INHIBITION OF THE INDUCTION OF HEAT SHOCK PROTEINS IN *DROSOPHILA MELANOGASTER* CELLS INFECTED WITH INSECT PICORNAVIRUSES

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1. Introduction

Infection of cells by mammalian picornaviruses results in an inhibition of 'shut-off' of host cell protein synthesis to varying extents depending upon the virus used [1]. The recently characterised picornaviruses of insects have been shown to act similarly, with Cricket paralysis virus (CrPV) being extremely efficient in inhibiting *Drosophila* cell protein synthesis [2,3], whereas the related *Drosophila* C virus (DCV) is relatively inefficient [4]. Effort has been concentrated on the elucidation of the shut-off mechanisms although this has not led to a satisfactory model being established. Work has been hindered by having to look at the inhibition of a broad spectrum of cellular proteins and also by the absence of a method to differentiate between the effects on mRNA existing before infection and de novo expression of genes during the course of infection. This means that while theories of shut-off at the level of translation may serve to explain events occuring early in the infection process, they may not necessarily be valid models for later shut-off. To overcome both these problems, we have utilised the phenomenon of heat-shock in *Drosophila* in which elevated temperature induces the expression of a novel set of genes. This was first recognised in vivo as a new puffing pattern in *Drosophila melanogaster* salivary gland chromosomes and has since been demonstrated in many of the tissues of this fly, as well as in *Drosophila* tissue culture cells [5]. Elevation of the temperature of *Drosophila* tissue culture cells shifts the pattern of protein synthesis from a normal broad spectrum of proteins to a small number of previously unexpressed proteins. This makes it possible to examine the effect of infection on a small number of proteins which are synthesised at a known chromosomal site in response to a specific stimulus. We report here that two insect picornaviruses affect the synthesis of heat-shock proteins (HSPs) in qualitatively different ways and suggest that this system can be used as a model for the study of shut-off mechanisms.

2. Methods

2.1. *Virus growth and titration*

CrPV and DCV were grown in confluent monolayers of *Drosophila melanogaster* tissue culture cells at 28°C as in [2]. Cells were grown to confluency in Schneider's medium containing 10% foetal calf serum and antibiotics while virus was grown in cells maintained on Schneider's medium containing 2% foetal calf serum and antibiotics [3]. Virus was titred as 50% tissue culture infectivity doses ($TCID_{50}$) in Falcon Microtest plates [2,4].

2.2. *Heat-shock and radiolabelling*

Cells in 25 cm^2 monolayers were infected with a minimum of 20 $TCID_{50}$/cell for 1 h at 28°C. Innocula were removed and the cells overlaid with Schneider's medium containing 2% foetal calf serum. Cells were incubated for 3–5 h (see figure legends) at 28°C, washed with methionine-deficient medium and incubated in the same medium for at least 30 min. Further details of infection periods, pulses and heat-shock times are given in the figure legends. The medium was replaced by Schneider's (methionine-deficient) at 37°C and the cells were incubated at 37°C, rather than the normal 28°C, to provide conditions of heat-shock.

2.3. *Slab gel electrophoresis*

Infected or mock-infected cells were scraped into

5 ml ice-cold medium and pelleted at 2000 × *g* for 10 min at 4°C. The drained pellet was boiled for 2 min into 250 μl 10% electrophoresis buffer containing 2% SDS, 2% 2-mercaptoethanol, 15% glycerol and 0.01% bromophenol blue. Electrophoresis was performed on 17.5%, 12.5%, 8% and 10–20% acrylamide gels in discontinuous buffer [6] and gels were impregnated with 2,5-diphenyloxazole [7].

3. Results

Increasing the temperature of the *D. melanogaster* tissue culture cells from 28–37°C resulted in the appearance of 5 proteins in uninfected cells (4,8, fig.1)

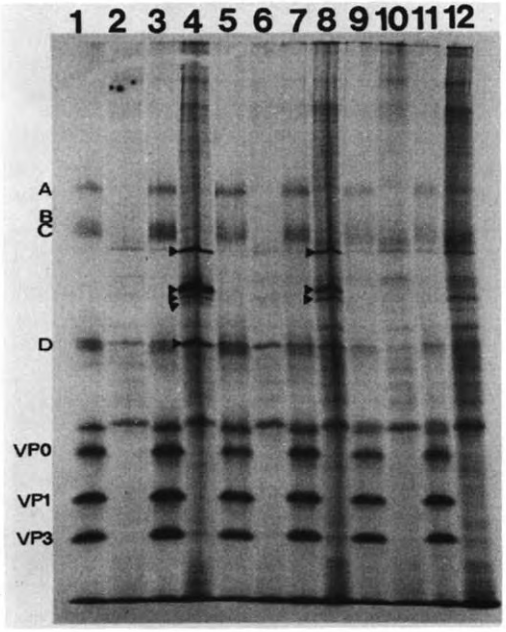

Fig.1. Electrophoresis of the [^{35}S]methionine-labelled proteins of heat-shocked CrPV-infected and mock-infected cells in the presence and absence of 5 μg/ml AMD. Monolayers of cells (25 cm^2) were washed with 2 × 2 ml methionine-deficient medium at 3.5 h post-infection. Methionine-deficient medium (1 ml) was added to each monolayer. Cells were heat-shocked at 37°C for 15 min (1–4) and 5 min (5–8) or maintained at 28°C (9–12) prior to pulsing with 200 μCi of ^{35}S for 15 min at 4.5 h post-infection. The numbers refer to the following treatment of monolayers: (1) infected + AMD; (2) control + AMD; (3) infected – AMD; (4) control – AMD; (5) infected + AMD; (6) control + AMD; (7) infected – AMD; (8) control – AMD; (9) infected + AMD; (10) control + AMD; (11) infected – AMD; (12) control – AMD. Virus-induced proteins are indicated on the left of the photograph while the arrows indicate the HSPs produced in the non-infected heat-shocked cells.

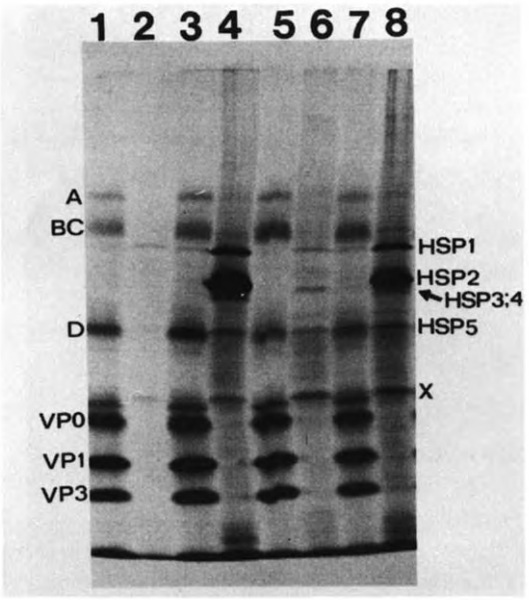

Fig.2. Electrophoresis of the [^{35}S]methionine-labelled proteins of CrPV infected and mock-infected cells heat-shocked for 60 min (1–4) and 30 min (5–8) before pulsing for 15 min at 4.5 h post-infection. The numbers indicate: (1) infected + AMD; (2) control + AMD; (3) infected – AMD; (4) control – AMD; (5) infected + AMD; (6) control + AMD; (7) infected – AMD; (8) control – AMD. Virus-induced proteins are indicated as are the heat-shock proteins (HSP 1–5). The major cellular protein apparent at 28°C is also indicated (X).

and the inhibition of much of the normal protein synthesis (12, fig.1). The two highest molecular mass heat-shock proteins (HSPs) have been reported to have M_r 82 000 and 70 000 (HSP1 and HSP2) [5]. In the presence of actinomycin D (AMD) no new proteins were induced by heat-shock (2,6, fig.1). Surprinsingly, with CrPV-infected cells in the absence of AMD, there was no appearance of the HSPs when the temperature was elevated (3,7, fig.1). The virus appears to be as effective as AMD in inhibiting the appearance of HSPs. With longer times of heat-shock (fig.2) the normal host cell protein synthesis was virtually eliminated in the presence of AMD (2, fig.2) and in the absence of AMD, the majority of the cells synthetic activity was switched to the production of HSPs (4, fig.2). Very small amounts of proteins comigrate with HSPs, even in AMD-treated and infected cells (fig.1,2). This is probably due to the translation of pre-existing base levels of HSP-mRNA in the cells [8]. Small amounts of normal uninfected cell proteins (fig.1,2) were synthesized even with long heat-shock.

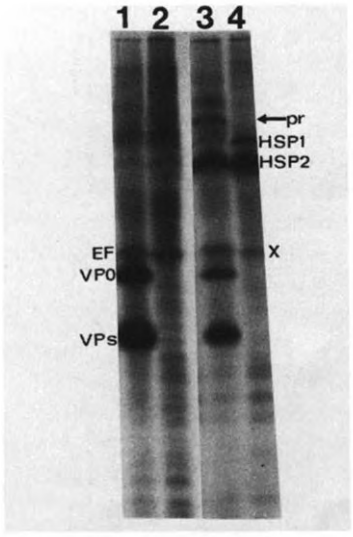

Fig.3. Electrophoretic analysis of control and DCV infected *Drosophila* cell proteins pulsed with [^{35}S]methionine in the absence of AMD during infection and pulse. The numbers refer to DCV-infected (1) and mock-infected cells (2) maintained at 28°C, and DCV-infected cells (3) and mock-infected cells (4) heat-shocked at 37°C for 30 min prior to the pulse. HSP1 and HSP2 are indicated as are the viral precursor proteins E, F VPO and the structural proteins (VPs). The major cellular protein X is indicated. An additional protein (pr) is found in the heat-shocked infected cells.

The pattern of virus-induced proteins was apparently unaffected by heat-shock, but densitometer scan comparison of the AMD treated infected cells with the parallel heat-shocked cells demonstrated that the relative amounts of precursor protein D were increased (not shown).

Heat-shocking DCV infected *Drosophila* cells resulted in an appearance of HSPs at only a slightly reduced level (fig.3). DCV fails to shut-off protein synthesis to the same extent as CrPV. Heat-shock of infected cells produced an additional protein (pr-precursor) not found in uninfected shocked cells or infected cells maintained at 28°C. This protein is a novel virus precursor attributed to the heat-mediated inhibition of protein processing of precursors to virus structural proteins [9]. This protein corresponds to the high molecular mass precursor (A3) obtained by iodoacetamide treatment of infected cells [4] as seen in fig.4 (2–5). Induced proteins A3 and D originally found in substantial amounts only with iodoacetamide treatment were found also in the heat-shocked cells (fig.4). Furthermore, all the virus-induced proteins were more apparent in the heat treated cells (6–8, fig.4) because of selective inhibition of host cell protein synthesis. Precursor proteins E, F and G were eliminated completely at high iodoacetamide concentration [4] but residual amounts of these 3 proteins were apparent after long heat-shock.

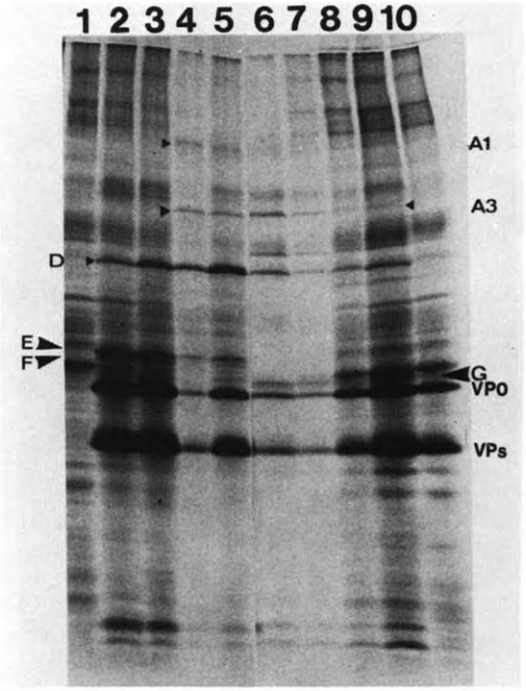

Fig.4. Electrophoretic analysis of DCV precursor polypeptides produced in iodoacetamide-treated and heat-shocked infected *Drosophila melanogaster* cells on a 10–20% polyacrylamide gradient gel. Actinomycin D (5 μg/ml) was included in all media during infection and pulse. Infected (2–10) and mock-infected cells (1) were washed with 2 × 2 ml of methionine-deficient medium at 4 h post-infection. Methionine-deficient medium (1 ml) was added to each bottle. The 10 channels are: (1) mock-infected cells pulsed with 100 μCi ^{35}S for 20 min at 4 h 50 min post-infection; (2–5) pulsed as above but pre-treated for 20 min with 0.1, 0.2, 0.5 and 0.75 mM iodoacetamide, respectively; (6–10) pulsed with 100 μCi of ^{35}S for 20 min at 5 h 20 min post-infection – cells were heat-shocked for 1 h 20 min (6), 50 min (7), 20 min (8) and 10 min (9) prior to pulsing at 37°C; (10) contains the proteins of infected cells maintained at 28°C. High molecular mass iodoacetamide 'induced' proteins are indicated by small arrows (A1, A3, D). Proteins A3 and D were induced by heat-shock. Precursor proteins E, F and G and VPO are also indicated as are the virus structural proteins (VPs).

4. Discussion

The above results clearly demonstrate that CrPV inhibits the production of HSPs while DCV infection of *Drosophila melanogaster* cells has little effect when the temperature of the cells is elevated. Similar inhibitory effects are found with normal (28°C) host cell protein sysnthesis; CrPV being by far the more effective in this capacity. Many conditions and chemicals have been shown to induce HSPs but relatively few stop their formation [5]. Actinomycin D stops both the formation of HSPs and the new puffing apparent on the chromosomes [5]. CrPV appears to work as efficiently as actinomycin D in stopping protein appearance but CrPV may not be acting at the level of transcription. The picornavirus literature suggests that inhibition of host cell protein synthesis may be attributable to inactivation of initiation factors or, alternatively, to competition between viral and host mRNAs. The system we have described provides a unique tool in the study of shut-off. It allows the examination of the effects upon a small number of easily identifiable proteins whose expression can be induced at any time before or during the course of infection. This makes it possible to determine if different mechanisms of shut-off are used by the virus during infection, e.g., mRNA competition early in infection and inhibition of transcription later in infection. The procedure of heat-shocking the virus infected cells in the presence of AMD serves the 2 additional functions of:

(i) Eliminating the majority of the host cell protein synthesis and allowing examination of the virus-induced proteins hidden by normal cellular synthesis;

(ii) Inhibiting the cleavages of the virus precursors hence producing the appearance of high molecular mass virus-induced proteins in DCV infected cells.

Based on the assumption that other viral RNAs will be transcribed at more efficient rates than host mRNAs at elevated temperatures the use of heat-shock also introduces the possibility of looking for inapparent infections in insect tissue culture cells and possibly in mammalian cells.

Acknowledgements

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