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Human NM23/Nucleoside Diphosphate Kinase Regulates Gene Expression through DNA Binding to Nuclease-Hypersensitive Transcriptional Elements

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NM23-H2/NDP kinase B has been identified as a sequence-specific DNA-binding protein with affinity for a nuclease-hypersensitive element of the c-*MYC* gene promoter (Postel *et al.*, 1993). The ability of Nm23-H2 to activate c-*MYC* transcription *in vitro* and *in vivo* via the same element demonstrates the biological significance of this interaction. Mutational analyses have identified Arg34, Asn69 and Lys135 as critical for DNA binding, but not required for the NDP kinase reaction. However, the catalytically important His118 residue is dispensible for sequence-specific DNA binding, suggesting that sequence-specific DNA recognition and phosphoryl transfer are independent properties. Nm23-H2 also has an activity that cleaves DNA site-specifically, involving a covalent protein–DNA complex. In a DNA sequence-dependent manner, Nm23-H2 recognizes additional target genes for activation, including *myeloperoxidase*, *CD11b*, and *CCR5*, all involved in myeloid-specific differentiation. Moreover, both NM23-H1 and Nm23-H2 bind to nuclease hypersensitive elements in the platelet-derived growth factor *PDGF-A* gene promoter sequence-specifically, correlating with either positive or negative transcriptional regulation. These data support a model in which NM23/NDP kinase modulates gene expression through DNA binding and subsequent structural transactions.

KEY WORDS: NM23; PuF; transcription; c-*MYC*; nuclease-hypersensitive; *PDGF*; differentiation.

INTRODUCTION

The first human *NM23* genes, *NM23-H1* and *H2*, were identified on the basis of their involvement in the metastasis (Steeg *et al.*, 1988; Stahl *et al.*, 1991, and pathogenesis (Hailat *et al.*, 1991) of tumors. Soon after their gene products were recognized as nucleoside diphosphate (NDP) kinases A and B, respectively (Gilles *et al.*, 1991), it was questioned whether NDP kinases, given their characteristically broad substrate specificities, were sophisticated enough to have gene regulatory properties. There has been considerable debate and activity in this area, as discussed elsewhere in this issue. The discovery of DNA binding and transcriptional activity of NM23-H2/NDP kinase B (Postel *et al.*, 1993; Berberich and Postel, 1995), suggested a model in which the regulatory properties of this enzyme could be explained by its ability to recognize and alter structural elements of DNA. Recent findings in our laboratories further support this hypothesis (Postel, 1999; Rooney *et al.*, 1999; Liu *et al.*, 1999). The present review summarizes our current understanding of the DNA binding and transcriptional properties of NM23-H1 and H2, and suggests a mechanism to explain gene activation and repression by these enzymes.

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DNA BINDING AND TRANSCRIPTIONAL ACTIVATION OF THE c-*MYC* GENE

Sequence-Specific Binding to a Nuclease-Hypersensitive Element of the c-*myc* Promoter

The c-*myc* gene encodes an oncoprotein with a diverse array of properties, suggesting that its main function is in the regulation of transcription in normal and neoplastic cells (Prendergast, 1997; Facchini and Penn, 1998). As part of an effort to identify components necessary for the expression of the c-*MYC* gene, a library screen was performed for proteins that bound to a 104-basepair (bp) c-*MYC* promoter fragment containing the GGGTGGG PuF recognition sequence (Postel *et al.*, 1989, 1993). The 104-bp DNA fragment was obtained by PCR to include a sequence that lies between −160 to −101 base pairs from the P1 transcription initiation site of the c-*myc* gene. While multiple positive and negative *cis*-acting elements have been localized upstream of the c-*myc* gene, the −160 to −101 sequence was recognized as an important positively acting regulatory region (Postel *et al.*, 1989). The sequence is comprised of a homopurine/homopyrimidine stretch with the potential to form non-B-like DNA structures and intermolecular triplexes (Boles and Hogan, 1987; Cooney *et al.*, 1988; Postel *et al.*, 1991; Mirkin and Frank-Kamenitskii, 1994). Because of the presence of a major DNase I (site III_1; Battey *et al.*, 1983) and several S1 nuclease-hypersensitive sites (Boles and Hogan, 1987), this Pur/Pyr sequence was termed the *n*uclease-*h*ypersensitive *e*lement, or NHE (Postel *et al.*, 1989). Although Pur/Pyr sequences appear frequently at functionally important sites in chromatin, including origins of DNA replication, recombination, and transcription, neither their structure *in vivo* nor their function in these processes is clearly understood.

The recombinant PuF protein was identified as Nm23-H2/NDP kinase B (Postel *et al.*, 1993) and its DNA-binding properties were investigated by electrophoretic mobility-shift assays (EMSAs). Both the DNA-binding affinities and specificities appeared moderate when measured in the presence of excess, nonspecific DNA. The apparent dissociation constant (K_d), determined from half-saturation points in EMSAs, was in the order of $\sim 10^{-6}$ *M* (Postel *et al.*, 1996). The affinity of binding also depended on the length of the substrate DNA. For example, a 104-bp DNA fragment bound NM23/PuF better than a 50-bp or a 34-bp duplex oligonucleotide; the 27-bp oligonucleotide used in earlier studies (Cooney *et al.*, 1988; Postel *et al.*, 1989), appeared, by comparison, to bind least well. This dependence of DNA binding on the length of fragment suggests that NM23 recognizes the DNA conformation assumed by the Pur/Pyr sequence.

The DNA-binding specificity of NM23-H2/PuF was tested using a series of oligonucleotides with internal deletions of different lengths. Under similar conditions, NM23-H2 did not bind to DNA with an unrelated sequence, or to DNA in which a substantial portion of the NHE was deleted (Postel *et al.*, 1993). Work is in progress to determine the relative importance of directly repeated elements in the NHE that surround and overlap the PuF-binding sites (Postel, 1999). The endogenous HeLa cell NM23/PuF had the same DNA-binding specificity as the recombinant protein, but it bound DNA more tightly, implying a requirement for modification and/or protein-binding partners for optimal interaction with DNA.

Under our standard EMSA conditions, which are near physiological in terms of pH and salt, the stoichiometry of Nm23-H2/NDP kinase B binding to single-stranded Py-rich (coding), Pu-rich (noncoding), and duplex NHE oligonucleotide substrates is rather similar (Postel *et al.*, unpublished results). The binding affinities of NM23-H2 to the *PDGF-A* silencer oligonucleotide sequences were also within the same order of magnitude (Liu *et al.*, 1999 and below). These results are at variance with Hildebrandt *et al.* (1995), who observed specific complex formation with double-stranded and with Pyr-rich single-stranded NHE oligonucleotides, but not with Pur-rich single-stranded oligonucleotides. The sources of these discrepancies may lie plausibly in the conditions of protein purification and other factors in the preparations, as well as on the length and stabilities of the different oligonucleotide substrates.

Like all sequence-specific DNA-binding proteins, NM23-H2/NDP kinase also binds to DNA sequence nonspecifically (Postel *et al.*, 1993) and particularly when the substrate DNA is single-stranded (Agou *et al.*, 1999). Contrary to suggestions in the literature that nonspecific DNA binding by NM23-H2 is incompatible with it being a transcription factor (Michelotti *et al.*, 1997), sequence-specific and sequence-nonspecific DNA-binding interactions are not mutually exclusive properties of DNA-binding proteins. Indeed, all sequence-specific DNA-binding proteins bind DNA nonspecifically first and as they switch and compete between nonspecific (largely electrosatic) and specific (hydrogen bond-based) binding modes in their path-

way toward specific complex formation (von Hippel, 1994). The extent to which one or the other binding mode predominates in an *in vitro* experiment is dictated by the conditions.

Transcriptional Activation of the c-*MYC* gene by NM23-H2/PuF *in vitro* and *in vivo*

In vitro reconstitution assays with recombinant NM23/PuF supported accurate transcription from the c-*MYC* P1 and P2 promoters, and, as in the DNA-binding assays, the recombinant protein was less active. Addition of a PuF-depleted fraction, which alone was unable to perform *in vitro* transcription, caused a large increase in Nm23/PuF transcriptional activity (Postel *et al.*, 1993). Given the highly regulated expression levels of *NM23* in the cell, the requirement for binding partners and modifiers is not surprising, and several laboratories are presently searching for such factors.

The studies on *in vitro* transcription were extended to determine whether NM23 also stimulated c-*MYC* transcription in transient cell transfection experiments that serve as model systems for *in vivo* transcriptional effects. Consequently, the NHE element was cloned into a plasmid upstream of the bacterial *CAT* gene and was then cotransfected into mouse fibroblast cells with a *NM23/PuF*-containing vector. Under these conditions, CAT activity was elevated 3- to 4-fold, relative to control plasmids without the NHE. Although the level of promoter activation was modest, these experiments confirmed the *in vitro* results, namely, that the NM23/PuF protein is capable of functioning as a transcription factor and that the NHE is necessary for the activation of the c-*MYC* gene by NM23-H2 (Berberich and Postel, 1995).

NM23/PuF also binds to the c-*MYC* promoter in Burkitt lymphoma cells *in vivo*, where it activates the translocated c-*MYC* allele via the NHE element (Arcinas and Boxer, 1994). In Burkitt lymphoma cells, one of the c-*MYC* genes is translocated through juxtaposition to immunoglobulin loci so that the translocated c-*MYC* gene is expressed at high levels, whereas the normal c-*MYC* allele remains silent. *In vivo* footprinting experiments by Ji *et al.* (1995) identified a footprint over the PuF-binding site in the NHE of the translocated c-*MYC* allele in these cells. In a series of electrophoretic experiments and in conjunction with UV-crosslinking and antibody analysis, they also demonstrated that the protein that binds to the NHE PuF site *in vivo* is NM23-H2/PuF. Their transfection experiments showed that the NHE PuF site functions as a positively acting regulatory element in the DNA of Burkitt lymphoma cells. Overall, the findings of Ji *et al.* (1995) confirmed our observations that DNA binding by NM23-H2/PuF to the PuF site of the c-*MYC* NHE element is relevant to its *in vivo* transcription. Negative results on transactivation of c-*MYC* by NM23/NDP kinase (Michelotti *et al.*, 1997), may be explained on the basis that these authors used fusion proteins in their cell transfection experiments. Such fusion proteins may not be able to fold properly into subunits or assemble into native hexamers—the biologically active conformation of NM23/NDP kinases. Indeed, our mutational analyses of DNA binding by NM23-H2 suggest an unusual DNA-binding surface comprised of at least two subunits of the hexamer (Postel *et al.*, 1996). In addition, NM23 may not directly interact with RNA polymerase or with coactivator complexes to stimulate transcription. Rather, the mechanism of c-*MYC* regulation by NM23 is suggested to entail recognition and structural modification of promoter DNA in order to facilitate binding of the transcriptional machine (see model in Appendix below).

MUTATIONAL ANALYSIS OF NM23-H2 NDP KINASE AND DNA-BINDING FUNCTIONS

The Role of the Catalytic His118 Residue in DNA Binding and Transcription

To understand the relationship beween DNA binding and the NDP kinase activities of NM23/PuF, the catalytically relevant and phylogenetically conserved His118 was replaced with phenylalanine by site-directed mutagenesis. As expected, the H118F mutant protein was inactive as an NDP kinase but bound to DNA normally, indicating that the His118 residue is not necessary for sequence-specific *in vitro* DNA-binding activity. Moreover, in a minimally reconstituted transcription assay in the presence of only recombinant NM23/PuF and a chromatographic fraction containing RNA polymerase, the H118F mutant was as active as the wild-type protein in stimulating *in vitro* transcription (Postel and Ferrone, 1994). It should be emphasized, however, that under these conditions only a basal level of transcriptional stimulation is detected, as fully activated transcription requires activator proteins present in additional chromato-

graphic fractions (Postel *et al.*, 1993), which were not included in this test. Nonetheless, the data presently on hand suggest that a functional His118 residue and the sequence specific recognition of DNA by NM23 are independent properties.

Identification of Amino Acids Critical for DNA Binding by NM23-H2

To identify residues and domains necessary for DNA binding by NM23-H2, additional site-directed mutagenesis was performed. The rationale for the design of mutations was based on the known three-dimensional crystal structure (Morera *et al.*, 1995; Webb *et al.*, 1995), on potential transcription factor motifs, and on the combined functional and immunological attributes of the protein. A most important consideration was the 12% sequence diversity between NM23-H1 and H2, for, despite this small difference, N23-H1 binds poorly or not at all to the c-*MYC* element as compared with NM23-H2 (Hildebrandt *et al.*, 1995; Postel *et al.*, 1996). The results of these mutational studies suggested that amino acid residues Arg34, Asn69, and Lys135 are critical for sequence-specific DNA binding (Postel *et al.*, 1996). Their location on the crystal structure implies a combinatorial DNA-binding surface consisting of a region around the two-fold axis of the hexamer (Postel *et al.*, 1996; Fig. 1). These mutants also confirmed our earlier findings with the H118F mutant that the DNA-binding surface on NM23-H2 is distinct from the phosphotransferase active site (Postel and Ferrone, 1994) and, indeed, all three mutant proteins retained normal NDP kinase activity.

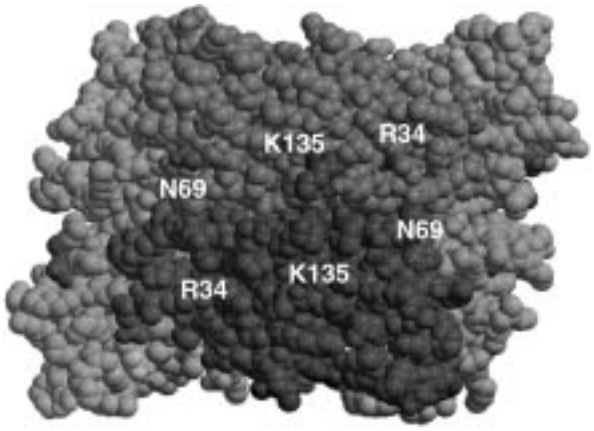

Fig. 1. Space-filling model of the NM23-H2 hexamer showing residues Asn69, Lys135, and Arg34 implicated in DNA-binding (red) and GDP bound to the NDP kinase active site (yellow) (after Postel *et al.*, 1966).

It is important to note that two of the three DNA-binding residues of NM23-H2, Asn69, and Lys135 are among the few that are not conserved between NM23-H1 and NM23-H2. This finding confirmed our hypothesis that the poor DNA binding of NM23-H1 to the c-*MYC* NHE is a question of sequence specificity (Postel *et al.*, 1996). This hypothesis is supported by two additional observations. First, NM23-H1 prefers the *PDGF-A* promoter sequence, whereas NM23-H2 binds to both c-*MYC* and the *PDGF* sequences (Liu *et al.*, 1999 and below). Second, when the NM23-H1 residues His69 and His135 are mutated to the NM23-H2 specific DNA-binding residues Asn69 and Lys135, the doubly mutated NM23-H1 protein now binds to the c-*MYC* sequence (Postel *et al.*, unpublished data). Thus, only these two mutations are required to convert the DNA-binding specificity of NM23-H1 to that of NM23-H2 with regard to the c-*MYC* sequence. These findings, together with the discovery of sequence-specific DNA binding by NM23-H1 to the *PDGF* promoter (Liu *et al.*, 1999; see below), argue against canonical DNA-binding residues in the NM23/NDP kinase family. It is very likely that other members of this protein family will also bind DNA but with different amino acid and nucleotide sequence specificities.

DNA BINDING BY NM23/NDP KINASE INDUCES REVERSIBLE CLEAVAGE OF DNA

NM23-H2 Cleaves Linear Duplex and Supercoiled Plasmid DNAs

In the course of the DNA-binding studies, it was noted that NM23-H2 has an activity that cleaves the bound DNA and produces double-stranded breaks. The cleavage reaction has no cofactor requirement, other than Mg^{2+} ions, is DNA-sequence dependent, and requires DNA binding by the protein. It was shown that NM23-H2 cleaves the c-*myc* NHE promoter element within the directly repeated 5′-CCTCCCCA motifs. The enzyme also cleaves negatively supercoiled plasmid DNA containing the NHE element, yielding linear, unit-length fragments and nicked circular products. Other experiments established that the cleaved DNA ends are staggered with single-stranded 3′-termini approximately 5 bp apart and that NM23 protein is found attached to the cleaved DNA strands at the 5′-phosphoryl end.

Upon further examining the role of metal ions, KCl was found inhibitory. Expectedly, when EDTA was included in the reaction, the cleavage of DNA was virtually abolished; however, when EDTA was added at the completion of the cleavage reaction, which was then followed by Proteinase K treatment, the DNA breaks were restored almost completely. Since a reversible DNA cleavage reaction requires breakage and religation of DNA strands through a covalent enzyme-DNA complex, these results suggested that DNA cleavage by NM23 was mediated by such a rare chemical reaction (Postel, 1999).

DNA Cleavage Proceeds through a Covalent Protein–DNA Complex

To determine if NM23-H2 indeed becomes covalently attached to DNA during the cleavage reaction, the enzyme was reacted with uniformly ^{32}P-labeled plasmid DNA, denatured, the bound DNA hydrolyzed, and the products analyzed in denaturing SDS-PAGE electrophoresis. It was clear from the results that the linkage between NM23-H2 and radiolabeled DNA is covalent, because heat and detergent-stable transfer of radioactivity from ^{32}P-radiolabeled DNA to NM23-H2 has occurred. Western blot analysis confirmed that the polypeptide in the purified protein–DNA complex was NM23-H2. Since covalent protein–DNA intermediates are common energy sources for strand breakage and reunion by DNA transaction enzymes, the ability of NM23 to form such bonds strongly suggests that NM23 is catalyzing DNA structural transactions. Experiments are under way to identify the nucleotide and the amino acid residues in the complex and to determine the chemical nature of their interaction. Whether the DNA cleaving activity and the covalent complex formation are pertinent to the biological function of NM23 is also under investigation. Other interesting questions are whether the NDP kinase and the DNA-cleaving activities of NM23 are related to each other *in vivo* or whether the enzyme performs two different functions in DNA metabolism.

TRANSCRIPTIONAL REGULATION OF ADDITIONAL GENES BY NM23/NDP KINASE

Activation of Genes During Myeloid Differentiation

In order to identify factors that regulate myeloid differentiation and function, we set out to isolate genes expressed early during macrophage differentiation (Rooney *et al.*, 1999). Macrophages play a critical role in development, physiology, innate and adaptive immunity, and in pathogenesis of many infectious, immunologic, and degenerative disease processes. They possess many specialized cellular functions, such as phagocytosis, chemotaxis, antibody-dependent cell cytotoxicity, antigen presentation, and specific expression of a repertoire of cytokines, chemokines, and cell surface markers (Gordon, 1995). Two established myelocytic cell lines, HL60 and U937, were chosen as model systems, which, when differentiated with a variety of agents, make the study of these diverse functions tractable. A representational difference analysis (RDA) was performed very early in the differentiation program for macrophages in order that the genes isolated by RDA would be causal and not merely correlative. In the course of these studies, one of the genes isolated was *NM23-H2/PuF*. Northern analyses using the HL-60 cell line indicated that *NM23-H2/PuF* levels are rapidly induced from undetectable levels within 30 min of phorbol ester treatment and are sustained throughout the differentiation program (Rooney *et al.*, 1999).

In order to identify downstream targets of *NM23-H2/PuF*, promoter regions of myeloid-specific genes were screened for homology to the known *NM23-H2/PuF* binding site, GGGTGGG (Postel *et al.*, 1989). The promoters of *myeloperoxidase* (the enzyme that generates peroxide free radicals; Austin *et al.*, 1995), *CD11b* (the receptor, along with CD18, for ICAM-1; Shelley and Arnaout, 1991; Hickstein *et al.*, 1992), and *CCR5* (the M-tropic chemokine receptor present in macrophages and activated T cells; Moriuchi *et al.*, 1997), were found to have perfect *NM23-H2/PuF* binding sites (Fig. 2). Luciferase reporter constructs were made containing regions of the *myeloperoxidase* promoter (−600 to + 25), the *CD11b* promoter (−345 to +40) and the *CCR5* promoter (−550 to +68), in the pGL2 luciferase construct. In transient transfection experiments in U937 cells, using these reporter constructs, phorbol ester treatment induced reporter activity eight-, six-, and twelvefold, respectively. Interestingly, coexpression of *NM23-H2/PuF* was

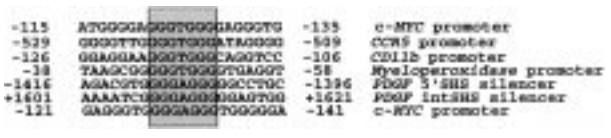

Fig. 2. Sequence alignments of NM23 interacting promoter elements. Bases were aligned with the Nm23-H2/PuF binding site GGGTGGG of the c-*MYC* promoter (Postel *et al.*, 1989).

found to augment this PMA induction of the *myeloperoxidase*, *CD11b*, and *CCR5* promoters six-, three-, and sixfold, respectively. To determine if this augmentation was dependent upon the *NM23-H2/PuF* binding sites (Fig. 2), these sites were mutated by base substitutions. In each of the mutated promoters, a complete loss of *NM23-H2/PuF* augmentation was seen. These results strongly implicate *NM23-H2/PuF* as an important determinant in myeloid differentiation (Rooney *et al.*, 1999). NM23/NDP kinases have previously been linked to differentiation in many organisms (Postel, 1998 and elsewhere in this issue).

Nm23-H1 and H2 Interact with Silencer and Promoter Elements in the *PDGF-A* Gene

Nuclease-Hypersensitive Transcriptional Elements Contribute to Both Repressive and Stimulatory Control of PDGF-A Gene Transcription

Overexpression of the genes encoding the A and B subunits of platelet-derived growth factor (*PDGF*) has been linked to both oncogenesis and tumor progression (Silver, 1992). An interesting aspect of the *PDGF-A* promoter is a preponderance of transcriptional elements exhibiting hypersensitivity to the nucleases S1 and DNase I, suggesting unusual non-B DNA conformations. The proximal promoter region (−100 to −40), which is required for basal transcription (Kaetzel *et al.*, 1994), is nuclease-hypersensitive over most of its length and, like similar regions in the c-*MYC* promoter, consists of polypurine/polypyrimidine tracts. In addition, two silencer elements have been located in the *PDGF-A* gene by virtue of their hypersensitivity to S1 nuclease that are also purine(Pu)- and pyrimidine(Py)-rich: one of these is in the 5′-flanking region (−1418 to −1388) referred to as 5′-*SHS* (Liu *et al.*, 1996) and the other in the the first intron (+1605 to +1630; Wang *et al.*, 1994) is termed *intSHS* (Fig. 2).

NM23-H1 and NM23-H2 Bind to the 5′-SHS Silencer with High Affinity

On the assumption that S1 nuclease-hypersensitivity detects single-stranded components in DNA (Boles and Hogan, 1987; Ulyanov *et al.*, 1994), single-stranded DNA probes were used to identify DNA binding by nuclear proteins. Since Py-rich strands of the silencers were bound with high affinity by a profile of nuclear proteins, a HeLa expression library was screened using a ^{32}P-labeled, single-stranded oligodeoxynucleotide probe derived from one such strand. Of three cDNA clones obtained, all three encoded the *NM23-H1* isoform (Liu *et al.*, 1999).

As expected, recombinant NM23-H1 bound the 5′-*SHS-Py* sequence avidly, as assessed by the ability of very low concentrations (IC_{50} of approximately 5 n*M*) of unlabeled 5′-*SHS-Py* to compete for radioactive complex formation. Binding was also nucleotide sequence-specific, as an unrelated sequence did not bind or compete. NM23-H1 also bound to the coding, Pur-rich strand (5′-*SHS-Pu*) with similar affinity, but bound less well to double-stranded 5′-*SHS*. Very similar binding profiles were observed with the NM23-H2 protein. Thus, the DNA binding specificities for the *PDGF-A* gene of NM23-H1 and NM23-H2 differ from those of the c-*MYC* gene (above). Preliminary studies also indicate that both NM23-H1 and NM23-H2 can bind to a DNA sequence derived from the nuclease-hypersensitive basal promoter element, a positive regulatory region (Fig. 2).

Both NM23-H1 and NM23-H2 contain a highly potent endonucleaselike activity with respect to the 5′-*SHS* sequences, similar to that observed for the c-*MYC* NHE substrates (Postel, 1999 and above). A comparison of cleavage sites within the 5′-*SHS* element with those seen in the c-*MYC* sequence will shed light on the structural characteristics of the cleavage substrates of this enzyme.

Nm23-H1 and H2 Are Functionally Involved in the Regulation of PDGF-A Transcription

Overexpression of Nm123-H1 or H2 repressed transcription driven by a *PDGF-A* proximal promoter fragment (−261 to +8), up to threefold. The repressive activity of Nm23-H1 and NM23-H2 is promoter-specific, as it is not seen with a control vector containing the cytomegalovirus LTR-promoter/enhancer element. The most likely target of this overexpression is the nuclease-hypersensitive region between −80 and −40, which harbors almost all of the basal transcription activity of the *PDGF-A* promoter. This region contains binding sites for several transcription factors, as well as a GGGGAGGGGG motif, which is central to the silencer element *intSHS* and 5′-*SHS* and in the c-*MYC* NHE (Fig. 2).

Overexpression of NM23-H1 and NM23-H2 also augments silencer activity of the upstream region of the *PDGF-A* promoter, which contains the 5′-*SHS* element. Substitution of an arginine residue at position 34 of the NM23-H2 molecule with alanine (R34A), a mutation which disrupted DNA-binding to c-*MYC* (Postel *et al.*, 1996), resulted in a complete loss of ability both to augment the upstream silencer and to repress the activity of the basal promoter. Taken together, the binding of NM23 to both positive and negative regulatory elements in the *PDGF-A* promoter has functional implications in transcriptional regulation.

NDP Kinase Is a Transcriptional Regulator in Other Eukaryotes

Zimmerman *et al.* (1999) identified a novel NDP kinase in *Arabidopsis*, NDP kinase Ia, that may be a potential transcriptional regulator of the UV-B light-mediated response in plants. NDP kinase Ia binds to double-stranded DNA comprised of the *HIS4* promoter of yeast sequence-specifically and induces *HIS4* transcription *in vitro*. Thus, NDP kinase Ia of *Arabidopsis* may be a functional homolog of the yeast *Gcn4* transcription factor. The residues crucial to the binding of human NM23-H2 NDP kinase B are conserved in *Arabidopsis* NDP kinase Ia. Interestingly, human NM23-H2/NDP kinase B also binds to double-stranded yeast *HIS4* promoter DNA sequence-specifically (Zimmerman *et al.*, 1999).

CONCLUSION

The evidence of transcriptional activity of NM23/NDP kinase with respect to several different genes appears very strong. It includes activation of the c-*MYC* gene *in vitro* (Postel *et al.*, 1993) and *in vivo*, in the context of two different cell systems (Berberich and Postel, 1995; Ji *et al.*, 1995). In addition, three different myeloid specific genes are known to be activated by NM23-H2, including *myeloperoxidase*, *CD11b*, and *CCR5* (Rooney *et al.*, 1999). Moreover, transcription of the *PDGF-A* gene can be both augmented and repressed through different elements in the promoter (Liu *et al.*, 1999). There is also evidence that NM23 regulates its own expression (N. Kimura, personal communication). The mechanism of gene regulation by NM23 was previously proposed to entail binding to DNA directly in order to modify its structure (Postel *et al.*, 1993; Postel, 1999). An updated version of this model is presented in the Appendix below.

APPENDIX: A MODEL FOR THE TRANSCRIPTIONAL ACTIVITY OF Nm23/NDP KINASE

Polypurine/polypyrimidine sequences such as those found in the promoters of c-*MYC*, *CCR5*, and *PDGF-A* genes appear frequently at functionally important sites in genomic DNA and are often associated with unusual (distorted, non-B) DNA structures (Wells, 1988). On the basis of the *in vivo* and *in vitro* evidence summarized in this paper, we suggest that the role of NM23 in transcription is the recognition and modification of such non-B like elements resulting in altered promoter activity. Distortion in a Pur/Pyr sequence could occur when two complementary DNA strands are shifted relative to each other, producing loops or flipped out structures, one in each of the complementary strands ("Gene Off" in the diagram in Fig. 3). This kind of a "slippage structure" is believed to form in DNA regions containing direct repeats of a Pur/Pyr sequence (Soyfer and Potaman, 1996), of which there are four to five in the c-*MYC* NHE sequence (Postel *et al.*, 1989; Postel, 1999). In addition, as a result of base pairing, the DNA loops themselves have the potential to interact with each other and form higher-order structures (Ulyanov *et al.*, 1994). Such tertiary structures are likely to be under superhelical stress.

Our data indicate that NM23-H1 and NM23-H2 have the ability to both stimulate and repress transcription through interactions with positive and negative transcriptional elements. Activation by NM23 in the present model involves removal of distorted and potentially repressive DNA structures, by mechanisms that may entail unwinding locally supertwisted DNA, or

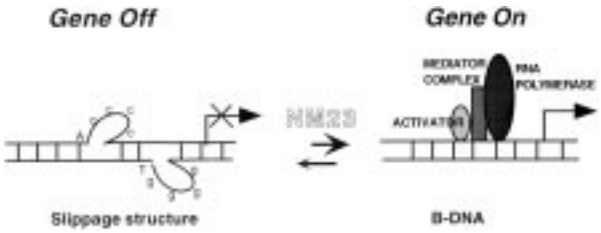

Fig. 3. Model for the activation and repression of gene transcription by NM23. Refer to text for additional details.

by excision of mismatched bases in the sequence, or, by a cut-and-paste recombinational activity. Removal of the inhibitory sequence would allow the transcriptional machinery, including RNA polymerase and activator complexes, access to promoter DNA that is now in a more typical B conformation ("Gene On," Fig. 3). A negative effect, such as the silencing of the *PDGF-A* gene, may originate from the reversal of this type of a mechanism, e.g., supercoiling or other changes that would close up promoter DNA and result in transrepression. Alternatively, activation and repression may be the consequences of similar mechanisms at the DNA structural level, but with the direction of promoter activity determined by the DNA sequence of the protein binding sites as well as the sequence of the flanking regions (Fig. 2), and by the overall promoter context. *In vivo*, the transcriptional properties of NM23 are also likely to be influenced by the relative abundance of transcriptional cofactors and their effect on the control regions of DNA, as well as by the presence of positive and negative modulators of NM23 in varying cellular contexts.

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