

# The repression of the reverse-oriented transcription from the adenovirus terminus by NFI in competition with TFIID

Ken Matsumoto<sup>1,2,3</sup>, Kyosuke Nagata<sup>1</sup>, Fumio Hanaoka<sup>2</sup> and Michio Ui<sup>3</sup>

<sup>1</sup>Department of Molecular Genetics, National Institute of Genetics, Mishima, Shizuoka 411, Japan, <sup>2</sup>Radiation Biology Laboratory, RIKEN Institute, Wako, Saitama 351-01, Japan and <sup>3</sup>Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo 113, Japan

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Nuclear factor I (NFI) represses the transcription which is promoted by the cloned adenovirus (Ad) type 5 DNA replication origin and is reverse-oriented with respect to the direction of the replication. The mechanism of this repression by NFI was investigated. In the cell-free transcription system, the repression was observed only when NFI was present during the formation of the transcription initiation complex. From the results of DNase I protection experiments, it was indicated that NFI bound to its binding site in the Ad replication origin prevents TFIID from proper binding to the adjacent AT-rich region and consequently represses the transcription.

Transcription repression: Nuclear factor I; TFIID; Adenovirus

## 1. INTRODUCTION

The adenovirus (Ad) type 5 DNA replication origin, when cloned in a plasmid, functions as a transcription promoter which directs the transcription oriented in the opposite direction with respect to that of DNA replication [1,2]. The major initiation site is located about 30 nucleotides downstream from the AT-rich region in the minimum origin, which is the putative TATA-box of this promoter. Adjacent to the AT-rich region is a binding site for a cellular factor, nuclear factor I (NFI), which is essential for Ad DNA replication [3,4]. We have shown previously that NFI and its cognate binding site are involved in the negative regulation of this reverse-oriented transcription in the cell-free transcription system [2]. In this paper we further investigated the mechanism of this transcription repression by NFI.

## 2. MATERIALS AND METHODS

The cell-free transcription using pSPAdori as template and HeLa nuclear extracts was performed under the reaction conditions described previously [2]. DNase I protection experiments were performed essentially as described [4] except that 0.015  $A_{260nm}$  unit of poly(dGdC)·poly(dGdC) was added. The double-stranded 212 bp *RsaI*-*HindIII* fragment of pSPAdori uniquely labeled with <sup>32</sup>P at the 5' end of the *HindIII* site was used as probe. Double-stranded oligodeoxynucleotides were prepared as described [5]. Oligo1 (5'-CCTTATTTTGGATTGAAGCCAATATGATA-3') corresponds to nucleotide positions 17–45 of Ad 5 DNA, and oligoFIB-2 (5'-GCTA

GGTCTGGCTTTGGGCCAAGAGCCGCT-3') is derived from human genomic DNA [5].

NFI was purified from HeLa nuclei [3] and the single-stranded DNA-cellulose fraction was used throughout this study. The yeast TFIID was a generous gift of Dr H. Handa (University of Tokyo).

## 3. RESULTS

The plasmid pSPAdori containing Ad 5 terminal 73 bp in pSP65 vector was used as template in the cell-free transcription, and the reverse-oriented transcription by RNA polymerase II was detected by the primer extension assay ([2] and Fig. 1A). It has been suggested that Sarkosyl separates cell-free RNA synthesis into two discrete steps, formation of initiation complex that is sensitive to Sarkosyl and elongation of initiated RNA chains that is resistant to Sarkosyl [6]. Based on this, the time course of the formation of the initiation complex in the reverse-oriented transcription was examined. The template DNA and HeLa nuclear extracts were incubated at 30°C in the presence of oligoFIB-2 which resumes the transcription from repression by the endogenous NFI in HeLa nuclear extracts. As shown in Fig. 1B, the formation of the initiation complex was completed in 30 min.

Next, we examined whether NFI represses the formation of the initiation complex (Fig. 2). When NFI purified from HeLa cells was present during the preincubation, the transcription was repressed (lane 3). Furthermore, when oligo1 was added during the preincubation to titrate the endogenous NFI, the level of the transcription was elevated (lane 7). In contrast, these effects could not be observed if NFI (lane 4) and oligo1

Correspondence address: K. Matsumoto, Radiation Biology Laboratory, RIKEN Institute, 2-1 Hirosawa, Wako, Saitama 351-01, Japan

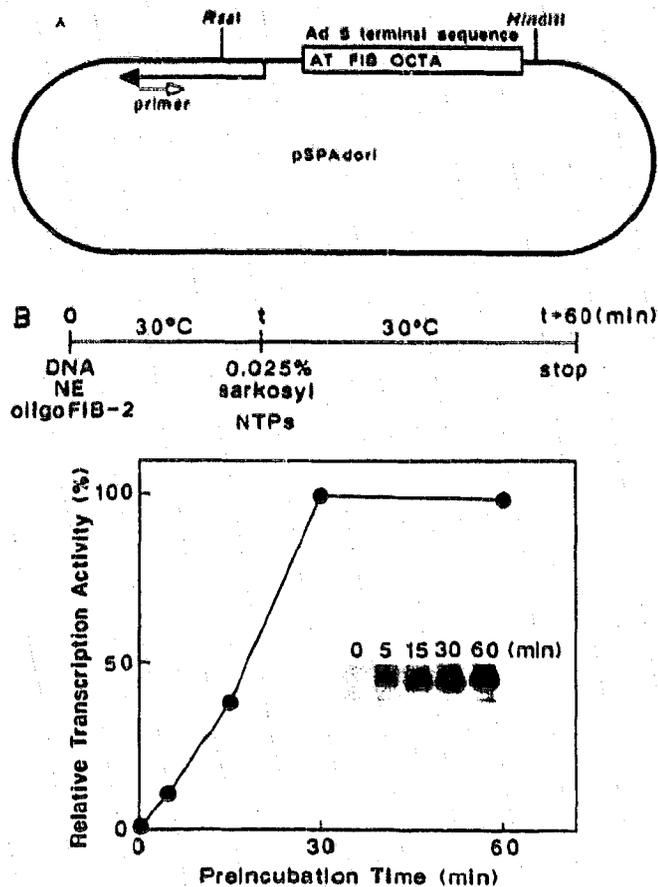


Fig. 1. The formation of the transcription initiation complex on the Ad reverse-oriented transcription promoter. (A) The plasmid pSPAdori. The transcript was analyzed by the primer extension analysis [2]. AT, AT-rich region; OCTA, nuclear factor III (NFIII) binding site. (B) The time course of the formation of the initiation complex. The supercoiled pSPAdori (0.5 pmol) was preincubated with HeLa nuclear extracts (60 µg protein) and 5 pmol of oligoFIB-2 at 30°C for 0, 5, 15, 30 and 60 min. Further formation of initiation complex was inhibited by adding Sarkosyl in a final concentration of 0.025% and simultaneously the transcription was started by adding 0.5 mM each of 4 NTPs. The level of the primer-extension product (inlet) was quantified by densitometric scanning.

(lane 8) was added together with Sarkosyl. These results indicate that NFI represses the formation of the transcription initiation complex on the reverse-oriented transcription promoter.

It has been reported that ATP destabilizes the initiation complex [7]. We thus examined the effect of ATP on the formation of the initiation complex of the reverse-oriented transcription. The addition of 0.5 mM ATP reduced the level of the transcription (Fig. 2, lanes 1 vs 2, and lanes 3 vs 5). The effect of oligoI was eliminated with the simultaneous addition of ATP (lanes 7 vs 9). These results confirmed that ATP inhibits the formation of the initiation complex or destabilizes the initiation complex in this promoter and that the

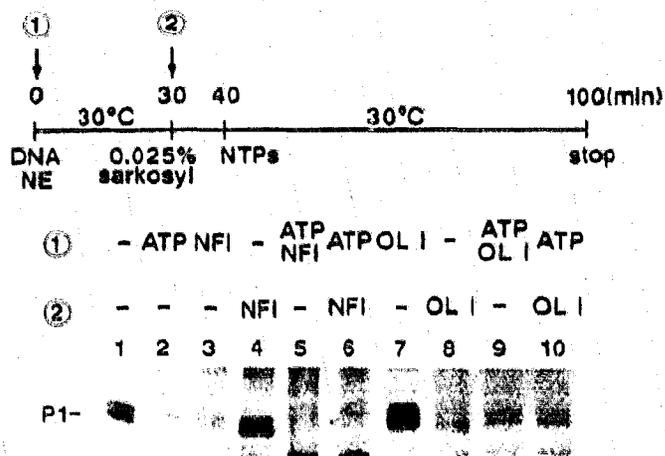


Fig. 2. Kinetic analysis of transcription repression by NFI. Transcription reaction was performed as described above without oligoFIB-2. Before or after the preincubation, the materials (ATP, 0.5 mM; HeLa NFI, 4 ng; oligoI, 5 pmol) were added where indicated.

repression by NFI takes place at the step of the formation of the initiation complex.

The formation of the initiation complex involves the binding of proteins to the region around the TATA-box and the initiation site. The effect of NFI on the binding profiles with nuclear proteins to the promoter region was investigated using DNase I protein experiments (Fig. 3A). Lane 7 shows that not only the NFI binding site (FIB site) but also the upstream region of the FIB site were protected from DNase I digestion with HeLa nuclear extracts. The region between the AT-rich region and the initiation site are slightly protected by the nuclear extracts. The addition of oligoI competed with a protein(s), presumably NFI, to bind to the FIB site and enhanced the protection around the AT-rich region (lane 8). TFIID, known as the TATA-box binding protein, is a good candidate for the protein binding to this AT-rich region [8]. Finally, we examined the effect of NFI on TFIID binding to the AT-rich region (Fig. 3B). Indeed, the purified yeast TFIID bound to the AT-rich region, indicating that this AT-rich region was recognized as the TATA-box of this promoter (lane 8). However, TFIID could not protect the AT-rich region in the presence of NFI (lane 7). It is noted that the DNase I hypersensitive sites generated by TFIID shown in lane 8 were also detected in lane 7. We concluded that TFIID does bind to the AT-rich region but does not properly bind when NFI binds to the adjacent FIB site.

4. DISCUSSION

In this paper we have investigated the mechanism of the transcription repression by NFI in the Ad 5 reverse-oriented transcription promoter. NFI has been originally identified from HeLa cells as a sequence-specific

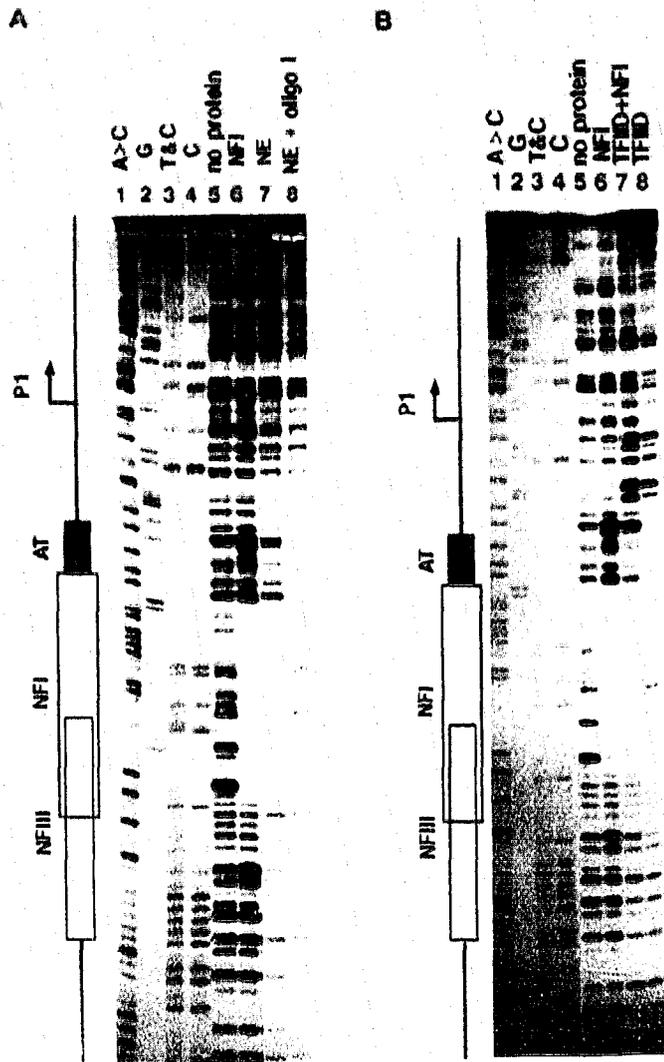


Fig. 3. DNase I protection experiments using nuclear extracts (A) and purified NFI and TFIID (B). 3.25 ng of HeLa NFI, HeLa nuclear extracts (7 µg protein), 200 ng of yeast TFIID and 1.5 pmol of oligo were added where indicated. Sequencing ladders (lanes 1-4 in both panels A and B) are electrophoresed in parallel. The DNase I protection regions by NFI (lane 6) and NFIII [14], AT-rich region and the transcription initiation site (P1) [2] are indicated.

DNA binding protein involved in the initiation of the Ad DNA replication [3,4]. Recent studies have revealed that NFI functions as an activator for transcription of several cellular and viral genes ([9,10] and references therein). Using the Ad reverse-oriented transcription system, we have shown that NFI is also involved in the negative regulation of transcription [2].

For the eukaryotic transcription repression, two mechanisms are proposed [11]. First, the transcription activator is inactivated by its associated with the other protein. Second, the repression is caused by the competition of a binding site between activator and repressor. In the regulation of interferon gene expres-

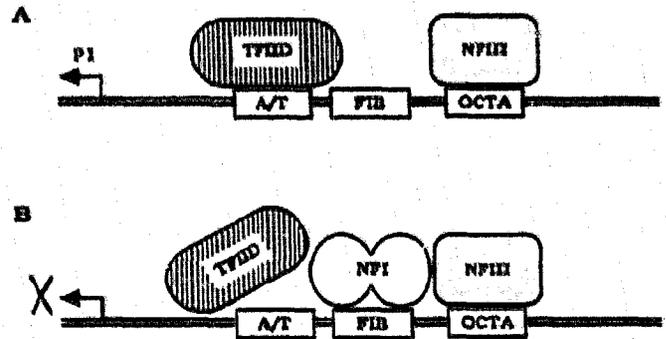


Fig. 4. Model of transcription repression by NFI. (A) In the absence of NFI on its binding site, TFIID can bind to the AT-rich region and the transcription (P1) occurs. (B) When NFI binds to its binding site, TFIID can not bind properly to the AT-rich region. Consequently, the transcription is repressed.

sion, an activator IRF-1 and a repressor IRF-2 compete for their binding sites [12]. A homeodomain protein, *engrailed*, binds also to the TATA-box and, as a result, inhibits the binding of TFIID to the TATA-box [13]. These studies showed the competition for the same binding site. We have shown here an alternative mechanism of transcription repression. NFI inhibited the proper binding of TFIID to the AT-rich region by its binding to the adjacent FIB site (Fig. 4).

This promoter can be functional, if at all, only when the Ad genome is integrated into cellular genome in front of a certain gene. Although the biological significance of this promoter is totally unknown, it remains possible that this reverse-oriented transcription plays a role in transformation by Ad. On these lines, it is hypothesized that the regulation of the level of NFI should be crucial in the integration and infection cycle of the Ad DNA. If the level of active NFI is reduced, for example in Ad 12 E1A expressing cells [10], the replication of the Ad DNA could be repressed and the transcription apparatus could be assembled on the Ad terminus. By defining the level of active NFI in a variety of cells we hope to gain insight into the regulation of integration and replication of the Ad DNA.

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