

PD-AFW-655

12906

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE:

8/25/87

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18
FROM: AID/SCI, Victoria Ose
SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No.

C 5-336

Progress - Jan 1, 87 - June 30, 87

Attachment

2 up

1/ PD-AAW-655

PROGRESS REPORT

January 1, 1987 - June 30, 1987

CS-336

Title: Preparation of a live vaccine against
the Caprine Arthritis-Encephalitis Virus (CAEV).

Project No. DPE-5544-G-~~55~~-6007-00

Investigators: A. Yaniv and A. Gazit
Department of Human Microbiology
Sackler School of Medicine
Tel-Aviv University
Ramat Aviv, 69978, ISRAEL

Rec'd in SC. AUG 21 1987

For the last six months we have been focusing on obtaining new molecular clones of CAEV, in order to get an infectious clone containing functional genes, that will enable the construction of CAEV env-vaccinia recombinant. Since our CAEV clone, that was molecularly cloned from the viral integrated form (provirus), was found to contain stop codons, presumably due to frameshift mutations, our new strategy was to molecularly clone the CAEV genome from its unintegrated circular form.

To this end, Himalayan tahr ovary cells were infected with CAEV. Following 14 days, when CPE was apparent, the infected cells were cocultivated with fresh tahr cells. At 24 h after cocultivation, extrachromosomal DNA was separated from chromosomal DNA by the method of Hirt, then extracted with phenol-chloroform, and ethanol precipitated. The purified DNA was digested to completion with Xba-I which cuts only once within the CAEV genome. The linearized permuted CAEV molecules were ligated to Xba-I-cleaved λ Wes B DNA using T4 DNA ligase. Following in-vitro packaging and propagation in Escherichia coli strain BNN 45, plaques were screened by hybridization with ^{32}P -labelled CAEV-DNA.

In these series of experiments, 15 CAEV clones were obtained. In order to search for a clone containing the entire viral genome, all clones were subjected to restriction endonuclease analysis using two methods:

a) Partial digestion of unlabelled - recombinant DNA followed by hybridization with ^{32}P -labelled oligonucleotide complementary to the left or right end of λ phage (using the phage λ mapping Quick - kit of Collaborative Research).

b) The restriction maps obtained were confirmed

by complete double enzyme digestions, and the maps were compared with the known restriction maps of our uninfected CAEV clone.

These restriction maps and especially the identifications of the deleted regions were further confirmed by hybridization to ^{32}P labelled probes obtained from various regions of CAEV genome.

As can be seen from the figure, all the clones obtained from CAEV unintegrated form, had deletions of various lengths, ranging in size from 1.3 to 7.0 kbp. Several major points can be withdrawn from these results:

1) All the clones had deletions extending from the LTR towards the env region, covering up to 6 kb of CAEV genome.

2) In several of the clones, containing large deletions (6-7 kb deletion) the deletions extended also downstream towards the gag gene covering regions no more than 0.3 kb downstream from the 5'LTR.

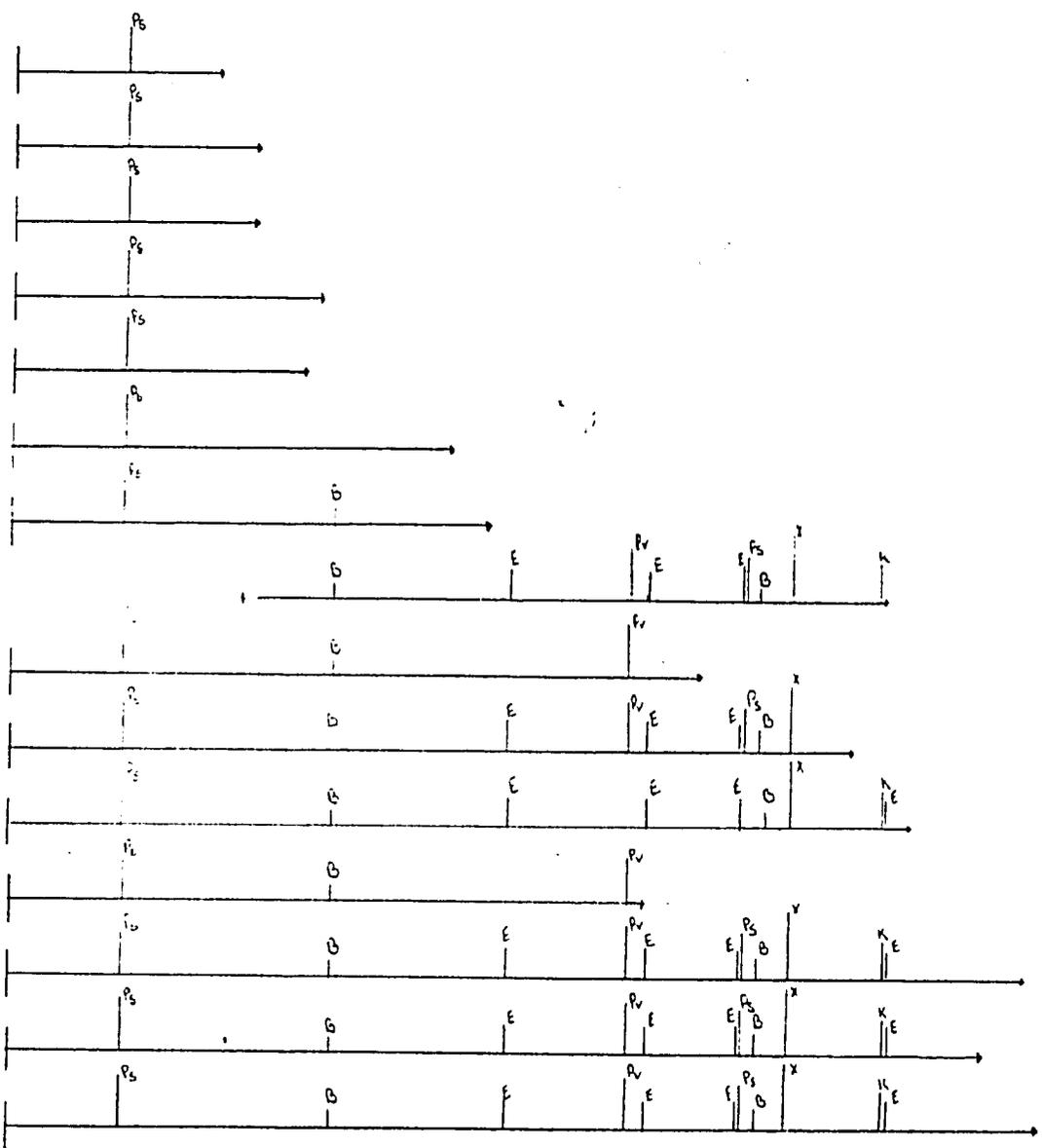
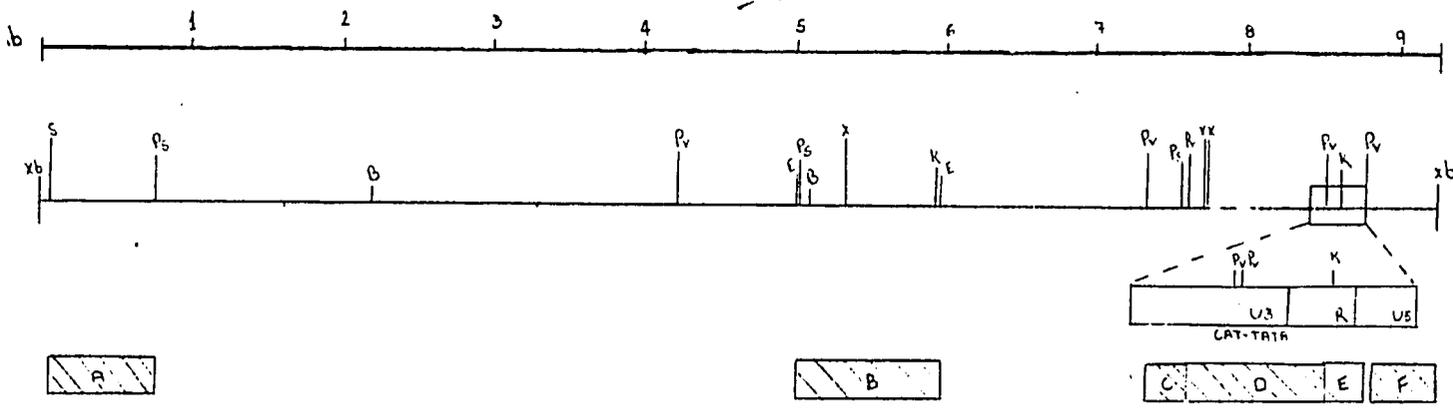
3) Data indicate that the starting point of the deletion is located within the LTR. The logic behind this conclusion is the following: In case the deletion starts within the LTR sequences, deletions extending further downstream (more than 0.5 kb into the gag region) would not be expected because clones were selected for XbaI site. Thus, the absence of internal deletions in the gag-pol regions, or env regions (except of one clone, No. 93) points to the conclusion that each deletion starts always within the LTR region, and extends mainly towards the env direction.

4) 11 out of the 15 clones contain part of the LTR region. In all of these, the R-U₅ sequences were totally preserved. Moreover, in all these clones also the Kpn I site was preserved, indicating the presence of promoter sequences.

The fact that all the clones obtained from the circular genomes were deleted, indicates the presence of very high proportion of deleted unintegrated form in CAEV-infected cells. If such a deletion event in circular DNA occurs prior to integration, perhaps by inappropriate cleavage by the int product, such a mechanism can interfere with efficient integration leading to aggregation of unintegrated forms, a fact which is characteristic to lentiviruses. Also, the presence of deleted genomes containing mainly U₅ sequences, can interfere with efficient virus production, possibly by competing with rate-limiting host factors essential for efficient virus expression. We are currently investigating this mode of restriction which may reveal mechanisms associated with the slow development of Lentiviral disease.

Nevertheless, since all the CAEV clones obtained from the unintegrated form suffer large lesions, it seems that cloning of an infectious molecule from the unintegrated DNA, is not feasible. Consequently, at present, attempts are being made to obtain infectious clones from the integrated form of CAEV.

5



- cl. 51 15kb
- cl. 59 17kb
- cl. 79 17kb
- cl. 127 24kb
- cl. 91 2.6kb
- cl. 55 3.5kb
- cl. 140 3.2kb
- cl. 148 5.0kb
- cl. 144 5.2kb
- cl. 116 6.3kb
- cl. 45 6.3kb
- cl. 93 6.3kb
- cl. 56 7.0kb
- cl. 142 7.0kb
- cl. 118 7.4kb

Enzymes: B-BglII E-EcoRI K-KpnI Ps-PstI Pv-PvuII S-SphI X-XhoI Xb-XbaI