

Investigating the effects of an auxiliary protein, Wrp, of the baculovirus *Autographa californica nucleopolyhedrovirus* during virus replication of cells in culture

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Introduction

Viruses encode several proteins that do not have known influence on their replication. However, these so called auxiliary proteins are of significant interest to the researchers as it is suspected that these proteins are responsible for the success or failure of the virus in its insect host.

Objective

The study was conducted to determine whether there is a significant difference between host cell survival rate in cultures infected with a mutant virus, lacking the ability to produce the Wrp protein, and a wild-type virus.

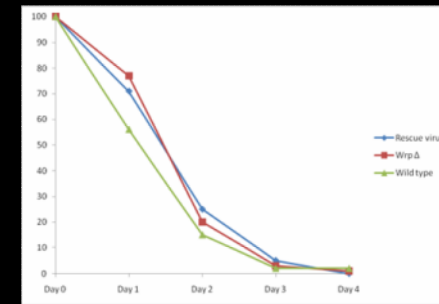
Methods

- Three virus samples were purified and amplified:
 - AcNPV (Wild type)
 - AcWrp.Δ (Wrp Δ - Deletion virus)
 - AcWrp.R (Rescue virus)
- Plaque assay was performed to calculate the values of pfu/ml of the virus samples.
- Sfg insect cell cultures were infected (10pfu/cell) and survival rates measured on four consecutive days using Trypan blue dye to distinguish between dead and living cells.

Results

Table 1. Viability and % viability of infected Sfg cell cultures.

Date	Virus	Day 0			Day 1			Day 2			Day 3			Day 4		
		Dead	Alive	%	Dead	Alive	%	Dead	Alive	%	Dead	Alive	%	Dead	Alive	%
21 July 2008	Rescue virus	39	74	65	31	77	72	30	98	77	71	77	71	77	77	71
	Wrp Δ	33	108	77	25	82	77	27	93	76	77	77	77	77	77	77
	Wild type	58	64	52	51	67	57	55	77	58	56	56	56	56	56	56
23 July 2008	Rescue virus	90	31	25	89	29	24	79	29	27	25	25	25	25	25	25
	Wrp Δ	87	24	22	89	14	13	85	27	24	20	20	20	20	20	20
	Wild type	100	17	14	115	17	13	92	19	17	15	15	15	15	15	15
24 July 2008	Rescue virus	94	5	5	107	9	8	103	4	3	5	5	5	5	5	5
	Wrp Δ	125	2	1	91	4	4	101	3	3	3	3	3	3	3	3
	Wild type	113	2	2	113	4	3	101	3	3	2	2	2	2	2	2
25 July 2008	Rescue virus	77	1	1	90	0	0	89	1	1	0	0	0	0	0	0
	Wrp Δ	75	1	1	90	1	1	93	1	1	1	1	1	1	1	1
	Wild type	86	2	2	120	3	2	93	1	1	2	2	2	2	2	2



Graph 1. Average % viability for each virus Infection on four consecutive days.

Conclusions

Results indicate that the Wrp protein does contribute to the survival rates of host insect cells. Clearly, the Wild type virus is responsible for more cell deaths especially in the early stages – up to 25% lower percentage viability in case of the Wild type virus on Day 1. However, the Rescue virus doesn't seem to be as effective, especially as of Day 2 onwards. This interesting trend requires further investigation as to what mechanisms are involved in the decrease of effectiveness of the Rescue virus.

Following the initial experiment a sample of the AcNPV (Wild type virus) was taken to investigate the effects of the virus *in vivo*. Photographic results are as follows:



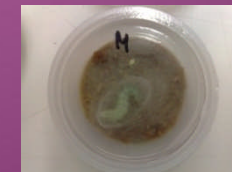
Picture 1. Healthy larvae.



Picture 2. Infected dead larvae.



Picture 3. Infected dead larvae (one visibly liquified).



Picture 4. Uninfected larva developed into pupa.