CHAPTER 6

Hepatitis Delta Antigen and RNA Polymerase II

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Abstract

Replication and transcription of HDV proceed via RNA-dependent RNA synthesis. These reactions are thought to be catalyzed at least in part by host RNA polymerase II (RNAPII). Hepatitis delta antigen (HDAg), which is critical for these processes, was recently proposed to function as a transcription elongation factor for RNAPII. The involvement of a DNA-dependent RNA polymerase in RNA-dependent RNA synthesis is itself intriguing and poses fundamental questions as to how RNA synthesis initiates, elongates, and terminates on an unusual HDV RNA template. In addition, the presence of a 'viral' transcription elongation factor is unprecedented in eukaryotes, whereas a few are known to exist in prokaryotes. Thus, the study of HDV replication and transcription should provide tremendous insight into the basic mechanism underlying RNAPII transcription.

Introduction

Three types of RNA-dependent RNA synthesis occur during the HDV life cycle: (i) antigenomic RNA synthesis from genomic RNA, (ii) genomic RNA synthesis from antigenomic RNA, and (iii) HDAg mRNA synthesis from genomic RNA (see Chapter 3 for details). The first and second types of reactions are steps in replication that are thought to proceed by a 'rolling cycle' mechanism. This mechanism is analogous to DNA replication of many plasmids and filamentous bacteriophages. As for the third type of reaction, based on the analysis of the mRNA's 5' end, it is assumed that the transcription is initiated from a position that is very close to an end of the rod-like structure of the HDV genome. By extension, the first type of reaction, which also utilizes genomic RNA as a template, may be initiated from the same position of the HDV genome. In this chapter, we refer to the three types of reactions simply as 'transcription'.

Several lines of evidence suggest that RNAPII is involved in HDV RNA transcription. First, viroid RNAs, infectious agents in plants that show structural similarity to HDV RNA, are reportedly transcribed by RNAPII in cell-free extracts.[1] Second, as reported by a few laboratories, HDV RNA can also be transcribed by RNAPII in vitro.[2-4] It should be noted, however, that the studies completed thus far have been unable to synthesize full-length complementary RNAs. In one report, for example, RNAPII in the nuclear extract of human HeLa cells directed a genomic strand synthesis of up to ~40 nt using an antigenomic fragment of HDV RNA as a

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template.[3] Third, HDAg, the sole HDV protein, directly binds to RNAPII and remarkably stimulates DNA-directed and HDV RNA-directed transcription in vitro.[5-7] The second half of this chapter deals with this topic. Fourth, HDAg mRNA is capped and polyadenylated at its 5' and 3' ends, respectively.[4] These processing events are tightly coupled to RNAPII transcription and occur in all the known mRNA species synthesized by RNAPII, with the exception of histone mRNA. Conversely, essentially no RNA species synthesized by other RNA polymerases are capped or polyadenylated. Fifth, in intact cells and in isolated nuclei, transcription of the HDV genome is reportedly sensitive to the mushroom toxin α-amanitin at concentrations low enough to selectively inhibit RNAPII.[8,9] One may need to view this with caution, however, because opposing results have been presented by another laboratory[10,11] (see Chapter 3 for more discussion). With these findings taken together, it should be reasonable to conclude that RNAPII is responsible at least in part for HDV RNA transcription.

Variation on a Theme: Initiation, Elongation, and Termination of HDV RNA Transcription

The idea that RNAPII, a DNA-dependent RNA polymerase, directs RNA-dependent RNA synthesis poses several interesting questions as to how RNA synthesis initiates, elongates, and terminates on an unusual HDV RNA template. From a mechanistic point of view, such an RNA-directed transcription seems quite a challenge to RNAPII, as discussed below. Elucidation of this mechanism may lead to the identification of new molecular targets to prevent the pathogenic virus. Furthermore, such knowledge should add insightful information on the basic mechanism of RNAPII transcription.

Before moving on to the central issue, we first overview the process of DNA-directed transcription by RNAPII. The transcription process comprises several distinct steps, including: (i) preinitiation complex assembly, (ii) promoter opening, (iii) transcription initiation, (iv) promoter escape, (v) transcription elongation, and (vi) transcription termination (Fig. 1A). The first four steps occur around transcription initiation sites. RNAPII alone is unable to initiate transcription. Instead it forms a preinitiation complex together with general transcription factors, including transcription factor (TF) IIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH, on a promoter.[12] Core promoter elements, such as TATA boxes and Inr elements, are important for the assembly. TFIIH then facilitates the conversion of a closed-to-open complex by its DNA helicase activity in an ATP-dependent manner (promoter opening).[12] Next, RNAPII starts to synthesize nascent RNA but immediately encounters a transcriptional block when it reaches 9~12 bp downstream. TFIIH helicase suppresses the block and facilitates the transition to the elongation phase (promoter escape).[13] This step is equated with the dissociation of RNAPII from promoter-bound transcription factors. During transcription elongation, RNAPII forms a ternary complex together with template DNA and nascent RNA. Within the 'transcription elongation complex', 12-15 bp of DNA are unwound to form a 'transcription bubble'. In addition, 8-9 nt of RNA in the 3'-end are contained by forming a hybrid with the template stand of DNA, with the growing 3'-end usually maintained at the active site of RNAPII.[14] Termination of premRNA synthesis is tightly but not entirely coupled to 3'-end processing.[15] The processing event, composed of transcript cleavage and polyadenylation, takes place 23 or 24 nt downstream of the AAUAAA sequence. Transcription termination seems to occur rather randomly between 200-2000 bp downstream of the poly(A) signal, triggered by preceding transcript cleavage and polyadenylation.

How is transcription initiated on the HDV RNA template? A few laboratories investigated the requirement for cis-acting elements in HDV transcription in vitro. According to these studies, small bulges close to the ends of the rod-like genome, where transcription is considered to be initiated, are important for efficient transcription.[2,3] These studies, however, were unable to establish the necessary and sufficient conditions for transcription initiation in