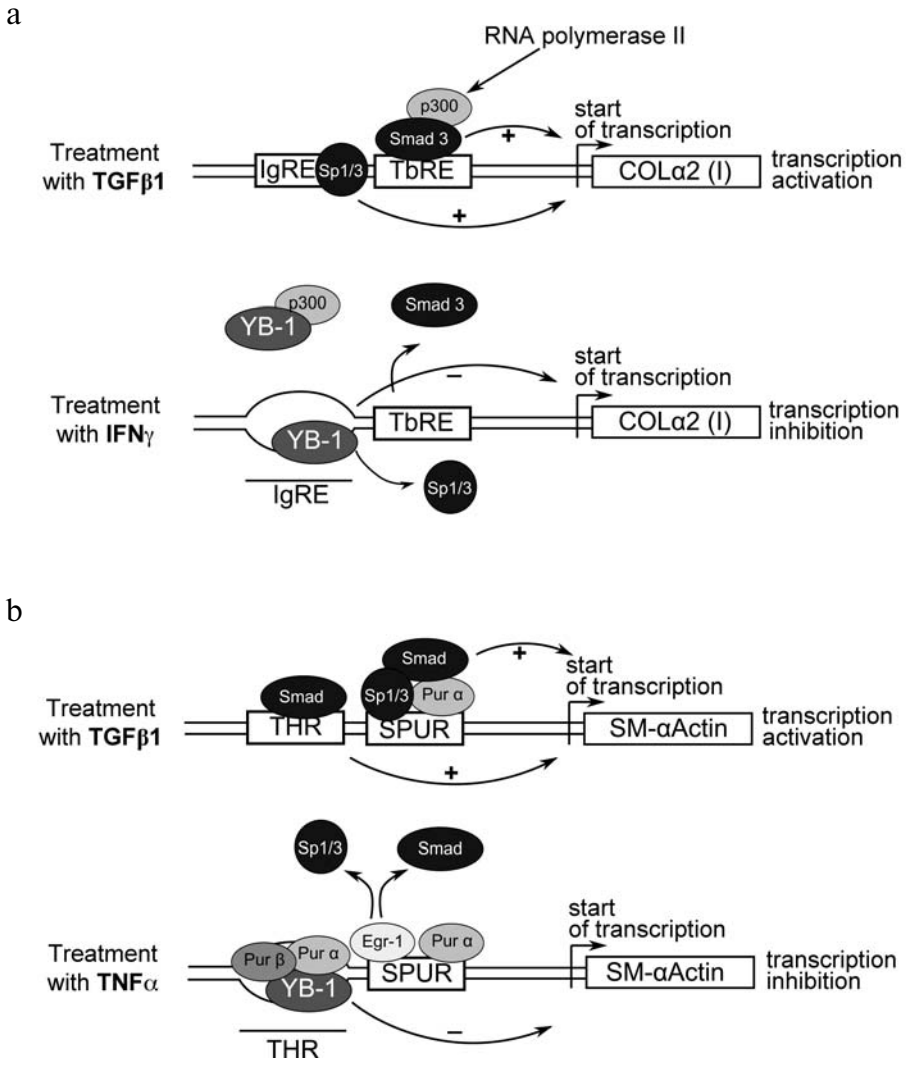

Fig. 4. Schemes of regulation of *COLα2(I)* (a) and *SMαA* (b) gene transcription.

So, YB-1 is involved in *MDR1* gene transcription regulation in complex with other proteins. In all probability, the activation of transcription is observed only under rigorously definite conditions when YB-1-interacting proteins are subjected to regulatory modifications.

Originally it was thought that the YB-1-mediated transcription is dependent on the YB-1 binding to the Y-box sequence in double-stranded regions of gene promoters. But there is an ever increasing body of data on the YB-1 binding to single-stranded sequences, including those distinct from Y-boxes. For the given type of transcription regulation, YB-1 binding sites are sequences greatly asymmetrical in distribution of purine and pyrimidine bases between the two DNA strands. This promotes the transition of the DNA to a single-stranded state under the action of YB-1 because the latter binds predominantly to the pyrimidine-rich DNA strand [31, 35-37]. Stabilization of the single-stranded state prevents binding of the transcription factors that interact with the double-stranded DNA.

An example of such a regulation is transcription regulation of α1- and α2-strands of *procollagen I* (*COLα1(I)* and *COLα2(I)*) genes. Type-I collagen is composed of two α1-strands and one α2-strand. The transcription of these genes is coordinated. It has been shown that the transcription of the *COLα1(I)* gene is YB-1-mediated, probably because YB-1 binds to the pyrimidine-rich single-stranded sequence in the promoter of this gene [114, 115]. The transcriptional regulation of the *COLα2(I)* gene has been studied in more detail (Fig. 4a). It was found that the *COLα2(I)* promoter has regions responsible for the TGFβ1-induced activation (TbRE) and IFNγ-induced inhibition of transcription (IgRE). Cell stimulation by TGFβ1 leads to accumulation of Smad3, which in complex with p300 binds to the TbRE. Protein p300 attracts RNA polymerase II and activates transcription.

Transcription factors Sp1/3 interacting with IgRE activate the *COLα2(I)* gene transcription as well. *YB-1* overexpression or treatment of cells with IFNγ results in the YB-1-mediated inhibition of transcription in two ways. On one hand, YB-1 binds to Smad3 and p300 (predominantly to p300) preventing their interaction with each other and association with the TbRE. On the other hand, YB-1 binds either to the pyrimidine-rich sequence or to the Y-box within IgRE, which leads to inhibition of transcription by an unidentified mechanism, possibly because of stabilization of the single-stranded state and decreased binding of the transcription factors Sp1/3 [66, 105, 114, 116, 117].

The *VEGF* gene transcription is precisely regulated depending on external conditions. It was found that YB-1 takes part in one mechanism of inhibition of this gene. YB-1 interacts with the noncoding DNA strand in the *VEGF* gene promoter, and the other Y-box binding protein (dbpA) interacts with the DNA coding strand. Stabilization of a single-stranded DNA state in the promoter most likely prevents the binding of other transcription factors, which results in the inhibition of transcription. After phosphorylation of YB-1 by ERK2 and GSK3β kinases, its binding to the promoter region increases, which should lead to more efficient inhibition of the transcription [82]. Phosphorylation of YB-1 at Ser102 enhances its binding to promoters of some genes such as *EGFR*, *PIK3CA*, and *MET* and stimulates their transcription by an unidentified mechanism [96, 97, 103, 136-138].

Stabilization of the DNA single-stranded state can bring about not only inhibition of transcription. Accordingly by binding with the single-stranded DNA in the *DNA polymerase* α gene promoter, YB-1 stimulates transcription. This may proceed in complex with other proteins [95].

Another example of the implication of YB-1 in the activation of transcription can be the c-*myc* gene. YB-1 binds to the CT-rich sequence in the promoter of this gene and probably to the TATA-binding protein (TBP), which results in docking of core transcription factors and RNA polymerase II. It should be noted that YB-1 is not the only protein binding to the CT-rich sequence in the promoter of the c-*myc* gene. A similar property is inherent to hnRNP K. When interacting with the same region, it activates transcription of the c-*myc* gene. Although proteins YB-1 and hnRNP K can form a complex with each other, they have no synergic or additive effect on the c-*myc* gene transcription, but on the contrary diminish to some extent the transcription induced by the partner protein. In all probability these proteins compete for the same region and may activate transcription of the c-*myc* gene under different conditions [56].

YB-1 can regulate transcription of genes in complex with other proteins. For example, it binds to a single-stranded region in the promoter of the *metalloproteinase 2* gene in complex with proteins AP-2 and p53, which causes activation of transcription [75, 92-94].

Other well-known YB-1 partners are proteins Purα and Purβ. In contrast to YB-1 that binds predominantly to pyrimidine-rich sequences, Purα and/or Purβ bind the opposite purine-rich DNA strand. The formation of such a complex impedes the binding of both activator and repressor proteins [74, 119]. For instance, two sequences were found in the promoter of α-*actin* gene (*SMαA* and *mVSMαA*), which are responsible for the TGFβ1-mediated activation of transcription. They are the asymmetric THR-element (or MCAT) and the GC-rich sequence SPUR (Fig. 4b). After stimulation with TGBβ1, Smad2/3/4 bind to the double-stranded THR sequence and Smad2/3/4, Sp1/3, and Purα bind to the SPUR sequence, which leads to transcription activation. If there is no stimulation with TGBβ1, the amount of Smad2/3/4 proteins is low, and they are unable to compete for the binding to THR with the YB-1/Purα/Purβ complex, the docking of which stabilizes the single-stranded state of DNA [74, 111-113]. It has been found recently that the treatment of cells with TNFα (the inhibitor of a TGFβ1-induced signaling pathway) also results in inhibiting the transcription of *SMαA*. In this case, the transcription factor Egr-1 binds to the GC-rich SPUR sequence and prevents the binding of Sp1/3 factors. Moreover, after treatment with TNFα the YB-1 binding to the THR element increases. This can be explained both by the decrease in competition of the proteins for the regulatory sequence and the modification of YB-1 or its partners in the MEK1, ERK1/2-signaling pathway [113].

It is remarkable that transcription of the *Fas receptor* gene from the TNFR/NGFR family is regulated in a very similar way. In the promoter region of this gene two adjacent regions were revealed: the inhibitor region to which YB-1, Purα, and Purβ are bound, and the activator region to which c-Fos and c-Jun are bound. In this case, Purβ is a functional antagonist of the YB-1/Purα complex that decreases its inhibiting effect. The binding of YB-1 and Purα to the opposite strands of the inhibitor region strongly stabilizes the single-stranded conformation, which may lead to destabilization of the double-stranded state of the nearby activator region [119].

Thus, by binding to a single-stranded DNA, YB-1 not only stabilizes it in this state in the region to which it is bound, but also can destabilize the double-stranded state of the adjacent regions. Perhaps this is the most prevalent mechanism of the YB-1-dependent regulation of transcription.

Interestingly, regulation of some virus gene transcription occurs in a similar way. So, in the human JCV polyoma virus a regulatory region is found which contains oppositely directed promoters of early and late genes and consists of a great number of binding regions of various protein factors. At different stages of virus development, diverse protein complexes assemble in the regulatory

region, the activity of promoters being dependent on their composition. At an early stage of the virus development, Purα binds to the purine-rich strand in the regulatory region and activates transcription of early genes, including that of *T-antigen*. After that, Purα interacts with YB-1 and stimulates its binding to the opposite pyrimidine-rich strand, which results in inhibiting the early genes transcription. At late stages of the virus development, the T-antigen displaces Purα and in complex with YB-1 inhibits transcription of the early genes as well as activates transcription of the late genes [63, 65, 72, 109, 110].

In the examples reviewed so far, YB-1 is involved in transcription regulation not only *per se* but also in complex with other proteins, although in both cases its binding to DNA is specific and dependent on the DNA sequence rather than on protein–protein interactions. At the same time, there are situations when the YB-1-recognized sequence is not found either in the gene promoter or gene itself, though YB-1 is implicated in regulation of transcription of this gene. For instance, YB-1 inhibits p53-induced transcription of gene *p21* when interacting with p53 [51].

Another mechanism of the effect of YB-1 on transcription must be mentioned here, though it is poorly studied yet. It is known that under certain conditions such as DNA-damaging stress or treatment of endothelial cells with thrombin, YB-1 can be cleaved by the 20S proteasome after Glu219 [85, 86]. The truncated protein lacking the cytoplasmic retention signal is translocated to the nucleus where it can be implicated in repair and transcription. It was revealed that after treatment of endothelial cells with thrombin, the truncated YB-1 stimulates transcription of the gene of the B-chain of platelet-derived growth factor PDGF. A detailed mechanism of this process is not known yet [86]. A transcriptome profiling showed that groups of genes whose transcription is affected by the truncated or full-length YB-1 are very similar; however, there are some distinctions [139]. In all probability the truncated YB-1 can influence the transcription both by the mechanism of a full-size protein and by its own mechanism, e.g. by recognizing other sequences or attracting other proteins.

YB-1 in DNA repair. The assumption on the involvement of YB-1 in DNA repair was made in 1991 when Lenz and Hasegawa with their colleagues identified YB-1 as a protein possessing a high affinity to DNA containing abasic sites [31, 38]. This assumption is also corroborated by the data that YB-1 has an increased affinity to DNA damaged with cisplatin or containing mismatches, as well as the data on the ability of YB-1 to efficiently melt such a DNA [36, 39, 40]. The hypothesis on the involvement of YB-1 in repair is also compatible with its exhibiting weak 3′-5′-exonuclease activity on single-stranded DNA and weak endonuclease activity on double-stranded DNA. It is believed that nuclease activity of YB-1 is comparable to that of p53 and should, presumably, be strongly dependent on the DNA sequence and structure [36, 40, 139]. It was also demonstrated that YB-1 promotes cell survival under stress conditions, is able to move to the nucleus and, possibly, activate transcription of some genes implicated in repair [95, 131]. In addition, YB-1 interacts *in vivo* and *in vitro* with various proteins involved in repair and can regulate the activity of some of them. It was shown that YB-1 interacts with the following proteins.

Proteins of base excision repair: PCNA [39], p53 [51], hNth1 [140, 141], NEIL-2 [142], DNA ligase IIIα [142], DNA polymerase β [142], DNA polymerase δ [40], WRN [40, 143], Ku80 [40], MSH2 [40], and APE-1 [100, 144].

Proteins of nucleotide excision repair: PCNA [39], p53 [51], DNA ligase IIIα [142], DNA polymerase δ [40], and APE-1 [100, 144].

Mismatch repair: PCNA [39], MSH2 [40].

Repair of DNA single-stranded breaks: DNA ligase IIIα [142], APE-1 [100, 144].

Repair of DNA double-stranded breaks: WRN [40, 143], Ku80 [40].

Recombination repair: WRN [40, 143], MSH2 [40].

So, YB-1 can be involved in practically all types of repair. It should be noted that YB-1 interacts with most of the proteins of base excision repair (Fig. 5), which makes it possible to suggest that YB-1 plays the most important role just in this type of repair.

It is established that mouse embryonic stem cells heterozygous at *YB-1* (*YB-1*$^{+/-}$) have an increased sensitivity to DNA cross-linking agents such as cisplatin and mitomycin C. However, their sensitivity to other types of DNA-damaging stress, such as treatment with etoposide and X-ray and UV radiation remains unchanged [145]. These data allow us to suggest that YB-1 can be directly involved in the repair of DNA adducts with cisplatin and mitomycin C. It is known that cisplatin- and mitomycin-C-modified DNA is repaired in the cell both by mismatch repair and base and nucleotide excision repair. The latter mechanisms are the most frequently used by the cell. YB-1 may be directly involved in the early stages of the given type of repair. As has been mentioned, YB-1 by binding to the damaged DNA can melt the duplexes containing mismatched or damaged nucleotides and has a rather low nuclease activity. Moreover, YB-1 enhances the enzymatic activity of DNA glycosylases hNth1 and NEIL-2 [140-142], interacts with their partner proteins PCNA and p53, and probably is implicated as a platform protein in the repair complex assembling.

YB-1 in DNA replication. It is believed that YB-1 can be involved in DNA replication. Some indirect data support this assumption. Thus, YB-1 is translocated to the nucleus in the cell-division cycle at the G1/S boundary [106]. The increase of the YB-1 amount in the cell correlates with the growing level of PCNA, DNA topoisomerase IIα, and DNA polymerase α [95, 106, 146, 147]. It was also shown that decreasing of the YB-1 amount in cells is accompanied by cessation of their proliferation [95, 148]. YB-1 can have a positive effect not only on the

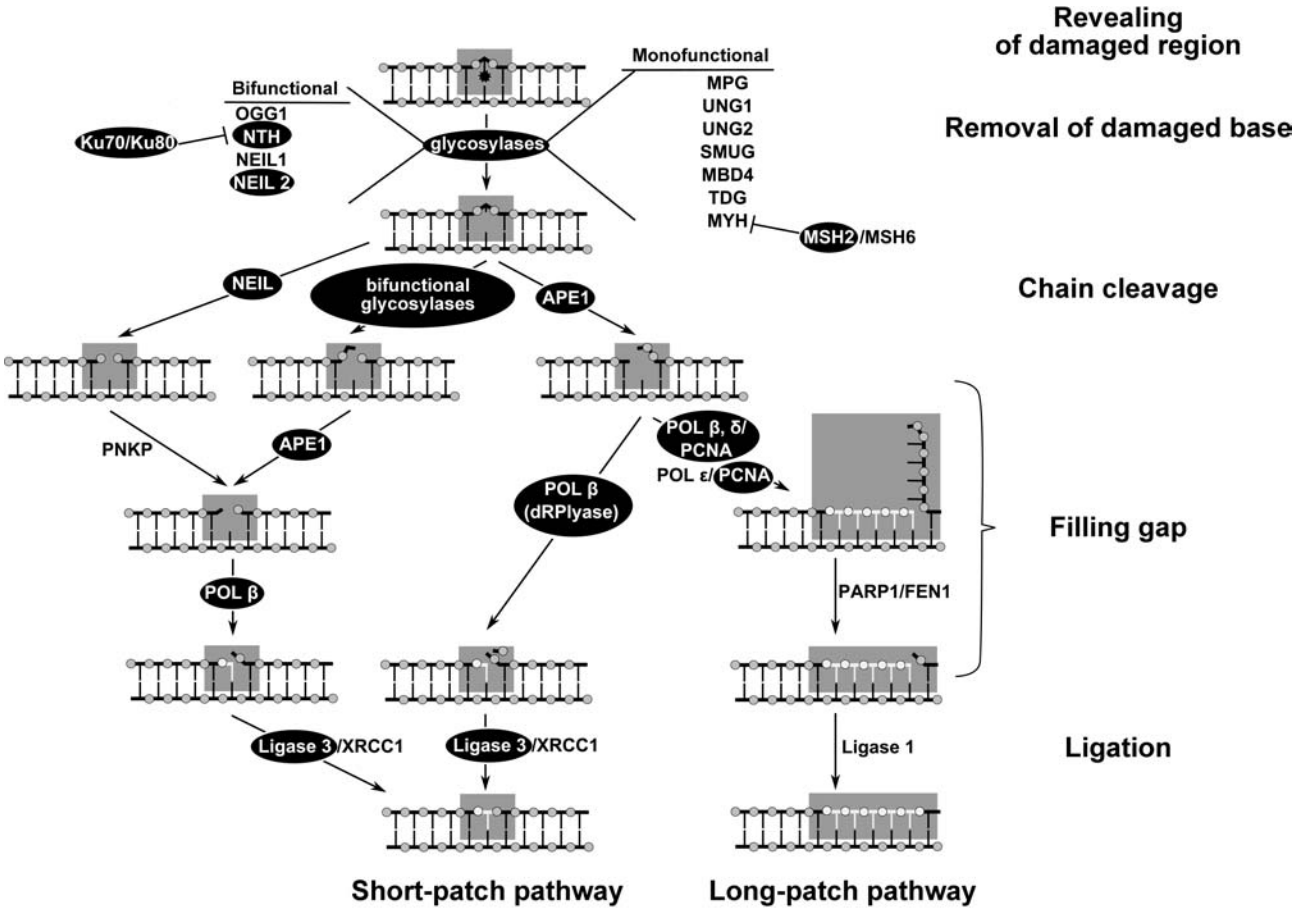

Fig. 5. Scheme of base excision repair. Names of YB-1 partner proteins are given in dark ovals.

replication of cell DNA, but also on replication of the adenovirus genome [107].

YB-1 in pre-mRNA splicing. YB-1 is involved in pre-mRNA splicing. It was found that homologs of YB-1 from *Ch. tentans* bind to pre-mRNA during transcription and are in complex with the latter at all stages of its maturation [149]. In mammals, YB-1 was discovered within spliceosomes during the A-complex formation (the ATP-dependent binding of snRNP U2 to the branch point) and the B-complex formation (the binding of the triple U4/5/6 snRNP complex) [150, 151].

As known, in eukaryotic cells, the splicing of pre-mRNA proceeds co-transcriptionally. YB-1 attaches to the transcription complex by interacting with protooncoproteins TLS and EWS. Their N-terminal parts bind to hyperphosphorylated RNA polymerase II, and their C-terminal parts bind to YB-1 and some other proteins within the spliceosome [62, 152, 153]. By attracting YB-1 to this complex, TLS promotes its involvement in the splicing of mRNA. In some types of cancer, as a result of mutations C-terminal parts of TLS and EWS are substituted by fragments of other proteins. Mutant proteins continue binding to RNA polymerase II, but do not bind YB-1 any longer. Such proteins hinder the binding of YB-1 and prevent the YB-1-mediated splicing.

Under DNA-damaging stress, the interaction of EWS with YB-1 declines greatly. This prevents involvement of YB-1 in splicing of various mRNAs, in particular, *MDM-2* mRNA [153]. In the absence of EWS, the same as under DNA-damaging stress, an alternatively spliced *MDM-2* mRNA deprived of some exons is formed. Such an mRNA is short-living, and a functional protein is not synthesized from it [153]. It should be mentioned that MDM-2 is a p53-specific ubiquitin ligase, and p53 is a transcription activator of the *MDM-2* gene. Under DNA-damaging stress, the amount of p53 grows probably because of the decreased amount of functionally active MDM-2 [153]. Earlier it was demonstrated that under such a stress, YB-1 interacts with p53 and can affect the p53-regulated transcription [51]. Within an YB-1 molecule the p53 and EWS binding sites overlap. Presumably the interaction of YB-1 and p53 may disturb the interaction of YB-1 with EWS, which prevents involvement of YB-1 in splicing, but makes it more available for implication in repairing and regulation of transcription of genes involved in DNA-damaging stress response.

It is not yet known at what stages of splicing YB-1 is involved. It may act through binding to some splicing factors such as SRp30c and SRrp86 [50, 154] or recognize certain sequences in pre-mRNA [155, 156].

The involvement of YB-1 in regulation of their alternative splicing was demonstrated for some pre-mRNAs [51, 62, 153, 155, 157]. YB-1 in complex with protein MBLN-1 can stimulate alternative splicing of *α-actinin* mRNA [158]. In *NF1* (neurofibromatosis 1) mRNA a region with a high mutation frequency was revealed in exon 37. Single-nucleotide substitutions in it resulted in skipping of exon 37 from the mRNA. It appeared that this exon contains the nucleotide sequence ACAAC interacting with YB-1 and p72 that stimulate inclusion of exon 37 in the mRNA. Mutations in this sequence lead to diminishing the binding of these two proteins to it and to stimulation of the binding of negative regulators DAZAP1 and hnRNP A1 and A2. But it is likely that there are other proteins – functional homologs of YB-1 and p72 – that promote the inclusion of exon 37 as well [156].

YB-1 stimulates the inclusion of exon v4 in *CD44* mRNA. It recognizes the A/C-rich sequence ACE (A/C-rich exon enhancer element) in exon v2 of this mRNA [155]. In addition to YB-1, p72 and Tra2-β1 promoting the inclusion of not only v4 but also v5 interact with this sequence. The functional activity of the splicing factor Tra2-β1 increases greatly in the presence of YB-1 [157]. In all probability, these proteins function within a united complex, though there has been no direct information on their interaction so far.

Therefore, the role of YB-1 in splicing most likely consists in recognition of specific sequences in pre-mRNA and attraction of splicing factors to them. Probably YB-1 is not a core component of the spliceosome and is required only for regulation of splicing of certain mRNAs.

YB-1 in disassembly of nucleoli. There are some reports on the involvement of YB-1-like proteins in the disassembly of nucleoli. The protein of amphibian oocytes FRGY2 was shown to be responsible for the reversible disassembly of nucleoli occurring after ovulation [159]. It was demonstrated on HeLa cells that YB-1 is also implicated in disassembly of nucleoli by binding to B23, which is one of nucleolar proteins. Protein B23 interacts with many proteins and pre-rRNA within the nucleoli and can be involved in the processing of pre-rRNA. In this case, the role of YB-1 may consist in carrying away B23 and its associated proteins from nucleoli, which leads to the subsequent disassembly of the nucleoli [160].

YB-1 FUNCTIONS IN THE CYTOPLASM

In the cytoplasm, YB-1 is the major packing protein of mRNPs, regulates mRNA translation, provides for its stability, and is involved in its localization. It should be noted that all these functions of YB-1 are interrelated.

YB-1 as a packing protein of mRNPs. As known, the entire mRNA in the cytoplasm of eukaryotic cells exists as mRNPs. These particles with unique physical and chemical characteristics fall into two classes: free cytoplasmic (non-translated) and translated mRNPs of polysomes [161-163]. Both classes of mRNPs have a narrow density distribution and a low buoyant density value in CsCl. In free mRNPs it is about 1.39 g/cm^3, which is in line with a very high protein/RNA ratio of 3 : 1 (nearly 75% protein). In polysomal mRNPs the buoyant density value is somewhat higher, 1.45 g/cm^3, which corresponds to the protein/RNA ratio of 2 : 1 (nearly 65% protein). The buoyant density value of mRNPs (the protein/RNA ratio) does not significantly depend on the mRNA size and, consequently, the protein should be more or less uniformly distributed along the whole length of the mRNA. In spite of the very high content of protein in mRNPs of both classes, their mRNA is extremely sensitive to endoribonucleases. This argues for the exposed, surface position of mRNA in the particles [164].

The composition of mRNPs includes a great variety of proteins recognizing specific sequences and/or specific elements of three-dimensional structure of individual mRNAs (mostly in 5′- and 3′-untranslated regions – UTRs). These proteins are responsible for selective translational control, regulation of the lifetime of individual mRNAs, and their specific intracellular distribution. Such proteins look like minors in protein preparations of total mRNPs.

Other mRNP proteins are represented as majors in total mRNP preparations. They are associated with the major part or even with all mRNAs and are present on them in a great number of copies. The most known are two major mRNP proteins of mammalian cells: YB-1 (or its homologs) and poly(A)-binding protein PABP [1-3]. PABP is associated mainly with poly(A)-tails of poly(A)$^+$ mRNA in polysomes. YB-1 is found in both polysomal and free mRNPs, in the latter its amount calculated per weight of mRNA being twice as large as that in polysomal mRNPs [165]. So, the mRNA transition to polysomes is accompanied by association with PABP and dissociation of about half of the initial amount of YB-1. It was demonstrated that YB-1 is one of the mRNP proteins most strongly associated with mRNA: a large portion of it remains on the mRNA at a high concentration of monovalent cations evoking dissociation of other mRNP proteins [41]. YB-1 makes about 0.1% total protein in Cos-1 and HeLa cells and on average from 5 to 10 YB-1 molecules fall per every cellular mRNA [166]. The amount of the protein associated with every specific mRNA depends on its length and translation status.

Thus, YB-1 and its homologs are universal proteins associated with all or many mRNAs existing both in untranslated and translated states. Accordingly, it was

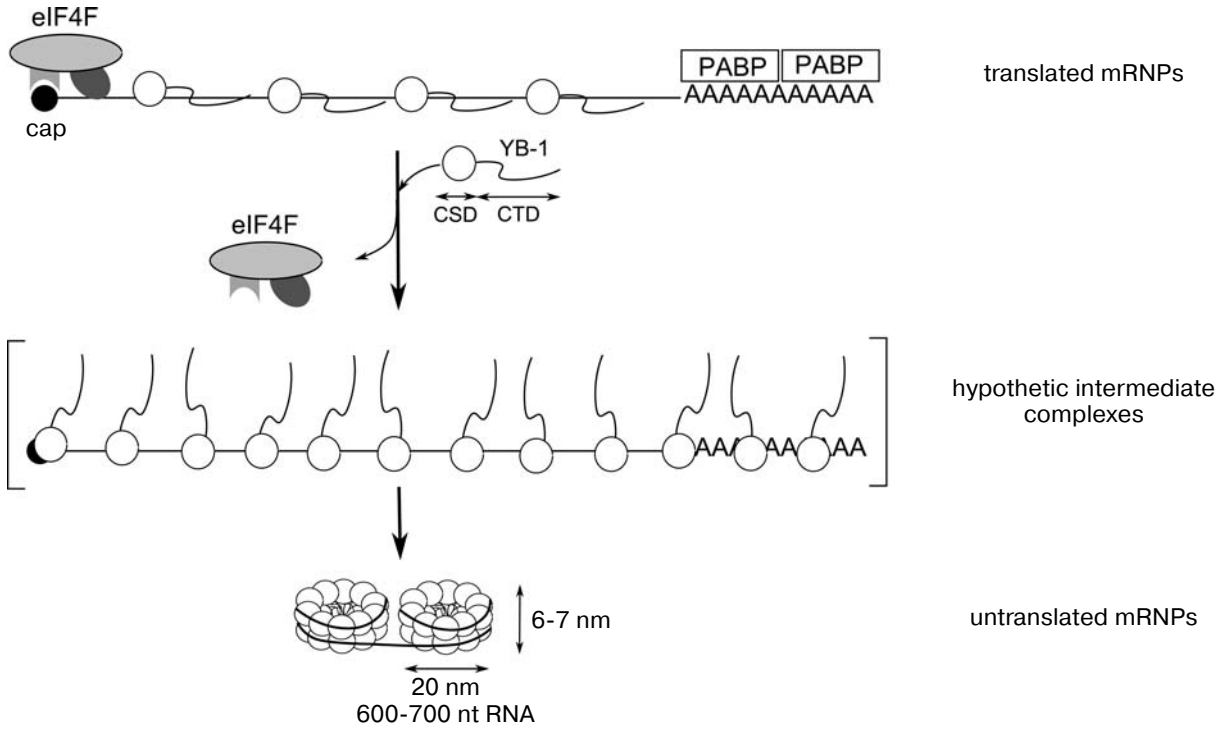

Fig. 6. Structural peculiarities of YB-1-unsaturated (translated) and YB-1-saturated (non-translated) mRNA in association with the major mRNP proteins and eIF4F. The transition of mRNP from the translated to the non-translated state is accompanied by a twofold increase in the number of YB-1 molecules, which displace eIF4F and PABP from mRNA, and by mRNA compactness due to YB-1 multimerization. The linear dimensions of the YB-1 multimer associated with mRNA in saturated non-translated complexes (as revealed by atomic-force and electron microscopy) and the size of an RNA segment packed on the surface of a multimeric protein globule are indicated (the figure is reproduced with modifications from [168]).

suggested that YB-1 determines unique physical and chemical properties of mRNPs. Indeed, it was shown on a model complex including YB-1 and mRNA that this protein alone can form particles that are very close to natural mRNPs as to their sedimentation coefficient and buoyant density [28]. The same as in natural mRNPs, mRNA in complexes with YB-1 is exposed on the surface since it had an enhanced sensitivity to endoribonucleases. The authors of that study did not reveal any appreciable ribonuclease activity of YB-1 *per se*.

Further analysis of YB-1 complexes with mRNA demonstrated that at a relatively low YB-1/mRNA ratio specific for polysomal translated mRNPs, YB-1 is bound to mRNA as a monomer by two RNA-binding domains – CSD and CTD. This leads to unfolding of mRNPs, which may render their mRNA accessible for interaction with translation initiation factors and ribosomes. At a high YB-1/mRNA ratio specific for free untranslated mRNPs, YB-1 molecules interact with mRNA mostly through CSD, whereas CTDs probably interact with each other resulting in the formation of large multimeric YB-1 complexes consisting of approximately 15-18 protein molecules. These multimers are about 20 nm in diameter and 7 nm high and pack an mRNA fragment of about 600-700 nucleotides on their surface. In such a complex, the mRNA ends possibly become inaccessible for interaction with proteins of the translation initiation apparatus and exonucleases [28]. As a result, by mRNA packing, YB-1 can influence its own translation and its lifetime in the cell (Fig. 6). Its effect on translation can be both positive and negative depending on the YB-1/mRNA ratio (see the following subsection).

It should be mentioned that only 18% of the whole diversity of mRNA species in complex with YB-1 were isolated from NIH3T3 cell extracts [83, 167]. This suggests that other mRNA species are either associated with YB-1 homologs or packed by other proteins.

Effect of YB-1 on translation. The effect of YB-1 on translation depends on the YB-1/mRNA ratio. At high ratios, YB-1 inhibits translation, and on the contrary, at low ratios it activates translation.

YB-1 is able to inhibit the translation process both in cell-free systems and in mammalian cell culture [13, 41, 166, 169]. Inhibition of translation is observed only at the initiation stage prior to association of mRNA with the small ribosomal subunit, so that mRNA is found as a constituent of free mRNPs. The inhibition is produced mainly by YB-1 CTD. This domain, like full-size YB-1, displaces translation initiation factor eIF4G from the complex with mRNA [170]. The CSD of YB-1 was shown to

interact with the cap-structure or with the adjacent region, which results in displacement of eIF4E, eIF4A and eIF4B. Thus, YB-1 can displace all subunits of the factor eIF4F (eIF4G, eIF4E, and eIF4A) from the complex with mRNA and inhibit translation at the initiation stage [170-172].

It is remarkable that when the YB-1 concentration in the cell-free translation system increases, PABP-mediated translation stimulation is enhanced [173]. This is evidently explained, on one hand, by the competition between YB-1 and eIF4F for the binding to mRNA, and on the other hand, by the interaction of PABP with eIF4G, which enhances the affinity of eIF4G to mRNA [174, 175]. At low YB-1 concentrations, eIF4F binds effectively to mRNA near the cap-structure and assures active translation of mRNA even at a low concentration of PABP. At high YB-1 concentrations, this protein displaces eIF4F from the complex with mRNA and inhibits translation. In this case, an increase in the PABP concentration and its interaction with eIF4G enhances the affinity of eIF4F to mRNA and, as a consequence, its competitiveness to bind to mRNA. As a result, eIF4F displaces YB-1 from the complex with mRNA, which leads to activation of translation under the action of PABP [173].

As mentioned above, the involvement of YB-1 in translation is not limited only by inhibition of this process. When YB-1 is removed from the lysate or high mRNA concentrations are achieved, the translation process is stopped. An addition of YB-1 to such lysates leads to activation of translation [41, 48, 176]. YB-1 stimulates the protein synthesis only at the initiation stage without any effect on elongation and termination [176]. The removal of YB-1 from rabbit reticulocyte lysates leads to accumulation of the 48S preinitiation complex, i.e. cessation of the protein synthesis in the lysate deficient in YB-1 may occur either upon association of the 60S ribosomal subunit with the 48S complex or at the previous stage of scanning the 5′ UTR of mRNA by the small ribosomal subunit up to the initiation codon [176]. The experiments on reconstruction of the 48S preinitiation complex using purified components (mRNA, 40S ribosomal subunits, and translation initiation factors) showed that at a limited amount of initiation factors, YB-1 stimulates the formation of the 48S preinitiation complex in which the small ribosomal subunit has completed scanning of the mRNA 5′ UTR and is located on the initiation codon [177].

There are a number of hypotheses explaining the promoting action of YB-1 on translation. According to the first of them, YB-1 prevents nonspecific binding of RNA-binding translation initiation factors through the whole mRNA molecule, which causes their concentration in the region of the cap-structure and the 5′ UTR [41, 178]. According to the second hypothesis, YB-1 facilitates the movement of the small ribosomal subunit along the 5′ UTR of mRNA to the initiation codon because of its ability to melt the secondary structure of mRNA [176, 177]. It was also found that the homolog of YB-1 from *Ch. tentans* interacts with the DEAD-box-containing RNA helicase (hrp84), and these two proteins are components of polysomal mRNPs. It can be suggested that the formation of such a complex activates translation due to the more efficient unwinding of the mRNA secondary structure, thus facilitating the 5′ UTR scanning [77].

So, YB-1 exerts a dual effect on translation of various mRNAs: at a relatively low ratio of YB-1 to mRNA (up to the ratio observed in polysomal mRNPs), YB-1 promotes translation, whereas at mRNA saturation with the protein (as in free mRNPs) it acts as a repressor of translation.

In addition to global protein synthesis regulation, YB-1 can be involved in selective translation regulation of certain mRNAs. It was demonstrated for *ferritin* mRNA that at a low concentration of iron ions IRP-2 binds to the IRE (Iron-responsive element) hairpin structure in the 5′ UTR of mRNA and selectively inhibits its translation. At a high iron concentration, YB-1 binds to the oxidized IRP-2 and prevents its interaction with the 5′ UTR of mRNA. Consequently, YB-1 abrogates the inhibiting effect of IRP-2 on translation of *ferritin* mRNA at a high concentration of iron ions [59].

It has been recently shown that YB-1 binds to a highly structured G/C-rich 5′ UTR of *TGFβ* mRNA. The predicted heat of melting of the secondary structure of this mRNA 5′ UTR is less than that required for preventing the scanning of the 5′ UTR by the 43S preinitiation complex. But sucrose gradient ultracentrifugation demonstrated that this mRNA exists predominantly as free mRNPs. It is assumed that the binding of YB-1 to the 5′ UTR stabilizes its secondary structure and prevents translation initiation [179].

The binding of YB-1 to the specific sequence in the 3′ UTR of some mRNAs also leads to selective inhibition of translation of these mRNAs at a relatively low concentration of YB-1, which does not yet result in significant inhibition of the total protein synthesis. An example of such a regulation is autoregulation of the synthesis of YB-1 *per se* [42, 180], that will be described in more detail in the section "YB-1 Gene Structure and Regulation of Its Expression". Under selective inhibition of translation of mouse protamine mRNA by MSY-2/MSY-4 proteins, which are mouse homologs of YB-1, these proteins bind to a specific sequence in the 3′ UTR of this mRNA [43]. By specifically binding to the ψ-sequence from the 5′ LTR (Long-terminal repeats) of Rous sarcoma virus, chicken proteins chk-YB-1 and chk-YB-2 selectively suppress translation of the reporter mRNA with this sequence in the 5′ UTR [45]. The cell culture experiments showed that cell infection with Dengue virus enhances greatly upon *YB-1* gene knockout. It was found that YB-1 is able to suppress translation of the Dengue

virus (+)RNA, specifically binding to the sequence 5′-UCCAGGCA-3′ in the hairpin structure in the 3′ UTR of this RNA [46].

There is another mechanism of the effect of YB-1 on translation. In NIH3T3 cells some YB-1-associated mRNAs encode growth factors as well as proteins synthesized in response to various stress conditions. These mRNAs are regarded as the so-called "weak" templates having a highly structured 5′ untranslated region and are translated only at a high concentration of translation initiation factors, especially eIF4E. In the absence of factors promoting cell growth, YB-1 inhibits translation of "weak" templates by displacing initiation factor eIF4E (within eIF4F) from the mRNA cap-structure. Upon activation of cell signaling pathways by external stimuli, many kinases are activated including kinase Akt that phosphorylates YB-1 at Ser102 in CSD. The phosphorylated YB-1 has a lower affinity to the cap-structure and/or to the adjacent mRNA region, which promotes the binding of eIF4E with the cap-structure of "weak" mRNAs and their involvement in translation [83].

YB-1 is able to influence cap-independent translation, including translation dependent on internal docking of the ribosome to a special site of mRNA enriched with secondary structure called IRES (Internal Ribosome Entry Site). It is established that YB-1 positively regulates translation of IRES-containing mRNAs of the *myc* family protooncogenes [181, 182]. Moreover, it was found that YB-1 is involved in regulation of translation of a number of mRNAs responsible for the epithelial–mesenchymal transition (EMT) such as *Snail1* mRNA [183, 184]. This mRNA has a highly structured 5′ UTR, and initiation of its translation proceeds by the cap-independent mechanism at higher YB-1 concentrations than the optimal ones for cap-dependent translation of most cellular mRNAs [184].

Hence, regulation of translation of "weak" templates by YB-1 can be most likely observed for a great variety of mRNAs translated by both the cap-dependent and cap-independent mechanisms.

YB-1-dependent mRNA stabilization. YB-1 and its homologs can efficiently stabilize mRNAs preventing their degradation in cells and cell lysates, its CSD playing the critical role in stabilization of mRNAs. The maximum stabilization of mRNA is achieved at a high YB-1/mRNA ratio, which is associated with the mRNA release from polysomes and cessation of their translation. It is notable that the efficient stabilization of mRNA was observed both for long-lived and short-lived mRNAs such as *TNFα* mRNA [171]. In other words, mRNA stabilization caused by the action of YB-1 proceeds by an universal mechanism independent of destabilizing AU-rich elements ARE (AU-rich element) in the 3′ UTR of mRNA but dependent on the YB-1/RNA ratio.

UV-cross-linking of YB-1 and mRNA with a radiolabeled cap-structure and affinity binding experiments on cap-Sepharose demonstrated that CSD, stabilizing mRNA, and probably the first half of the C-terminal domain of YB-1 interact with the cap-structure and/or its adjacent region [171, 172]. At first glance, stabilization of mRNA caused by YB-1 looks paradoxical, since, as mentioned above, mRNA within mRNPs is exposed and highly sensitive to endoribonucleases [13]. The paradox can be explained if we remember that mRNA in the cell is usually disrupted by exoribonucleases at two termini [185], and assume that the structure of mRNPs in which mRNA is saturated with YB-1 is such that upon general exposure of mRNA both its termini are hidden and inaccessible for the action of exoribonucleases the same as for interaction with other proteins including translation initiation factors.

In addition to the general stabilization of mRNA, YB-1 is able to selectively protect some mRNAs from degradation. So, it can enhance the stability of *renin* mRNA by specifically binding to CU-rich elements in the 3′ UTR of this mRNA together with five other proteins: nucleolin, hnRNP E1, αCP (or PCBP), hnRNP K, dynamin, and MINT-homologous protein. An increase in the intracellular amount of cAMP (after forskolin treatment) results in a high content of both YB-1 and the other five proteins, which is accompanied by an increase of the life-time of *renin* mRNA [186].

The stability of *IL-2* mRNA grows under activation of the JNK-signaling pathway. It was found that JRE (JNK-response element) in the 5′ UTR of *IL-2* mRNA is responsible for this stabilization. Two proteins (YB-1 and nucleolin) bind to this element. It is believed that during activation of the JNK-signaling pathway, in addition to these two proteins a third protein, either modified or newly synthesized, which implements interconnection between the 5′ JRE and destabilizing AU-rich elements in the 3′ UTR, is involved in the stabilization [187].

The stability of the vascular endothelial growth factor (*VEGF*) mRNA increases in response to cytokine stimulation and stress conditions (for example, hypoxia). As demonstrated earlier, YB-1 and PTB (Polypyrimidine tract binding protein) bind to their specific overlapping sequences both in the 5′ and 3′ UTR of *VEGF* mRNA [44]. It is supposed that stabilization of *VEGF* mRNA is a result of such a binding.

Moreover, it was shown that YB-1 interacts with the ARE-element in the 3′ UTR of granulocyte and macrophage colony-stimulating factor (*GM-CSF*) mRNA in eosinophiles stimulated with TNFα and fibronectin. An increase in the content of YB-1 in eosinophiles leads to stabilization of *GM-CSF* mRNA and enhancement of viability of these cells. It is assumed that YB-1 is involved in the stabilization of *GM-CSF* mRNA when in complex with other proteins such as HuR and hnRNP C [188, 189].

Thus, YB-1 can stabilize mRNA in two ways. First, YB-1 forms saturated complexes with mRNAs in which

5′- and 3′-termini of molecules are buried inside protein globules and are inaccessible to exoribonucleases. Second, YB-1 recognizes specific sequences in some mRNAs, and when in complex with other proteins it stabilizes them by an unknown mechanism.

CHANGING THE INTRACELLULAR LOCALIZATION OF YB-1

As a rule, the major part of YB-1 is within the cytoplasm in association with mRNA. However, in response to some intra- and extracellular signals a significant portion of YB-1 can move to the cell nucleus.

Experiments with YB-1 fragments showed that a molecule of this protein contains two sequences regulating its distribution between the nucleus and the cytoplasm. This is the nuclear localization signal (NLS) between amino acid residues 186 and 205 and the cytoplasmic retention site (CRS) between residues 267 and 293 [106, 190]. It should be noted that the effect of CRS prevails over that of NLS, and therefore YB-1 is typically in the cytoplasm. The N-terminal part of the protein (A/P-CSD) also has a slight tendency to nuclear localization [190]. A mutation analysis demonstrated that YB-1 NLS is noncanonical because positively charged amino acids residues are not required for its action [190].

Redistribution of YB-1 between the cytoplasm and the nucleus. Quite long ago it was suggested that YB-1 and its homologs can move from the nucleus to the cytoplasm in complex with the newly synthesized mRNA [191]. A decrease in the concentration of mRNA in the cytoplasm due to inhibition of its synthesis and/or enhancement of its degradation could promote release and back transfer of these proteins to the cell nucleus, which should activate the transcription process. By this mechanism YB-1 and its homologs can maintain the required ratio between the synthesis rate of some mRNAs and the content of mRNAs in the cytoplasm. It was demonstrated that in mature oocytes from *X. laevis*, in which a large amount of masked mRNA is accumulated in the cytoplasm, and the transcriptional activity in the nucleus is low, the major part of FRGY2 is localized in the cytoplasm [192]. The following evidence supports the retention of YB-1 in the cytoplasm due to its binding to mRNA. First, the predominant part of cytoplasmic YB-1 is found in complex with mRNA in HeLa cells [166]. Second, the replacement of phenylalanine and tyrosine residues in the RNP-1 consensus sequence of YB-1, decreasing greatly the protein binding to RNA in cell lysates, causes the transition of the mutant protein from the cytoplasm to the nucleus [190]. However, the authors of the cited paper did not perform direct experiments on the binding of the mutant protein to RNA *in vitro*. At the same time, it is likely that YB-1 can be retained in the cytoplasm because of the interaction with partner proteins. So, for example, FRGY2 fragments which are incapable of binding to mRNA still remain in the cytoplasm [48].

Transition of YB-1 from the cytoplasm to the nucleus is observed in the following cases. In the first place, it occurs at a definite moment of the cell cycle, namely at the G1/S phase interface [106]. CSD and amino acid residues 171-225 are important for this transition. In the nucleus YB-1 promotes transcription of cyclins A and B1 genes, which supports progression of the cell cycle [106]. In the second place, the transition takes place during cells treatment with UV-radiation, DNA-damaging agents, and upon oxidative stress and hyperthermia [99, 142, 193, 194]. In these cases, YB-1 in the nucleus stimulates transcription of genes of multidrug resistance and is involved in repairing process, thus enhancing cell viability. In the third place, YB-1 is found in the nucleus after transfection of cells with adenoviruses [107]. Then nuclear YB-1 stimulates transcription of late virus genes, replication of virus genome, and virion assembly. Finally, the transition of YB-1 to the nucleus can be promoted by some growth factors and cytokines, for example, fetal bovine serum and IFNγ [66, 195].

The transition of YB-1 from the cytoplasm to the nucleus may also occur as a result of its interaction with other proteins. For example, YB-1 may interact with the splicing factor SRp30c [50]. It was demonstrated on HeLa and HEK-293 cells that upon overexpression from the plasmid, SRp30c is predominantly localized in nuclei and distributes in speckles. YB-1 synthesized from the plasmid has a primarily diffused localization. Upon simultaneous overexpression of the two proteins, YB-1 moves to the nucleus and is co-localized with SRp30c, the distribution of both proteins being diffused. If the cells are subjected to heat shock, at which SRp30c moves to stress-induced Sam68 nuclear bodies (SNB), the interaction of YB-1 with SRp30c is impaired and YB-1 returns to the cytoplasm. This shows that SRp30c promotes the transition of YB-1 to the nucleus, and in this case the nuclear localization of YB-1 is maintained through the interaction with SRp30c [50].

Another known protein that may be involved in the transport of YB-1 to the nucleus is p53. It was demonstrated on some cell lines that the nuclear localization of YB-1 correlates with overexpression of *p53*, it being essential that p53 should be functionally active (mutant forms of p53 lacking the transcription factor activity do not stimulate YB-1 transition to the nucleus) [196, 197]. During UV radiation the p53-dependent YB-1 transition into the nucleus is facilitated appreciably [143].

Data are available on the relation of the transition of YB-1 to the nucleus with its posttranslational modifications. Indirect evidence is that upon stimulation with IFNγ, the transition of YB-1 to the nucleus of human fibroblasts is suppressed with inhibitors of Jak1/Jak2 kinases and casein kinase II, and the transition caused by ultraviolet light – with the protein kinase C inhibitor [66,

193]. There is inconsistency in the data on the affect of YB-1 phosphorylation with Akt kinase on its nuclear–cytoplasmic distribution. Experiments with MCF-7 and SCOV-3 cells show that phosphorylation induces transition of YB-1 to the nucleus [53, 195], while results obtained for NIH3T3 and CEF fibroblasts are evidence for the absence of such a dependence [83, 198].

Another regulatory mechanism for nuclear–cytoplasmic transport of YB-1 associated with its proteolytic processing was discovered quite recently. Thrombin-stimulated transition of YB-1 to the nucleus in endothelial cells is accompanied by cleavage of YB-1 into two fragments, during which the larger N-terminal protein fragment is transferred to the nucleus [86, 199]. Then the cleavage site on YB-1 (before Gly220) was found, and the protease responsible for this process was determined [85]. This protease is the 20S proteasome that ubiquitin- and ATP-independently splits off the cytoplasmic retention site from YB-1, which provides its transition to the nucleus [85]. Limited proteolysis of YB-1 with the 20S proteasome is induced in response to the DNA-damaging stress. The truncated YB-1 probably has functions distinct from those of the full-length protein. Thus, most likely the development of multidrug resistance correlates with the appearance of a truncated rather than full-length YB-1 in the nucleus [85].

The mechanism of YB-1 transport to the nucleus and from the nucleus is insufficiently studied. It was demonstrated that YB-1 can bind to karyopherin β2, which is a factor enabling import to the nucleus of some proteins involved in mRNA processing [200]. As shown, the export of YB-1 from the nucleus is independent of Crm1, a protein of the exportin family responsible for export from the nucleus of numerous nucleocytoplasmic proteins [190].

Localization of YB-1 in the cytoplasm and nucleus. The major part of YB-1 in the cytoplasm is associated with mRNA and is diffusely distributed both within translated polysomal mRNPs and free untranslated mRNPs.

The A/P domain of YB-1 has a motif typical of actin-binding proteins. YB-1 can interact with both monomeric and polymerized actins and in model experiments decorates actin microfilaments within the cell [49]. The protein retains its capability to bind to actin being in complexes with mRNA at a relatively low ratio of YB-1 to mRNA characteristic of translated mRNPs. However, YB-1 loses its capability to interact with actin within complexes in which mRNA is saturated with this protein, i.e. within untranslated free mRNPs. This means that YB-1 can localize the translated mRNAs on the actin cytoskeleton.

On the other hand, it was shown that YB-1 can interact with both the αβ-tubulin dimer and microtubules and is capable of accelerating the microtubule assembly from the tubulin dimers. At rather low YB-1/mRNA ratios, mRNA competes with tubulin for YB-1. But when these ratios are high, YB-1 promotes association of mRNA with microtubules [69]. It is proposed that YB-1 can localize mRNAs, existing as free untranslated mRNPs, on the tubulin cytoskeleton and be involved in transport of such mRNPs along microtubules.

Besides, YB-1 was detected within centrosomes of both interphase and mitotic cells [201, 202]. YB-1 is probably associated with centrosomes due to its affinity to tubulin.

YB-1 can be found within cytoplasmic granules. At present localization of YB-1 has been established in two types of such granules – stress granules (SGs) and processing bodies (PBs). Under stress conditions, a great part of YB-1 is associated with SG.

SGs are formed in the cell in response to heat shock, hypoxia, oxidative stress, viral infection, etc. In experimental conditions, the formation of such granules is stimulated with arsenic salts. The formation of SGs is initiated by eIF2α phosphorylation, which leads to inhibition of the protein synthesis at the initiation stage. Apart from YB-1 and mRNAs, such granules contain translation initiation factors eIF2α, eIF3, eIF4E, eIF4A, eIF4G, proteins G3BP, PABP, HuR, and TIAR, 40S ribosomal subunits, etc. It is assumed that these granules represent a type of mRNA storage where the latter is protected from ribonucleases. These mRNAs can be used once again for translation upon restoration of favorable conditions [203, 204].

PBs are present in the cell almost constantly. The protein composition of these granules varies greatly from that of SG. PBs include proteins involved in mRNA degradation: exoribonuclease Xrn1, decapping proteins DCP1/DCP2 and their coactivators Hed1s/GE-1, proteins involved in degradation of aberrant mRNAs (UPF1, SMG5, SMG7, and Lsm1), proteins involved in microRNA-dependent degradation of mRNAs (GW182, RISC, Ago2), and others. It is believed that these granules are the place of mRNA degradation. Under normal growth conditions they also contain YB-1 but in a lower amount than in SGs [203, 204].

To localize YB-1 within PBs, the A/P domain, CSD, and a fragment of the C-terminal domain (205-281) are required. To localize it within SGs, a fragment of CSD (44-128) is required [204]. In contrast to RAP55 (RNA-associated protein 55), which swiftly moves from SGs to PBs upon cessation of stress, YB-1 remains bound to SGs for some time. It is possible that in this way YB-1 assists the restoration of translation and facilitates the transition of mRNAs from SGs to polysomes [204].

Studies of the formation of SGs demonstrated that at first a great number of very small SGs are formed, and then they merge into larger granules, the merging process being active (not because of free diffusion) and depending on the assembly and disassembly of microtubules. It is thought that basic proteins of mRNPs, such as YB-1 and PABP, can facilitate association of stress granules with microtubules [205].

In the cell nucleus, Y-box binding proteins can be located on chromatin both as a result of interaction with DNA in promoters and in damaged regions under reparation, or due to its association with newly synthesized mRNA. In the latter case, Y-box binding proteins will leave chromatin upon treatment with endoribonucleases, which destroy the growing pre-mRNAs [149].

SECRETION AND EXTRACELLULAR FUNCTIONS OF YB-1

Frye et al. reported not long ago that YB-1 can be secreted from cells under inflammatory stress when treated with lipopolysaccharide, hydrogen peroxide, platelet-derived growth factor PDGF-BB, or TGFβ [87]. In line with microscopy data, YB-1 is secreted not by the classical mechanism of protein secretion, i.e. not via the Golgi apparatus and the endoplasmic reticulum. This is additionally supported by (1) the absence from YB-1 of the canonical N-terminal signaling sequence required for secretion by the classical mechanism, (2) retention of the molecular mass (i.e. the absence of the N-terminal truncation) of secreted YB-1, and (3) lack of the effect of brefeldin A (an inhibitor of classical protein secretion) on YB-1 secretion.

YB-1 secretion from cells is suppressed by reserpine, which is an inhibitor of the ATP-dependent packing of bioamines in vesicles. Under inflammatory stress YB-1 is co-localized with protein RAB7, a marker of vesicle transport. This is evidence that similar to some antiinflammatory proteins (IL-1β, MIF, HMGB1, and FGF2), YB-1 is secreted by a non-classical mechanism within endolysosomal vesicles.

YB-1 secretion strictly depends on the presence of lysines 301 and 304 in its CTD, and substitution of alanine for these residues completely inhibits YB-1 export from the cell. It is presumed that acetylation of lysine residues 301 and 304 plays an essential role upon YB-1 secretion from the cell [87].

YB-1 and its 16-amino acid fragment from CSD (73-88) can function as a growth factor. When added to cultured cells in nanomolar concentrations, it stimulates proliferation and migration of rat mesangial cells and human kidney cells [87]. YB-1 was found to interact with EGF-like repeats of the Notch-3 receptor extracellular domain. After interaction the Notch-3 the intracellular domain moves to the nucleus, where in complex with the transcription factor RBP-Jκ (Recombination signal binding protein for immunoglobulin kappa J) it activates target genes such as transcription factors of the HES family (Hairy and enhancer of split). It is remarkable that using a rat model of mesangioproliferative nephritis high levels of both the Notch-3 receptor and YB-1 were found in kidney glomeruli. Moreover, on the 7-th day of the disease, YB-1 and Notch-3 extracellular domain were detected in urine of the afflicted animals. So, the presence of YB-1 in urine may be a diagnostic indication of mesangioproliferative diseases [206]. It has been discovered recently that extracellular YB-1 can downregulate the *Notch-3* expression by an unspecified mechanism [207]. It is worth mentioning that Notch receptors play a vital role in the embryonic development of an organism and their overexpression leads to various diseases including cancer transformation [208]. However, it is not clear yet what role extracellular YB-1 and its interaction with Notch-3 play in this event.

YB-1 INVOLVEMENT IN EMBRYONIC DEVELOPMENT

The experiments on *YB-1* gene knockout in rat embryos demonstrated that *YB-1*$^{-/-}$ embryos develop normally up to 13.5 days (stage E13.5); then the embryo growth is drastically retarded and there appear anomalies in the neural tube formation and other distortions [209, 210]. If animals with such a genotype are ever born, they are unviable and rapidly perish. These data indicate an important role of YB-1 in late embryogenesis. It could have been expected that knockout of *YB-1* gene – which involved in such vital cellular processes as transcription and translation – should result in serious distortions in cell functioning. However, during analysis of fibroblasts prepared from *YB-1*$^{-/-}$ embryos at stage E13.5 no noticeable changes in transcription and translation were revealed. Such fibroblasts possessed only enhanced sensitivity to different stress types (oxidative and genotoxic stress) and reduced ability to grow and divide [209, 210]. In this connection, it is interesting that cultured chicken cell line DT40 with *YB-1* gene knockout (*YB-1*$^{-/-}$) do not differ from control cells under optimal growth conditions, but their division completely stopped at a suboptimal temperature [58].

It was proposed that functions of YB-1, when it is absent, can be performed by its paralogs MSY-3/4 and/or MSY-2. It should be remembered that in an adult organism, *MSY-2* and *MSY-3/4* are expressed only in germ cells (although MSY-3/4 is synthesized yet in embryonic cells, this protein disappears utterly by the moment of birth). This suggests that in *YB-1*$^{-/-}$ embryos, MSY-3/4 proteins perform not only their own functions but up to a certain moment they can compensate for the absence of YB-1. To verify this assumption, *MSY-3/4* gene knockout mice were obtained, as well as *MSY-3/4* and *YB-1* genes knockout mice. It turned out that *MSY-3/4*$^{-/-}$ mice have no pathologies except loss of fertility. Double mutant embryos (*YB-1*$^{-/-}$ and *MSY-3/4*$^{-/-}$) have serious distortions and perish by day 8.5-11.5 of embryonic development [209]. The results of such experiments lead to a conclusion that indeed at early stages of embryonic development, MSY-3/4 can functionally substitute YB-1, but for normal development at later stages additional func-

tions of YB-1 are required, which probably cannot be performed by its paralog. Of interest is also the circumstance associated with the affect of *YB-1* gene knockout on the formation of the neural tube. A key stage in this process is EMT, i.e. the transition in the cell phenotype typical of epithelial cells to the mesenchymal one [211]. This transition is accompanied with enhanced cell motility and migration required for successful neural tube formation. Inasmuch as it was established that overexpression of *YB-1* in Ras-transformed cells causes EMT (for more detail, see section "YB-1 and Oncological Diseases"), it can be assumed that disorders in the neural tube formation upon knockout of the *YB-1* gene are associated with the involvement of YB-1 in EMT. This assumption is also indirectly supported by the fact that another key participant of the EMT process, Twist, is a transcription regulator of *YB-1* gene expression.

YB-1 AND ONCOLOGICAL DISEASES

The investigation of mechanisms of oncogenesis is a principal direction in medical and biological studies. Taking into account the variety of YB-1 functions, it can be expected to be involved in tumor cell phenotype formation. More than 10 years ago, the first data on the amount and localization of YB-1 in breast cancer cells were obtained and suggested to be used for survival prognosis [212]. Since then the number of publications on the role of YB-1 in oncogenesis has been increasing.

At present all the data on the involvement of protein YB-1 in progression of malignant tumors can be divided in two groups. In accord with the first group of data, YB-1 can be considered as an oncoprotein that stimulates cell proliferation, enhances multidrug resistance, and promotes metastasis. But other studies show that in a number of cases YB-1 can be a tumor suppressor. Let us analyze these data in more detail.

YB-1 as an oncomarker. It is known that the amount of *YB-1* mRNA and YB-1 protein are frequently increased in tumors of different origin, including malignant ones [146, 212-222]. Many authors believe that the appearance of YB-1 in cell nuclei or its enlarged content in tissues adjoining the tumor is an indication of more aggressive and advanced tumors [213, 214, 217, 218, 220, 223]. Therefore it is suggested to regard YB-1 as a prognostic marker of disease aggressiveness and tumor resistance to chemotherapy, at least in the case of breast cancer [212, 214, 222, 224]. But not in all cases it is possible to reveal a correlation between the *YB-1* expression and clinical–pathological symptoms of tumor, such as its size, ability to metastasize, etc. [214, 225, 226]. The data on the role of YB-1 in malignant neoplasms were last summarized in 2005 [227]. The basic conclusions have not changed since then, however the information has widened greatly (Table 3).

Role of YB-1 in the formation of multidrug resistance. A significant obstacle in treating oncological diseases is the fact that cells acquire resistance to drugs.

YB-1 decreases the cell sensitivity to different types of chemical agents used in treating oncological diseases [85, 98, 145, 219, 240-242]. The mechanism of this process is not completely clear. It is thought that YB-1 can act via proteins ensuring multidrug resistance or it can be directly involved in DNA repair (see section "YB-1 Functions in the Nucleus"). The probability of curing various types of cancer without relapses after chemotherapy is lower if *YB-1* was overexpressed in the neoplasm and/or localized in the nucleus [213, 214, 220, 223, 233, 237]. In light of the latest discoveries, of special importance is the capability of YB-1 to protect stem cells from drug effect, including also tumor stem cells, which may be a reason for a cancer relapse [91, 145].

Molecular mechanisms for development of multidrug resistance are diverse. One of them is an enhanced synthesis of transporter proteins from the ABC family (ATP-binding cassette), in particular, P-glycoprotein (*MDR1* gene product).

Some researchers associate multidrug resistance of cells in culture and tumors with protein YB-1. So, it was found that YB-1 nuclear localization or *YB-1* gene overexpression correlates with P-glycoprotein increase in different types of cancer [194, 212, 217, 220, 224, 225, 234, 235, 243]. But the analyses of the colorectal carcinoma and ductal breast carcinoma cases did not reveal such a correlation [146, 226].

The mechanism that YB-1 uses to regulate the P-glycoprotein level is of great interest. YB-1 was isolated as a protein that binds to the regulatory region of the *MDR1* gene promoter [98]. It is postulated that YB-1 is involved in the activation of transcription of this gene, mainly, under stress conditions [99]. For some cell lines it is recognized that with *YB-1* knockout the cell sensitivity to DNA-damaging drugs increases [98, 219]. And *vice versa*, upon overexpression of *YB-1* in epithelial breast cells their sensitivity to doxorubicin decreases [212]. In some papers it was demonstrated that YB-1 can stimulate transcription of reporter genes that are under control of the *MDR1* gene promoter [133]. Yet there is no direct experimental data on the involvement of YB-1 in transcription of the *MDR1* gene; what is more, there are reports disproving this hypothesis. Thus, according to some authors, YB-1 has not been found within DNA–protein complexes that assemble in nuclear cell extracts on double-stranded oligonucleotides corresponding to *MDR1* gene promoter regions [134, 135]. Mutations in *MDR1* promoter diminishing its transcription activity have a slight effect on its binding to YB-1. In addition it was shown that neither short-term nor long-term suppression of *YB-1* expression in gastric and pancreatic carcinoma cells affected the sensitivity of these cells to medical preparations and the amount of P-glycoprotein [244]. Therefore,

the mechanism by which YB-1 stimulates the *MDR1* expression remains unknown. Apparently, it is not directly involved in transcription of the *MDR1* gene or is not a key factor in transcription, at least in some cell types. It is likely that transition of YB-1 to the nucleus or an enhancement of its expression and activation of *MDR1* are independent events occurring in cells in response to stress. Besides, it is possible that YB-1 stimulates expression and activity of P-glycoprotein at the posttranscriptional level.

There are several publications on the role of YB-1 in activation of other genes mediating multidrug resistance. It was shown that on transient overexpression of *YB-1* the amount of mRNA and protein LRP/MVP (Lung resistance-related protein/Major vault protein) in cells increases, and the increase in the amount of YB-1 is accompanied by enhancement of transcription reporter constructs under control of *LRP/MVP* promoter [102, 245]. Similar data were also obtained on the effect of YB-1 on the amount of *MRP1* (Multidrug resistance-associated protein 1) and *BCRP* mRNA [99, 225, 245]. Yet the mechanism of YB-1 action in these cases has not been found either.

YB-1 as an oncoprotein. So, in cells that have undergone oncogenic transformation protein YB-1 either is frequently localized in the nucleus, or its content in the cells is increased. Many researchers consider such changes not only as a consequence of oncogenic transformation of cells but as one of significant reasons for this phenomenon. Let us analyze some examples.

At present the available numerous data demonstrate that YB-1 promotes cell proliferation. Initially it was noted that an increased amount of YB-1 in clinical samples or its nuclear localization correlate with the expression of proliferation markers [146, 235]. Then it was clearly shown that the time of cell doubling in culture and in organism grows both upon transient and stable suppression of the *YB-1* expression, the portion of cells in G1 phase being increased in the population [58, 209, 210, 237, 240, 246, 247]. At the same time, *YB-1* overexpression results in hyperplasia [248]. It is especially remarkable that upon suppression of the *YB-1* expression cancer cells of many types lose their ability for anchorage-independent growth and soft agar colony formation [96, 230, 240, 249].

A number of mechanisms were proposed according to which YB-1 could influence cell proliferation. First, YB-1 can be involved in regulation of synthesis of proteins directly involved in DNA replication. It was demonstrated that upon suppression of YB-1 synthesis the transcription of reporter genes, which are under control of the *DNA topoisomerase IIα* gene promoter, decreases [146]. Similar results were obtained with the *DNA polymerase α* gene promoter [95]. Moreover, it was demonstrated that YB-1 is found within the complex, which is assembled on this promoter after serum stimulation of the cells, and it may be responsible for the transition of this promoter to a single-stranded state. Second, YB-1 can regulate the amount of proteins promoting the cell cycle progression. As known, YB-1 moves into the cell nucleus at the G1/S interface and activates *cyclin A* and *B1* genes transcription [106]. In addition, YB-1 activates transcription of the *CDC6* (Cell division control protein 6) gene, which controls the process of mitosis [250]. YB-1 participates in the IRES-dependent translation of mRNA of the c-*myc* protooncogene that is capable of stimulating cell proliferation [181]. It was also reported that suppression of *YB-1* expression decreases the amount of cyclin D1 and increases the amount the cell cycle inhibitor $p21^{CIP1}$ by an undefined mechanism [219, 241, 250].

In addition to stimulation of proliferation, YB-1 can protect cancer cells of several types against apoptosis [230, 241, 247]. At the moment we know several different mechanisms of such protection. The first is that YB-1 can modulate the activity of the apoptosis regulator p53. In its turn, dependent on the conditions p53 can activate two groups of genes: the genes responsible for the cell cycle arrest and proapoptotic genes. YB-1 prevents p53-dependent activation of transcription of proapoptotic genes and, to a lesser extent, activation of the genes of the first group [196, 197]. Not long ago another mechanism of YB-1-dependent inhibition of apoptosis was suggested. It was noticed that during suppression of the *YB-1* expression in breast cancer cells overexpressing the HER-2 receptor, the PTEN/mTOR/STAT3-pathway typically preventing apoptosis is inactivated [230]. Moreover, YB-1 is a repressor of transcription of the *Fas-receptor* gene, from which the proapoptotic signaling pathway begins [119]. In all probability, inhibition of apoptosis with protein YB-1 is not a universal mechanism but most likely is specific for a small number of cell lines, because in most cases a decrease in the amount of YB-1 merely results in a decreased level of cell proliferation rather than cell apoptosis.

Special attention is being focused on studying the role of YB-1 in progression of breast cancer. Experiments with transgenic mice demonstrated that *YB-1* overexpression is a reason for malignant transformation of breast tissues [248]. As clarified, an elevated amount of YB-1 in a cell leads to mitosis anomalies connected with abnormal state of centrosomes: there emerge cells that contain several nuclei and an aberrant set of chromosomes [202, 248].

The experimental data obtained recently suggest that YB-1 can be involved in transcription activation of some components of signaling pathways that are of great importance for progression of breast cancer. It was shown that overexpression of *YB-1* is accompanied by increase of the EGFR, PIK3CA, MET, and HER-2 levels [96, 97, 103, 136-138]. YB-1 stimulates transcription of genes of these proteins only when it is phosphorylated at Ser102. Therefore, it was proposed to consider YB-1 as a poten-

Table 3. YB-1 in human malignant tumors

Examined tumor	Probable involvement of YB-1 in tumor progression	References
Breast tumors (classification according to histology)		
Invasive ductal and lobular carcinomas	Nuclear localization of YB-1 correlates with P-glycoprotein synthesis	[212]
	YB-1 stimulates synthesis of EGFR and HER-2	[137]
	Presence of YB-1 is obvious adverse prognostic factor	[228]
	High content of *YB-1* mRNA correlates with aggressive progression of disease – early development of metastasis in remote organs	[229]
Cancer (histological subtype is not determined)	Increased content of YB-1 correlates with higher risk for relapse after surgery	[214]
	Nuclear localization of YB-1 positively correlates with synthesis of HER-2 and negatively with synthesis of ERα and CXCR4 and indicates to lower survival probability	[138]
Invasive ductal carcinoma	Nuclear localization of YB-1 correlates with synthesis of progesterone receptor and P-glycoprotein but is not associated with survival	[216]
	Without adjuvant chemiotherapy, the relapse frequency is lower at a low level of YB-1	[226]
	Higher content of YB-1 correlates with loss of cadherin E and is a factor of high probability of metastasis and poor prognosis	[184]
	Enhanced *YB-1* expression correlates with metastasis of small-size tumors in remote organs. Nuclear localization of YB-1 correlates with tumor dimensions exceeding 5 cm	[222]
Cancer (different subtypes)	YB-1 nuclear localization correlates with P-glycoprotein synthesis, impedes treatment with paclitaxel	[194]
Breast tumors (classification according to expression of biological markers)		
Basal cancer	YB-1 activates *EGFR* transcription, decreases cell sensitivity to inhibitor of EGFR (Iressa) tyrosine kinase activity	[96]
Tumors with amplification of the *HER-2* gene	YB-1 stimulates proliferation of cells and protects them from apoptosis	[230, 231]
Cancer (luminal A, luminal B, basal and HER2/neo-positive)	Cytoplasmic localization of YB-1 in small-size tumors correlates with expression of several genes of multidrug resistance. After neoadjuvant chemiotherapy, YB-1 may pass to cell nuclei in case of large and small tumors	[232]
Ovary tumors		
Serous adenocarcinoma	Nuclear localization of YB-1 correlates with poor prognosis	[233]
Epithelial cancer of different histological subtypes	YB-1 is mostly present in secondary centers rather than in primary ones	[215]
Carcinomas (mainly serous ones)	Nuclear localization of YB-1 correlates with the content of P-glycoprotein, LRP/MVP and pAkt as well as with poor prognosis	[234]
Bone tumors		
Different histological subtypes	Nuclear localization of YB-1 correlates with content of P-glycoprotein and accelerated proliferation	[235]
	Intestine tumors	
Colorectal carcinoma	Amount of YB-1 correlates with expression of proliferation markers	[146]

Table 3. (Contd.)

Examined tumor	Probable involvement of YB-1 in tumor progression	References
Skin tumors		
Melanoma	Progression of disease increases amount of YB-1, which stimulates proliferation and drug resistance	[219]
Lung tumors		
Squamous cell carcinoma	Nuclear localization of YB-1 is associated with clinic-pathological factors and poor prognosis	[213]
Non-small cell carcinoma	Nuclear localization of YB-1 correlates with metastasis in remote organs, stage of disease, and poor prognosis Tumor cells contain decreased content of *YB-1* mRNA	[223] [236]
Adenocarcinoma	Nuclear localization of YB-1 is associated with lower survival probability	[237]
Soft tissue tumors		
Synovial sarcoma	Nuclear localization of YB-1 correlates with enhanced expression of *MDR1* and *MRP1* and poor prognosis	[225]
Embryonic rhabdomyosarcoma	Nuclear localization of YB-1 correlates with increased content of MDR1 and LRP/MVP and accelerated proliferation	[238]
Nervous system tumors		
Glioblastoma in children	*YB-1* is overexpressed in such tumor cells	[239]
Multiform glioblastoma	*YB-1* is overexpressed in tumor cells as compared to normal cells	[240]
Prostate tumors		
Adenocarcinoma	Progression of disease increases amount of YB-1 and it passes to the cell nucleus	[217]
Liver tumors		
Hepatocellular carcinoma	Increased content and nuclear localization of YB-1 correlates with invasion to portal and liver veins, later stage of disease, and poor prognosis	[218]
Neoplasms of hemopoietic system		
Multiple myeloma	YB-1 synthesis is associated with high ability to proliferate	[241]
Diffuse large B-cell lymphoma	Nuclear localization of YB-1 correlates with increased content of P-glycoprotein, later stage of disease, and poor prognosis	[220]

tial target in therapy of various types of breast cancer [138, 230].

YB-1 is not only involved in malignant transformation of cells, but also facilitates subsequent metastasis. It is known that upon suppression of *YB-1* expression in cancer cells, their capability for migration and invasion is reduced [103, 219, 240]. This might be associated with the fact that YB-1 can activate transcription of the metalloproteinase *MMP-2* (Matrix metalloproteinase 2) gene, which cleaves components of the basal membrane [75]. Besides, YB-1 stimulates the activity of MT1-MMP (Membrane type 1 matrix metalloproteinase), which can

mediate invasion of cancer cells [251]. YB-1 is believed to modulate recirculation of this enzyme in the membrane.

The mechanism by which YB-1 stimulates metastasis of breast cancer was described in detail [184]. It is recognized that a significant stage in tumor metastasis is the so-called epithelial–mesenchymal transition (EMT). In the course of this process, cells lose their cuboidal shape, apical basal polarity, intercellular contacts and capability to form acinar-like spheroids, acquire fibroblast-like morphology, and more actively migrate in the gel matrix. Such a transformation is accompanied by almost entire loss of E-cadherin and other markers of epithelial cells. Instead of this, cells synthesize N-cadherin and a number of other markers of mesenchymal cells. It was demonstrated recently that human mammary epithelial cells (HMLE) that have undergone EMT have the properties of stem cells, i.e. have a higher proliferative potential and ability to differentiate (though restricted only to two types of myoepithelial cells) [183].

The overexpression of *YB-1* in the cytoplasm of MCF10AT breast epithelial cells with an activated Ras-MAPK-signaling pathway results in epithelial–mesenchymal transition. The molecular mechanism of EMT in this case was disclosed [184]. At the first stage the activated Ras-MAPK-signaling pathway is required. Apparently at this stage, genes of transcription factors regulating EMT such as *Snail1*, *HMG*, *Lef-1*, *TCF4*, *Zeb2/Sip1*, *HIF1α*, *bHLH*, *Twist*, etc. are activated. At the second stage, EMT requires a high concentration of YB-1 in the cytoplasm that stimulates cap-independent synthesis of at least one of the EMT regulators (Snail1) and possibly of some other required proteins. At the next stage, Snail1 and other transcription factors trigger global phenotypic alterations typical of EMT. It is notable that an increase in the YB-1 content brings about a decreased amount of proteins responsible for proliferation (in particular cyclins). As a result, the *YB-1* overexpressing cells have a low frequency of division. A proliferation decrease produced by YB-1 probably contributes to cell viability in conditions of broken cell contact with the extracellular matrix. It is in these conditions that the metastatic cells are in the circulation.

When injected in mammary fat pads of mice, in three months the *YB-1* overexpressing MCF10AT cells produced small tumors with metastatic spreads in various organs, whereas cells with a normal level of YB-1 formed larger tumors localized in the injection site [184].

So, YB-1 facilitates acquisition by tumor cells of an invasive phenotype at the expense of EMT, slows down their proliferation, and makes them more resistant to apoptosis, i.e. endows the cells with properties allowing them to pass from the original tumor to other organs and to form micrometastatic spreads. Moreover, such a set of properties protects cells from the action of most anticancer drugs because first of all they affect actively proliferating cells. One of the important conclusions made by Evdokimova et al. is that at least for some types of breast cancer the *YB-1* overexpression stimulates tumor metastasis but at the same time suppresses tumor growth. Therefore, in some cases suppression of YB-1 synthesis may lead not to recovery but to activation of proliferation of metastatic cells. This should be taken into account when creating curing schemes. These results are also supported by other research teams. For example, it was established that small-size mammary cancer tumors overexpressing *YB-1* have predisposition to early metastasis [222].

YB-1 as an antioncoprotein. Although most researchers agree that YB-1 has an oncogenic effect, there are reports clearly demonstrating that in some cases this protein, being in the cytoplasm, can behave as an antioncoprotein. It was found that the amount of *YB-1* mRNA drops at oncogenic transformation of chicken embryo fibroblasts caused by overexpression of oncoproteins PI3K and Akt, and additional synthesis of YB-1 from the plasmid prevents such a transformation [172]. Studies of the mechanism of this phenomenon revealed that YB-1 does not directly affect the activity of Akt kinase, and its action is exerted during inhibition of translation [83, 190]. It appeared that in normal conditions a great number of weak templates, among them those are associated with cell growth and division, are within YB-1 containing untranslated mRNPs. YB-1 phosphorylation with Akt kinase at Ser102 results in decreasing its affinity to the cap-structure and/or the cap-adjoining region of mRNA. As a result, the YB-1 inhibiting effect on the cap-dependent translation by displacing translation initiation factor eIF4E from the cap-structure diminishes [83, 198]. During this, the earlier repressed weak mRNAs encoding proliferative factors pass into a translated state, which leads to uncontrolled cell division. Upon *YB-1* overexpression from the plasmid, the content of non-phosphorylated YB-1, first of all inhibiting translation of weak matrices, enlarges, which, in turn suppresses uncontrolled cell division caused by hyperactivation of the PI3K-Akt-kinase pathway.

YB-1 GENE STRUCTURE AND REGULATION OF ITS EXPRESSION

Given that YB-1 regulates multiple DNA- and mRNA-dependent processes, it should be expected that its content and functional activity in the cell are strictly controlled.

The first studies of regulation of YB-1 synthesis started from the analysis of the *YB-1* gene structure. The human *YB-1* gene is located in the first chromosome (1p34), consists of 19 thousand base pairs, and includes eight exons. And after splicing, the *YB-1* mRNA has the length of just about 1500 nucleotide residues. It was clarified that the *YB-1* gene promoter has no regulatory

sequences most specific of the largest number of genes: the TATA box and the CCAAT element. But it has several E-boxes (CATCTG) and the beginning of the first exon (from +24 to +281) contains many CG-repeats as well as GATA motifs that are required for *YB-1* gene transcription [252].

Northern-blot analysis demonstrated that the *YB-1* mRNA is unequally distributed over different tissues. It was found to be abundant in testicles, skeletal muscles, and spleen, and in small amounts in kidneys, liver, and lungs [253]. Other researchers revealed high amounts of the *YB-1* mRNA only in kidneys and testicles [17]. It was also reported that the *YB-1* mRNA level in fetal liver cells is far higher than in adult liver cells [32, 253]. The amount of the *YB-1* mRNA may vary under different stresses [254] and during stimulation of cell proliferation [32, 253]. The data on the *YB-1* expression on microchip Affymetrix U133 (http://biogps.org/#goto=genereport&id=4904) showed that the amount of the *YB-1* mRNA is much higher in testicular cells, fetal brain cells, early precursors of erythroid and lymphoid cells, and in some others. The amount of the YB-1 protein in different tissues also varies greatly. The protein is abundant in testicles, liver, and spleen. It is almost absent in skeletal muscles, heart, and lungs [255]. Besides, it was found that the amount of YB-1 in mouse tissues can change with aging [255]. The amount of YB-1 in mouse brain, heart, and muscles decreases during several weeks after birth, while in testicles, spleen, kidney, and lungs the decrease is observed only during aging. Meanwhile, the amount of YB-1 in the liver remains unchanged through the lifespan of mice. The observed distribution of YB-1 over tissues may be associated with the proliferative activity of these tissues. Unfortunately, no systematic studies of the simultaneous determination of the amount of both the YB-1 protein and the *YB-1* mRNA have been performed yet. But the available incomplete data allow us to conclude that the amount of the YB-1 protein in tissues does not frequently correlate with that of the *YB-1* mRNA. In other words, regulation of *YB-1* gene expression can be realized not only at the level of transcription, but at the posttranscriptional level as well.

It was experimentally shown on KB cells that on their treatment with cisplatin the *YB-1* mRNA amount increases six-fold because of the activation of transcription of the gene. The activation requires the presence in the gene promoter of the E-box along with two mutually interacting proteins – p73 and c-Myc. In the presence of cisplatin, p73 stimulates interaction of c-Myc with protein Max and facilitates the binding of this complex to the E-box leading to activation of transcription [256]. It was shown recently that *YB-1* mRNA synthesis is promoted upon binding of another transcription factor, Twist, to the E-box [249]. Shiota et al. found [242] that protein PCDP4 (Programmed cell death protein 4) can bind to the DNA-binding domain of Twist and prevent activation of *YB-1* gene expression. Another Twist-binding protein, PCAF (p300/CBP-associated factor), can promote activation of *YB-1* expression [257]. As reported not long ago [258], *YB-1* expression is negatively regulated by protein Foxo3a and decreases upon integrin-linked kinase (ILK) inhibition [259]. In these cases it is assumed that the synthesis of YB-1 is regulated by a Twist-mediated mechanism.

To transcribe the *YB-1* gene in K562 cells, the presence of a GATA motif in the gene promoter is required. The binding of GATA-1 and GATA-2 transcription factors to it results in activation of *YB-1* gene transcription. The synthesis of mRNA enhances at the proliferative stage of differentiation of K562 cells committed to erythropoiesis [260, 261].

It is remarkable that the level of the *YB-1* mRNA in some tissues and cells is directly connected with the expression level of transcription factors interacting with the *YB-1* gene promoter. For instance, in early precursors of erythroid cells the transcription factors GATA-1 and GATA-2 are expressed far stronger than in other cells of the organism [261]. Protein Math2 (Neurod6), which is a transcription factor binding to the E-box of the *YB-1* gene promoter, is found only in fetal brain, where a high level of *YB-1* mRNA is observed [262].

Data on the regulation of the YB-1 synthesis at the posttranscriptional level have appeared quite recently and are associated with the involvement in this process of both the 3′ UTR [180] and 5′ UTR of the *YB-1* mRNA [263].

Fukuda et al. [263] demonstrated that in human KB3-1 and H1299 cells, YB-1 inhibits its own synthesis by specific binding with the 5′ UTR of the *YB-1* mRNA. It turned out that the 5′ UTR of the *YB-1* mRNA studied in the cited paper is 200 nucleotides longer than the 5′ UTR of the human, mouse, rat, and rabbit *YB-1* mRNA from the PubMed database (for example, NM_004559.3 for the human *YB-1* mRNA). It is with this additional sequence at the 5′ terminus that YB-1 interacts, which is why these results are somewhat dubious. At the same time, these results can be explained by a varying starting point of *YB-1* gene transcription in different cell lines or types of tissues, which in turn suggests the presence of another possible regulatory mechanism of YB-1 synthesis.

On the other hand, our experiments in a cell-free translation system showed that regulation of the YB-1 synthesis takes place with the involvement of an approximately 80-nucleotide regulatory element in the 3′ UTR of the *YB-1* mRNA. It is with this element that two major proteins of cytoplasmic mRNPs interact specifically: the YB-1 itself and PABP. It was found that PABP stimulates translation of the *YB-1* mRNA even in the absence of the 3′ terminal poly(A)-sequence [180], and YB-1 selectively inhibits it at rather low concentrations optimal for translation of other cellular mRNAs [264]. Both proteins have their positive or negative effect at the stage of translation initiation, at the step of the mRNA binding to the small

ribosomal subunit, or at an earlier step of the mRNA interaction with translation initiation factors. Footprinting analysis demonstrated that the regulatory element contains two sequences (UCCAGCA and UCCAACA) specifically interacting with YB-1 (1137-1144 and 1164-1171), as well as an A-rich (about 50% A) region, which is a site of binding of two PABP molecules (1149-1205). The binding sites of these two proteins overlap, which explains their competition for the binding to the regulatory element [42]. Due to this, the inhibition of the *YB-1* mRNA translation caused by YB-1 is overcome at an increased concentration of PABP in the translation system.

It is notable that the *PABP* mRNA translation is negatively autoregulated through the specific binding of PABP to the 60-nucleotide A-rich element in the 5′ UTR of its own mRNA [265].

Altogether, the described regulatory mechanisms of YB-1 and PABP synthesis allow maintaining the concentration and ratio of the two proteins at the level optimal for translation of other cellular mRNAs.

It has become firmly established recently that microRNAs (miRNA) play a vital role in regulation of gene expression [266]. Regulation proceeds either as repression of translation or at the expense of mRNA destabilization. In both cases the 3′ untranslated region of mRNA is involved. Many specialized databases and Internet search services predict microregulatory RNAs for almost any mRNA including the *YB-1* mRNA. Of potential importance for the regulation of translation or stability of the *YB-1* mRNA may be about 20-30 microRNAs, but until recent time there were no data supporting experimentally such regulation. In 2010 it was reported [267] that as a result of 24-h stimulation of mesangial cells with the TGFβ factor, the amount of the YB-1 protein and the *YB-1* mRNA decreased sharply. At the same time, the expression of the *miR-216a* microRNA having a potential binding site in the *YB-1* mRNA enhances. Experiments with *luciferase* reporter mRNA containing either the 3′ UTR of the *YB-1* mRNA or the 3′ UTR of the *YB-1* mRNA with mutations at the *miR-216a*-binding site supported the assumption of regulation of stability of the *YB-1* mRNA with the involvement of this microRNA. It is presumed that TGFβ-induced degradation of the *YB-1* mRNA and consequently cessation the YB-1 synthesis are compulsory for the involvement of YB-1-repressed mRNAs (specifically *Tsc22* mRNA) in translation. As a result, the expression profile of the genes is changed in accord with the received signal (TFGβ), in particular, the synthesis of extracellular matrix proteins including collagen enhances. Interestingly, the excess synthesis of these proteins caused by overexpression of *TGFβ* in diabetic nephropathy correlates with decreased expression of *YB-1*. From this point of view, YB-1 can be regarded as a potential therapeutic target in treating similar diseases.

USE OF YB-1 FOR THERAPEUTIC PURPOSES

Clarifying the mechanisms of the involvement of YB-1 in oncogenic transformation of cells and in other pathologies has made it possible to suggest several promising approaches not only to diagnostics but also to therapy of cancer and some other diseases.

Accordingly for treating breast cancer, Dunn et al. propose to use the YB-1 fragment conjugated to the peptide, which enables its transport to the cell [268]. This fragment of CSD contains phosphorylated Ser102 and in line with molecular modeling is a suitable substrate for RSK, Akt, and PKC kinases. As mentioned above, enhanced phosphorylation of YB-1 may lead to an uncontrolled synthesis of some oncoproteins [83, 97] and a more aggressive progression of the disease. It can be expected that the YB-1 fragment that penetrated into the cell would compete with the full-length protein for the binding to kinases, prevent phosphorylation of YB-1 and thus alleviate the negative effect of the phosphorylated YB-1. The experiments demonstrated that the YB-1 fragment decreased by 90% the rate of growth of cultured breast and prostate cancer cells and enhanced their sensitivity to trastuzumab without suppressing the growth of normal cells.

Other authors [269, 270] propose to use for cancer therapy the property of YB-1 to maintain replication of adenovirus with mutation at the *E1A* gene [107]. Such a mutant adenovirus is reproduced only in cells with a high concentration and nuclear localization of YB-1, which are most specific of cancer cells. Consequently, the lytic effect of adenovirus is selectively directed to cancer cells.

Studying the synthetic low molecular weight substance HSc025 as a means against liver fibrosis, the teams of Hasegawa and Higashi discovered that it interacts with YB-1 and stimulates the transition of YB-1 from the cytoplasm to the nucleus. It is assumed that YB-1 is a molecular target for HSc025. Having passed to the nucleus, YB-1 binds to the promoters of collagen genes *COL1A1* and *COL1A2* and suppresses their transcription, which prevents progression of fibrosis [271, 272].

The use of YB-1 in therapy of viral diseases based on its ability to differently affect the translation of cellular and viral RNAs is quite promising. As mentioned above, YB-1 suppresses the Dengue virus RNA translation stronger than the translation of most cellular mRNAs because of its higher affinity to specific sequence in the 3′ UTR of the viral (+)RNA strand [46].

CONCLUSION

Seven years have elapsed since the publication of the last detailed review on Y-box binding proteins [273]. It can be boldly claimed that during this period our knowledge on the functions of these proteins has greatly increased.

The data reviewed herein demonstrate a wide range of YB-1 functions in the organism. It was shown that YB-1 is involved in practically all DNA- and mRNA-dependent processes in cells. It is a system regulator of gene expression at different levels both in the nucleus and in the cytoplasm. YB-1 packs mRNAs into mRNPs, can participate in the localization of mRNPs at various components of the cytoskeleton, and may be involved in regulation of the formation of the cytoskeleton *per se*. This protein should be given special attention owing to its participation in oncogenic transformation of cells, tumor metastasis, inflammatory processes, and viral infections. An increasingly more evident applied medical component is a characteristic feature of such studies.

A number of fundamental discoveries have been made recently in studying YB-1. The unexpected and much promising are first of all the data on the YB-1 secretion and its extracellular effects. Quite intriguing is the involvement of YB-1 in the processes of early embryonic development. Significant success has been achieved in studying the mechanism of the involvement of YB-1 in the PI3K/Akt-mediated translation regulation of some vital cell proteins. Finally, a mechanism of YB-1 action in the process of epithelial mesenchymal transition has been proposed that is of great outcome not only for oncology but also for cell differentiation and morphogenesis.

The involvement of YB-1 in various cellular processes is a prerequisite for further investigations of this protein in a number of directions. One of them is the analysis of a multiplicity of genes regulated by YB-1 in accord with its intra- and extracellular content, intracellular distribution, covalent modifications, and cell context. Notwithstanding a certain progress in studying regulatory mechanisms of gene expression caused by YB-1 both at the level of transcription and translation, the results are only initial steps on the lengthy way of clarifying the probable entire variety of such mechanisms. Studies of the mechanisms regulating nucleocytoplasmic transport and secretion of YB-1 are another vital task, the solution of which would find approaches to the control of intracellular localization of YB-1, its secretion and, which is more important, regulation of cellular processes with the involvement of YB-1. Further search for protein partners of YB-1 and clarifying the functional role of the interaction of YB-1 with these partners seems to be a necessary link for understanding the role of such interactions in the cell life span and the organism in general. Studying the molecular structure of YB-1 in complex with different partners is also a promising direction of future work, since the structure of YB-1 as a smart representative of natively unstructured proteins may be varying and dependent on partners in complexes. As the processes in which YB-1 and its homologs are involved are dependent on the concentration of these proteins, it is necessary to continue studying the mechanisms regulating their content in the cell.

In general, the solution of the above fundamental problems would not only enrich our knowledge on the regulation of cellular events in which YB-1 is involved, but would also have a great practical value both for physiology and medicine.

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