

MHV Recombination In Vitro and In Silico

Karen A. Duca

Dept. of Electrical Engineering
and Computer Science
Tufts U.; Medford, MA
kad@eecs.tufts.edu

Anselm Blumer

Dept. of Electrical Engineering
and Computer Science
Tufts U.; Medford, MA
ablumer@eecs.tufts.edu

Mary A. Lokuta

Dept. of Pediatrics
U. of Wisconsin Medical School
Madison, WI
malokuta@facstaff.wisc.edu

ABSTRACT

MHV-JHM variant 2.2-v-1 and MHV-A59 are neurotropic strains of murine hepatitis virus that cause a demyelinating disease in mice similar to multiple sclerosis in humans [1]. We examined conditions that kinetically favor or disfavor the viral recombination between the two strains and quantified the outcomes of competition experiments using a modification of an assay developed to measure viral propagation rates. Infection was initiated at a specific central location in replicate cultures with both strains simultaneously, with staggered addition of the second virus, and with a single virus. Monolayers were fixed at set time intervals from 12 to 96 hours post inoculation. Two viral proteins (N and S) were detected by direct and indirect immunofluorescence using strain specific antibodies. The areas infected by each strain individually and both strains together (as indicated by the presence of viral protein) were calculated and expressed as a percent of total infected area. Since MHV replicates in the cytoplasm of infected cells, recombination between two viral strains must occur within a common cytosol. Therefore, both viruses must be present and actively replicating in that space. In our simultaneously infected cultures, we actually saw dramatic partitioning of both viruses into sectors radiating out from the center of the infection and very little evidence of co-infection. The dual infection was limited to regions at the intersection of singly infected sectors. Given this unexpected result, we constructed a rule-based infection simulation to determine whether the pattern of infection could result from stochastic processes, given the geometry of the initial infected region.

The simulation was written as a Java applet that models the spread of MHV-JHM and MHV-A59 on a hexagonal grid of cells (so every cell has six immediate neighbors). The hexagonal grid is 200 cells on each side, so there are approximately 120,000 cells. Normal cells are represented as

white, and infected cells are represented as green (JHM infected) or red (A59 infected) or a combination (co-infected, equal parts green and red producing yellow). Initially, a hexagonal region in the center is infected at random with JHM and A59 at equal rates. The size of this region and the initial infection rate can be specified by the user. We chose values that approximate the actual ratio of viruses to cells used in the experiments. The user can also specify the rate at which normal cells become susceptible to infection (acquire the MHV receptor), the number of time steps for which the virus lives in infected cells, and the number of time steps the simulation should run before displaying the next cell states. When an uninfected cell becomes susceptible, both the six immediate neighboring cells and the twelve neighbors one step further are checked for live viruses. Live viruses propagate at random, but in a five times greater quantity from the immediate neighbors than the cells one step away. The number of virions of JHM and A59 from these cells is summed and determines the nature of the infection of the susceptible cell. In a version with no superinfection possible, the majority type (JHM or A59) determines the infection type, otherwise the cell is infected proportionally by the two types. Using simulation parameters approximating our experimental conditions, we were able to reliably reproduce our experimental results with and without an assumption of superinfection. These results have implications for the resurrection and recombination of viruses persisting in the central nervous system.

ADDITIONAL AUTHORS

John O. Fleming (Dept. of Neurology; U. of WI; Madison, WI; fleming@neurology.wisc.edu).

REFERENCES

- [1] Johnson, R.T. The Virology of Demyelinating Diseases. *Annals of Neurology*, 36 (1994), S54-S60.