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Nuclear Myosin I is Necessary for the Formation of the First Phosphodiester Bond During Transcription Initiation by RNA Polymerase II

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Abstract The nuclear isoform of myosin, Nuclear Myosin I (NMI) is involved in transcription by RNA polymerase I. Previous experiments showing that antibodies to NMI inhibit transcription by RNA polymerase II using HeLa cell nuclear extract (NE) suggested that NMI might be a general transcription factor for RNA polymerases. In this study we used a minimal in vitro transcription system to investigate the involvement of NMI in transcription by RNA polymerase II in detail. We demonstrate that NMI co-purifies with RNA polymerase II and that NMI is necessary for basal transcription by RNA polymerase II because antibodies to NMI inhibit transcription while adding NMI stimulates transcription. Further investigation revealed that NMI is specifically involved in transcription initiation. Finally, by employing an abortive transcription initiation assay, we demonstrate that NMI is crucial for the formation of the first phosphodiester bond during transcription initiation. J. Cell. Biochem. 99: 1001–1009, 2006.

Key words: Nuclear Myosin I (NMI); RNA polymerase II; Transcription Initiation

The presence of an isoform of myosin I in the nucleus has been demonstrated [Nowak et al., 1997]. Nuclear myosin I (NMI), the specific myosin found in the nucleus, is an isoform of myosin IC, a sub-family of unconventional myosins [Pestic-Dragovich et al., 2000; Gillespie et al., 2001]. NMI contains a unique NH_2-terminal extension that appears to be important for the translocation to or retention of NMI in the nucleus [Pestic-Dragovich et al., 2000]. Immunoelectron microscopy [Nowak et al., 1997] and indirect immunofluorescence microscopy [Fomproix and Percipalle, 2004] demonstrated the presence of NMI in nucleolar structures that contain RNA polymerase I (Pol I). The physical association of NMI with rRNA genes and Pol I was revealed by co-immunoprecipitation and Chromatin immunoprecipitation (ChIP) assays [Fomproix and Percipalle, 2004; Philimonenko et al., 2004; Percipalle et al., 2006]. In addition, recent studies demonstrated an important role for NMI in transcription by Pol I [Philimonenko et al., 2004; Percipalle et al., 2006].

NMI also plays an important role in transcription by RNA polymerase II. It was shown that NMI co-localizes and co-immunoprecipitates with RNA polymerase II and antibodies to the NH_2-terminal extension inhibit transcription by RNA polymerase II in an in vitro assay using HeLa NE [Pestic-Dragovich et al., 2000]. The notion of NMI being a general transcription factor is also supported by a recent study by Kysela et al. [2005] where it was shown that NMI upon activation of human lymphocytes localizes to transcriptionally actives sites.

In this study we performed a detailed analysis of the role of NMI in transcription by RNA polymerase II. We found that NMI co-purifies

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with RNA polymerase II. Moreover antibodies to NMI inhibit transcription in vivo as well as in a minimal in vitro transcription system consisting of recombinant TATA-binding protein (TBP), TFIIF, TFIIB and purified RNA polymerase II. Other experiments showed that adding purified NMI stimulated transcription in this assay. Further investigations of the different stages of transcription showed that NMI is required for transcription initiation and specifically for the formation of the first phosphodiester bond during transcription initiation.

MATERIALS AND METHODS

Used Antibodies and Proteins

The anti-NMI antibody is a rabbit antibody to the NMI specific 16 amino acid N-terminal peptide. The antibody was purified on a affinity column made by coupling the peptide to Sepharose 4B as described [Pestic-Dragovich et al., 2000]. The anti-myosin II antibody is a rabbit antibody that recognizes specifically smooth muscle myosin II [de Lanerolle et al., 1982]. Tracheal muscle myosin II was purified and an antibody to this myosin was produced as described in de Lanerolle and Stull [1980]. To affinity purify the antibody, we first made an affinity column. Briefly, purified tracheal muscle myosin II was dialyzed in 8M ultrapure urea, 5 mM $MgCl_2$, 2.5 mM dithiothreitol (DTT), 100 mM NaCl, 20 mM Mops, pH 7.0 overnight at 4°C. The dialysate was applied to a DEAE-Sepharose column equilibrated in the same buffer, washed with the same buffer and column fractions were assayed using the Bradford protein assay. Flow through fractions that contained protein were pooled and dialyzed in 10 mM NaCl, 2.5 mM DTT, 20 mM Mops, pH 7.0 overnight at 4°C to precipitate the myosin II heavy chain. The dialysate was then spun at 50,000*g* for 10 min. The supernatant was discarded and the pellet was resuspended and dialyzed in 500 mM NaCl, 2.5 mM DTT, 20 mM Mops, pH 7.0. SDS–PAGE followed by Coomassie blue staining showed that this fraction was >98% myosin II heavy chain. The purified heavy chain was coupled to Sepharose beads using standard techniques. Serum from rabbits immunized with the myosin II protein was then applied to the column and the column was washed extensively in 150 mM NaCl, 20 mM Tris-HCl, pH 7.5. Bound antibody was eluted in 8M urea, 150 mM NaCl, 20 mM Tris-HCl, pH 7.5. Fractions containing protein were pooled, concentrated in sucrose and dialyzed in 150 mM NaCl, 20 mM Tris-HCl, pH 7.5. The affinity purified antibody was stored frozen at −80°C. Western blot analyses have shown that this antibody is highly specific for smooth muscle myosin II.

The monoclonal antibody to β-actin (Sigma, St. Louis, MO) is an anti-peptide antibody that recognizes only the β-actin isoform. The antibody was purified on Protein A-Sepharose prior to use. The 8WG16 monoclonal antibody to the C-terminal domain of RNA polymerase II was obtained from BAbCO (Richmont, CA). The rabbit antibodies, specific to the RAP 30 subunit of TFIIF, to TFIID (TBP) and to TFIIB, were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The peroxidase conjugated anti-mouse or anti-rabbit antibodies were acquired from Vector Laboratories (Burlingame, CA) or from Research Diagnostics, Inc. (Flanders, NJ), respectively.

RNA polymerase II was purified from isolated HeLa cell nuclei [Dignam et al., 1983] as described [Reinberg and Roeder, 1987] using ammonium sulfate precipitation and sequential DEAE cellulose, DEAE-Sephadex A-25 and phosphocellulose column chromatography. At the last step the transcriptionally active fractions were pooled and used for the transcription assays.

NMI was immunopurified from COS cells overexpressing C-terminally Flag-tagged NMI [Pestic-Dragovich et al., 2000]. Nuclei were isolated as described [Dignam et al., 1983], Flag-NMI was bound to Anti-Flag® M2 Affinity Gel and eluted with Flag peptide (Sigma, St. Louis, MA) following the manufacturers protocol.

In Vitro Transcription Assays

In vitro transcription assays using purified transcription factors were carried out as described [Kugel and Goodrich, 2000, 2003; Hofmann et al., 2004]. Briefly, transcription reactions contained 5 ng of TATA binding protein (TBP), 10 ng TFIIB, 6 ng TFIIF, 70 ng of RNA polymerase II, 1 nM DNA template. The DNA template in all experiments was negatively supercoiled plasmid DNA, containing the adenovirus major late promoter (AdMLP), fused to a 380 base pair G-less cassette [Kugel and Goodrich, 2000].

Purified transcription factors with purified RNA polymerase II and template DNA were incubated with 2 μg (or as indicated in the respective figures) of the anti-NMI or the anti-

myosin II antibodies for 30 min on ice. Nucleotides were then added to initiate transcription. For transcription of full length RNA the nucleotides were 625 μM UTP, 625 μM α-^{32}P-CTP (5 μCi per reaction), 625 μM ATP. For transcription of the 15 nt RNA the nucleotides were 625 μM UTP, 625 μM [α-^{32}P] CTP (5 μCi per reaction), 625 μM ApC. For abortive transcription initiation nucleotides were 625 μM ATP and 625 μM [α-^{32}P] CTP (5 μCi per reaction). The total volume per transcription assay was 25 μl. Following incubation for 20 min, the transcription products were extracted and separated by 6% (to monitor 390 nt RNA), 18% (to monitor 15 nt RNA) or by 20% (to monitor 2 nt RNA) polyacrylamid, 7M urea denaturing gel electrophoresis and visualized using a PhosphorImager (Molecular Dynamics). To identify the 15 nt RNA product the Decade^{TM} Marker system (Ambion) was used. To identify the 2 nt RNA the dinucleotide ApC (Sigma) was loaded as a size marker and visualized by UV shadowing.

In all experiments, the densities of the bands representing the transcription products were quantified using ImageQuant software (Molecular Dynamics) and expressed as a fraction of the band in the control lane. The mean fractional activity is shown at the bottom of each lane of the relevant figures.

Pre-Initiation Complex Formation

Pre-initiation complex (PIC) formation assays were carried out as described previously [Hofmann et al., 2004] with minor modifications. Briefly, PCR was used to create a linear, 5′ biotinylated DNA template that contained the AdMLP. In addition, this template contains a recognition site for the HindIII restriction enzyme between the Biotin-tag and the beginning of the promoter. The DNA template was bound to streptavidin-magnetic beads (Promega, Madison, WI) and washed in Transcription Buffer (TB) (10 mM Hepes, pH 7.6, 100 mM K-Glutamat, 2.5 mM $MgCl_2$, 3.5% Glycerol). PICs were assembled on the immobilized DNA template by incubating with HeLa NE in TB for 30 min at 30°C. HeLa NE were incubated with either buffer, 4 μg myosin II antibody, or 4 μg NMI antibody for 30 min on ice prior to incubation with the DNA template. The beads were washed with 50 volumes of ice-cold TB containing 0.025% sarcosyl. The DNA template was then removed from the beads by cutting with the HindIII restriction enzyme for 1 h at 37°C. Supernatant that contained the DNA with the bound proteins and beads were separated and SDS sample buffer was added to the supernatant. After boiling, the proteins that bound to the DNA were analyzed by protein immunoblotting.

Microinjection Experiments

Microinjection experiments were performed exactly as described previously [Hofmann et al., 2004]. Briefly, HeLa cells grown in DMEM with 10% FBS were grown on fibronectin-coated glass coverslips (BD Biosciences, Palo Alto, CA). The cells were grown overnight before injection in 0.5% FBS in DMEM. Nuclei were microinjected with an Eppendorf automated microinjection system linked to an Olympus IX70 microscope. Injections were performed on a heated stage at 37°C. The antibodies were dialyzed in PBS, concentrated and combined with rhodamine-labeled dextran. The dextran was used to identify injected nuclei. The final concentrations were 6.8 mg/ml HUC 1-1 antibody, 3 mg/ml NMI antibody, and 5 mg/ml rhodamine-dextran. About 50 fl were injected into each nucleus and about 20 nuclei were injected per coverslip in 30 min. The microinjected cultures were incubated for 1 h at 37°C. The cells were washed and in vivo transcription assays were performed in the presence of Br-UTP for 10 min at 35°C. Nascent transcripts were visualized with an Alexa Fluor 488 conjugated monoclonal antibody to BrdU (Molecular Probes, Eugene, OR). After treatment with DAPI, to visualize the nuclei, the coverslips were mounted in ProLong Antifade (Molecular Probes) and examined using an Olympus IX70 microscope equipped with a Cooke Sensicam. Slidebook software from Intelligent Imaging Innovations (Denver, CO) was used for the capture and deconvolution of the images.

RESULTS

NMI Co-purifies With RNA Polymerase II

Initial studies have indicated that NMI plays an important role in transcription by RNA polymerase II in mammalian cells because antibodies to NMI inhibit transcription in an in vitro assay using HeLa cell NE [Pestic-Dragovich et al., 2000]. To analyze whether

NMI is directly involved in the basic transcription process, we used a minimal transcription system consisting of recombinant transcription factors and purified RNA polymerase II. To obtain the RNA polymerase II, we purified RNA polymerase II from HeLa cells according to described methods [Reinberg and Roeder, 1987]. Purification through DEAE-Cellulose, DEAE-Sephadex and phosphocellulose column chromatography was monitored using a monoclonal antibody to the C-terminal domain of the large subunit of RNA polymerase II (data not shown). After the last column the transcriptionally active fractions were pooled. Western blot analysis of the purified RNA polymerase II with antibodies to NMI showed the presence of a

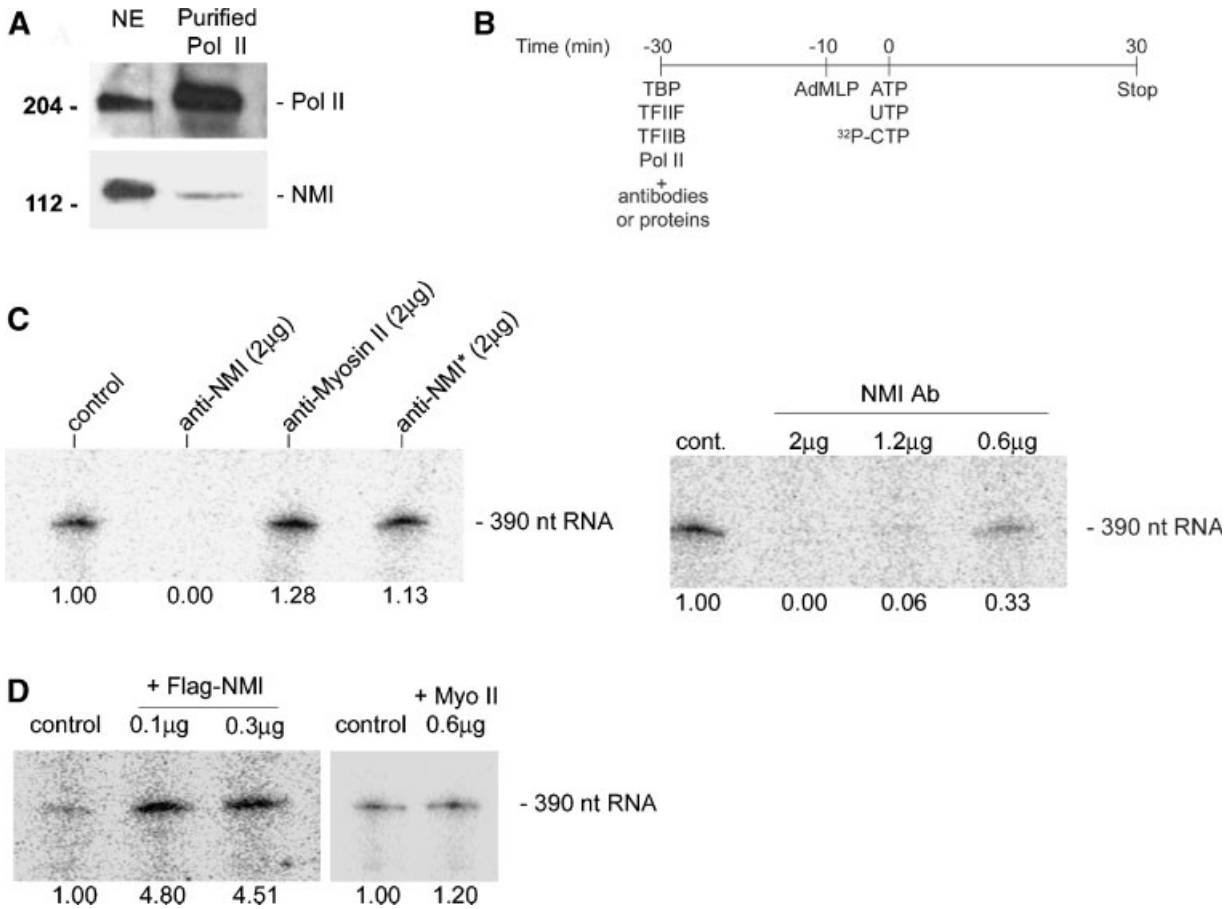

Fig. 1. NMI co-purifies with RNA polymerase II and is required for transcription in a purified system. **A**: Protein immunoblot analysis of purified RNA polymerase II. RNA polymerase II was purified from HeLa cells as described [Reinberg and Roeder, 1987]. Approximately 0.5 μg of RNA polymerase II was resolved. Immunoblotting with antibodies to NMI and to RNA polymerase II show that NMI co-purified with RNA polymerase II. HeLa nuclear extracting was used as positive control. The migration of the relevant molecular weight marker is shown on the left. **B**: Schematic presentation of the Transcription Assay: Recombinant transcription factors TBP, TFIIF, TFIIB, and purified RNA polymerase II were incubated with the indicated amount of NMI or myosin II antibodies or proteins for 20 min. Template DNA containing the AdML promoter was added and the reaction mixture was incubated for another 10 min. Transcription was initiated by adding rNTPs. The reaction was stopped after 30 min and analyzed as described in Materials and Methods. **C**: Effect of NMI antibodies on transcription. The left side shows that incubating with 2 μg of antibodies to NMI inhibited transcription of the full length RNA in this purified system. In contrast, incubating with either 2 μg of affinity purified antibodies to smooth muscle myosin II or with 2 μg of heat inactivated antibodies to NMI (NMI*) had no effect. The right side shows that transcription inhibition by antibodies to NMI occurs in a concentration dependent manner. Control represents samples incubated with buffer only. **D**: Stimulation of RNA polymerase II activity by NMI. Flag-tagged NMI was expressed in COS cells and purified as described in Materials and Methods. Purified RNA polymerase II and the recombinant transcription factors were incubated with 100 or 300 ng of Flag-NMI or with 600 ng of purified myosin II prior to initiation of transcription. Incubating with the Flag-NMI led to a ~4.5-fold stimulation of transcription with a standard error in case of 100 ng of 0.16 and in case of 300 ng of 1.08. In contrast, incubating with myosin II had no effect. Control represents samples incubated with buffer only. Each experiment was repeated three times. The fractional activities are given at the bottom and the 390 nt RNA product is identified.

small amount of NMI in the purified RNA polymerase II fraction (Fig. 1A).

Antibodies to NMI Inhibit In Vitro and In Vivo Transcription

We next tested whether the NMI that co-purifies with RNA polymerase II is necessary for transcription using an in vitro transcription assay. In this assay, bacterially expressed, recombinant transcription factors TBP, TFIIB, TFIIF and RNA polymerase II purified from HeLa cell nuclei, were incubated with a negatively supercoiled DNA template containing the Adenovirus major late (AdML) promoter coupled to a 380 nt long G-less cassette [Kugel and Goodrich, 2000]. Transcription was then started by adding the nucleotides ATP, UTP, and 2-^{32}P-CTP. To analyze the importance of the NMI that co-purifies with the RNA polymerase II, we pre-incubated the transcription factors and RNA polymerase II with antibodies to NMI prior to start of transcription. Figure 1B shows a schematic of the transcription assay. As shown in Figure 1C NMI that co-purifies with RNA polymerase II is apparently essential for transcription because antibodies to NMI inhibited transcription in a concentration dependent manner. Affinity purified rabbit antibodies to myosin II [de Lanerolle and Stull, 1980; de Lanerolle et al., 1982], which were used as a control as well as heat inactivated anti-NMI antibody had no effect on transcription. Previously it was shown that antibodies to NMI inhibit in vitro transcription using HeLa NE as a source of transcription factors [Pestic-Dragovich et al., 2000]. Now we show that NMI is involved in basic transcription by RNA polymerase II. To confirm that this role for NMI in transcription is also true in vivo, we microinjected antibodies to NMI or as control antibodies to muscle actin (HUC 1-1), together with rhodamine-labeled dextran, into the nuclei of HeLa cells (see Supplementary information, Fig. S1). After 1 h incubation in vivo transcription assays were performed. As previously shown [Hofmann et al., 2004], microinjection of HUC 1-1 antibody had no effect on in vivo transcription (88% of the injected cells showed nascent transcripts). In contrast, microinjecting antibodies to NMI inhibited transcription because only 17% of cells injected with the NMI antibody showed nascent transcripts in the nucleoplasm (see Supplementary Information, Fig. S1).

Exogenous NMI Stimulates In Vitro Transcription

We next investigated if adding exogenous NMI to the transcription assay stimulates transcription. Flag-tagged NMI was expressed in COS cells and purified using a FLAG column. As a control we added purified smooth muscle myosin II [de Lanerolle and Stull, 1980; de Lanerolle et al., 1982] (Fig. 1D). Adding 100 or 300 ng of Flag-NMI to the transcription assay stimulated transcription about 4.5-fold. In contrast, adding 600 ng smooth muscle myosin II had no effect on transcription. Because antibodies to NMI inhibit transcription while addition of NMI stimulates transcription, we conclude that NMI indeed plays a cardinal role in transcription by RNA polymerase II.

Antibodies to NMI Inhibit Transcription Initiation

Having established that NMI is necessary for basic transcription we next attempted to define which step of transcription NMI is involved in by performing a transcription initiation assay. The DNA template we use contains an A at position +1. The next A is at position +16. When the dinucleotide ApC is used as a priming dinucleotide instead of ATP, the polymerase stops at position +15 and produces a 15 nt long RNA product [Kugel and Goodrich, 2000]. To determine if NMI is involved in the production of these first 15 nucleotides, we pre-incubated the general transcription factors and the RNA polymerase II with antibodies to NMI or with antibodies to myosin II. Transcription was then initiated by adding UTP, 2-^{32}P-CTP, and ApC. As shown in Figure 2, antibodies to NMI inhibited the production of the 15 nt RNA. In contrast, antibodies to myosin II had no effect on transcription. These data indicate that NMI is involved at the initiation or pre-initiation state of transcription by RNA polymerase II.

NMI is not Necessary for Pre-initiation Complex Formation

Recently, we have shown that nuclear β-actin is required for PIC formation by demonstrating that antibodies to β-actin prevented PIC formation [Hofmann et al., 2004]. Because myosins work together with actin in the cytoplasm, one would predict that actin and NMI also work together in the nucleus. Therefore we analyzed whether NMI is similarly required for PIC

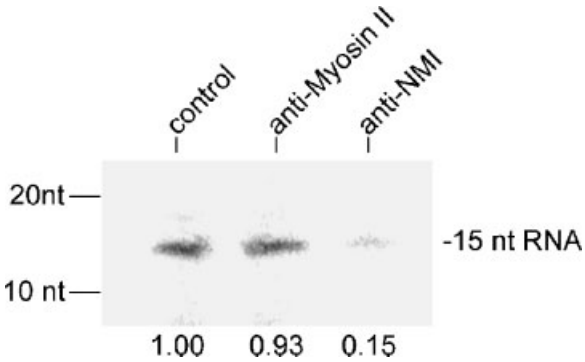

Fig. 2. Antibodies to NMI inhibit transcription initiation. The transcription assay was carried out as shown in Figure 1B with the exception that the dinucleotide ApC was substituted for ATP. Purified RNA polymerase II, recombinant transcription factors and the DNA template were incubated with antibodies to NMI or myosin II. Transcription up to position +16 was initiated by adding ApC, UTP, and 2-^{32}P-CTP. Antibodies to NMI clearly inhibited transcription of the first 15 nucleotides while antibodies to myosin II had no effect on transcription. Control represents samples incubated with buffer only. Each experiment was repeated three times. The fractional activities are given at the bottom and the 15 nt RNA product is indicated. The positions of the RNA size markers are indicated on the left.

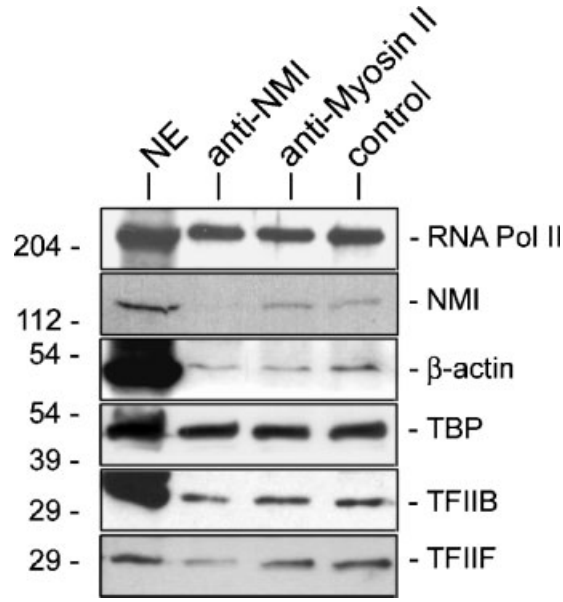

Fig. 3. NMI is not required for pre-initiation complex formation. A biotinylated DNA template containing the AdML promoter was coupled to streptavidin coated magnetic beads and incubated with HeLa nuclear extract for 30 min at 30°C to allow PIC formation in the presence of antibodies to NMI or smooth muscle myosin II. Control represents samples incubated with buffer only. The beads were then washed and the eluted complexes were analyzed by immunoblotting with antibodies to RNA polymerase II, NMI, β-actin, TBP, TFIIF, and TFIIB. The antibodies to NMI or myosin II had no effect on PIC formation, indicating that NMI is not required for the assembly of the PIC. HeLa nuclear extract (NE) was used as positive control. Molecular mass standards are indicated in kDa on the left.

formation. For this a biotinylated DNA template containing the AdML promoter was immobilized on Streptavidin coated magnetic beads. The beads were then incubated with HeLa NE in the presence of antibodies to NMI or as control to myosin II to form PICs. The bound complexes were eluted from the DNA and analyzed with antibodies to RNA polymerase II, NMI, β-actin, TBP, TFIIF, and TFIIB (Fig. 3). Not surprisingly, antibodies to myosin II had no effect on the PIC assembly. Surprisingly however, antibodies to NMI also had no effect on PIC formation. That is, removal of all but a trace of NMI with antibodies to NMI did not proportionately decrease the presence of RNA polymerase II, TBP, TFIIB or TFIIF in the assembled PICs, thus demonstrating that NMI is not stoichiometrically involved in PIC assembly. These data rule out the possibility that the observed inhibition of the production of the first 15 nucleotides is due to an effect on PIC assembly.

NMI is Necessary for the Formation of the First Phosphodiester Bond

The early phase of RNA synthesis from the AdML promoter can be divided into several steps. During PIC formation, the general transcription factors and RNA polymerase II are assembled at the promoter to form a stable PIC. When provided with a limiting set of nucleotides, the PIC transitions into an initiation complex and 2–3 nucleotide long transcripts are produced. During the next step, Escape Commitment, stable ternary complexes are formed. Promoter Escape occurs when a 15 nt transcript is synthesized [Kugel and Goodrich, 2003].

The data shown in Figure 2 clearly demonstrate that NMI is involved in transcription initiation but the PIC assembly assay in Figure 3 shows that it is not involved at the pre-initiation stage of transcription. This suggests that NMI is involved at some step between PIC formation and the transition to elongation. To analyze this we used an abortive transcription initiation assay as described by Kugel and Goodrich [2003]. This assay, shown schematically in Figure 4A, is based on the fact that after forming a functional initiation complex, RNA

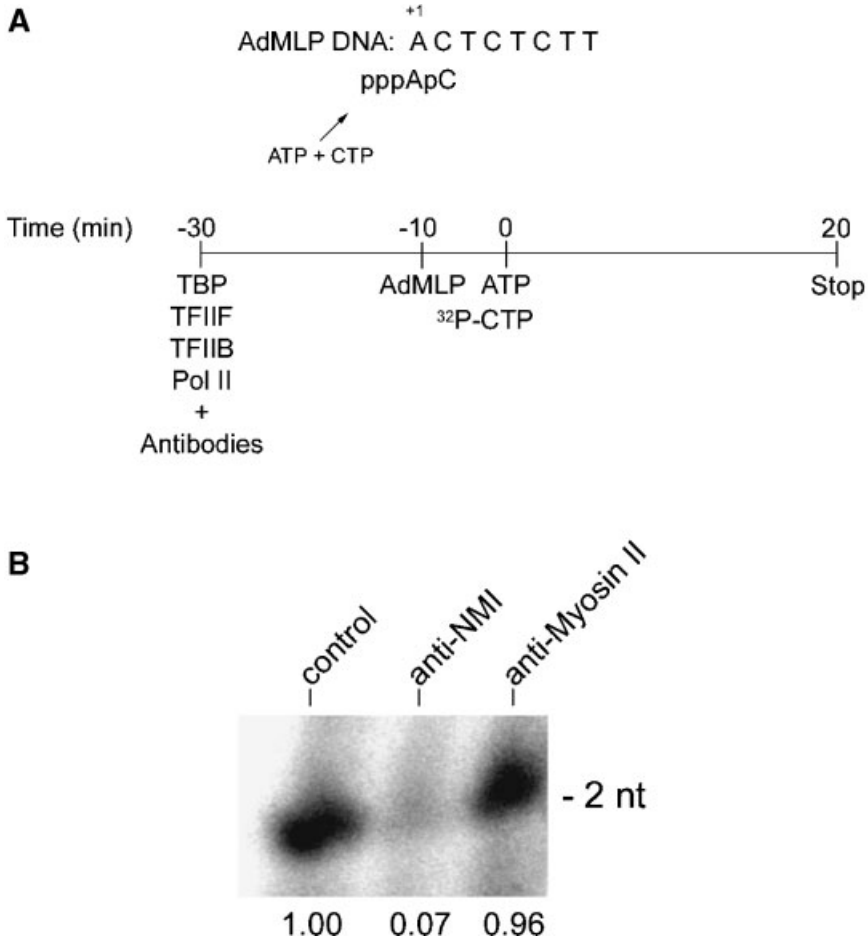

Fig. 4. Antibodies to NMI inhibit formation of the first phosphodiester bond during transcription initation. **A**: DNA template sequence and schematic of the transcription assay. The sequence of the non-template strand is shown. A dinucleotide is produced when the rATP and rCTP are added as nucleotides. **B**: Purified RNA polymerase II, recombinant transcription factors and DNA template were incubated with antibodies to NMI or as control with antibodies to myosin II. Production of the first dinucleotides was initiated by adding ATP and 2-^{32}P-CTP. Antibodies to NMI inhibited production of the first dinucleotide while antibodies to myosin II had no effect on transcription. Control represents samples incubated with buffer only. Each experiment was repeated three times. The fractional activities are given at the bottom. The 2 nt RNA product is indicated and was identified by UV shadowing using unlabeled ApC.

polymerase II produces 2–3 nt long RNA products when provided with a limiting set of nucleotides specific to the transcription start site of a promoter. In case of the AdML promoter, the addition of ATP and CTP leads to the formation of the dinucleotide pppApC. To analyze the role of NMI during the formation of this first dinucleotide bond, purified transcription factors and purified RNA polymerase II were incubated with antibodies to NMI or to myosin II as control. Transcription was then initiated by adding the AdML promoter containing DNA template, ATP and 2-^{32}P-CTP and the production of the dinucleotide ApC was monitored. As shown in Figure 4B, antibodies to NMI, but not antibodies to myosin II, inhibited the synthesis of the first dinucleotide during transcription initiation.

DISCUSSION

We have employed a highly purified in vitro transcription assay to analyze whether NMI plays a direct role in transcription by RNA polymerase II as well as the specific function of NMI during transcription by RNA polymerase II. Earlier studies have already suggested a role for NMI in transcription by RNA polymerase II because antibodies to NMI inhibit transcription by RNA polymerase II in an in vitro assay

using HeLa cell NE [Pestic-Dragovich et al., 2000]. In addition, a recent study by Kysela et al. [2005] shows by immunoelectron microscopy that NMI can be found at sites of active transcription. In this study we establish NMI as a transcription factor that has an essential function in basic transcription by RNA polymerase II in vivo and in vitro. Our data suggest that NMI and RNA polymerase II strongly interact because NMI co-purifies with RNA polymerase II. The purification procedure involves multiple chromatography steps and the presence of NMI following these procedures suggests a significant interaction. Despite the relative low abundance of NMI in this RNA polymerase II prep, the fact that antibodies to NMI inhibit transcription shows that this NMI is crucial for transcription. The functional significance of this observation is also supported by the fact that adding exogenous NMI to this transcription assay stimulated transcription almost fivefold. In addition, we show that microinjected antibodies to NMI also inhibit in vivo transcription by RNA polymerase II, emphasizing a role for NMI in transcription.

A detailed analysis of the different steps of early transcription demonstrated that NMI is necessary during transcription initiation, specifically during the formation of the first phosphodiester bond, a step that follows PIC assembly. This conclusion is based on the following data: (a) Antibodies to NMI inhibit synthesis of a 15 nt RNA (Fig. 2); (b) Antibodies to NMI do not inhibit PIC formation (Fig. 3); (c) Antibodies to NMI inhibit production of the first 2 nt after start of transcription (Fig. 4).

A recent study on the role of NMI in transcription by Pol I, Philimonenko et al. [2004] demonstrated an association of NMI with initiation-competent Pol I and TIF-IA, a factor that is required to activate transcription by Pol I. It was also shown that the binding of the NMI-TIF-IA complex apparently activates Pol I which places a role for NMI, besides possible other functions, at the initiation stage of transcription. Our data demonstrate that NMI is also required at the initiation stage of transcription by RNA polymerase II. NMI is required during transcription initiation to form the first nucleotide bond in the growing RNA chain indicating that it is required for activating RNA polymerase II. Therefore it seems that NMI is involved at the early stages of transcription by RNA polymerase I and II though the mechanism by which it functions seems to be different.

In this respect it is also noteworthy that both β-actin and NMI interact with RNA polymerase II [Pestic-Dragovich et al., 2000; Hofmann et al., 2004; Kukalev et al., 2005]. However, it was shown that β-actin is required for PIC formation [Hofmann et al., 2004]. NMI on the other hand is not required for PIC formation but for the activation of the PIC. Consequently, β-actin and NMI seem to have different roles during transcription initiation.

Finally, we have demonstrated an important function for NMI at the initiation step of transcription. Our data do not rule out a possible role for NMI at later steps of transcription. However, additional studies are needed to analyze whether and how NMI is also required for, i.e., transcription elongation.

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