WHY STUDY RETROVIRUSES?

* **PATHOGENIC CONSEQUENCES:** A HISTORY OF DISCOVERY...

EQUINE ANEMIA 1904 (EIAV)

CHICKEN LEUKOSIS 1907 (ALV)

CHICKEN SARCOMA 1911 (RSV)

MOUSE MAMMARY CARCINOMA 1930'S (MMTV)

MOUSE LEUKEMIA 1951 (MLV)

ADULT T CELL LEUKEMIA/LYMPHOMA 1980 (HTLV)

ACQUIRED IMMUNODEFICIENCY SYNDROME 1983 (HIV)

(OTHER PATHOLOGY INCLUDES ARTHRITIS, NEUROLOGICAL DISEASES, OSTEOPETROSIS, MANY CANCERS, ETC.)

* **UNUSUAL LIFE CYCLE**

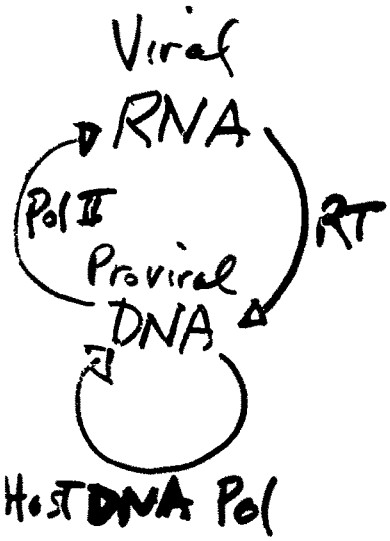
TEMIN'S PROVIRUS HYPOTHESIS:

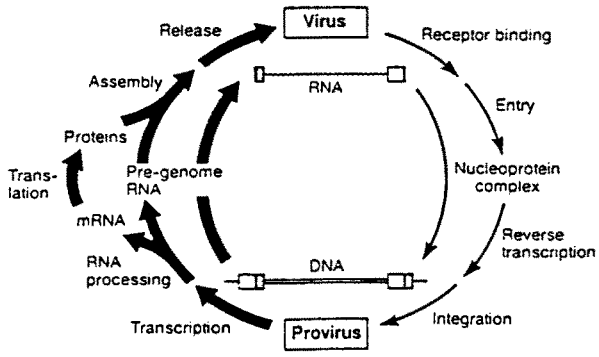
THE CLUES: INHIBITORS OF DNA SYNTHESIS AND OF DNA -DIRECTED RNA SYNTHESIS BLOCK INFECTION OF AN RNA VIRUS

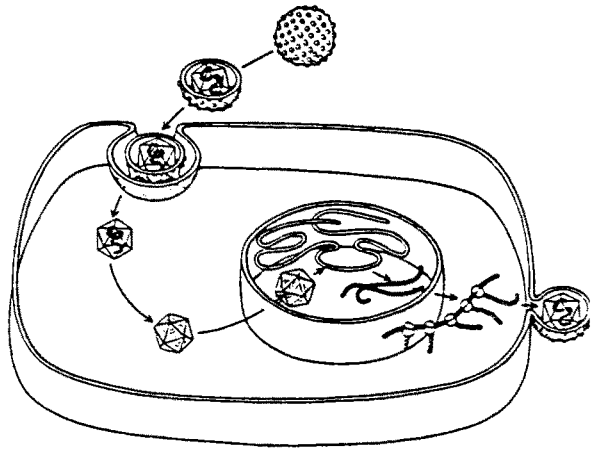
THE EVIDENCE: REVERSE TRANSCRIPTASE IN VIRIONS

TS MUTANTS OF POL GENE

DNA IN INFECTED CELLS (HYBRIDIZATION. TRANSFECTION, CLONING)



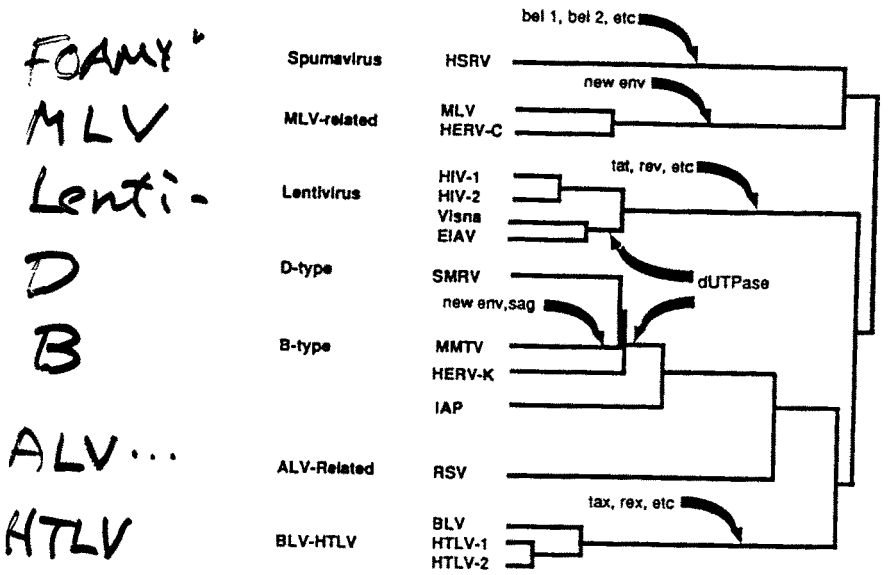




WHY STUDY RETROVIRUSES?

* WIDE DISTRIBUTION IN NATURE...THE SEVEN CLASSES

BASED ON SEQUENCE



(OTHER MEANS OF CLASSIFICATION: PATHOLOGY, PARTICLE TYPE, HOST)

TRANSMISSION: BLOOD, MILK (MMTV, HTLV), CONGENITAL INFECTION,

VIA GERM LINE (ENDOGENOUS PROVIRUSES)

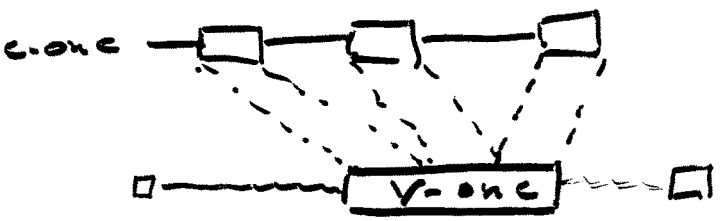
* EVOLUTIONARY IMPLICATIONS OF ENDOGENOUS PROVIRUSES,

OTHER RETRO-ELEMENTS (NEXT LECTURE)

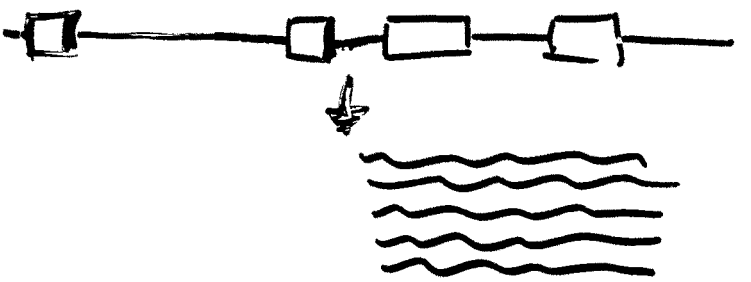
* NOVEL STRATEGIES FOR EXPRESSION (TAT, REV, FRAMESHIFTING)

* ONCOGENIC MECHANISMS:

TRANSDUCTION



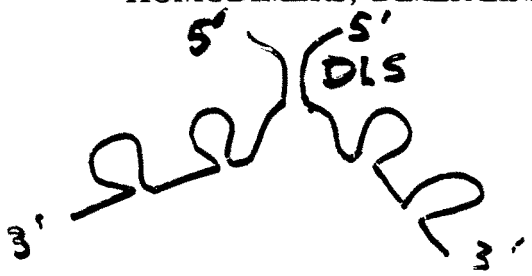
INSERTIONAL ACTIVATION



* UTILITY AS GENETIC VECTORS

RETROVIRAL GENOMES....

HOMODIMERS, DIMER LINKAGE, ASSOCIATED tRNA



NON-RANDOM tRNA, MAINLY PRIMER

IMPLICATIONS OF "PSEUDODIPLOIDY": HETEROZYGOSIS, RECOMBINATION

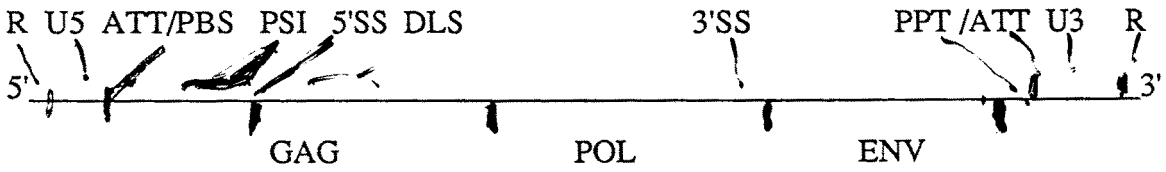
2 RNA subunits → 1 PROVIRUS

COMMON ELEMENTS

CIS ACTIVE: SITES FOR PRIMING RT AND FOR INTEGRATION (ATT SITES);

PROMOTERS, ENHANCERS, AND RNA PROCESSING SIGNALS (SPLICING AND POLYADENYLATION);

TRANSLATION, DIMERIZATION, PACKAGING SIGNALS.



TRANS ACTIVE: GAG----> MATRIX, CAPSID, NUCLEOCAPSID

POL----> PROTEASE, RT, INTEGRASE

ENV-----> SURFACE, TRANSMEMBRANE GP

IDIOSYNCRATIC ELEMENTS:

TRANSCRIPTIONAL REGULATORS: TAX, TAT, BEL GENES AND TARGETS

REGULATORS OF RNA METABOLISM: REX AND REV AND TARGETS

OTHER LENTIVIRUS GENES: NEF, VIF, VPU, VPR, VPX

SUPERANTIGEN (SAG) GENE OF MMTV; dUTP-ASES OF LENTIVIRUSES

TRANSDUCED GENES (MOSTLY ONCOGENES)

HIV-1

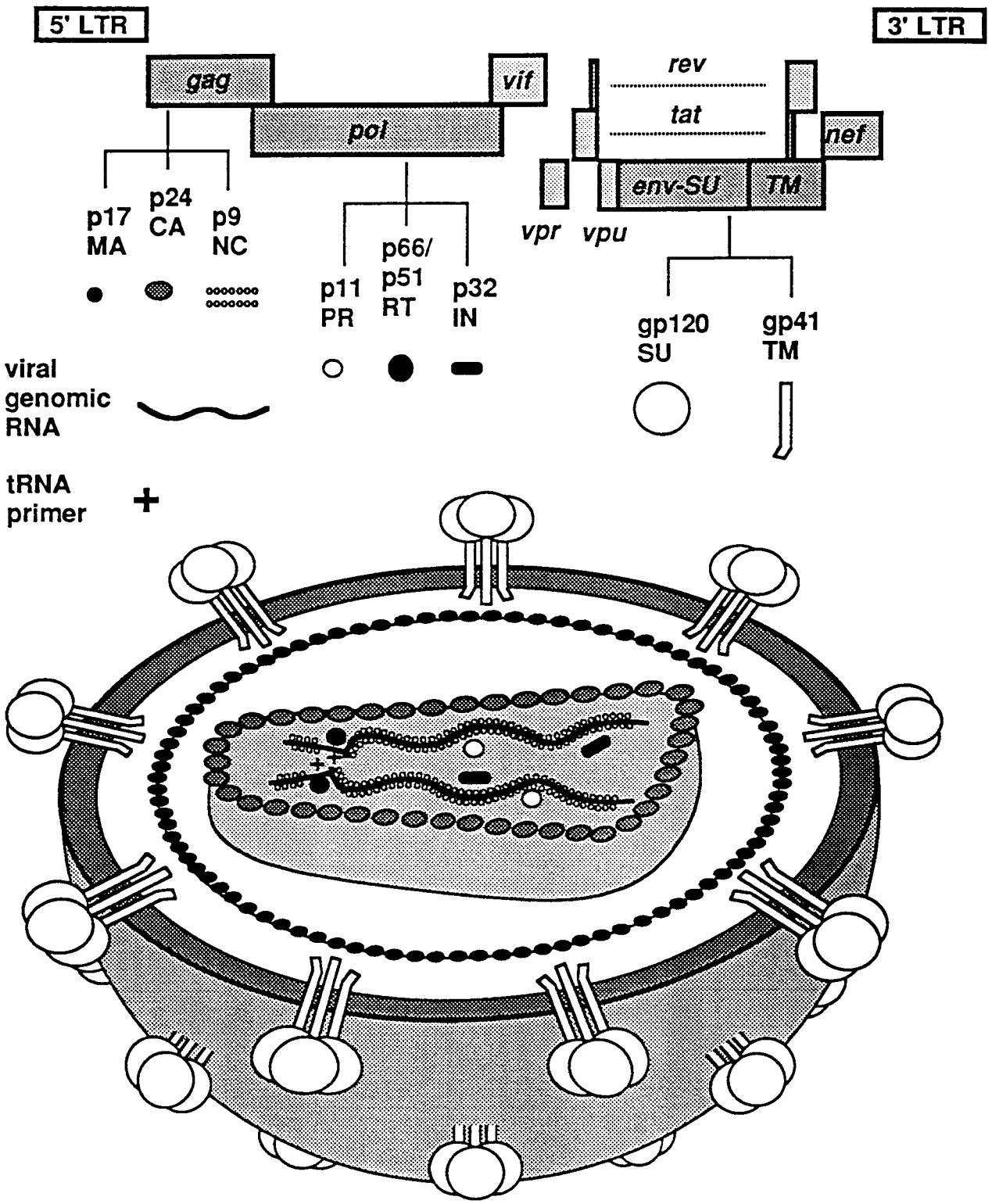


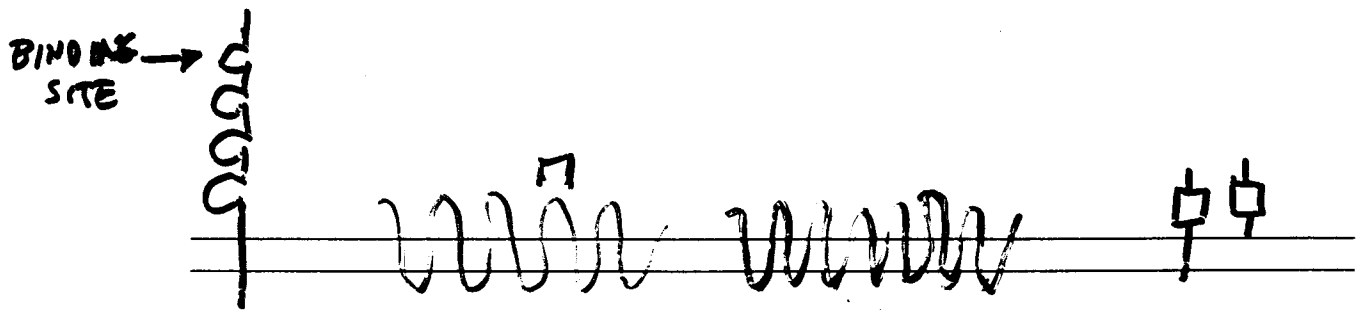
FIGURE 3. Virion morphology.

COURTESY OF PAUL LUCIW

DETERMINANTS OF VIRUS ENTRY AND HOST RANGE

ENTRY MEDIATED BY VIRAL SURFACE ENV PROTEIN AND VARIETY OF CELL SURFACE RECEPTORS WITH WIDE RANGE OF NORMAL FUNCTIONS...

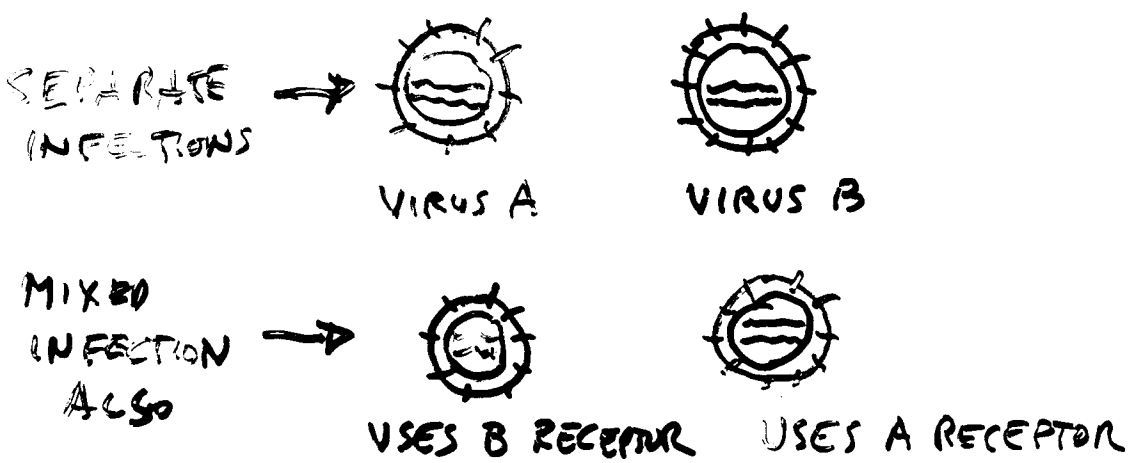
INFECTION BLOCKED BY: ABSENCE OF RECEPTOR OR CO-FACTOR
HOMOLOGOUS INTERFERENCE, DOWN REG.
ANTIBODY AGAINST SU-ENV OR RECEPTOR
SOLUBLE RECEPTOR



VIRUS :	HIV	GALV/FLV-B	MLV-ECO	RSV-A
RECEPTOR :	CD4 (REQUIRES COFACTOR)	?TRANSPORTER	BASIC AA PERMEASE	LDL-R RELATED
pH :	INDEP.	?	DEP.	INDEP.

SURFACE INTERACTIONS ARE MAJOR DETERMINANT OF VIRAL HOST RANGE AND TISSUE TROPISM

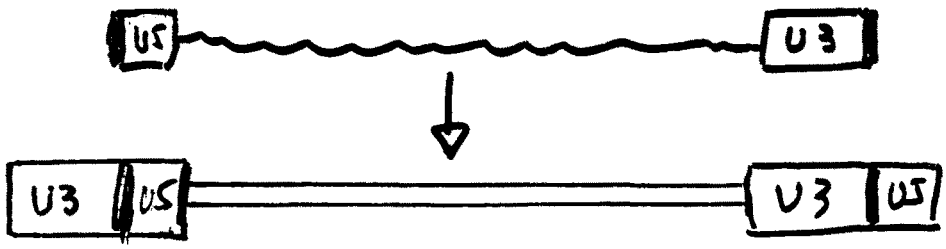
RESTRICTION CAN BE OVERCOME BY FORCED FUSION, PROVIRAL TRANSFECTION, AND PSEUDOTYPE FORMATION



EARLY EVENTS: THE NUCLEOPROTEIN COMPLEX

A PRODUCT OF **ENTRY AND UNCOATING,**
COMPOSITION AND STRUCTURE UNCERTAIN

VEHICLE FOR **REVERSE TRANSCRIPTION** IN CYTOPLASM, TO MAKE FULL-LENGTH LINEAR DUPLEX WITH **LONG TERMINAL REPEATS**

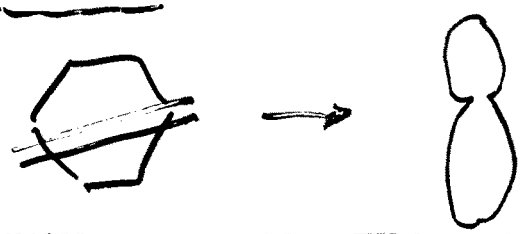


MIGRATION TO THE NUCLEUS:

* REQUIREMENT FOR NUCLEAR MEMBRANE BREAKDOWN IN MITOSIS (MLV) VS. DIRECT ENTRY (HIV)

* PRESUMPTIVE DEAD ENDS: CIRCLES WITH ONE OR TWO LTRS

INTEGRATION MACHINE FOR INSERTING DNA INTO CHROMOSOMES



HOST RESTRICTION VIA NPC: SOME **FV-1** ALLELES RESTRICT GROWTH OF SOME STRAINS OF MLV (VIA CAPSID DETERMINANT), OPERATING AFTER ENTRY AND BEFORE INTEGRATION....

PRINCIPLES OF RETROVIRAL REVERSE TRANSCRIPTION

*** PROPERTIES OF ENZYME....RNASE H AND DNA POLYMERASE**

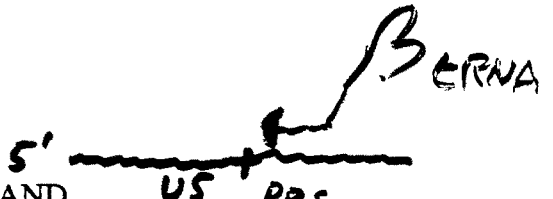
HOLOENZYME (INTRINSIC TEMPLATE:PRIMER, DISRUPTED VIRIONS)

ISOLATED ENZYME (FROM VIRIONS, MADE IN BACTERIA)

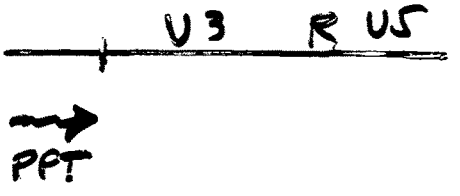
*** HETERODIMERS (HIV, RSV) VS. MONOMERS (MLV)**

	RT	RNASE H	IN
RSV			
HIV			
MLV			

*** TEMPLATES AND PRIMERS**



NATURAL PRIMERS: tRNA FOR FIRST (MINUS) STRAND
POLYPURINE OLIGO-RNA FOR PLUS STRAND



EXOGENOUS TEMPLATE-PRIMERS: mRNA-OLIGO dT
GAPPED DNA
POLY rA: dT OR POLY rC: dG

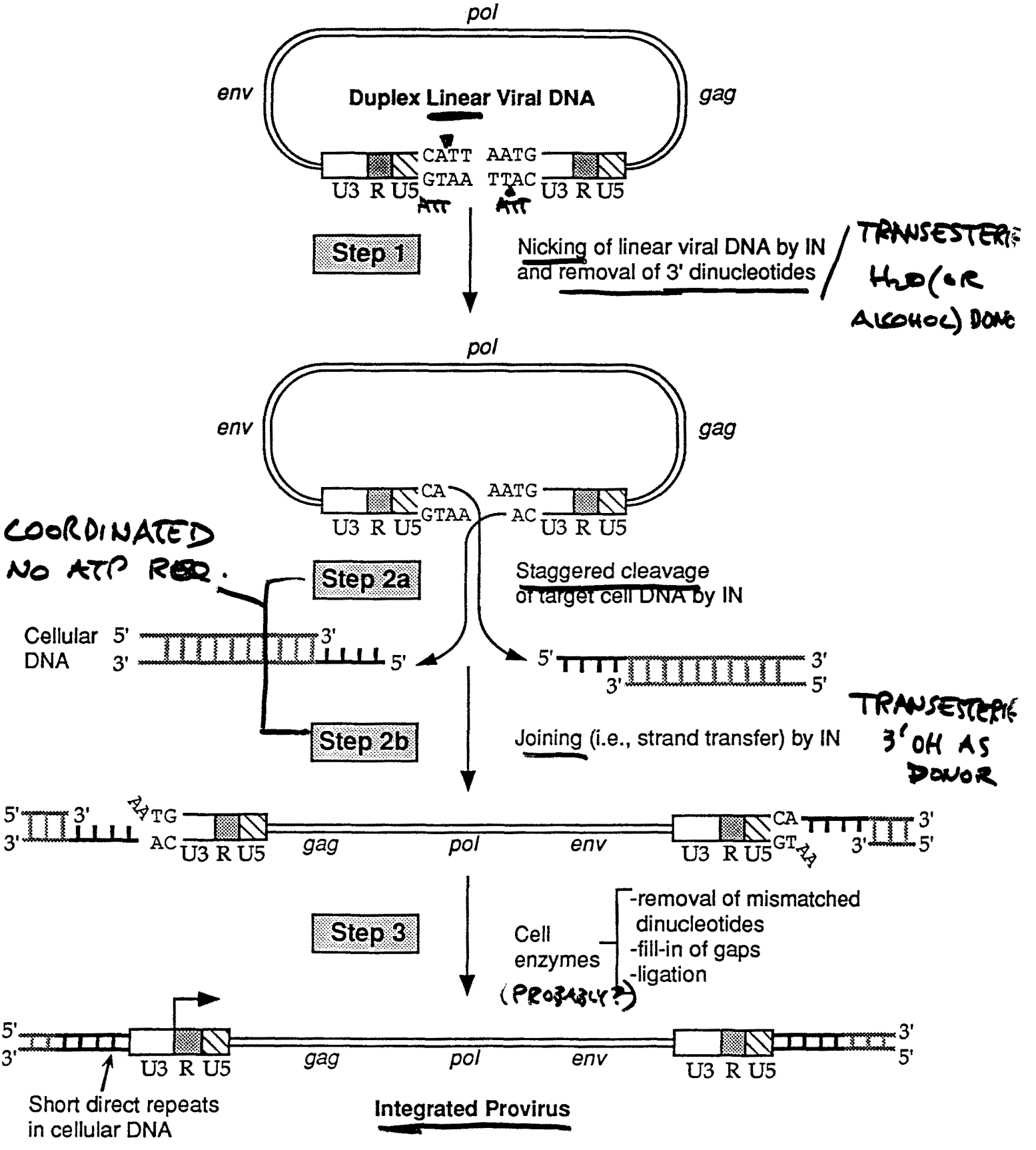
*** JUMPS, METASTABILITY, ERRORS**

*** INHIBITORS: NUCLEOSIDE ANALOGS, OTHERS**

AZT
ddI
ddC

Nevirapine
Pyridinone
TIBO
etc.

CURRENT VIEW OF INTEGRATION



PRINCIPLES OF RETROVIRAL INTEGRATION

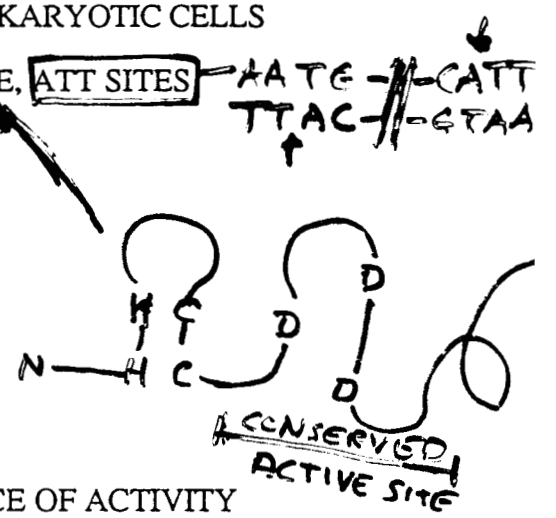
THE BEST UNDERSTOOD RECOMBINATION EVENT IN EUKARYOTIC CELLS

MACHINERY: NUCLEOPROTEIN COMPLEX, INTEGRASE, ATT SITES

PRECISION AT VIRAL ENDS, MANY SITES IN TARGET

NO REQUIREMENT FOR ENERGY SOURCE

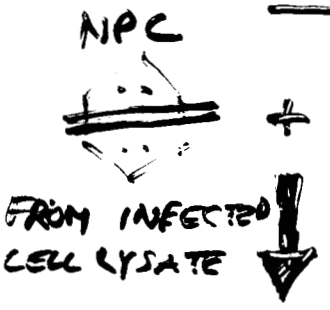
ESSENTIAL FOR REPLICATION AND PERSISTENCE



ASSAYS:

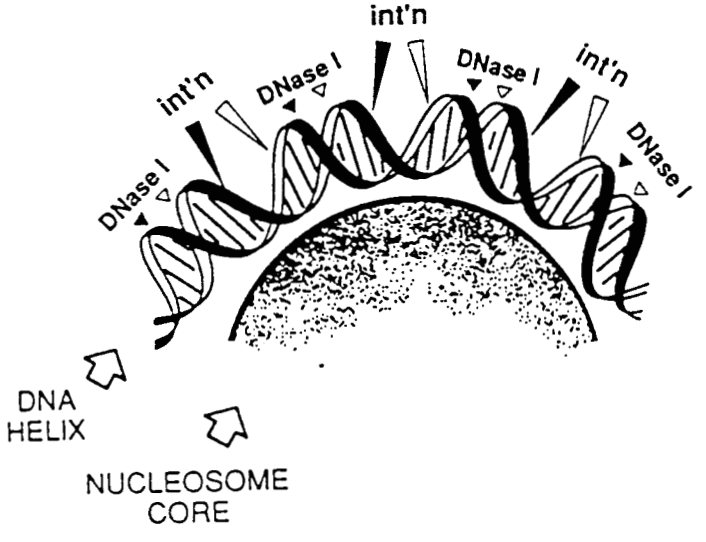
INFECTED CELLS....MAPPING, CLONING, PCR

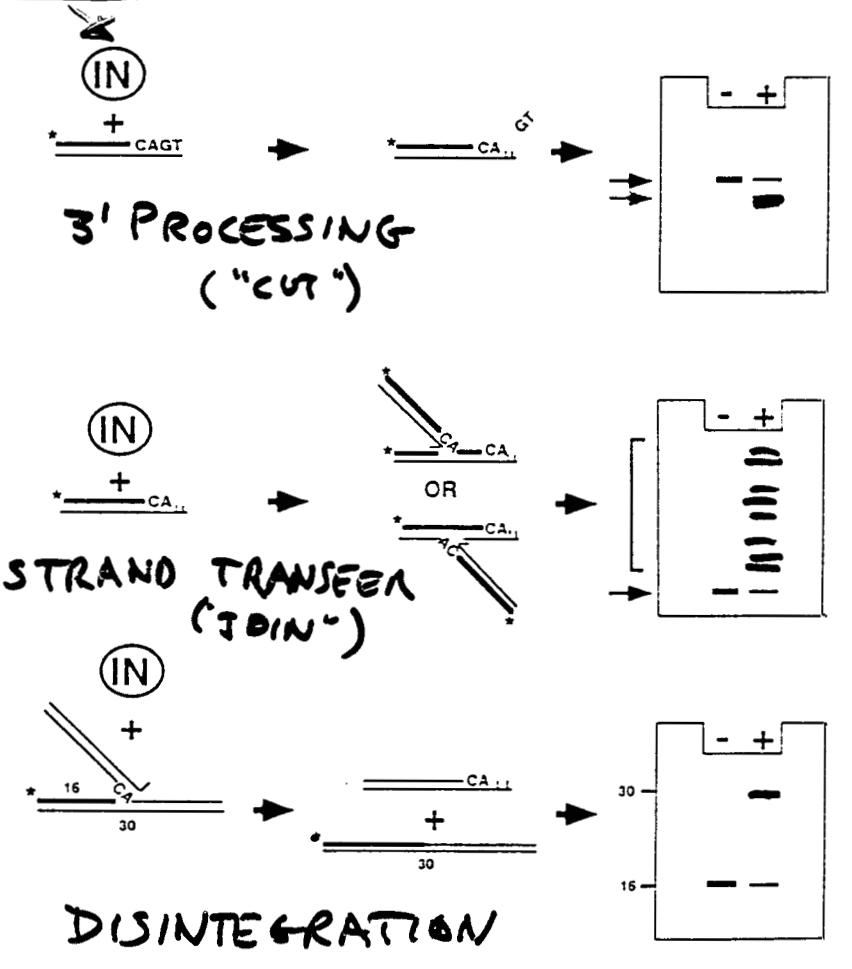
IN VITRO...NPC VS. PURIFIED INTEGRASE AS SOURCE OF ACTIVITY



PHYSICAL OR GENETIC
TEST FOR RECOMBINANTS.

ROLE OF NUCLEOSOMES





CONTROL OF PROVIRAL TRANSCRIPTION

1) HOST TRANSCRIPTION FACTORS INTERACT WITH TARGETS IN U3

* DETERMINES LEVEL OF EXPRESSION

* INFLUENCES TISSUE TROPISM AND PATHOGENIC TARGET

MLV: ERYTHRO VS. T CELL LEUKEMIA

MMTV: MAMMARY VS. T CELL TUMORS

2) VIRAL FACTORS AS TRANSCRIPTIONAL CO-ACTIVATORS

* TAX OF HTLV/BLV

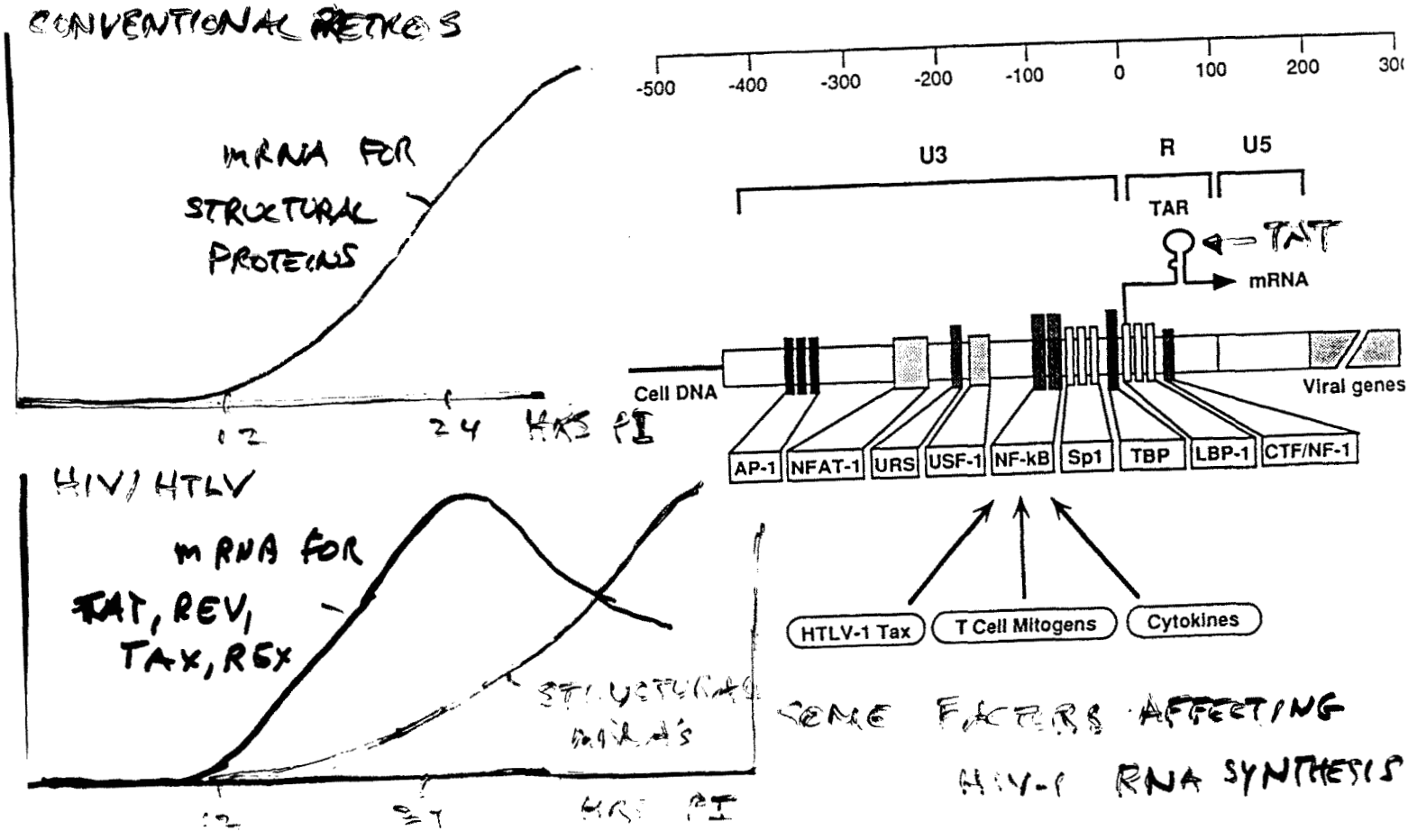
* BEL OF FOAMY VIRUSES

3) VIRAL FACTORS AS REGULATORS VIA VIRAL RNA TARGET

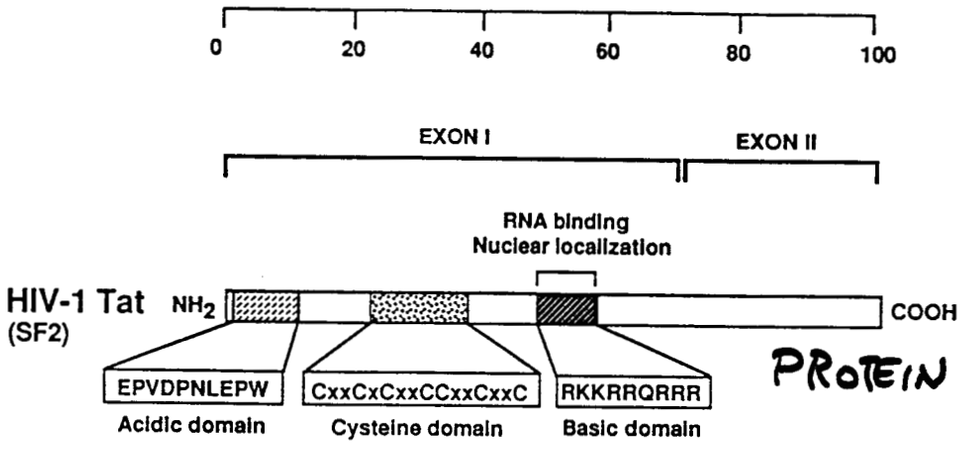
* TAT/TAR OF HIV + SIV

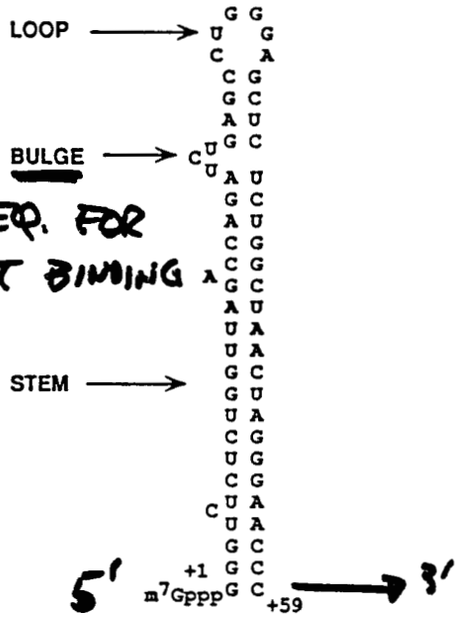
* ABSOLUTE REQUIREMENT FOR VIRUS PRODUCTION

* ROLE IN TEMPORAL CONTROL OF LATE EVENTS (AND LATENCY?)



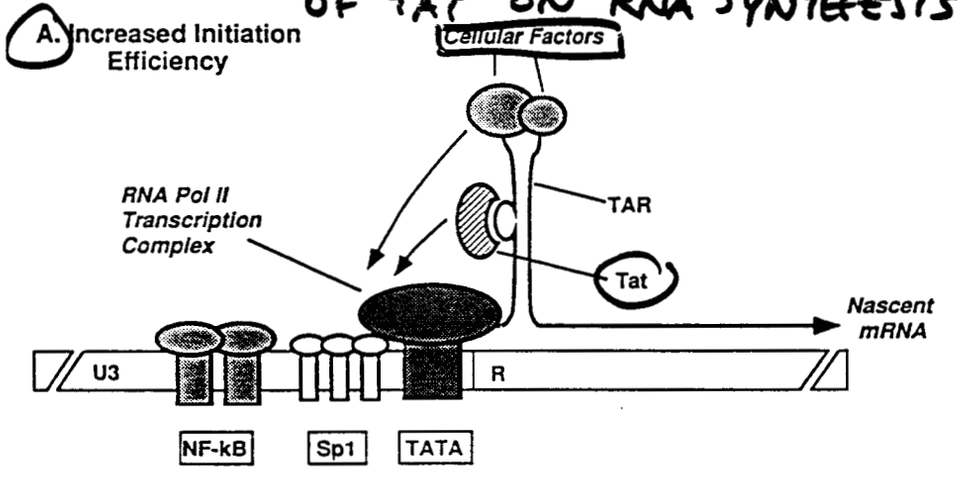
TAT & TAR

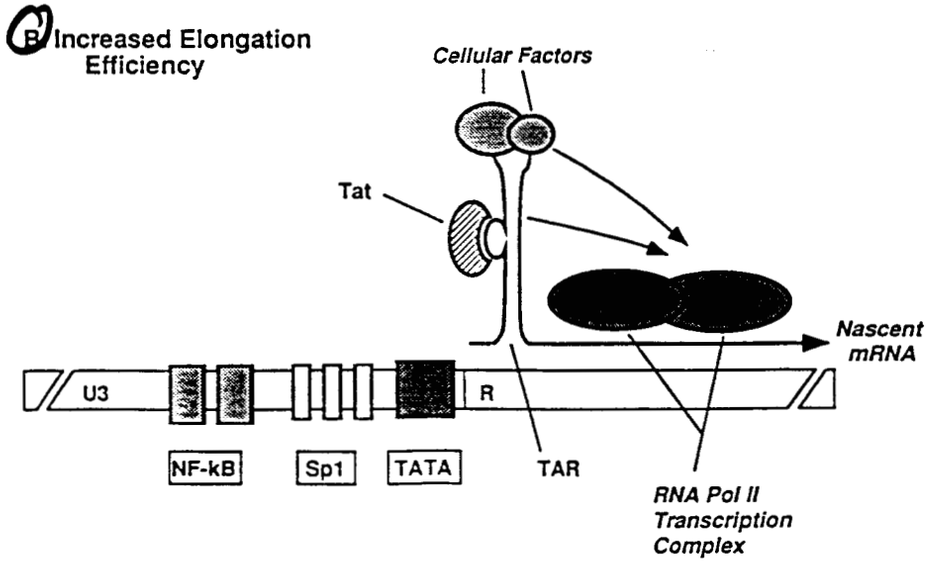


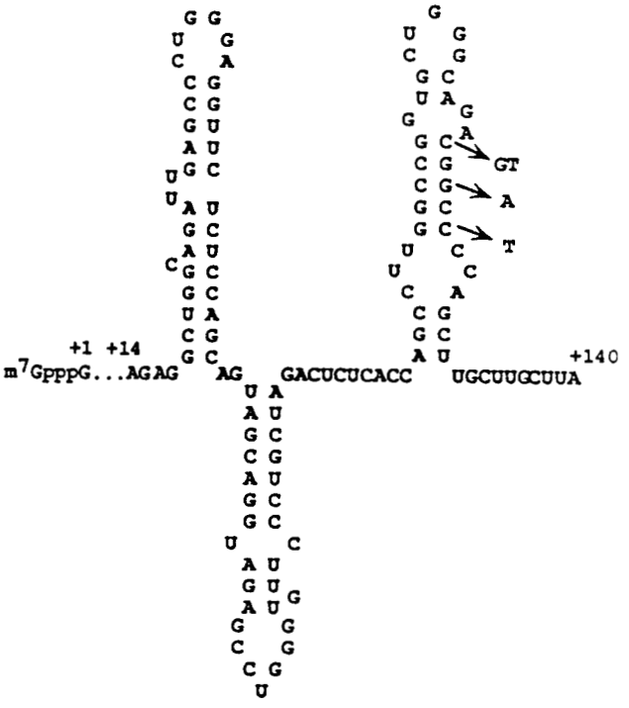


HIV-1 TAR

MODELS FOR RNA-MEDIATED ACTION OF TAT ON RNA SYNTHESIS





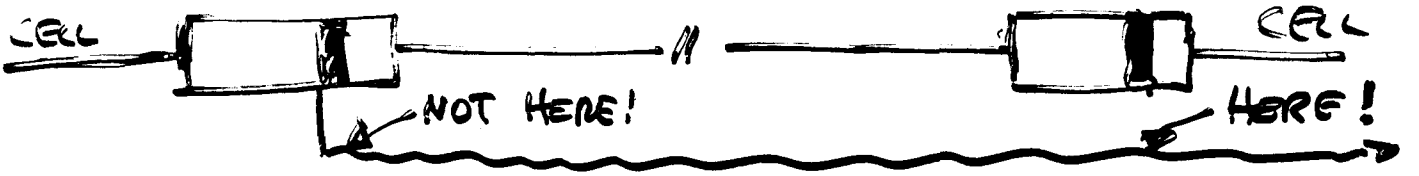


HIV-2 / SIV_{mac} TAR

PROCESSING OF RETROVIRAL RNA

CAPPING 5' ENDS

POLYADENYLATION: RESPONDING TO SIGNALS FROM 3' (NOT 5') LTR



SPLICING AND TRANSPORT TO CYTOPLASM:

- MAINTAINING AN APPROPRIATE RATIO OF RNA'S

  GENOME (GAG mRNA) vs ENV mRNA vs OTHER mRNA

  (N.B.: PRE-mRNA IS GENOME AND ALSO mRNA)
- FOR SIMPLE GENOMES: CIS SIGNALS SUFFICE
- FOR COMPLEX GENOMES:

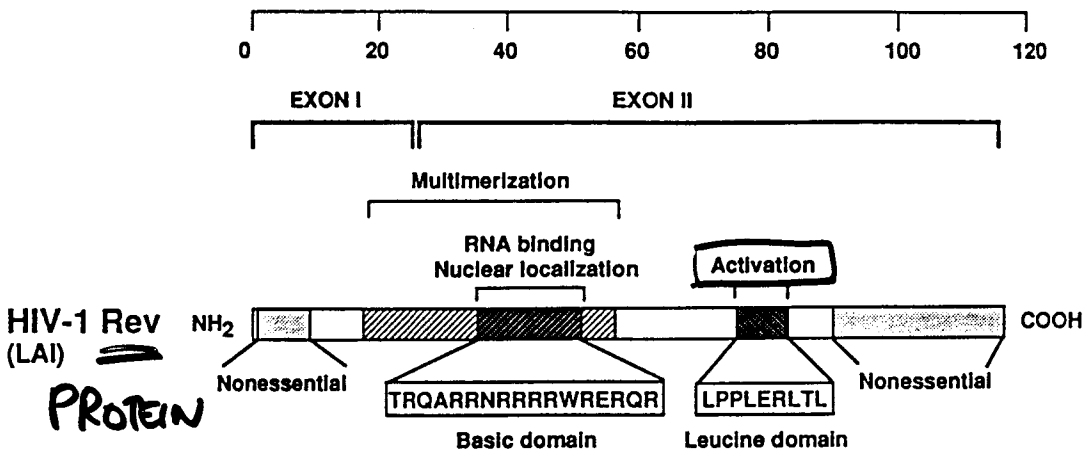
  APPEARANCE OF INCOMPLETELY SPLICED mRNA IN

  CYTOPLASM GOVERNED BY REX OR REV VIA RXRE OR RRE

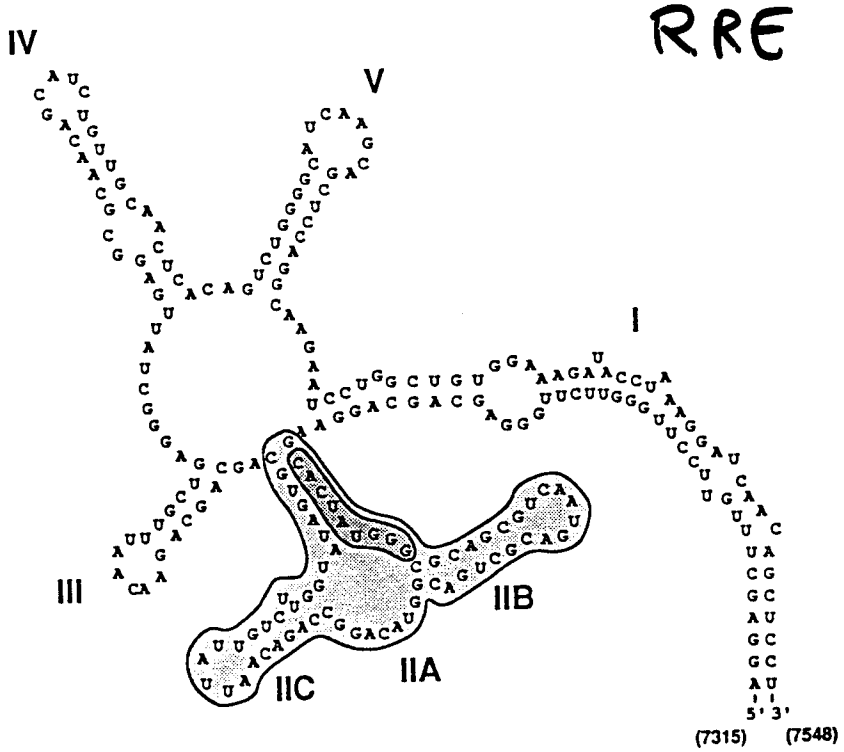
CYTOPLASMIC RNA
NORTHERNS

	MLV, ALV, etc.	HIV REV+	HIV REV−
GENOMIC gag mRNA	—	—	
ENV mRNA (spliced)	—	—	
TAT, REV, etc.	—	—	[redacted]

REV AND RRE OF HIV-1



RRE (IN ENV REGION)



CONTROL OF RETROVIRAL TRANSLATION

EXPRESSION OF POL GENES VIA TRANSLATIONAL READ THROUGH

- AVOIDS SPLICING OR INTERNAL START
- MAINTAINS RATIO GAG/GAG-POL
- PACKAGING POL

1) NONSENSE SUPPRESSION (MLV)

PR

----------GAG----------UAG----------POL----------

AA4 GLN

2) MINUS ONE FRAMESHIFTING---ONCE (RSV, HIV) OR TWICE (MMTV, HTLV)

RSV, HIV ----------GAG----------

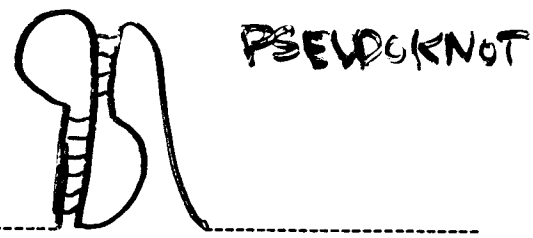
----------POL----------

MMTV, HTLV ----------GAG----------

-----PRT-----

----------POL----------

SIGNALS: SHIFTY SITE, RNA STRUCTURE 3' OF SITE, SPECIAL tRNA



----------X XXY YYZ----------

MECHANISM: SIMULTANEOUS SLIPPAGE AFTER DECODING YYZ

FREQUENCY: 3% TO 20%

OTHER VENUES: CORONOVIRUSES, RETROTRANSPOSONS, YEAST DS RNA, BACTERIAL TRANSPOSONS, PLANT VIRUSES, E.COLI DNA X.....

NO EUKARYOTIC HOST GENES AS YET

RULES GOVERNING RETROVIRAL ASSEMBLY

CAPSID-CAPSID INTERACTIONS DRIVE ASSEMBLY OF CORE

GAG PRECURSOR SUFFICES

INTERACTIONS WITH CA PORTION OF GAG-POL INCLUDE POL

MATRIX DETERMINES SITE OF ASSEMBLY

ACETYLATION/MYRISTYLATION REQUIRED TO GET TO MEMBRANE

MATRIX VARIANTS FOR ASSEMBLY AT PLASMA MEMBRANE (MOST),

CYTOSOL (TYPE D), OR ER (MUTANTS)

NUCLEOCAPSID REGION INTERACTS WITH PSI SITE ON VIRAL RNA,

AND FACILITATES DIMERIZATION

RT PROMOTES INCLUSION OF APPROPRIATE TRANSFER RNA (PRIMER)

ENV PROTEINS MODIFIED, OLIGOMERIZED IN ER AND GOLGI

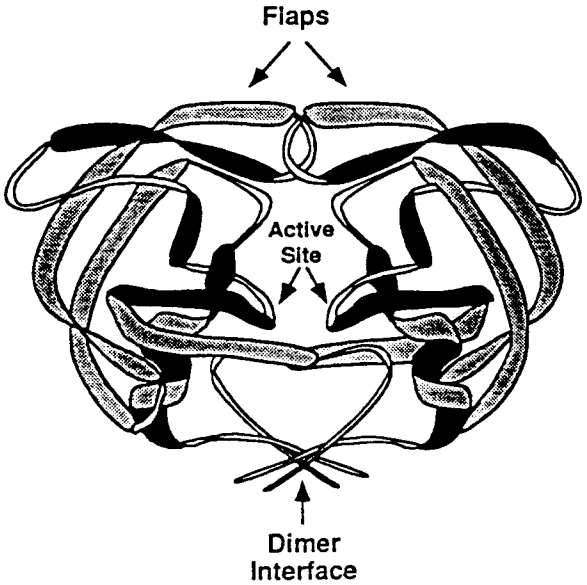
BUDDING AT SITE OF ENV PROTEIN: MOST HOST TM PROTEINS EXCLUDED

(?) HOW DOES CORE RECOGNIZE ENV-RICH PLASMA MEMBRANE?

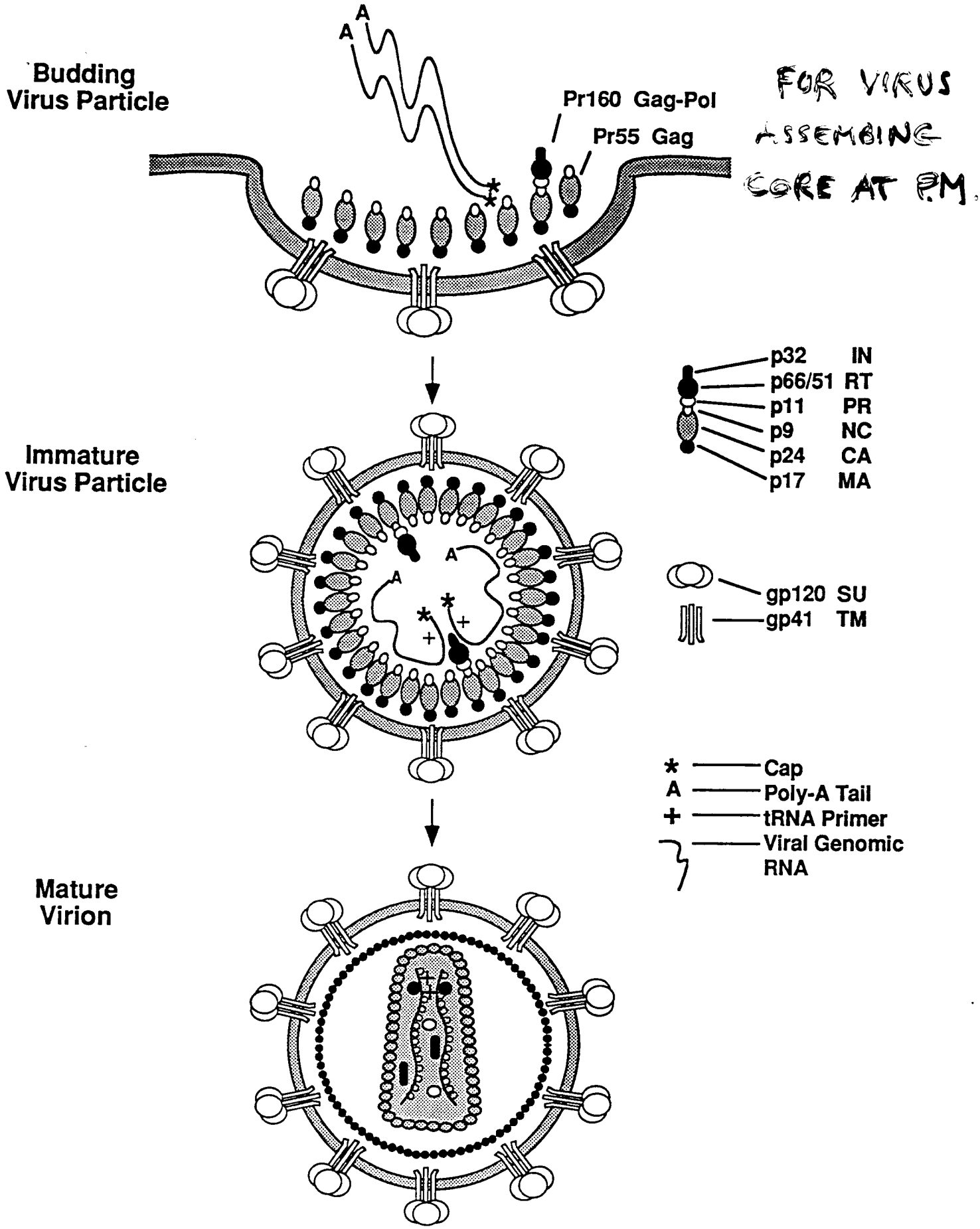
MATURATION: DIMERIZATION OF **PROTEASE** REQUIRED FOR ACTIVITY,

CLEAVES GAG AND GAG-POL AT SPECIFIC SITES

COINCIDENT WITH CORE CONDENSATION, INFECTIVITY, RT ACTIVITY



	Sites		Sequence						
RSV	junction		P4	P3	P2	P1	P1'	P2'	P3'
gag	MA	-	Gly	Thr	Ser	Cys	Tyr	His	Cys	Gly
gag		- p10	Pro	Pro	Tyr	Val	Gly	Ser	Gly	Leu
gag	p10	- CA	Pro	Val	Val	Ala	Met	Pro	Val	Val
gag	CA	-	Ile	Ala	Ala	Ala	Met	Ser	Ser	Ala
gag		- NC	Gln	Pro	Leu	Ile	Met	Ala	Val	Val
gag	NC	- PR	Pro	Pro	Ala	Val	Ser	Leu	Ala	Met
pol		- RT	Arg	Ala	Thr	Val	Leu	Thr	Val	Ala
pol	RTα	- IN	Thr	Phe	Gln	Ala	Tyr	Pro	Leu	Arg
pol	IN	-	Ser	Pro	Leu	Phe	Ala	Gly	Ile	Ser
HIV										
gag	MA	- CA	Val	Ser	Gln	Asn	Tyr	Pro	Ile	Val
gag	CA	-	Lys	Ala	Arg	Val	Leu	Ala	Glu	Ala
gag		- NC	Thr	Ala	Thr	Ile	Met	Met	Gln	Arg
gag	NC	-	Arg	Pro	Gly	Asn	Phe	Leu	Gln	Ser
gag		- p6	Glu	Arg	Gln	Ala	Asn	Phe	Leu	Gly
pol		- PR	Val	Ser	Phe	Asn	Phe	Pro	Gln	Ile
pol	PR	- RT	Cys	Thr	Leu	Asn	Phe	Pro	Ile	Ser
pol	RT66	-IN	Ile	Arg	Lys	Ile	Leu	Phe	Leu	Asp



. Model for virion assembly.

RETROVIRAL GENETICS

EXTREME CASES: HELPER CELLS FOR RV VECTOR

COMPLEMENTATION

HELPER GENOMES FOR GAG, POL, ENV COMPLEMENTATION

ENV PSEUDOTYPES (BETWEEN ANY TWO RETROVIRUSES, RETROVIRUSES AND VSV) AFFECT HOST RANGE

MUTATION

RATES SIMILAR TO OTHER RNA VIRUSES

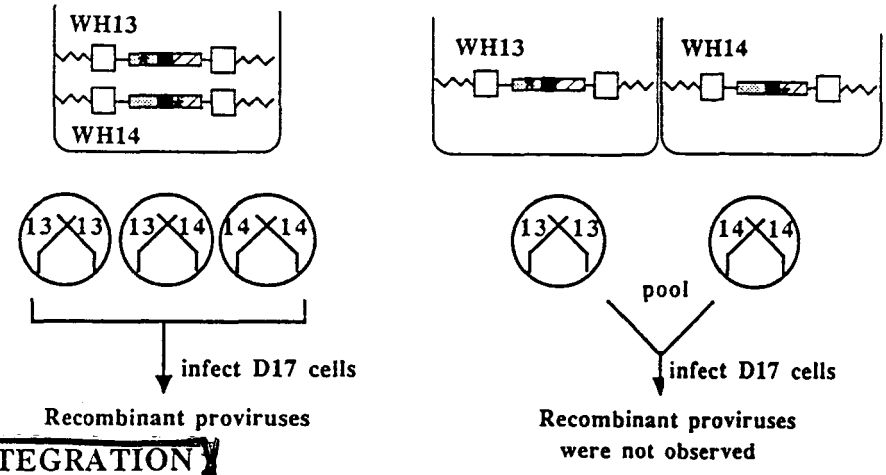
RECOMBINATION

HIGH FREQUENCY (TOO HIGH FOR GENETIC MAPPING!)

HETEROZYGOTES ARE OBLIGATE INTERMEDIATES

RECOMBINANTS FORMED DURING REVERSE TRANSCRIPTION

HU & TEMIN 1990



CONSEQUENCES OF INTEGRATION

INSERTION MUTATIONS (ACTIVATING OR INACTIVATING)

INTRODUCTION INTO GERM LINE

→ TRANSDUCTION OF HOST GENES...

→ ENDOGENOUS RETROVIRUSES

ENDOGENOUS PROVIRUSES

LEVELS OF COMPETANCE

* ENCODE INFECTIOUS VIRUS (MMTV, XENOTROPIC MLV, RD114 ETC)
* ENCODE VIRAL PROTEINS ONLY (GAG OR ENV)
* NO PRODUCTS

NUMBERS AND ORIGINS

* RECENT ENTRY (POST-SPECIATION)...VARIATION AMONG INDIVIDUALS, 0-10 COPIES (ECOTROPIC MLV, MMTV, ALV)
* ANCIENT SURVIVORS....UNIFORM IN SPECIES, MAY BE 100 TO 1000 COPIES (XENOTROPIC MLV, IAP'S, ENDOGENOUS HUMAN PV'S)

OBSERVED GERM LINE INSERTIONS

* SOME VIRUS-PRODUCING MOUSE LINES
* INFECTION OF PRE-IMPLANTATION EMBRYOS
* ??INFECTION OF MALE GERM CELLS

MUTAGENIC POTENTIAL (EXAMPLES)

* IN GERM LINE: HPRT, DILUTE (REVERTS BY LTR-LTR EXCISION) COLLAGEN ALPHA 1A (MOV 13), OTHER DEVELOPMENTALS
* MOVEMENT IN SOMATIC CELLS: IAP INTO PROTO-ONCOGENES

ROLE IN EVOLUTION ?