ENGINEERING VIRUS GROWTH BY GENE-ORDER PERMUTATION

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Abstract

How does the growth of an organism depend on the linear arrangement of its genes? For viruses, changes in gene order may inhibit or enhance growth, advancing their use as live attenuated vaccines, gene-therapy vectors or anti-tumor therapeutics. We have developed a kinetic model for the intracellular growth of vesicular stomatitis virus (VSV), an RNA virus that encodes five genes: 3´-N-P-M-G-L-5´. Our model accounts for the 120 possible permutations of these five genes and correctly predicts the growth ranking of virus strains having four of those permutations, NPMGL (wild-type), PNMGL, PMNGL, and PMGNL, confirmed by single-cycle growth experiments in the laboratory. Simulated growth yields for virus strains that carry the 120 permuted genomes span a factor of more than 1000, and wild-type VSV is predicted to grow with the third highest yield. By ranking the effects of gene position on growth we found that VSV growth is most enhanced by permutations that reduce the expression of protein L, the least abundant protein in wild-type VSV infections, or increase the expression of protein N, the most abundant protein in wild-type infections. This study shows how altering the expression profiles of the same set of five genes can dramatically affect the yield of progeny viruses.

Keywords

Gene order, permutation, genotype-to-phenotype, virus, model, kinetics

Introduction

What forces influence the linear order of genes within the genome of a virus? Answering this question may advance fundamental insights into how the expression profile of each virus gene contributes to the integrated development of virus progeny. Moreover, such insights can have practical consequences in the design of viruses for use as live vaccines, gene-therapy vectors and anti-tumor treatments. Previous work has shown how relocation of the gene encoding the RNA polymerase of bacteriophage T7 can influence its growth (Endy et al., 2000). However, phage T7 encodes 56 genes which has 56! ($\approx 10^{74}$) possible linear permutations of its genes, so only a vanishingly small fraction of total genome-design space can be studied by either experiments or simulations. Here we consider vesicular stomatitis virus (VSV), a negative-sense single-stranded RNA virus that has been well characterized (Rose and Whitt, 2001). VSV causes a disease similar to foot-and-mouth disease in livestock, and VSV has applications in the development of live vaccines (Rose et al., 2001; Wertz et al., 1998) , anti-tumor therapies (Barber, 2004), and in the quantitative characterization of innate immune responses (Ishitsuka et al., 1977). VSV encodes five genes that define only 120 gene-order permutations. The wild-type virus has a gene order of 3´-N-P-M-G-L-5´, where the letters refer to genes that encode nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and large protein (L). In addition to the wild-type virus, eight of the 120 gene-order permuted strains have been created using reverse genetics, changing the order of genes

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encoding N, P, M and G proteins (Ball et al., 1999; Wertz et al., 1998), as shown in Figure 1.

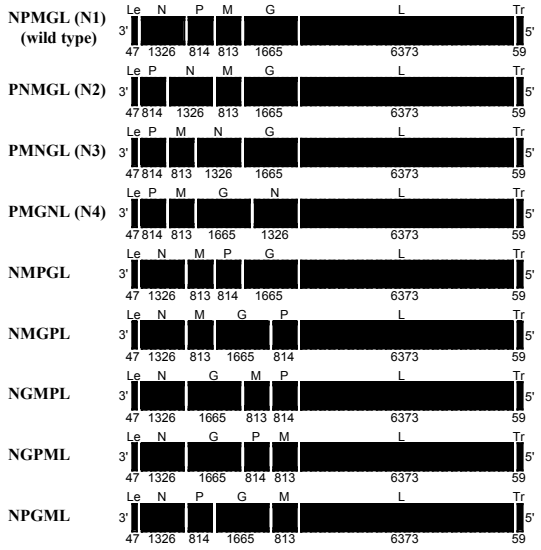

Figure 1. Genome structures of wild-type and engineered VSV strains. The number under each gene segment indicates the length [nucleotides (nt)] of the gene. Le and Tr denote the leader (47 nt) and trailer regions (59 nt) of VSV genome, respectively.

We probe the effects of gene order by developing a data-driven kinetic model for the intracellular growth cycle of wild-type VSV and extend our model to account for all 120 possible gene-order permutations of the VSV genes. The five VSV genes play well-established roles in the development of virus progeny, as summarized in Figure 2. Very briefly, the entering negative-sense RNA genome is transcribed by virion-associated VSV polymerase (proteins P and L). A controlled attenuation of transcription occurs in each inter-genic region, producing mRNA levels that progressively decrease from N to L. Hence, the expression level of any gene depends on its position within the genome; moving genes toward the 3′ or 5′ end of the genome respectively increases or reduces their level of expression. As N proteins accumulates in the cytoplasm, their association with newly synthesized or nascent anti-genomic RNA enables the elongating polymerase to bypass transcription attenuation signals, causing a switch from transcription to genome replication. Further, as M proteins accumulate, they associate with and condense the genomic nucleocapsid, diverting it away from transcription and replication processes, while directing it toward the formation of progeny virus particles. Finally, particle budding from the cellular plasma membrane incorporates protein G (not shown) into the surface of progeny viruses.

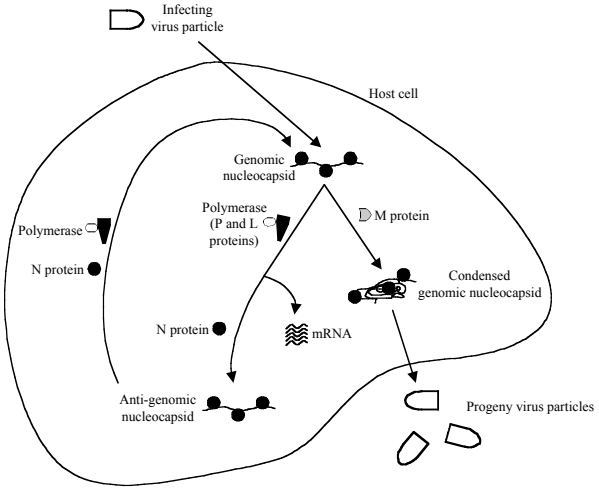

Figure 2. Simplified VSV growth cycle.

Results and Discussion

We fitted our model to one-step data (Lam et al., 2005) for the growth of wildtype VSV, N1 (NPMGL) and found that the model correctly predicted the progressive attenuation in growth for gene-order strains N2 (PNMGL), N3 (PMNGL) and N4 (PMGNL), as shown in Figure 3.

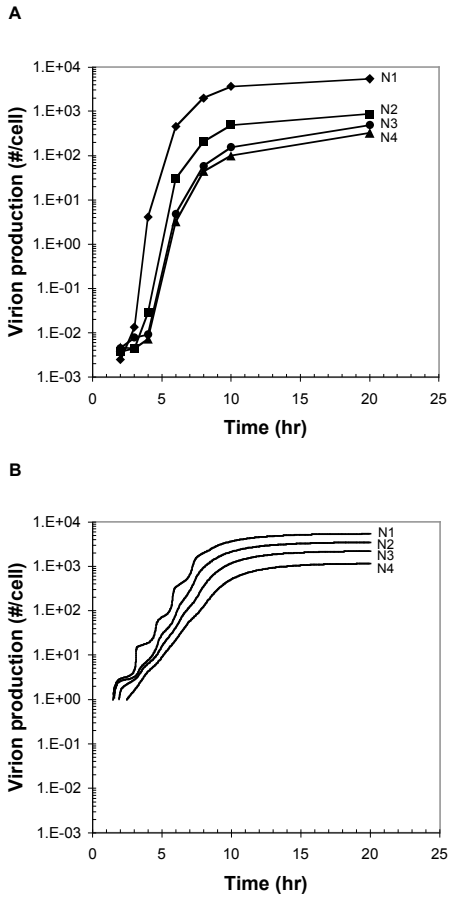

Figure 3. The growth of gene-rearranged VSV strains in BHK cells. (A) Experimental data (Lam et al. 2005). (B) Simulation results. The growth of the N1 strain (NPMGL, wt) is the fitting result, but the growth of the other three strains (N2 (PNMGL), N3 (PMNGL) and N4 (PMGNL)) is the model estimation result.

Although these rankings were correct, it is clear from a comparison of the experimental data and model predictions that quantitative features of the experimentally observed virus production are not yet fully captured; observed production of the different strains increases more rapidly and with greater differences in final yields than their simulated growth. Hence, this theoretical study of gene-order effects on virus growth offers a preliminary glimpse of potential trends. We used our model to estimate one-step growth yields for all 120 gene-order permuted genomes of VSV (Table 1). The wild type virus produced the third highest yield. Forty percent of the strains produced negligible progeny, giving yields less than the initial multiplicity of infection (MOI = 3), defined by the average initial number of infectious virus particles per cell. To correlate the genome organization of each strain with its progeny production, we partitioned the 120 strains into multiple groups based on the location of a specific gene (e.g., five groups based on the location of N gene, 3´-N-n-n-n-n-5´ (N1), 3´-n-N-n-n-n-5´ (N2), 3´-n-n-N-n-n-5´ (N3), 3´-n-n-n-N-n-5´ (N4), 3´-n-n-n-n-N-5´ (N5), where n encodes P, M, G, or L), and then averaged the progeny yields of the 24 strains in each group (Table 2). VSV growth appears to be most sensitive to gene-order permutations that increase expression of protein L, which is needed for RNA-dependent RNA polymerase activity and located at position 5 in the wild-type genome. Strains of the L5 group appeared to grow with significantly higher yields than strains of the other four groups (L1 ~ L4), consistent with the experimental observation that overexpression of L protein inhibits virus growth (Banerjee and Barik, 1992). VSV growth also appears to be sensitive to permutations that reduce expression of gene N, which codes for the nucleocapsid protein and is located at position 1 in the wild-type genome. This trend is consistent with the experimental and simulation results in Figure 3 as well as the experiments by Wertz and co-workers, who engineered the N-gene rearrangement strains (Wertz et al., 1998). In general, structural proteins (N, M and G) are in a greater demand than the enzymatic protein (L) during the viral infection cycle. Interestingly, gene P is second only to gene N in its level of transcription, yet the average progeny yield for the P2 group, which includes the wild-type virus, increased as P was moved toward the 5´ end, progressively reducing its level of transcription. This result suggests that VSV growth is relatively insensitive to the developmental context of P; only small amounts of P protein, made at any time, are adequate to supply the transcription and replication needs for the infection cycle and the low content of P protein in virus progeny particles.

Table 1. Ranking of gene-permuted VSV str ains based on progeny yield per BHK cell at 20 hr post infection.
** refers to recombinant VSV strains (Ball et al. 1999; Wertz et al. 1998).*

Ranking	Strain	Yield	Ranking	Strain	Yield
1	NMPGL*	6000	61	NPLMG	180
2	NMGPL*	5700	62	LMNPG	150
3	NPMGL (N1)*	5400	63	MLPNG	120
4	NGMPL*	5100	64	LNPMG	91
5	NPGML*	4900	65	PNLMG	83
6	NGPML*	4800	66	MPLNG	74
7	MNPGL	3800	67	LMPNG	61
8	MNGPL	3600	68	PMLNG	43
9	NGMLP	3600	69	LPNMG	36
10	NGLMP	3500	70	LPMNG	32
11	NMGLP	3500	71	PLNMG	21
12	PNMGL (N2)*	3500	72	PLMNG	19
13	NMLGP	3300	73	MGLPN	< 2
14	NLGMP	3100	74	NGLPM	< 2
15	NLMGP	3100	75	MLGPN	< 2
16	PNGML	3000	76	NLGPM	< 2
17	GNMPL	2800	77	GMLPN	< 2
18	GNPML	2500	78	MLPGN	< 2
19	MPNGL	2300	79	MGPLN	< 2
20	GNMLP	2300	80	LMGPN	< 2
21	GNLMP	2300	81	MPGLN	< 2
22	MNGLP	2300	82	GMPLN	< 2
23	PMNGL (N3)*	2200	83	MPLGN	< 2
24	MNLGP	2100	84	NLPGM	< 2
25	MGNPL	2100	85	GLMPN	< 2
26	NMLPG	2100	86	LGMPN	< 2
27	GMNPL	1900	87	LMPGN	< 2
28	GMNLP	1600	88	GNLPM	< 2
29	MGNLP	1500	89	PMGLN	< 2
30	PGNML	1500	90	NGPLM	< 2
31	GPNML	1400	91	GPMLN	< 2
32	LNMGP	1400	92	PMLGN	< 2
33	MNLPG	1400	93	NPGLM	< 2
34	LNGMP	1300	94	PGMLN	< 2
35	MPGNL	1300	95	GLPMN	< 2
36	MGPNL	1200	96	LGPMN	< 2
37	PMGNL (N4)*	1200	97	LPMGN	< 2
38	GMPNL	1100	98	NPLGM	< 2
39	GLNMP	1000	99	GNPLM	< 2
40	NLMPG	1000	100	LNGPM	< 2
41	MLNGP	980	101	GPLMN	< 2
42	PGMNL	970	102	PNGLM	< 2
43	GPMNL	930	103	LPGMN	< 2
44	LMNGP	930	104	PLMGN	< 2
45	LGNMP	890	105	PGLMN	< 2
46	NMPLG	830	106	GLNPM	< 2
47	GMLNP	660	107	LGNPM	< 2
48	GLMNP	650	108	LNPGM	< 2
49	MNPLG	630	109	GPNLM	< 2
50	MGLNP	630	110	PGNLM	< 2
51	LGMNP	580	111	PNLGM	< 2
52	MLGNP	580	112	PLGMN	< 2
53	LMGNP	570	113	GLPNM	< 2
54	NPMLG	460	114	LGPNM	< 2
55	NLPMG	440	115	LPNGM	< 2
56	MLNPG	290	116	GPLNM	< 2
57	MPNLG	270	117	LPGNM	< 2
58	PNMLG	240	118	PGLNM	< 2
59	PMNLG	190	119	PLNGM	< 2
60	LNMPG	190	120	PLGNM	< 2

Conclusions

For a well-characterized virus that encodes five genes we can experimentally and computationally elucidate how virus growth will depend on alternative patterns of gene expression, achieved by changing the linear sequence of genes in the viral genome. The simulated one-cycle growth of vesicular stomatitis virus is most adversely affected by permutations that overall increase the expression of the gene L or reduce expression of gene N, genes that are expressed in low and high abundance in wild-type VSV infections, respectively. This work shows how virus growth can be exquisitely sensitive to perturbations in expression of some of its genes, such as L and N, while being insensitive to perturbations in the expression of other genes.

Table 2 Effects of gene position on progeny yield of VSV.

Ranking	VSV strain group	Yield
1	L5	2900
2	N1	2400
3	P5	1800
4	G4	1500
5	N2	1400
6	M3	1300
7	M2	1300
8	G3	1300
9	M4	1300
10	G2	1100
11	P4	1100
12	M1	1100
13	P3	890
14	N3	810
15	G1	790
16	L4	730
17	P2	720
18	L3	680
19	P1	540
20	L2	470
21	N4	440
22	G5	370
23	L1	260
24	N5	< 1
25	M5	< 1

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