

The importance of molecular diagnostics in HBV treatment

Will Irving

University of Nottingham

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Molecular diagnostics

- HBV DNA viral load is measured as a way of monitoring response to therapy
- Quantitative HBsAg measurement may also be important
 - (but not really a molecular diagnostic!)
 - (and how many labs do this?)

Alternative Title

The molecular virology of hepatitis B
virus

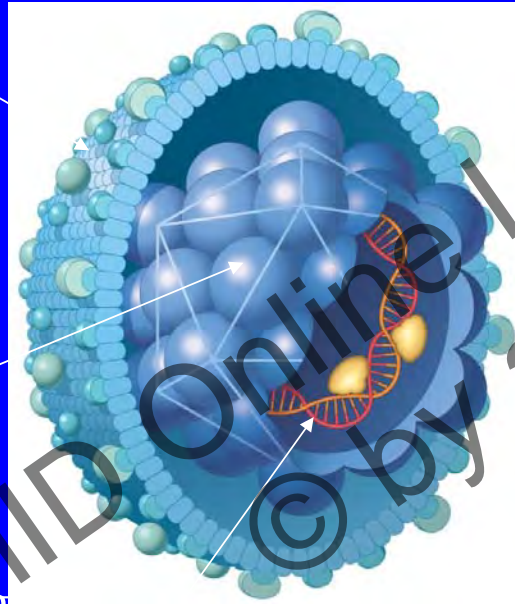
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Content

- HBV replication
- HBV mutants and their clinical significance
 - Precore
 - Pol
 - Surface
- HBV Genotypes
- Molecular Virology and the Pathogenesis of HCC

Hepatitis B Virus (HBV)

Surface Ag

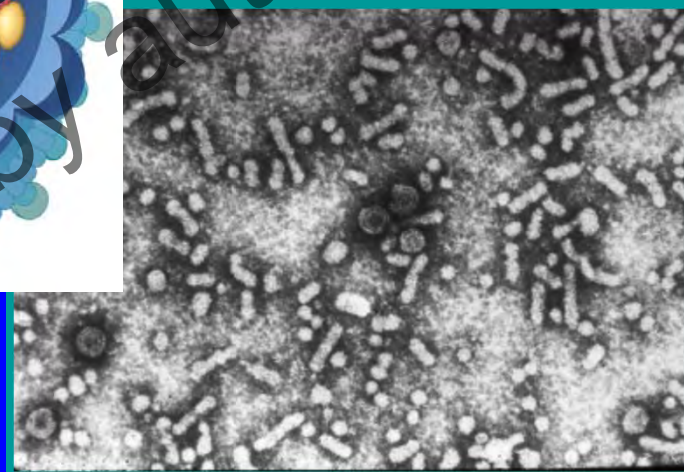


Core Ag

Also eAg

DNA

- Discovered in 1965 (Blumberg et al)
- Hepadnavirus (DNA)

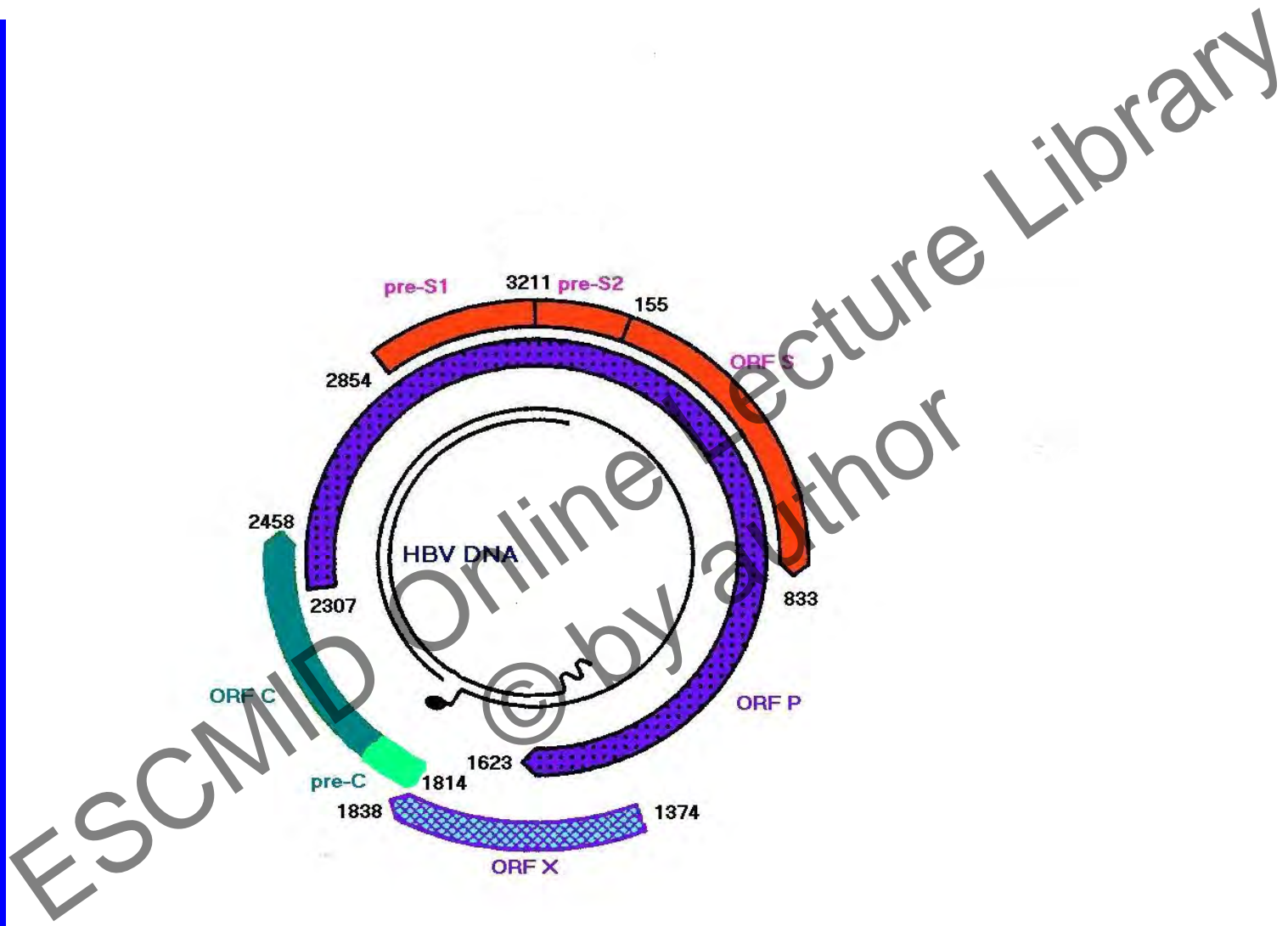


Source: Center for Disease Control and Prevention

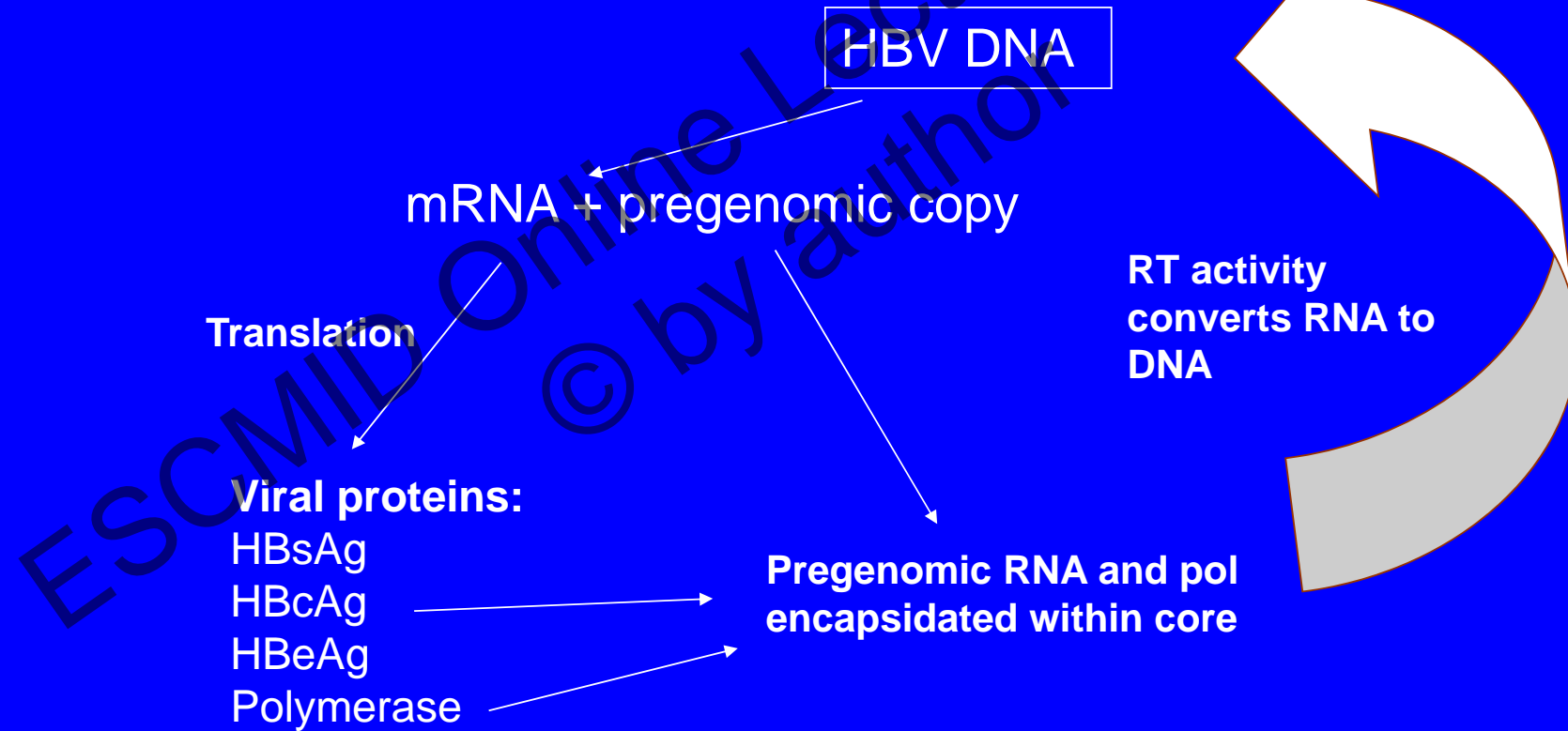
Genome of Hepatitis B

Partially
double
stranded
DNA
3,200 bp





HBV Replication Cycle



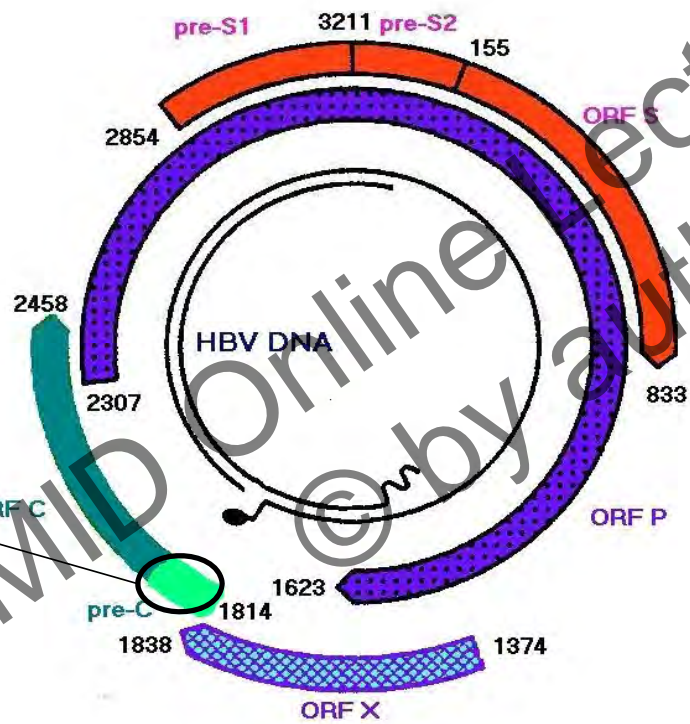
HBV replication

- Enters cell as partially ds DNA
- 2nd strand is completed → covalently closed circular DNA (cccDNA)
- Synthesis and packaging of pregenomic RNA
- Reverse transcription of pregenomic RNA within capsid → partially dsDNA

Hepatitis B mutants

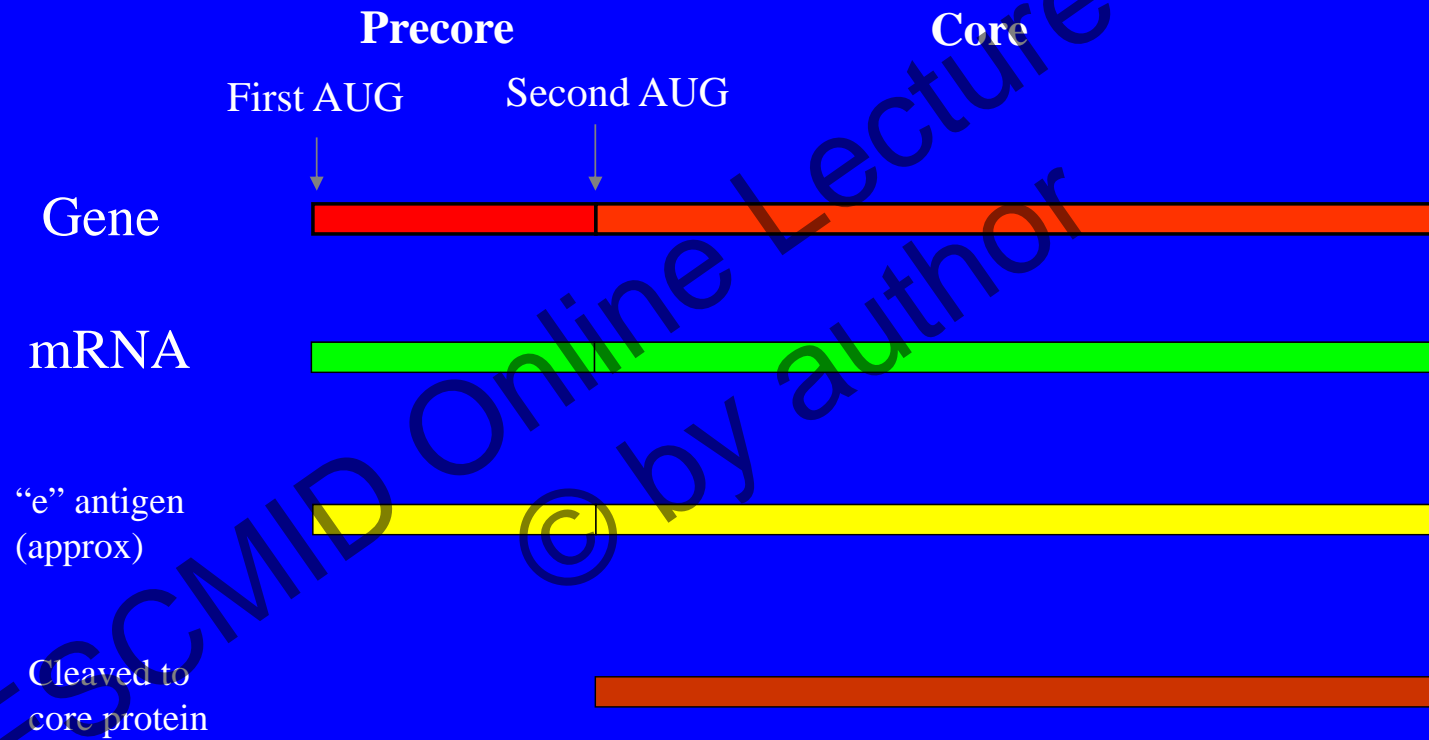
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Pre-core mutants

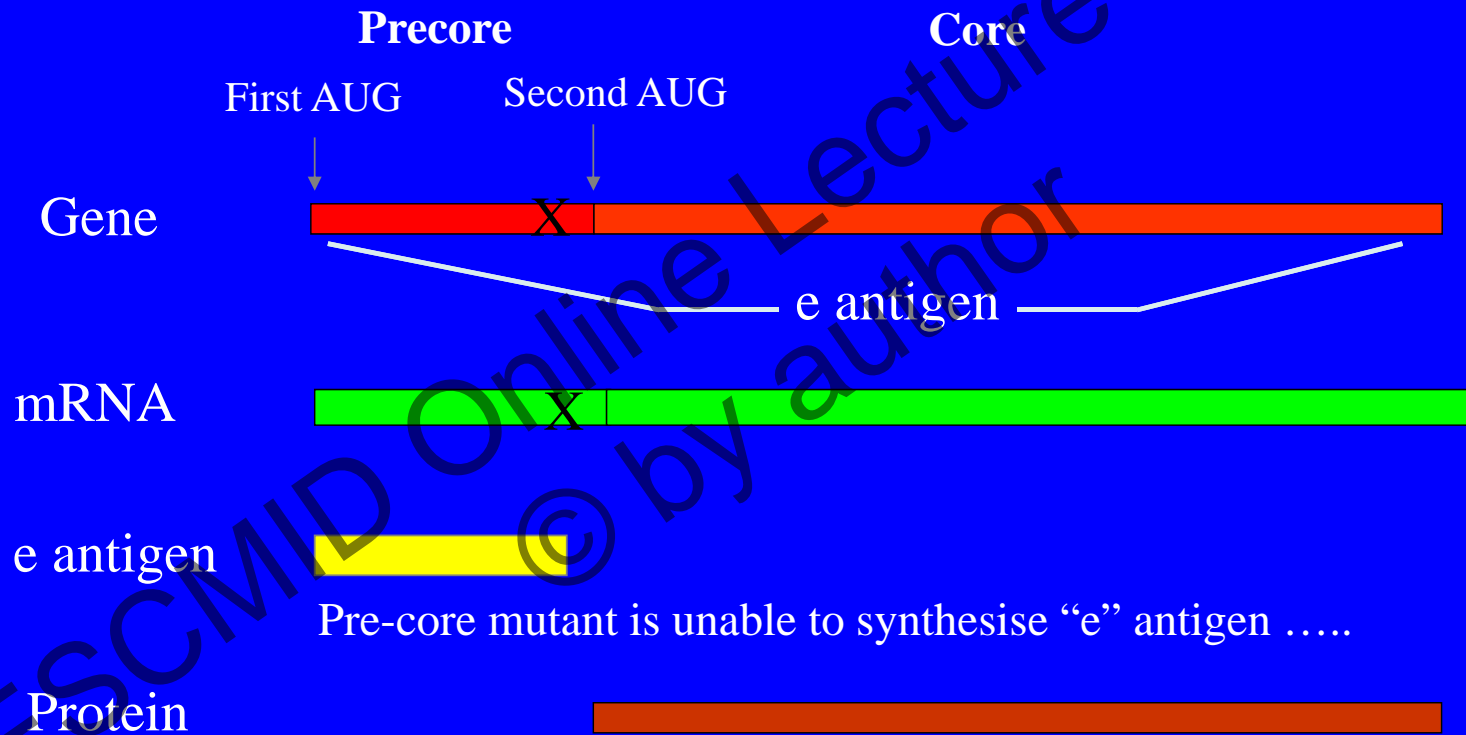


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Pre-core mutations



Precore mutants

Virus unable to synthesise e antigen

Replication competent

HBeAg negative profile, which usually indicates

low infectivity

low risk of chronic liver disease

may therefore be misleading

Precore mutants: Lab diagnosis?

Measure HBV DNA levels

Suspect if HBeAg negative, HBV DNA ++

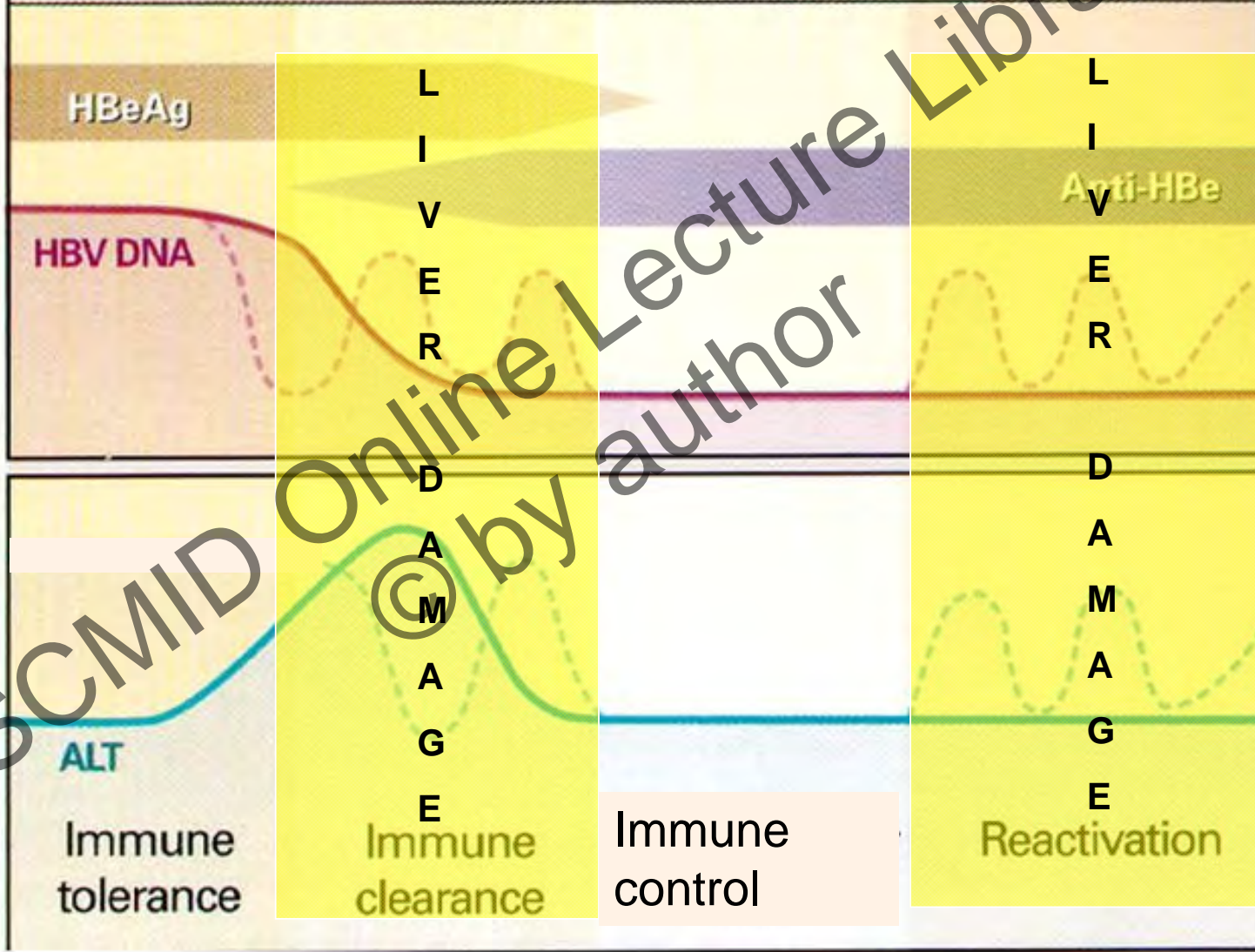
Precise sequencing may or may not be helpful

Pre-core mutants: Clinical significance

- Reactivation of virus with pre-core mutants means that, worldwide, HBeAg negative disease is the commonest form of HBV associated chronic liver disease

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Natural Course of Chronic HBV Infection



Pre-core mutants: Clinical significance

- Responsible for eAg chronic hepatitis B disease
- Implications in monitoring response to therapy and long-term natural history
 - eAg/anti-HBe status is not sufficient – need HBV DNA levels

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Pre-core mutants: Clinical significance

- Responsible for eAg chronic hepatitis B disease
- Implications in monitoring response to therapy and long-term natural history
- Impact on strategies for prevention of mother-to-child transmission
 - HBIg may be reserved for eAg +ve mothers only, but MTCT will arise from anti-HBe +ve mothers with high viral loads

Pre-core mutants: Clinical significance

- Responsible for eAg chronic hepatitis B disease
- Implications in monitoring response to therapy and long-term natural history
- Impact on strategies for prevention of MTCT
- Impacts on strategies for prevention of health-care worker to patient transmission of HBV
 - Most fitness-to-practice guidance is predicated on excluding eAg +ve HCWs from performing EPPs BUT

The New England Journal of Medicine

Established in 1812 as THE NEW ENGLAND JOURNAL OF MEDICINE AND SURGERY

VOLUME 336

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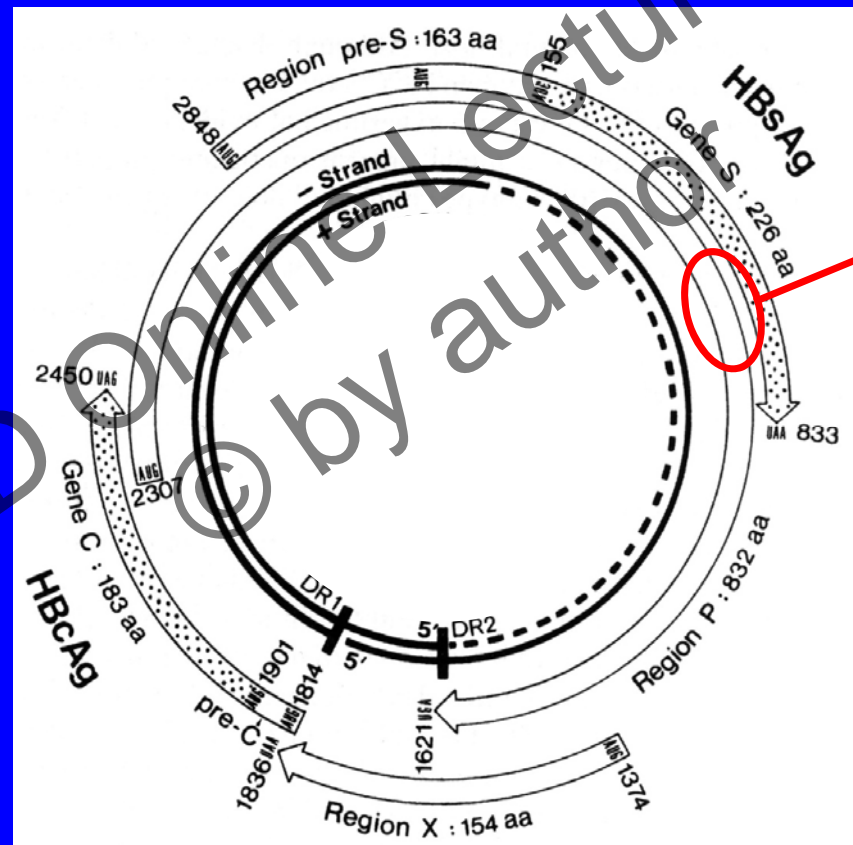
NUMBER 3

**TRANSMISSION OF HEPATITIS B TO PATIENTS FROM FOUR INFECTED
SURGEONS WITHOUT HEPATITIS B e ANTIGEN**

THE INCIDENT INVESTIGATION TEAMS AND OTHERS*

Therefore UK guidance stipulates HBV DNA testing of anti-HBe +ve HCWs, and sets a threshold limit (10^3 gEq/ml, 200 IU/L) above which operators are banned

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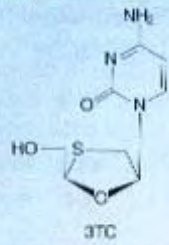
"YMDD"

POLYMERASE MUTANTS

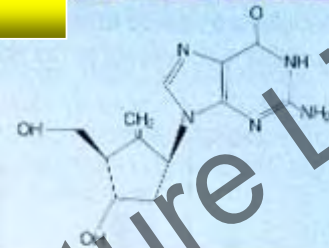
Resistance to therapy

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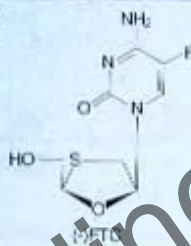
**Pyrimidine analogues
(L-nucleosides)**



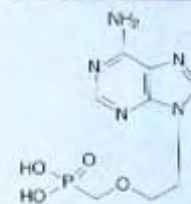
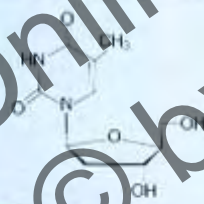
**Purine analogue
(cyclic D-nucleoside)**



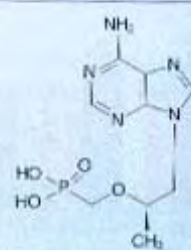
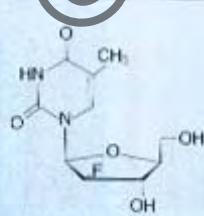
Emtricitabine



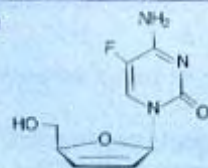
**Purine analogue
(acyclic D-nucleotides)**



Clevudine

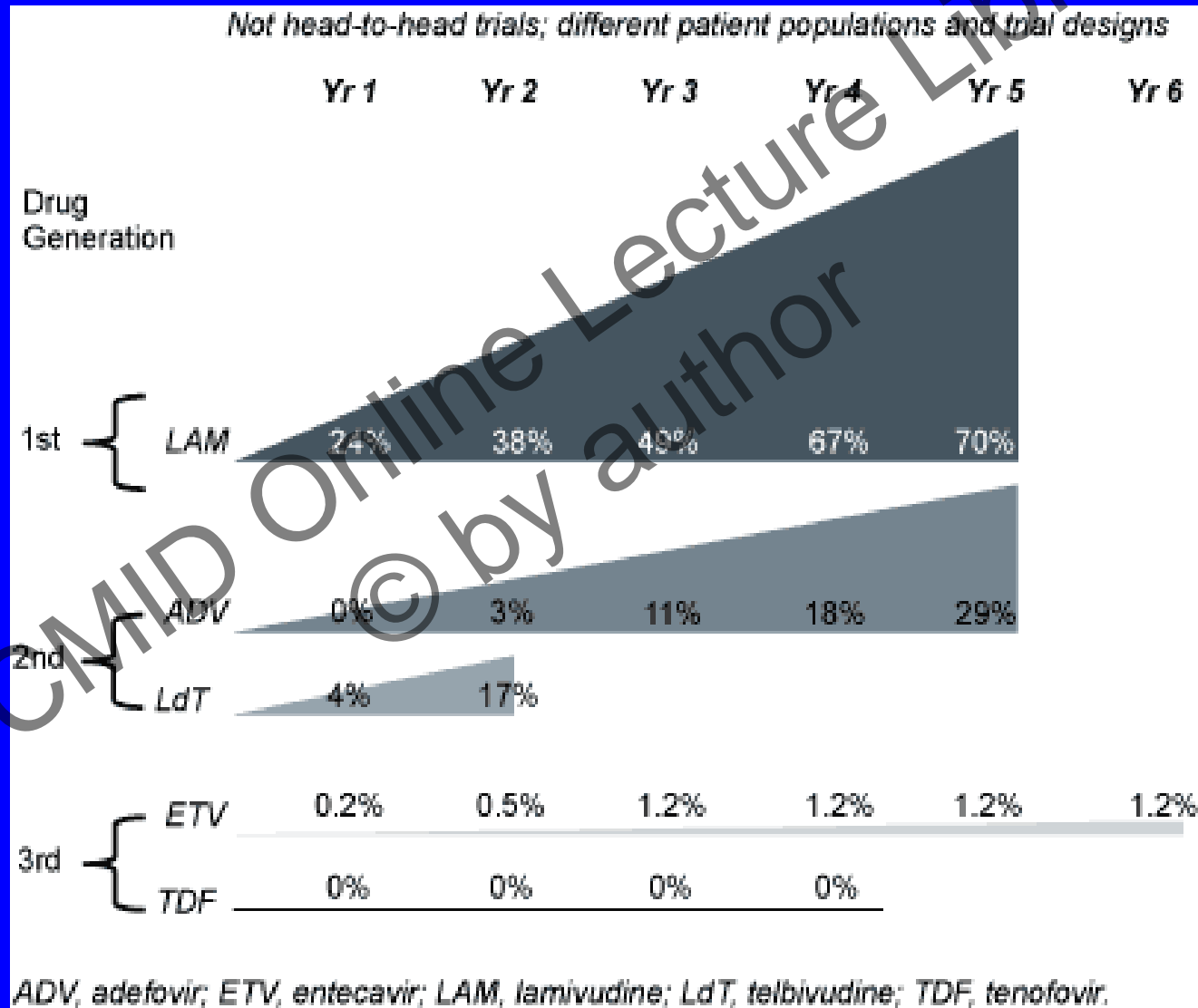


Elvucitabine

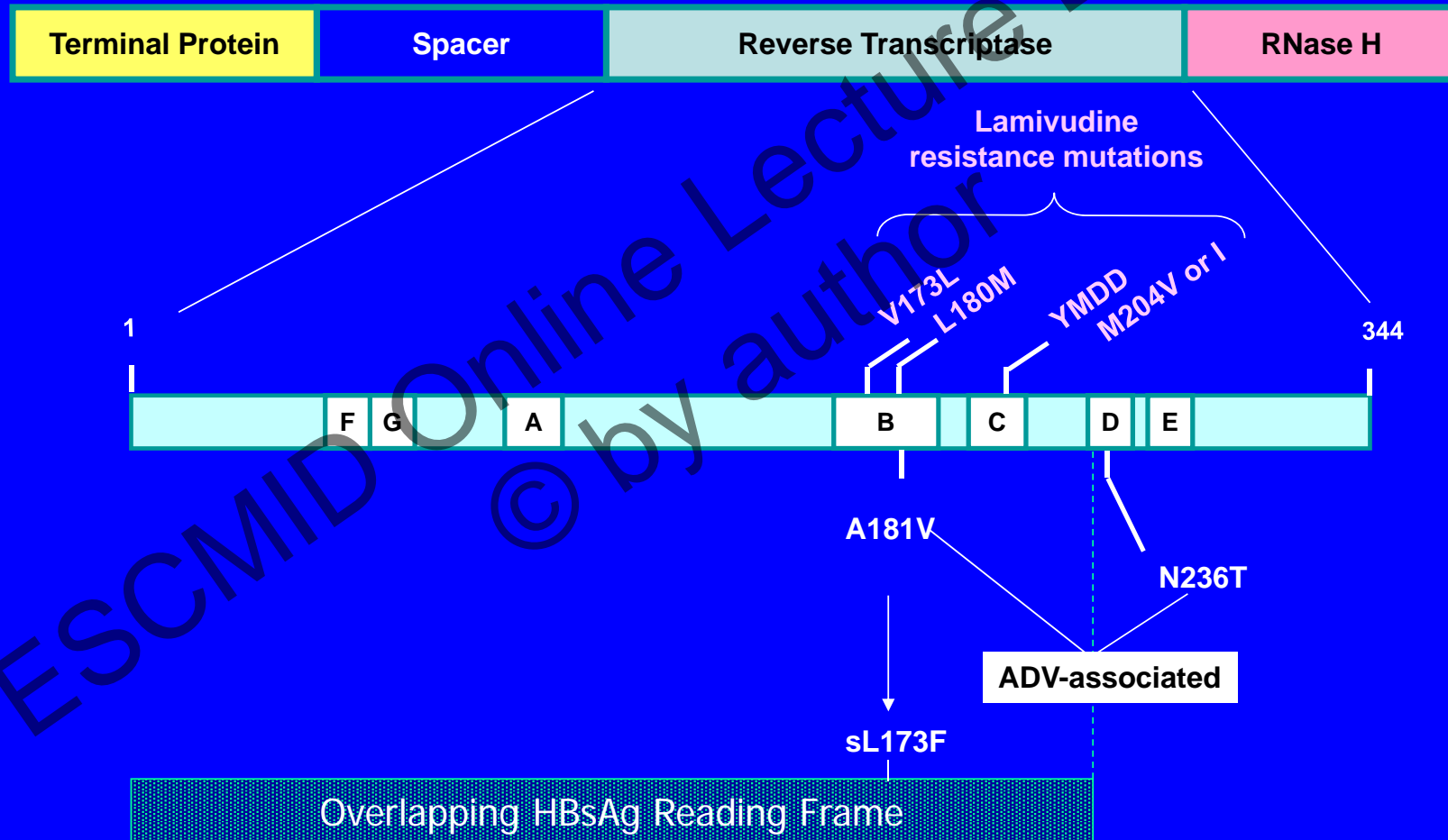


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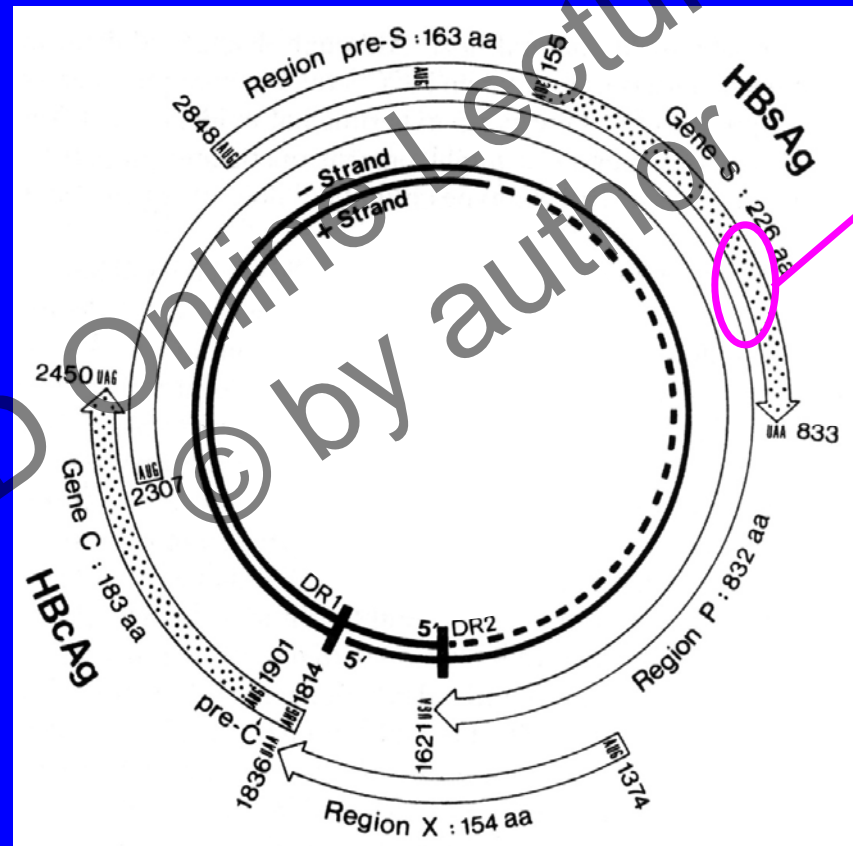
Antiviral therapy for HBV: emergence of resistance



Location of ADV-Associated Mutations in HBV Polymerase



Open reading frame S



"HBs"
G145R

S Gene Mutants: Clinical significance

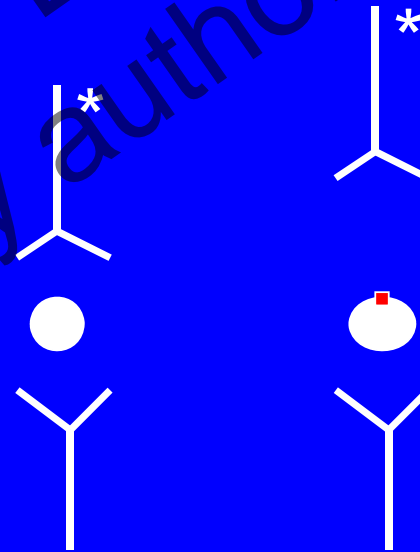
- Escape from neutralising anti-HBs
 - HBIg in transplant recipients
 - Vaccine escape mutants - MTCT
- Diagnostic difficulties – may explain some “occult” infection

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S gene mutations: diagnostic difficulties

Conjugated anti-HBs

Capture anti-HBs

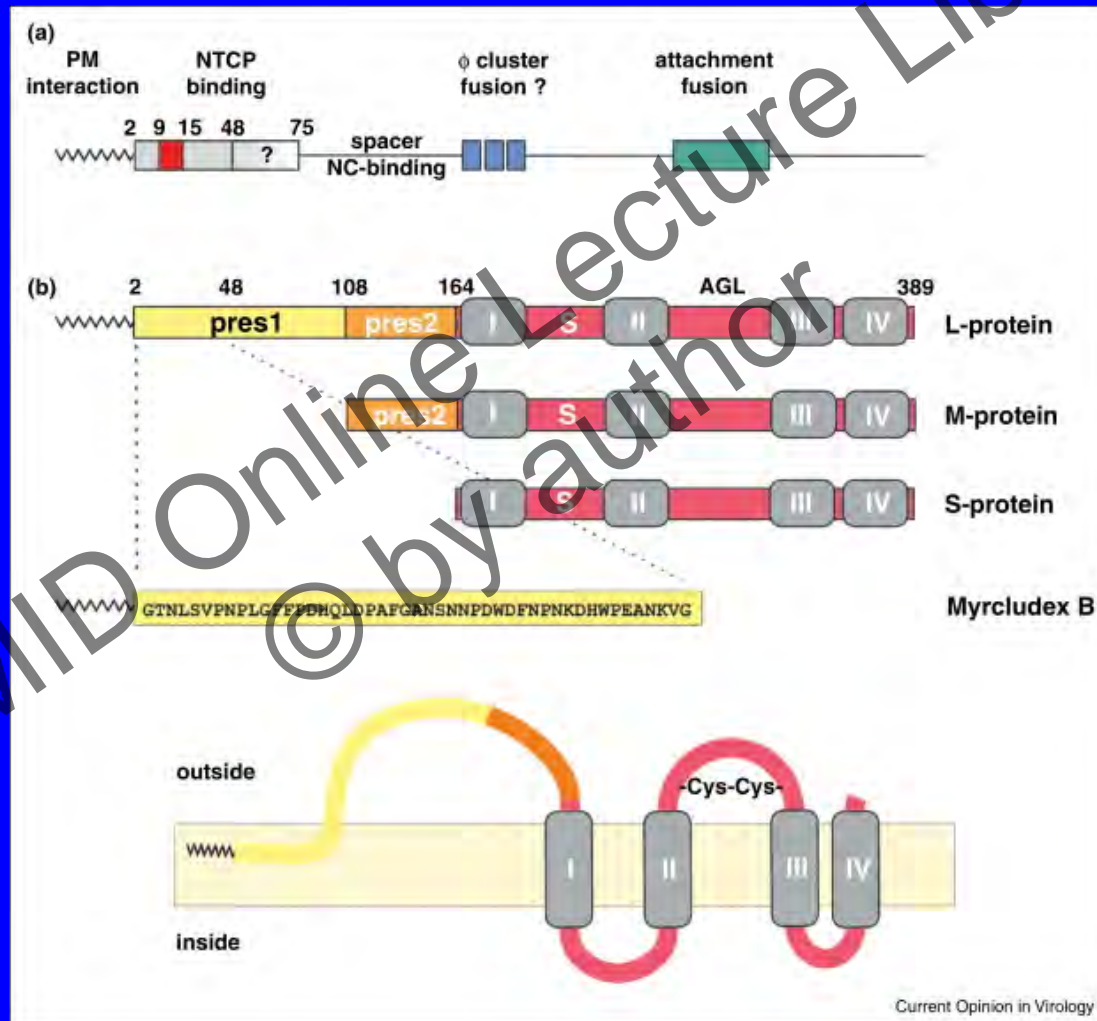


S Gene Mutants: Clinical significance

- Escape from neutralising anti-HBs
- Diagnostic difficulties – may explain some “occult” infection
- May impact on HBsAg quantification

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Structure of the HBV S antigen



HBV Genotypes

- Genotyping based on up to 10% sequence variation
- Genotypes may be geographically distributed
- Genotypes may differ in disease severity, likelihood of generation of precore and pol mutants, and rate of response to IFN therapy
- In clinical practice, is it useful to test your patients to see what genotype they are infected with?

Molecular Virology of HBV and development of HCC

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Integration of HBx in LINE1 regions as a marker for HCC

Cancer Cell

Article

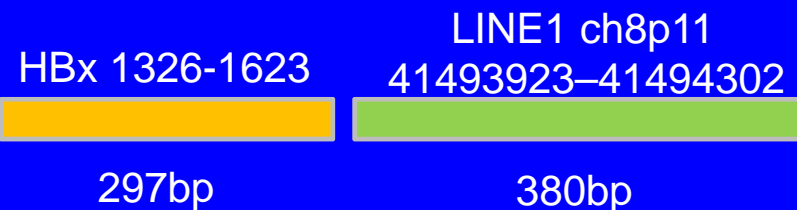
2014

Viral-Human Chimeric Transcript Predisposes Risk to Liver Cancer Development and Progression

Chi-Chiu Lau,¹ Tingting Sun,¹ Arthur K.K. Ching,¹ Mian He,¹ Jing-Woei Li,^{1,2} Alissa M. Wong,¹ Ngai Na Co,¹ Anthony W.H. Chan,¹ Pik-Shan Li,³ Raymond W.M. Lung,¹ Joanna H.M. Tong,¹ Paul B.S. Lai,⁴ Henry L.Y. Chan,⁵ Ka-Fai To,^{1,6,7} Ting-Fung Chan,^{2,7} and Nathalie Wong^{1,6,7,*}

Identified integration of HBV into host genomes in repeated LINE1 elements

This results in generation of a chimeric virus-human RNA transcript – HBx-LINE1



Integration of HBx in LINE1 regions as a marker for HCC

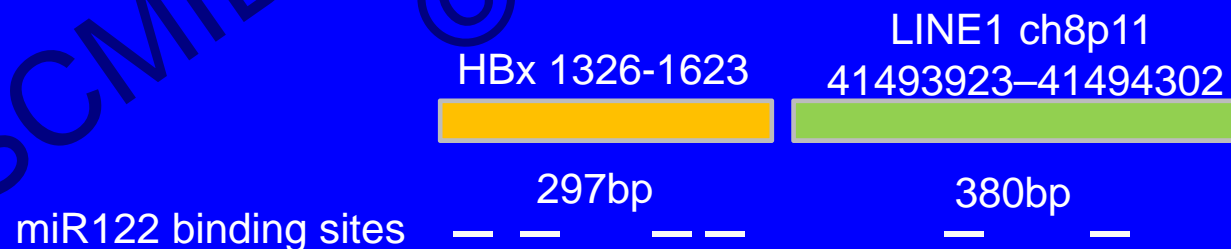
Research Article 2016



Hepatitis B virus-human chimeric transcript HBx-LINE1 promotes hepatic injury via sequestering cellular microRNA-122

Hong-Wei Liang^{1,†}, Nan Wang^{1,†}, Yanbo Wang^{1,†}, Feng Wang^{2,†}, Zheng Fu¹, Xin Yan³, Hao Zhu⁴, Wenli Diao¹, Yitao Ding^{5,*}, Xi Chen^{1,*}, Chen-Yu Zhang^{1,*}, Ke Zen^{1,*}

The HBx-LINE1 transcripts possess 6 miRNA-122 binding sites



Integration causes dysregulation of miR122-mediated regulation pathways

Summary

- Understanding the molecular virology of HBV has had enormous impact on diagnosis and patient management
- Routine diagnostic assays include
 - Serology – sAg/anti-HBs, anti-HBc, eAg/anti-Hbe, quant sAg
 - Molecular – HBV DNA viral load, sequencing to assess drug resistant variants, (genotyping)