Homologous Viral Interference in Trout and Atlantic Salmon Cell Cultures Infected with Infectious Pancreatic Necrosis Virus

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The occurrence of homologous interference in the replication of infectious pancreatic necrosis virus was demonstrated after successive passages of partially purified virus at high input multiplicities in trout and Atlantic salmon cell cultures. Pretreatment of cell cultures with interfering virus inhibited the replication of homologous standard infectious virus but not unrelated viruses. The ability of infectious pancreatic interfering virus to interfere with homologous virus was abolished with UV irradiation, immune serum, and freeze-thawing.

Infectious pancreatic necrosis (IPN) virus causes a highly contagious and destructive disease of young trout. The virus has also been isolated from Atlantic salmon (7, 13; Roger Dexter, personal communication) and Coho salmon (14). Fish that survive infection often become carrier fish spreading the virus through feces and ovarian and seminal fluids (2, 16). Persistently infected carrier cell cultures have also been developed in the laboratory (unpublished data).

The nucleic acid of IPN virus has been established as RNA (1, 3, 5, 10); however, conflicting reports have been published concerning its structure. Argot and Malsberger (1) and Cohen et al. (3) have presented evidence that the RNA is double stranded and that IPN virus is a reo-like virus. However, Nicholson (10) and Kelly and Loh (5) have suggested that the RNA is single stranded.

In many viral systems, continuous passaging of virus leads to the accumulation of defective interfering (DI) particles and the development of homologous interference (4). Malsberger and Cerini (8) reported that the yield of stock IPN virus decreased with successive passages. This paper presents evidence for the occurrence of homologous interference in the replication of IPN virus.

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The continuous rainbow trout gonad (RTG-2) (15) and Atlantic salmon (AS) (11) cell lines were propagated as described previously (10-12). The Dry Mills strain of IPN virus was used throughout this study. This strain of the virus was originally isolated from an outbreak of infectious pancreatic necrosis at the Dry Mills Hatchery, Gray, Me. in 1967 and has been maintained in this laboratory for several years.

Infection of RTG-2 and AS cell cultures with undiluted stock IPN virus obtained by several passages at high viral input multiplicities resulted in little, if any, cytopathic effect (CPE) and reduced virus yields. Infection of susceptible cell cultures with appropriate dilutions of such interfering virus resulted in extensive CPE and increased virus yields (Table 1). AS cells infected with undiluted stock virus prepared by three successive passages at high input multiplicities yielded no CPE in 72 h, slight CPE in 120 h, and a virus yield of $10^{7.0}$ tissue culture infective doses ($TCID_{50}$) per ml. Replicate cultures infected with lower input multiplicities (1:30 dilution of stock virus) showed moderate CPE at 72 h and complete destruction of the monolayer at 120 h postinfection (PI). In addition, at 120 h PI, these cultures yielded a virus titer of $10^{8.4}$ $TCID_{50}$ per ml.

To determine if the depression in synthesis of IPN virus was a result of an abortive replication associated with the virus particles or was a result of an interfering component such as interferon being carried in the medium, the virus was partially purified by ultracentrifuga-

tion (5) after each successive undiluted passage. Depressed viral yields were again obtained after several passages of partially purified virus at high input multiplicities in RTG-2 cell cultures. Repeated passage of partially purified virus at low input multiplicities (less than 1 TCID₅₀ per cell) always resulted in extensive CPE and high virus yields. In addition, interferon assay of virus-free medium from cell cultures infected with interfering IPN virus indicated that IPN virus may induce some interferon production (unpublished data); however, the level of inhibition resulting from interferon production was negligible in comparison to that observed with partially purified virus.

To determine if interfering IPN virus obtained from successive high input multiplicity passage (HMP-IPN) could interfere with the replication of standard infectious virus from low input multiplicity passage (LMP-IPN), replicate RTG-2 and AS cell cultures were exposed to HMP-IPN virus and then challenged with LMP-IPN virus at varying times from 0 to 24 h post initial infection. The results in Table 2

TABLE 1. *Homologous interference in Atlantic salmon cell cultures after infection with IPN virus obtained by several passages at high virus input multiplicities*

Time (h)	Input virus, dilution of stock			
	Undiluted		1/30	
	CPE ^a	Virus yield ^b	CPE	Virus yield
24	0		1+	
72	0		2+	
120	1+	7.0	4+	8.4

^a 4+, Complete cytopathic effect (CPE) (complete destruction of monolayer); 3+, extensive CPE; 2+, moderate CPE; 1+, slight CPE; 0, no CPE.

^b Log₁₀ of TCID₅₀ per 1.0 ml.

indicate that RTG-2 and AS cells infected with HMP-IPN virus and challenged with LMP-IPN 2 h later showed no CPE 72 h PI and resulted in virus yields in AS cells of 10^{6.7} TCID₅₀ per ml. Control RTG-2 and AS cell cultures infected with only LMP-IPN virus showed complete CPE 48 h PI and virus yields in AS cells of 10^{8.4} TCID₅₀ per ml. It was also observed that the level of homologous interference, although substantial when interfering virus and standard virus were added simultaneously, was increased greatly when cells were treated with interfering virus prior to being challenged with standard virus. Maximal interference was achieved when cultures were exposed to interfering virus 24 h prior to infection with standard virus.

Irradiation of HMP-IPN viral stocks with UV light destroyed their ability to interfere with homologous standard virus (Table 3). In addition, treatment of interfering virus with specific antiserum abolished or greatly diminished the ability of the virus to interfere with standard virus. A single cycle of freeze-thawing also destroyed interfering ability.

Preparations of interfering IPN virus of the Dry Mills strain were found to interfere with the replication of at least two other strains of the virus (Berlin, N.H. strain and ATCC VR no. 299 strain). IPN virus, however, did not interfere with the replication of the unrelated infectious hematopoietic necrosis (IHN) virus and the bluegill myxovirus (BMV). IHN and BMV produce characteristic types of CPE quite distinct from each other and that caused by IPN virus. Pretreatment of cell cultures with interfering IPN virus did not prevent the development of extensive characteristic CPE by either IHN virus or BMV.

In summary, the results demonstrate that IPN virus prepared by successive passages at high input viral multiplicities can induce ho-

TABLE 2. *Interference by HMP-IPN virus with the replication of standard IPN virus obtained by LMP*

Time (h)	RTG-2 cell cultures				AS cell cultures			
	HMP ^a treated		Untreated		HMP ^a treated		Untreated	
	CPE	Virus yield	CPE	Virus yield	CPE	Virus yield	CPE	Virus yield
24	0		0		0		0	
48	0		3+		0		3+	
72	0	6.9	4+	8.5	0	6.7	4+	8.4

^a After exposure to HMP-IPN virus for 2 h, the cultures were challenged with LMP-IPN virus at an input multiplicity of approximately 1 TCID₅₀ per cell.

^b 4+, Complete CPE (complete destruction of monolayer); 3+, extensive CPE; 0, no CPE.

^c Log₁₀ of TCID₅₀ per 1.0 ml.

TABLE 3. *Effect of UV irradiation on homologous interference of IPN virus^a*

Time after challenge with standard IPN virus (h)	Interfering IPN and standard IPN		UV-irradiated interfering IPN ^b and standard IPN		UV-irradiated standard IPN ^b and standard IPN		UV-irradiated standard IPN only	
	CPE ^c	Virus yield	CPE	Virus yield	CPE	Virus yield	CPE	Virus yield
24	0		3+		3+		0	
48	1+	7.3 ^d	4+	8.5	4+	8.4	0	2.3

^a RTG-2 cell cultures were exposed to interfering IPN virus for 2 h. After adsorption of interfering virus, the cultures were washed and, when appropriate, challenged with standard IPN virus at an input multiplicity of approximately 1 TCID₅₀ per cell.

^b A 2-ml sample of stock interfering or standard IPN virus was irradiated for 20 min in an open petri dish by using a UV lamp (GE-68T5-USA) positioned 15 cm above the virus suspension. Standard IPN virus, with an infectivity titer of 10^{8.1} TCID₅₀/ml prior to irradiation, exhibited an infectivity titer of 10^{4.2} TCID₅₀/ml after UV irradiation.

^c 4+, Complete CPE (complete destruction of monolayer); 3+, extensive CPE; 2+, moderate CPE; 1+, slight CPE; 0, no CPE.

^d Log₁₀ of TCID₅₀ per 1.0 ml.

mologous interference. The virus did not interfere with the replication of unrelated viruses. On the basis of physical and biological properties the interference cannot be attributable to interferon. Susceptibility to immune serum, UV irradiation, and sedimentation properties indicate that the interfering component is similar to that of the IPN virus particle.

DI virus particles have been described for many viral systems (4). DI particles exhibit the following properties: they possess normal viral structural proteins; they lack some segment of the viral genome; they reproduce only in the presence of standard infectious virus; and they interfere specifically with the intracellular growth of homologous standard virus. When DI particles are present in virus stocks, diluted passage in susceptible cell cultures results in the production of more infectious standard virus particles than when passed undiluted (4). Our results suggest that IPN virus is, quite likely, a DI particle-producing system similar to that described for other viruses. It is necessary, however, to directly demonstrate the existence of DI particles in interfering IPN virus stocks. The elucidation of the phenomenon of homologous interference in the replication of IPN virus may help in understanding the establishment of carrier cultures in the laboratory and the role of interfering particles in the disease process.

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LITERATURE CITED

- Argot, J., and R. G. Malsberger. 1972. Intracellular replication of infectious pancreatic necrosis virus. *Can. J. Microbiol.* **18**:865-867.
- Billi, J. L., and K. Wolf. 1969. Quantitative comparison of peritoneal washes and feces for detecting infectious pancreatic necrosis (IPN) virus in carrier brook trout. *J. Fish Res. Bd. Can.* **26**:1459-1465.
- Cohen, J., A. Poinard, and R. Scherrer. 1973. Physicochemical and morphological features of infectious pancreatic necrosis (IPN) virus. *J. Gen. Virol.* **21**:485-498.
- Huang, A. S. 1973. Defective interfering viruses, p. 101-117. *In* M. P. Starr, J. L. Ingraham, and S. Raffel (ed.), *Annual review of microbiology*. Annual Reviews Inc., Palo Alto, Calif.
- Kelly, R. K., and P. C. Loh. 1972. Electron microscopical and biochemical characterization of infectious pancreatic necrosis virus. *J. Virol.* **10**:824-834.
- Lightner, D., and G. Post. 1969. Morphological characteristics of infectious pancreatic necrosis virus in trout pancreatic tissue. *J. Fish. Res. Bd. Can.* **26**:2247-2250.
- Mackelvie, R. M., and H. Artsob. 1969. Infectious pancreatic necrosis virus in young salmonids of the Canadian maritime provinces. *J. Fish. Res. Bd. Can.* **26**:3259-3266.
- Malsberger, R. G., and C. P. Cerini. 1963. Characteristics of infectious pancreatic necrosis virus. *J. Bacteriol.* **86**:1283-1287.
- Moss, L. H., and M. Gravell. 1969. Ultrastructure and sequential development of infectious pancreatic necrosis virus. *J. Virol.* **3**:52-58.
- Nicholson, B. L. 1971. Macromolecule synthesis in RTG-2 cells following infection with infectious pancreatic necrosis (IPN) virus. *J. Gen. Virol.* **13**:369-372.
- Nicholson, B. L., and C. Byrne. 1973. An established cell line from the Atlantic salmon (*Salmo salar*). *J. Fish. Res. Bd. Can.* **30**:913-916.
- Piper, D., B. L. Nicholson, and J. Dunn. 1973. Immunofluorescent study of the replication of infectious pancreatic necrosis virus in trout and Atlantic salmon cell cultures. *Infect. Immunity* **8**:249-254.
- Wolf, K. 1966. The fish viruses. *Advan. Virus Res.* **12**:36-95.
- Wolf, K., and L. L. Pettijohn. 1970. Infectious pancreatic necrosis virus isolated from Coho salmon fingerlings. *Progr. Fish Cult.* **32**:1218.
- Wolf, K., and M. C. Quimby. 1962. Established eurythermic line of fish cells *in vitro*. *Science* **135**:1065-1066.
- Wolf, K., M. C. Quimby, and L. L. Pettijohn. 1968. Virology section: progress in sport fishery research. *Bur. Sport Fish. Wildl.* **77**:136-141.