A human NDP-kinase B specifically binds single-stranded poly-pyrimidine sequences

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ABSTRACT

Recently, a DNA binding protein 'PUF' was purified that binds to a poly-pyrimidine rich element in the human *c-myc* promoter. Cloning of the corresponding gene surprisingly identified this putative transcription factor as isoform B of the enzyme nucleoside diphosphate kinase (NDPK-B) [Postel *et al.* (1993) *Science*, 261, 478–480], the product of the potential metastasis suppressor gene *nm23-H2*. Using different recombinant NDP kinases, we demonstrate by electrophoretic mobility shift analysis (EMSA) that the NDP kinase DNA binding properties are predominantly observed with human isoform B. Unlike typical DNA binding proteins that are involved in transcriptional regulation, binding occurs to single-stranded DNA rather than to a double-stranded oligonucleotide. As a consequence, complexes of single-stranded DNA and NDPK-B are generated from double-stranded oligonucleotide hybrids in an ATP independent manner. In addition to the *c-myc* element, NDPK-B is binding *in vitro* to a variety of poly-pyrimidine rich sequences including dC or dT homo-oligomers, (CT)_n dinucleotide repeats, the initiator region of the Adenovirus major late promoter and even poly-pyrimidine rich RNAs. The possible consequences of these findings in understanding the multiple roles of NDP kinase are discussed.

INTRODUCTION

Nucleoside diphosphate (NDP) kinase is the enzyme that exchanges a phosphate between (deoxy-) nucleoside triphosphates and (deoxy-) nucleoside diphosphates (2). Usually, ATP serves as the phosphate donor. NDP kinases have been cloned and sequenced from a number of species including procaryotes and eucaryotes. All NDP kinases are oligomers consisting of 17–20 kDa polypeptides. The crystal structures of the eucaryotic enzymes from *Dictyostelium discoideum* (3) and *Drosophila melanogaster* (4) have been solved at high resolution and demonstrate that these NDP kinases are hexamers (5). In humans, two distinct NDP kinases, NDPK-A and NDPK-B, have been

described. The two types of monomers share 88% sequence identity which can combine to form different types hybrid hexamers with variable ratios of each subunit (6). NDP kinases from other eucaryotic species are also highly conserved with identities of 72 and 62% with NDPK-A for the *Drosophila* and *Dictyostelium* enzymes, respectively.

There are indications that NDP kinase is directly involved in several developmental processes. In *Drosophila*, mutations in the gene encoding NDP kinase (*awd*) cause developmental phenotypes: while a null mutation leads to abnormal wing disc development (7,8), the dominant mutation *killer of prune* (*K-prn*) corresponding to a P97S substitution causes a conditional lethal phenotype in the genetic background of a *prune* null mutation (9). In humans, the two isoforms NDPK-A and NDPK-B are identical to the products of the human genes *nm23-H1* and *nm23-H2* (5). *nm23-H1* was originally isolated by differential screening as a potential metastasis suppressor gene (10), whereas *nm23-H2* was isolated due to its homology to *nm23-H1* (11). Reduced mRNA levels of *nm23-H1* and, to a minor extent, of *nm23-H2* have been reported in highly metastatic cell lines (12,13). A decreased Nm 23 protein level in invasive tumors has also been observed (12,14) although this is not always the case (15–18). In contrast, a correlation between an increased level of *nm23* proteins and proliferation has been observed in all tissue types analyzed including normal proliferating or leukemic lymphocytes (19), and several other types of tumors (20,21).

Independently, a DNA binding protein PUF has been isolated, which recognizes an element at position –115 to –145 in the *c-myc* promoter, consisting of a poly-pyrimidine rich tract in the 'coding strand' and a complementary poly-purine rich tract in the 'noncoding strand' (1). This and other similar homo-pyrimidine/homo-purine sequences are known to be able to form triple helix and other unusual DNA structures *in vitro* (22) and presumably *in vivo* (23). Thereby nuclease hypersensitive sites are created (24,25). A cDNA encoding the factor that binds to the corresponding *c-myc* element (= NHE element) was cloned by functional screening of an expression library and surprisingly identified NDPK-B as the putative binding protein (1). The recombinant protein has analogous DNA binding properties as the previously purified factor, including the capacity to stimulate *in vitro* transcription from the *c-myc* promoter.

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Table 1. Sequences recognized by NDPK-B

ssPy ₃₀ (<i>c-myc</i> promoter):	CAC	CTT	CCC	CAC	CCT	CCC	CAC	CCT	CCC	CAT
ssPy ₁₇ (<i>c-myc</i> promoter):			CCC	CAC	CCT	CCC	CAC	CC		
ssY (AML promoter):	GCG	TTC	GTC	CTC	ACT	CTC	TTC	CGC	ATC	GTG
CT (CT-repeat):	CTC	TCT	CTC	TCT	CTC	TCT	CTC	TCT	CTC	TCT
Oligo-T		TTT	TTT	TTT	TTT	TTT	TTT	TT		
Oligo-C		CCC	CCC	CCC	CCC	CCC	CCC	CC		
TTCC-oligo:	CAT	ATG	CCT	CCT	TCT	TCC	TC			
TTTCC-oligo:	CAT	ATG	CTT	TCC	TTT	CCT	TTT	C		

In this study we have investigated the potential of NDP kinase to recognize and interact with different species of poly-pyrimidine rich single stranded DNA and RNA sequences.

MATERIALS AND METHODS

Expression vectors for mammalian NDP kinases

The coding regions of *nm23-H1* and *nm23-H2* were amplified by polymerase chain reaction (PCR) from cDNA obtained by reverse transcription of RNA purified from the human hepatoma cell line HepG2. Primers were designed according to the published sequences (10,11): CCCCGAATTCCATGGCCAACCTGTGAGCGT (forward) and TTTTCCCGGGCCCTCTGTTCATTCATAGAT (reverse) were used for the NDPK-A and CCCCGAATTCCATGGCCAACCTGGAGCGC (forward) and TTTTCCCGGGTCCACCTCTTATTCATAGAC (reverse) for the NDPK-B, respectively, and contained an *EcoRI* site 6 bp upstream of the initiating codon and a *SmaI* site 9 bp downstream of the stop codon. After digestion with *EcoRI* and *SmaI*, the 0.48 kb amplification product was inserted at the corresponding sites of the pPROK-1 procaryotic expression vector (Clontech) and subsequently used to transform the *E.coli* host strain DH5 α .

Protein purification

NDPK-B was purified by a four step procedure: recombinant bacteria were lysed by sonication in 50 mM Tris-HCl pH 8, 1 mM EDTA and 5 mM β -mercaptoethanol. After centrifugation the supernatant was loaded on a DEAE-Sephacel (Pharmacia) column equilibrated at pH 8 with the same buffer. The flow through (depleted of the bacterial NDP kinase) was collected and loaded on a Blue-Sepharose (BioRad) column. The enzyme was eluted by 2 M NaCl and concentrated by ammonium sulfate precipitation. The precipitate was resuspended in buffer H (50 mM Hepes pH 6.8, 5 mM β -mercaptoethanol), dialysed, and loaded onto a S-Sepharose FF column (Pharmacia) washed with the same buffer. The enzyme was eluted by buffer H containing 0.2 M NaCl. To eliminate traces of contaminating proteins, the pooled fractions were dialysed against 10 mM sodium phosphate buffer, pH 7, 5 mM β -mercaptoethanol and loaded on hydroxyapatite (5 ml, Bio-Rad HTP). Elution with a linear gradient of 10–400 mM sodium phosphate yielded a pure NDPK-B. For purification of recombinant NDPK-A, a bacterial extract obtained by sonication was centrifuged and the supernatant was loaded on a DEAE-Sephacel equilibrated in Tris 50 mM buffer pH 8.0

containing 1 mM EDTA and 1 mM β -mercaptoethanol. The enzyme was eluted at 0.5 M NaCl, and after a 2-fold dilution in distilled water, absorbed onto a hydroxyapatite column. After washing with 0.12 M phosphate buffer pH 7.0, the enzyme was eluted with 0.3 M of the same buffer. Purification of *Drosophila* and *Dictyostelium* wild-type and mutant proteins was done as described previously (9,26). The different preparations were homogenous as checked on SDS-polyacrylamide gels (not shown). Protein concentrations were calculated on the basis of their catalytic activity in a coupled enzyme assay using a value of 800 U/mg (6) for the molecular specific activity of the enzymes.

EMSA: probes and radioactive labelling

Two complementary 30mer oligonucleotides ssPY: CAC CTT CCC CAC CCT CCC CAC CCT CCC CAT and ssPU: TTA TGG GGA GGG TGG GGA GGG TGG GGA AGG represent the NHE element at position -145 to -115 in the *c-myc* promoter. From these two oligonucleotides, three different radioactive probes were prepared: (i) ssPY (poly-pyrimidine strand); (ii) ssPU (poly-purine strand): single-stranded oligonucleotides were labelled by a T4-PNK reaction with radioactive [γ -³²P]ATP without previous hybridization; and (iii) ds*: hybrids 1 μ g of each strand was used for hybridization in a total volume of 40 μ l by heating for 2 min at 95°C and subsequent slow cooling to room temperature within ~5 h. Labelling afterwards was performed by a Klenow fill-in reaction in the presence of radioactive [α -³²P]dATP.

The two complementary oligonucleotides ssY: GCG TTC GTC CTC ACT CTC TTC CGC ATC GTG and ssR: CAC GAT GCG GAA GAG AGT GAG GAC GAA CGC are representing the initiator region from -12 to +18 of the AML promoter. Both single strands were labelled as described above. dsY is a double-stranded probe that was generated by hybridizing a T4 PNK labelled ssY-oligonucleotide to its (unlabelled) complementary ssR strand. Single-stranded oligonucleotides 18, 30 and 36 are (CT)₉, (CT)₁₅ and (CT)₁₈ dinucleotide repeats.

rPyr represents an RNA with the length of 198 nt, identical to the poly-pyrimidine rich nucleotide sequence of the 3' region of intron 6B from the human β -tropomyosin gene (27). The probe was generated by SP6 *in vitro* transcription in the presence of [α -³²P]CTP from a DNA fragment cloned in Plasmid BluGem 3.

After labelling, all probes were cleaned by phenol-chloroform extraction followed by ethanol precipitation and were resuspended at a final concentration of ~1 ng/ μ l.

Figure 1. Recognition of single-stranded poly-pyrimidine DNA by NDPK-B. (a) Competition of binding to the *c-myc* NHE element. 300 ng NDPK-B was used in an EMSA with the single-stranded NHE poly-pyrimidine tract (ssPy) as a probe. 10- or 50-fold excess of single-stranded oligonucleotides corresponding to the *c-myc* NHE element (ssPy, ssPu), the AML element (ssY, ssR) or ssdIdC were added as competitors before addition of the protein. Competitor DNA was added to the radioactive probe prior to the protein in order to allow hybridization of complementary sequences. This experiment was performed without a basal concentration of nonspecific competitor. (b) NDPK-B binding to the AML element. 300 ng NDPK-B was analyzed in an EMSA for its ability to bind different oligonucleotides in presence of 50-fold excess of different single-stranded competitors representing either the *c-myc* NHE element (ssPy, ssPu) or the AML promoter element (ssY, ssR). Complementary oligonucleotides representing either the poly-pyrimidine strand (ssY) or the poly-purine strand (ssR) of the AML element were used as probes. Competitor DNA was added to the radioactive probe prior to the protein in order to allow hybridization of complementary sequences. (c) NDPK-B binding to a (CT)_n dinucleotide repeats. 300 and 600 ng NDPK-B were subjected to an EMSA with single-stranded (CT)₉ = '18', (CT)₁₅ = '30' and (CT)₁₈ = '36' oligonucleotides as probes.

EMSA: incubation and gel electrophoresis

Incubation conditions were those typical for the analysis of mammalian DNA binding proteins: ~1 ng labeled DNA or RNA was incubated with recombinant protein for 20 min on ice in a total volume of 20 μ l with final concentrations of 12.5 mM Hepes pH 7.9, 10 mM MgCl₂, 50 mM KCl, 0.05% NP-40, 0.5 mM DTT and 12.5% glycerol. A typical assay contained 300 ng of protein in 20 μ l corresponding to a concentration of 125 nM, and 1 ng of a 30 base oligonucleotide corresponding to a concentration of 5 nM. Unless otherwise indicated, 100 ng double-stranded dIdC for competition of double-strand binding or 100 ng single-stranded dIdC for single-strand binding were added as nonspecific competitor. Unspecific single-stranded competitor was prepared by denaturing dIdC for 10 min at 95°C, followed by a rapid cooling to 0°C. For RNA gel shifts, 300 ng heparin was added instead of dIdC. Samples were separated on native 4% polyacrylamide (19:1) mini-gels (1.5 mm $\times$ 8 cm $\times$ 10 cm) under low ionic strength conditions in the presence of 0.25 $\times$ TBE buffer. After a pre-run at 200 V for 15 min, the separation was performed for ~30 min at 170 V.

Single-strand generation assay

For this assay, a 5-fold molar excess of a 34mer oligonucleotide representing the AML element with the sequence GG GCG TTC GTC CTC ACT CTC TTC CGC ATC GTG GG was hybridized

under the conditions described above to a T4-PNK-labelled complementary 18mer with the sequence GCG GAA GAG AGT GAG GAC. This substrate was incubated for 30 min at room temperature with NDPK-B in the same buffer that had been used for the gel shift assays. Afterwards, the reaction was stopped by addition of 10% volume loading buffer containing 0.5% SDS in order to separate the bound protein from the oligonucleotide. The samples were loaded on native 12% polyacrylamide (19:1) mini-gels (1.5 mm $\times$ 8 cm $\times$ 10 cm) in the presence of 0.5 $\times$ TBE buffer.

RESULTS

NDPK-B binds preferentially to single-stranded poly-pyrimidine tracts

Performing EMSAs with recombinant NDPK-B and double-stranded oligonucleotides representing the *c-myc* NHE element, we observed that the retardation of the double-stranded probe was competed by an excess of single-stranded specific competitor (not shown). Subsequently, we tested the protein for its ability to bind single-stranded DNA. Surprisingly, under various conditions, we consistently observed a specific complex formation between the polypyrimidine tract of the NHE element, but never with the complementary purine strand. Moreover, this poly-pyrimidine specific interaction was also observed with a second promoter element found at position -12 to +18 in the initiator region of the

Adenovirus major late promoter (AML). As shown in Table 1, this element is also pyrimidine rich in its coding strand, but its sequence differs completely from the *c-myc* NHE element.

Figure 1a shows a typical experiment, where the radioactive poly-pyrimidine oligonucleotide corresponding to the *c-myc* NHE element was used as a probe. Complex formation could be abolished with excess of the unlabelled NHE and AML pyrimidine strands (lanes 3 and 4, and 7 and 8) as well as with excess of the complementary poly-purine oligonucleotide from the NHE element (lanes 5 and 6), but not by the AML purine strand (lanes 9 and 10). When the oligonucleotides corresponding to the AML element were used as radioactive probes, a specific binding could be demonstrated with the poly-pyrimidine tract (Fig. 1b) that again was competed by both its complementary poly-purine sequence (lane 4) and the NHE poly-pyrimidine sequence, but not the NHE poly-purine oligonucleotide (lane 6). Specific retardation of the radioactively labelled poly-purine probe was never observed. Therefore, the observed inhibition of complex formation in presence of excess non-labelled poly-purine competitors is not due to a direct interaction with the protein. Instead, an enhanced hybrid formation between the radioactive probe and complementary specific competitor affects the formation of the complex.

Binding of NDPK-B to other pyrimidine rich oligonucleotides including homopolymeric oligo-dT and oligo-dC was also tested (Table 1). All of these probes were recognized by NDPK-B in the presence of a 100-fold excess of single-stranded dIdC used as unspecific competitor. The strongest binding was observed with a 30mer single-stranded probe consisting of 15 CT dinucleotide repeats (CT)₁₅. Binding to this oligonucleotide was barely affected by a 50-fold excess of the poly-pyrimidine oligonucleotide corresponding to the NHE element (not shown). In contrast, attempts to obtain a retardation with complementary poly-purine sequences failed constantly. We conclude that binding of NDPK-B to its target DNA is dependent mainly on the poly-pyrimidine content of the DNA rather than its actual sequence.

In order to obtain information about the number of nucleotides involved in the interaction with the NDP kinase protein, (CT) dinucleotide repeats varying in length were tested in an EMSA (Fig. 1c). One retarded band was obtained with oligonucleotides between 18 and 30 residues, whereas an EMSA with a 36mer revealed two retardation bands indicating the presence of two DNA-protein complexes. We conclude that NDPK-B covers between 15 and 20 nt.

Comparison of double-stranded and single-stranded interaction

In order to compare directly the binding of single-stranded and double-stranded probes in the same experiment, an aliquot of the labelled poly-pyrimidine oligonucleotide corresponding to the AML element was hybridized with its complementary strand. Subsequently, both single-strand and hybrid were tested in an EMSA with NDPK-B. Figure 2a shows that the retardation signals obtained with the double-stranded DNA were always much weaker than the signals observed with the single-stranded probe over the complete range of protein concentrations tested.

In order to exclude that the retardation signals obtained with the double-stranded probes could result from a contamination of single-stranded DNA which was not completely hybridized during the preparation of double-stranded probes, we prepared a double-stranded hybrid and subsequently labelled it by a

Figure 2. Interaction of NDPK-B with double-stranded DNA. (a) Direct comparison of single-strand and double-strand binding. An aliquot of radioactively labelled single-stranded poly-pyrimidine oligonucleotide (ssY) corresponding to the AML element was hybridized to its complementary strand (dsY). Afterwards, identical amounts of radioactivity corresponding to ~1–2 ng oligonucleotide were subjected to an EMSA with the indicated amounts of NDPK-B. 100-fold excess of single-stranded dIdC was added to each sample as unspecific competitor. (b) Proof of ds recognition: 300 or 600 ng NDPK-B was tested for binding to the *c-myc*-NHE double-stranded oligonucleotides labelled by a Klenow Polymerase reaction (ds*). Competition was carried out with additional 40-fold excess of dIdC or 40-fold excess of unlabelled specific double-stranded hybrid.

radioactive Klenow-fill-in reaction. A specific retardation signal occurred (Fig. 2b), showing that NDPK-B can interact with both double-stranded and single-stranded DNA.

This result could be explained in two ways: either NDPK-B is able to form a complex with both single-stranded and double-stranded DNA which would imply a high affinity interaction with the poly-pyrimidine strand and a low affinity interaction with the poly-purine complement; or alternatively, NDP kinase could be able to separate the two strands because of its high affinity to the poly-pyrimidine tract. These two possibilities were investigated by a specific oligonucleotide melting assay. A short radioactive oligonucleotide was (i), hybridized under usual conditions to a longer, non-labelled oligonucleotide or (ii), hybridized to a plasmid as done in the typical annealing step prior to a sequencing reaction (see Materials and Methods). After incubation with NDPK-B, melting of the hybrids was analyzed by separation on a 12% native polyacrylamide gel. Indeed, in presence of molar excess of NDPK-B, a complete strand separation occurs (Fig. 3). Unlike the strand separation of the SV40 large T antigen (28) and others, this process does not require the addition of exogenous ATP.

Predominantly isoform B of human NDP kinase interacts with poly-pyrimidine rich DNA and RNA sequences

We tested other purified NDP kinases including recombinant human NDPK-A, *Drosophila* NDP kinase and recombinant *Dictyostelium* NDP kinase for single-stranded binding of poly-pyrimidine rich oligonucleotides. None of these proteins interacted

Figure 3. Single-strand generation in presence of NDPK-B. (a) Single-strand generation from oligonucleotide hybrids: a 34 mer oligonucleotide representing the AML element was hybridized to a radioactively labelled 18mer oligonucleotide. The partial hybrid was incubated with various amounts of NDPK-B and with or without ATP. The upper band (*) represents the partial 34mer/18mer hybrid; the lower band (>) represents the single-stranded 18mer. (b) Oligonucleotide liberation from a plasmid: ~1 ng T4-PNK labelled (CT)₁₈ oligonucleotide was annealed to a plasmid containing the same sequence in a typical ds-sequencing annealing reaction and subsequently tested in the single-strand generation assay. (–) without NDPK-B (+) in presence of 300 ng NDPK-B.

with single-stranded or double-stranded poly-pyrimidine rich DNA probes (not shown). When binding of NDPK-B and NDPK-A to single-stranded poly-pyrimidine DNA were compared, a retardation signal was detectable using as little as 5 ng of NDPK-B (Fig. 4a), whereas binding to NDPK-A was only observed with $\geq 4 \mu\text{g}$ protein (Fig. 4b).

Other proteins that previously have been described to interact with single-stranded poly-pyrimidine tracts, are known to interact also with poly-pyrimidine rich RNA. For example, PYBP, the major protein interacting with the pyrimidine rich element at the initiator region of the Adenovirus major late promoter (29), has been shown to be identical with PTB (pyrimidine tract binding protein), the major factor that binds to most poly-pyrimidine sequences present in the 3' half of introns in pre-mRNAs (30). Therefore, we tested various NDP kinases in an RNA-EMSA. As a target, we used an RNA *in vitro* transcribed from 3' end of intron 6b from the human β -tropomyosin gene (27). Figure 5 shows that NDPK-B interacted specifically with this pyrimidine rich 198 nt long RNA sequence. The binding was not competed by excess of d(CT)₁₈ deoxynucleotide, which indicates at least *in vitro*, a high affinity of NDPK-B towards RNA. Again, only isoform B of human NDP kinase showed a specific interaction.

DISCUSSION

In this study, we have shown that human NDPK-B binds not only to the NHE motif of the *c-myc* promoter but also to other single-stranded poly-pyrimidine tracts with completely different sequences. This includes the poly-pyrimidine rich element at the

Figure 4. ssDNA binding of human NDPK-A and NDPK-B. Different amounts of NDPK-A and NDPK-B were tested in an EMSA for their ability to bind to the ssPy oligonucleotide in the presence of 100-fold ssdIdC. (a) 20 and 5 ng NDPK-B. (b) 300 ng NDPK-B and 300 ng or 4 mg NDPK-A.

initiator region of the AML promoter as well as homopolymers of oligo-dC or oligo-dT and poly-pyrimidine rich RNA. When NDPK-B interacts with double-stranded targets containing a poly-pyrimidine tract in one strand, an ATP independent strand-separation is observed. Therefore, there is no intrinsic ATPase activity of NDP kinase involved and NDP kinase cannot be classified as a typical helicase protein (31). In contrast, the observed inhibition of strand separation by molar excess of ATP or other nucleotides might be due to an overlap between the DNA binding site and the nucleotide binding pocket.

A saturated retardation signal was never obtained in our experiments even if excess of recombinant enzyme was applied. Similar results were previously obtained by Postel *et al.* (1). A possible explanation would be, that NDPK-B is capable of binding DNA only in a yet unidentified, less abundant conformation, or that the recombinant, bacterially expressed NDP kinase is slightly differently folded or post-translationally modified as compared with the endogenous protein. Alternatively, high affinity binding of NDPK-B to the NHE element *in vivo* may require a second DNA binding factor as suggested previously (1).

Neither NDP kinase from *Drosophila* nor NDP kinase from *Dictyostelium* are able to bind single-stranded poly-pyrimidine DNA, although, like NDPK-B, they have very basic isoelectric points. It is interesting to note that in contrast to vertebrates, *Drosophila* and *Dictyostelium* possess only one gene encoding a cytoplasmatic NDP kinase. Thus, the DNA binding property of the human isoform-B might have appeared during evolution upon duplication of the gene and the subsequent generation of a second isoform. Although both human NDP kinases share 88% sequence homology, NDPK-A only binds weakly to the NHE element and other pyrimidine sequences and it is unlikely that it also might serve as a transcription factor. However, since hybrid hexamers are

Figure 5. Recognition of poly-pyrimidine rich RNA by NDP kinase. A poly-pyrimidine rich, radioactively labelled 198 nt RNA representing the 3' end of intron 6b from the human β -tropomyosin gene (27) was used as a probe for an RNA EMSA with recombinant *Dictyostelium* wild type and P105G mutant NDP kinase, *Drosophila* NDP kinase, and both human isoforms A and B. 10 ng, 100 ng and 1 μ g d(CT)₁₈ oligonucleotide were used for specific competition.

formed in the cell which contain various numbers of NDPK-A and NDPK-B subunits (5), amplifications or mutations of NDPK-A could influence the DNA binding of homohexameric NDPK-B through modulating its concentrations by a titration effect.

Recently, single-stranded DNA binding activity has been demonstrated for other metabolic enzymes involved in the synthesis of (deoxy-) nucleosides: adenylosuccinate synthase which is necessary for the *de novo* synthesis of adenylylate, was found to be one of three major proteins that bind to a dT rich sequence in the core consensus of the autonomously replicating sequence (ARS) from *Saccharomyces cerevisiae* (32). The multi-functional enzyme tetrahydrofolate synthase from *Schizosaccharomyces pombe* and *Clostridium acidurici*, which is involved in the *de novo* synthesis of thymidylate, has been shown to bind single-stranded DNA without any sequence specificity (33). In addition, other metabolic enzymes that bind nucleotides either as substrates or as cofactors have also the capability to recognize RNA (reviewed by Hentze, 34).

The *in vitro* stimulation of *c-myc* transcription by a catalytically inactive mutant of NDPK-B argues for a function in gene regulation unrelated to its enzymatic activity (35). There are two possibilities how NDP kinase might serve as a transcription factor: (i) in addition to its DNA binding site, the NDP kinase protein may have a transactivation domain which interacts with the transcription initiator complex or (ii) the protein does not possess a transactivation domain but rather changes the structure of its target DNA so that other typical transcription factors can interact with the RNA polymerase. Taken together, the unusual structure of the nuclease hypersensitive NHE element as the

cis-acting target and the capacity of NDPK-B to separate *in vitro* double-stranded oligonucleotides support the latter possibility.

The *c-myc* NHE promoter element is found at position -115 to -145 (25), whereas the AML element is located around the transcriptional initiation site (36). If binding of NDPK-B to the AML element also stimulates transcription as discussed previously (25), NDPK-B would be able to enhance transcription independently from the position of its corresponding *cis*-acting target. The potential of NDP kinase to bind poly-pyrimidine tracts from different promoters regardless of their sequence suggests a pleiotropic role in the regulation of gene expression. Because of its increased abundance in highly proliferative cells (16,20), NDP kinase might especially enhance the transcription of a number of genes that, like the *c-myc* oncogene, are induced during proliferation. Nevertheless, it remains to be understood how a protein with preferential single-stranded DNA binding activity and a broad range of target sequences might affect transcription from specific promoters. The definitive proof for a role of NDPK-B in transcriptional activation will await *in vivo* transfection studies, where vectors expressing NDPK-B confer transcriptional induction to *c-myc*- and AML-reporter constructs.

The possible role of NDPK-B as a RNA binding protein deserves discussion. It is interesting to note that two other RNA binding proteins, hnRNP A1 and hnRNP K, are activating transcription of the *c-myc* gene through binding to NHE promoter element (37). Thus, it is not exceptional that NDPK-B recognizes both DNA and RNA poly-pyrimidine tracts without a strict specificity. In addition, another single-stranded polypyrimidine DNA binding protein FBP is interacting specifically with the far upstream element (FUSE) of the same promoter (38).

The crystal structure of NDP kinase has revealed an α/β structure with a central motif of four antiparallel β -strands (3), that bears significant structural homology to the RRM motif found in many single-stranded RNA binding proteins (39). The crystal structure of the U1 small nuclear ribonucleoprotein A (40) and data from mutational analysis of other RNA binding proteins suggest an interaction of the nucleic acid with the β -sheet. The possibility that RNA binding proteins and NDPK-B share a common mode of nucleic acid recognition remains to be investigated.

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