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

Piotr Henryk Wachowicz dr. Caroline Griffiths School of Life Sciences, Oxford Brookes University

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.
Sf9 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 Sf9 cell cultures.

22 July 2008							Sample 2					Sargle 3				
		average for grid 1 & 2					average for grid 1 & 2				average for grid 1 & 2					
		deal cells	iving cells	dead cells	ining cells	% vability	dead cells but	ng cells	dead cells	bing cells	% viet by	deal cells live	g cells dead ce	is living cells	5 vability	Average % visibility
Fleticue who	10 1210 1 12111 7			39	74	65	27	76	31	77	72	20	-# 30	98	77	71
WpA	121611	11	100	33	108	77	25	67	25	82	77	29	1 27	93	78	77
Wild type	2101 2102	58 37	42 65	58	64	52		64 69	51	67	57		- 7 55	77	58	56
23 July 2008		Sample 1								Gargio 3						
		average for grid 1.6.2				average for grid 1 & 2				average for grid 1 & 2				Annual Property		
Plancial viru	m gad t	and CERS	30	90	31	25	72	27	89	29	24	80	20 79	29	27	25
WpA	2101		- 2	87	24	22	100		89	14	13		85	27	24	20
Wild type	gnd 1	47	19	100	17	14	111	100	115	17	13	90	92	19	17	15
24.14	2008															
24 007 2000		warage for grid 1 & 2				everage for grid 1 & 2				www.age.for.grid 1.6.2				Average No. of Mark		
Persone who	n 2101	72	-	94	5	5	167	-1	107	9	8	112	100	3 4	3	5
Wp 5	29101	114	1	125	2	1	88	6	91	4	4	935	10	3	3	3
stild type	2112	118		113	2	2	100	- 2	113	4	3		contante	ded .		2
25 July 2008		Sample 1 Inverse for grid 1.6.2				Sample 2				Sample 3 Investment for origit 1.5.2						
Dencue vin	in last t	dead cells	hing cette	cheed ceth	hing celu	Swabits	dead cetta bei	ng ceth	dead ceta	hing cate	N VID P2	dead cetts live	in cetta chead co	to hime cette	5 waters	Average Novietilly
illing a	2102	72		11	1	1	85		90	0	0	99	89	1	1	0
and y	210 2			75	1	1	66	- 1	90	1	1		93	1	1	1
Veid type	12102	86 2 2				120 3 2				CONTRACTING				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 liquidised)



Picture 4. Uninfected larva developed into pupa.