中文摘要

D 型肝炎病毒(HDV)是由四種成份所組成：第一種成份為病毒外套膜上之 B 型肝炎病毒(HBV)表面抗原(HBsAg)、第二及第三種成份為大型及小型 delta 抗原(L-& S-HDAg)、第四種成分則為 D 型肝炎病毒之 RNA 基因體 (RNA geome)。為了研究 D 型肝炎病毒 RNA 基因體的包裹(Packaging)機制，我們發展並應用了一個四質體共同轉染系統：在此系統中，病毒顆粒各成份各由一特定質體表現。將這四個不同的質體共同轉染至 HuH-7 人類肝癌細胞株中，收集釋放出來之 D 型肝炎病毒顆粒，發現 D 型肝炎病毒 RNA 可以被包裹進入病毒顆粒，故知此四質體共同轉染系統可以用來探討 D 型肝炎病毒 RNA 基因體的包裹機制。

在第一部份的實驗中，我們探討包裹 RNA 基因體所需要的病毒蛋白質及所需要的蛋白質功能區。我們發現在沒有小型 delta 抗原存在的情形下，大型 delta 抗原單獨存在已可帶出 D 型肝炎病毒 RNA，惟包裹效果較差; 分析一系列大型 delta 抗原的突變株，得知大型 delta 抗原之 RNA 結合區域對 RNA 的包裹是必須的。另一方面，當加入小型 delta 抗原時，病毒 RNA 包裹的效果將加強三到四倍; 分析一系列小型 delta 抗原突變株，得知小型 delta 抗原不但可以直接與 D 型肝炎病毒 RNA 結合，且其上之 RNA 結合區域對其結合 RNA 的能力為必須的。進一步探討小型 delta 抗原如何加強病毒 RNA 的包裹效率時，發現其上的 RNA 結合區域及 coiled-coil 結構對促進病毒 RNA 包裹是必須的。經由以上的研究，我們推測小型 delta 抗原之所以加強 RNA 包裹的效率，可能是因為小型 delta 抗原與 D 型肝炎病毒 RNA 直接結合，然後此 RNA-蛋白質複合體再被包裹進入病毒顆粒所致。本實驗結果令我們瞭解大型及小型 delta 抗原在包裹 D 型肝炎病毒 RNA 時所扮演的角色，並令我們瞭解 delta 抗原上何功能區對包裹病毒 RNA 是重要的。

在第二部分的實驗中，我們試圖鑑定 D 型肝炎病毒 RNA 上重要的 *cis*-elements 位於何方。我們利用 Linker insertion mutagenesis 的方法將變異引入 D 型肝炎病毒 RNA，保持其獨特的桿狀二級結構，並將這些變異型 D 型肝炎病毒的 cDNA 或其體外合成的 RNA 轉染至會穩定表現小型 delta 抗原的細胞株中，分析在 D 型肝炎病毒 RNA 上不同位置接入 Linker 各會造成什麼影響。結果顯示 (i):在不同位置接入 Linker 的 D 型肝炎病毒 RNA 仍可自我剪接而產生 1.7 Kb 的單套體，並且這些單套體 RNA 的穩定性亦相似，而且環繞在核酸酵素(Ribozyme)區域附近的序列對雙套體 RNA 的自我剪接是重要的。(ii):這些單套體 RNA 的包裹能力並未受顯著影響。(iii): D 型肝炎病毒 RNA 的複製在許多突變株中大幅降低或完全消失，顯示病毒 RNA 中大部份的區域對於複製都是必須的。(iv):在某些突變株中，接入的 Linker 選擇性地抑制反基因體 RNA 的複製，但是對基因體 RNA 的複製影響不大，暗示這兩個重要生理步驟所需要的 *cis*-elements 為可分開的。(v): 在核 酸 1625 (ScaI)至核 酸 431(NheI)這一連續區中接入 Linker，會影響 D 型肝炎病毒 mRNA 的產生，顯示此區對病毒 RNA 的轉錄是重要的。(vi): 以往的報告曾經推測 D 型肝炎病毒的複製及轉錄均是由同一個啟動子所控制的，然而本實驗中有兩個突變株其 mRNA 的生成雖受抑制，但病毒的複製卻仍然活躍，暗示病毒的複製可自病毒其他地方起始。本實驗令我們瞭解病毒複製及轉錄所需的 *cis*-elements，並增進

我們對 D 型肝炎病毒生活史之了解。

Abstract

Hepatitis delta virus (HDV) is composed of four specific components. The first component is the envelope protein which contains hepatitis B surface antigens (HBsAgs). The second and third components are nucleocapsid proteins, referred to as small and large hepatitis delta antigens (HDAgs). The final component is a single-stranded circular RNA molecule known as the viral genome. To study the mechanism of HDV RNA packaging, a four-plasmid cotransfection system, in which each viral component was provided by a separate plasmid, was employed. After cotransfection, the virus-like particles released from the HuH-7 hepatoma cells were collected and were found to contain the HDV RNA along with three viral proteins. Therefore, the four-plasmid cotransfection system could lead to successful HDV RNA packaging in vitro. The system was then used to show that the large HDAg alone was able to achieve a low level of HDV RNA packaging. Analysis of a variety of large HDAg mutants revealed that the RNA-binding domain was essential for viral RNA packaging. By increasing the incorporation of small HDAg into virus-like particles, we found a three-to fourfold enhancement of HDV RNA packaging. The S-HDAg was found to interact with HDV RNA directly, and the RNA-binding domain was essential for such interaction. Furthermore, the coiled-coil domain of S-HDAg, together with the RNA-binding domain, was found to be essential for the effective RNA packaging. Therefore, the enhancement effect of HDV RNA packaging was probably through a direct binding of HDV RNA, independent from that of large HDAg, with the small HDAg, then the RNA-protein complex was packaged into viral particles. The results provided insight into the roles and functional domains of small and large HDAgs in HDV RNA packaging.

To define the important cis-elements in HDV RNA, the viral genome was mutated by a linker-scanning mutagenesis strategy to maintain the native rod-like structure of HDV RNA Mutant HDV cDNAs or their corresponding RNA transcripts were transfected into a HuH-7-derived cell line which continuously expressed small HDAgs to study the viral replication and transcription. Here we report the following findings. (i) Although most of the mutant RNAs could self-process to generate the 1.7-Kb genomic RNA and all their stabilities were similar, positions which surround the genomic ribozyme domain were found to be important for the self-processing of the dimeric RNA. (ii) All of the resulted monomeric RNAs could be packaged into the virus-like particles. (iii) The replication of viral RNA was greatly diminished in many mutants, suggested that multiple regions in HDV RNA were required for replication. (iv) In certain mutants, replication of the HDV antigenomic RNA was selectively abolished but that of the genomic RNA was not. Therefore, this was the first report to show that the cis-elements needed for the replication of genomic or antigenomic HDV RNA could be different. (v) A continuous region (nt. 1625 to nt. 431), spanning the HDAg mRNA initiation site and containing the *in vitro*

identified RNA promoter, was found to be important for mRNA production *in vivo*. (vi) The HDV RNA replication and transcription was previously proposed to be govemed by a single “ double-acting promoter ” . However, two mutants which were deficient in mRNA synthesis still retained active viral RNA replication. It suggested that the HDV replication could initiate from sites other than this single promoter. This study therefore provided an insight into the cis-elements required for HDV RNA replication and transcription and further coutributed to our understanding of HDV life cycle.