Harold Varmus 12/14/92

(see Q's posed to Baltimore)

11) site — Cell (July?)
chromatin / insert.

viral DNA → chromosomes
in vitro search / viral integration
the process isn't just copy of DNA — it's actually a "machine": DNA of virus to be integrated
"viral integration machines" → mediate entry of DNA then carry ⇒ foreign DNA
machine: RTAase
Integrase
viral core components
virus has just infected cell
↳ get RTAase rxn in cytoplasm
↳ complex gets into nucleus
↳ gets DNA into chromos.

set up in vitro:
- made chromatin for in vitro use
- viral integ. machinery has pref. for nucleosome sites
↳ prior argument
- they saw that when DNA wrapped into nucleosomes becomes more reactive
(nucleosome ~140 bp DNA) wrapped DNA twice
- can see histones almost always in specific sites
↳ and are preferred integ.

sites
The DNA is bent around histones
the conformation altered
and becomes good site
for integrase
Can simulate this by just
bending the DNA
(not chemical re: the histones)

PCR to analyze insert. events =>
@ single bp insert. => zipper
declares there is a path

Is not random

But there are lots of sites
probably 100s of 1000s of sites

Correlate bends & bends w/ host
promoters, etc?
↳ no evid. nucleosome-free
sites are preferred integ.
sites

Can inject cells w/ SV40 → then
use as a target for integ.
↳ not a physiological
signif. insert. site

Abil. virus to induce a tumor
↳ any gene → cell will dominate
over other cells & overgrow
neighbors

@ first level of viral activity
required for virus = insert
@ proto oncogene
↳ next evol. level is for

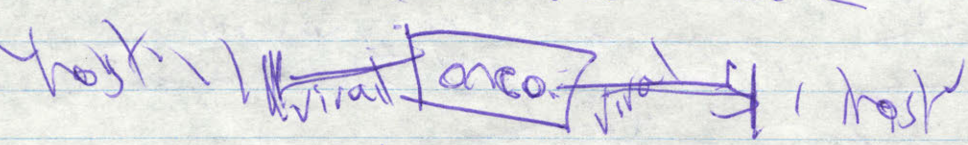
②

virus to capture the protooncogene
→ virtually all oncoviruses have been generated in nature
→ find them in the tumors

~1/2 oncogs. we work w/ are found by taking tumors from animals in poultry farms & look for virus that can transform the oncogenic trait to other cells
then look in tumors to see where the pro-gene lodges

All evid. favors idea that capture is initiated by the protooncogene, itself
→ not a direct effect
but the oncogen stims. tumor
→ tumor provides 10^8-10^9 cellular factories
→ ↑ statistical poss. for genetic events to occur

Need rare events to occur for capture to occur:
- viral seg. 5' stream of oncogene segs. and downstream



↑ get capture

RTase

<u>RTVs are diploid</u>

means recomb. more likely → every virus particle has 2 nonidentical genomes
↳ so won't selective copy occurs (which genome)
↳ and selected copy dominates in infection
↔ is a time when both could get copied, both inserted,
One way to get that capture event
But not only way
Howard Temin, John Coffin (in Bost.)
↳ call him $$ TUFTS

Randomness: still needs to be further understood
- ~~they~~ he's looking @ MTV to see freq. of insertion to stimulate specific genes
↳ assay prob., so no answer

Jumping genes, transposable elements in virus
- has been one in cancer system (retrotransposons)
over evolution our genomes have many 1000s of transposable elements in them to influence gene express.
One type of ~~hem~~ hemophilia is so
There aren't good assays for determining how our genomes are influenced by the elements w/in them.

3

Rivet to me: conservation of properties of these elements:
- integrases (transposases) fruit fly ones are amazingly similar to RTV one
- ~~shift of~~
- RTVases have problem making RTase: pol gene doesn't have own promoter; requires a frame shift to trigger

~~gag~~ pol overlap; shift back to get read

~~also found in bacterial transposons~~

Used widely in transposable

these elements don't have conventional introns, are very small & compact

Cytokine — Cell (see review of issue)
→ my assumpt. is that were see products of evolut. that have devel'ed for a reason

RTVs mutate rapidly → if function unnecess. would be lost

Harder to see where the functions come from

How arise? Where tat or rev come from?
dunno
some primordial gene

HTLVs & HIVs are suscep. to cytokines for their replicate.
↳ may be true in other systems, too - just hasn't been studied heavily
Work w/ HIV & HTLV → intense interest → regulation of virus
Latency of disease key: how sustain latency

virus gets genes from host - how?
Unclear. Incomplete knowledge. Most agree that:
- has to be recomb. viral & host genet. info. Not independent traitors → direct covalent linkage
- recomb. by integration of viral DNA next to host DNA of interest
- has to be 2nd recomb. event → in a virus particle → dimeric genome (wt + chimera derived from region of chromosome the viral DNA is adjacent to. Recomb. by moving the templates... generates recomb. events
John Coffin & Howard Temin have worked on this
↳ see his paper in Science on this
see Varmus/Bishop review on this 10 yrs ago