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MODULATION IN RESPONSE TO TEMPERATURE OF MAYARO VIRUS PROTEOSYNTHESIS IN *AEDES ALBOPICTUS* CELLS

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Summary. — Incubation of *Aedes albopictus* cells infected with Mayaro virus at 37 °C causes inhibition of virus replication. During the first hour post infection (p.i.) incubation at 37 °C inhibited cellular and virus proteosynthesis. A preferential translation of heat shock proteins 82 kD and 70 kD was observed. After incubations longer than 1 hr at 37 °C, a switch to normal pattern of cell protein synthesis occurred without recovery of virus proteosynthesis. In addition, preferential synthesis of three major virus proteins of 62 kD, 50 kD and 34 kD was observed, when infected cells incubated at 37 °C were shifted down to 28 °C

Key words: *Mayaro virus*; *heat shock*; *Aedes albopictus; protein synthesis*

Introduction

Incubation of cells at elevated temperature causes alterations in the gene expression. A group of highly evolutionary conserved proteins referred to as heat shock proteins (hsps) is being induced, whereas the translation of most cellular proteins ceases. The function of heat shock proteins in response to stress is unknown (Schlesinger *et al.*, 1982). Recent studies have shown that infection of cells by several viruses also induces the synthesis of cellular proteins related to heat shock proteins (hsp) (Peluso *et al.*, 1978; Hightower and Smith, 1979; Nevins, 1982; Collins and Hightower, 1982; Notarianni and Preston, 1982; Garry *et al.*, 1983; Khandjian and Turler, 1983; Kao and Nevins, 1983). Virus replication itself also damages the host cells; both conditions, temperature shift and virus infection, involve a reprogramming of gene expression in host cells. As reported by Gillies and Stollar (1982) *Aedes albopictus* (clone LT-C7) infected with vesicular stomatits virus and maintained at 34 °C in the presence of 10 % serum, revealed inhibition of protein synthesis and cytopathic effect (CPE). However, cells maintained at 28 °C in the presence of 2 % serum show little or no CPE. As they suggest

the increase of temperature (from 28 ° to 34 °C) elicit inhibition of proteosynthesis by increasing the amount of viral gene products. Recently, we have investigated the effect of high temperature on the replication of Mayaro virus. Approximately 100 fold reduction of virus titre was observed in cells maintained at 37 °C, as compared to those kept at 28 °C (Carvalho *et al.*, 1987). Mayaro virus (*Alphavirus*, *Togaviridae*) was first isolated in Trinidad in 1954 from the blood of febril patients (Anderson *et al.*, 1957). In Brazil it was isolated during an outbreak in a rural community (Causey and Maroja, 1957) 120 miles east of the city of Belém.

Two structural proteins of Mayaro virus with apparent molecular mass of 34 kD and 50 kD were identified (Mêzencio *et al.*, manuscript in preparation). In addition, two nonstructural proteins (62 kD and 110 kD) were found. Togaviruses have an icosahedral capsid enveloped and containing a positive single-standed RNA (Kääriänen and Söderbund, 1978). In this communication we studied the effect of high temperature on Mayaro virus replication in *Aedes albopictus* cells. We analyse the changes in the pattern of virus and in cellular proteins synthesized in infected cells shifted to elevated temperature at early or at late intervals p.i. We found that the pattern of proteins induced by Mayaro virus infection could be reverted by incubation of these cells at higher temperature (37 °C).

Materials and Methods

Virus and cell cultures. Aedes albopictus, clone C6/36 used in these studies (Igarashi, 1978) was a gift from Dr. R. E. Shope, Arbovirus Research Unit, Yale University, U.S.A. The cells were grown in 60 cm^2 glass bottles at 28 °C. The growth medium consisted of Dulbecco's modified Eagle Medium supplemented with 0.2 mmol/l non-essential amino acids, 2.25 % $NaHCO_3$, 2 % foetal calf serum, penicillin (500 U/ml), streptomicin (100 µg/ml) and amphotericin B (fungizone 2.5 µg/ml). For subcultivations, confluent monolayers containing 1.5×10^7 cells/bottle were gently washed with Dulbecco's phosphate-buffered saline (PBS) and after a short trypsinization the cells were suspended in the culture medium. The monolayers, grown in scintilation vials were seeded with 2×10^5 cells and the culture incubated at 28 °C in 5 % CO_2 atmosphere.

Mayaro virus was obtained from American Type Culture Collection, Rockville, M. D., U.S.A. The virus stock was prepared from BHK-21 cells and stored at -60 °C. Infectivity titrations of Mayaro virus were performed by plaque assay in L-A9 cells as described by Volkmer and Rebello (1981).

Confluent monolayers of 3×10^6 *Aedes albopictus* cells growing in scintilation vials (5 cm^2 of area) were infected with Mayaro virus at a multiplicity of 5 or 100 PFU/cell. After the adsorption period, the inoculum was aspirated, and to each culture dish growth medium was added. The cells were incubated at 28 °C or 37 °C, respectively.

Heat shock treatment and labelling of cultures with (^{35}S)*-methionine. A. albopictus* cells growing in scintilation vials (5 cm^2 of area) were preincubated for 30 min at 37 °C, in Eagle's Minimal Essential Medium in the absence of serum and methionine. After this period, the medium was supplemented with 7.5 277.5 kBq/ml of ^{35}S-methionine and the incubation was continued. One hour later, the medium was removed, and monolayers suspended in 70 µl of loading buffer (62.5 mmol/l Tris-HCl, pH 6.8, 2 % SDS, 10 % glycerol, 5 % 2-mercaptoethanol and 0.001 % bromophenol blue).

Analysis of (^{35}S)*-methionine labelled proteins by SDS-polyacrylamide gel electrophoresis* (*SDS-PAGE*). Cells in 70 µl of loading buffer were heated for 5 min at 95 °C and subjected to electrophoresis on one-dimensional 12.5 % polyacrylamide gels using the SDS buffer system of Laemmli (1970) at room temperature. The dried gels were exposed to Kodak X-Omat (YAR-S).

Results

Shift from viral mRNA to heat shock mRNA translation

Aedes albopictus cells growing in scintilation vials were infected with Mayaro virus at 100 PFU/cell and incubated at 28 °C for 16 hr. Then the cells were shifted at 37 °C, labelled with ^{35}S-methionine, and analysed in SDS-PAGE and by autoradiography. The results (Fig. 1) show that during the first 16 min at 37 °C, an inhibition of viral and cellular proteosynthesis occurred. Preferential translation of heat shock messengers was observed since the beginning of exposition of infected cells to elevated temperature. The synthesis of heat shock proteins 70 kD and 82 kD was concomitant with inhibition of the others cellular or viral proteins. The viral 34 kD protein is the most resistent to temperature inhibition. However, a significant inhibition of its synthesis was observed as compared with infected cells not submitted to heat treatment. For incubations longer than two hr at 37 °C, decreased inhibition of proteosynthesis was noticed; in addition, a shift to preferential translation of normal cellular mRNAs occurred. Another two heat shock proteins 90 kD and 76 kD were synthesized (Fig. 1C). When cells were infected with 5 PFU/cell, preferential translation of a protein of apparent m.m. of 40 kD was observed. However, after 3 hr of incubation of cells at 37 °C, no significant difference in the pattern of protein synthesis between control and infected cells was detected (Fig. 2).

Effect of different heat shock pulses, early or late post-infection, on the extent of viral protein synthesis

If infected cells exposed to different incubation times at 37 °C had recovered, we investigated the translation of viral mRNAs after shifting to 28 °C. The effect of duration of exposition to 37 °C, before shifting back to 28 °C, was also analysed. Cells infected for 16 hours were exposed to 37 °C for 1, 3 and 6 hr. After this time they were returned to 28 °C for 24 hr and their proteosynthesis was analysed. As shown in Fig. 3A, three viral proteins of 62 kD, 50 kD and 34 kD were synthesized in cells infected with Mayaro virus at the multiplicity of 100 PFU/cell. Only the viral protein of 34 kD was detected in cells infected with 5 PFU/cell (Fig. 3B). No detectable interference in the synthesis of viral protein was observed, independently of the duration of exposition to 37 °C. In this experiment the cells were previously infected before being submitted to heat stress. We next investigated whether elevated temperature could interfere with the recovery of viral proteosynthesis in cells heat-stressed after virus adsorption. The cells were infected with Mayaro virus at a multiplicity of 100 or 5 PFU/cell. Then the cells were incubated at 37 °C for 2 hr. After exposition to high temperature, the infected cells were returned to 28 °C and further incubated for 24 hr. The results presented in Figs. 3 and 4 show that, under either conditions, in early heat shock (Fig. 4) or late (Fig. 3) post-infection, no modification in the pattern of viral proteosynthesis occurred. These results suggest that the conditions necessary for inhibition of viral proteosynthesis and virus repli-

cation (Carvalho *et al.*, 1987) were maintained in infected cells at a supra-optimal temperature (37 °C). In addition the modifications in the proteosynthesis control induced by viral infection could be reverted by temperature elevation.

Modification in the cellular pattern of proteosynthesis after virus adsorption

The modifications occurring in the pattern of proteosynthesis after virus adsorption were analysed in the absence or in the presence of 1 μg/ml of Actinomycin D (Act D). Act D was used because a constant expression of some cellular functions was shown to be required for Sindbis virus multiplication in invertebrate cells (Erwin and Brown, 1983). Sindbis virus is a prototype alphavirus which growth in *Aedes albopictus* cells was shown to be sensitive to the presence of Act D. The results presented in Fig. 5A show that in cells infected with Mayaro virus at multiplicity of 100 PFU/cell, viral protein 33 kD occurred during the first hour p.i. No apparent inhibition of the other cellular proteins was observed. The synthesis of 34 kD protein was slightly inhibited in the presence of Act D. Five hr p.i. this protein showed an increase apparent m.m. by 1 kD. Other proteins 110 kD and also 62 kD were also synthesized independently of the presence of Act. D. No changes in the pattern of proteosynthesis were observed during the first 5 hr p.i. when cells were infected at the multiplicity of 5 PFU/cell (Fig. 5B). These data suggest that temperature could modulate the translation of virus messenger RNAs.

Discussion

The presented data show that *Aedes albopictus* cells infected with Mayaro virus and incubated at 37 °C inhibited of virus protein synthesis. However, this inhibition could be reverted by shifting the temperature from 37 °C to 28 °C. The results support the previous observations from our laboratory that the replication of Mayaro virus in *A. albopictus* cells was shown to be inhibited at 37 °C (Carvalho *et al.*, 1987). This inhibition in invertebrate cells at 37 °C could be associated with some nuclear function as described for Sindbis virus (Erwin and Brown, 1983). Observations from our laboratory show that incubation of *A. albopictus* cells at 37 °C results in inhibition of cell growth. As reported by Gillies and Stollar (1982), *Aedes albopictus* cells infected with VSV presents cytopathic effect if the cells were incubated at 34 °C in the presence of 10 % serum. The latter temperature is not sufficient to inhibit the cellular growth, and the cells grow as well as at 28 °C. As Gillies and Stoller suggest the development of CPE in *A. albopictus* cells appears to be associated with the inhibition of proteosynthesis. We have shown that after incubation of cells at 37 °C for periods longer than two hours, a recovery of the cellular proteosynthesis occurred (Figs. 1, 2), which could favour the inhibition of virus proteosynthesis and the absence of the CPE. Because during incubation of cells at supra-optimal temperature (37 °C) the cellular growth was inhibited, we assume that a nuclear function neces-

sary for virus replication could be involved in the translational control of viral or cellular mRNAs.

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References

Anderson, C. R., Downs, W. G., Wattley, G. H., Ahin, N. W., and Reese, A. J. (1957): Mayaro virus: a new human disease agent. II. Isolation from blood of patients in Trinidad, B.W.I. *Am. J. trop. Med. Hyg.* **6**, 1012–1016.

Carvalho, M. G. C., Rebello, M. A., and Mezêncio, J. M. (1987): Effect of high temperature in *Aedes albopictus* cells infected with Mayaro virus. *Brazilian J. med. Biol. Res.* **20**, 857–860.

Causey, O. R., and Maroja, O. M. (1957): Mayaro virus: a new human disease agent. III. Investigation of an epidemic of acute febrile illness on the river Guamá in Pará, Brazil and isolation of Mayaro virus as a causative agent. *Am. J. trop. Med. Hyg.* **6**, 1017–1023.

Collins, P. L., and Hightower, L. E. (1982): Newcastle disease virus stimulates the cellular accumulation of stress (heat shock) mRNAs and proteins. *J. Virol.* **44**, 703–707.

Erwin, C., and Brown, D. T. (1983): Requirement of cell nucleus for Sindbis virus replication in cultured *Aedes albopictus* cells. *J. Virol.* **45**, 792–799.

Garry, R. F., Ulug, E. T., and Bose Jr., H. R. (1983): Induction of stress proteins in Sindbis virus and vesicular stomatitis virus-infected cells. *Virology* **129**, 319–332.

Gillies, S., and Stollar, V. (1982): Conditions necessary for inhibition of protein synthesis and production of cytopathic effect in *Aedes albopictus* cells infected with vesicular stomatitis virus. *Mol. Cell Biol.* **2**, 66–75.

Hightower, L. E., and Smith, M. D. (1979): Effects of canavanine on protein metabolism in Newcastle disease virus infected and uninfected chicken embryo cells, pp. 395–405. In B. W. J. Mahy and R. D. Barry (Eds): *Negative-strand Viruses and the Host-Cell*, Academic Press, (London) New York.

Igarashi, A. (1978): Isolation of a singh's *Aedes albopictus* cell clone sensitive to Dengue and Chikungunya viruses. *J. gen. Biol.* **40**, 532–544.

Kääriäinen, L., and Söderlund, H. (1978): Structure and replication of α-viruses. *Curr. Topics Microbiol. Immunol.* **82**, 15–69.

Kao, H. T., and Nevins, J. R. (1983): Transcription activation and subsequent control of the human heat shock gene during adenovirus infection. *Mol. Cell Biol.* **3**, 2058–2065.

Khandjian, E. W., and Turler, H. (1983): Simian virus 40 and polyoma virus induce synthesis of heat shock proteins in permissive cells. *Mol. Cell Biol.* **3**, 1–8.

Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature (London)* **227**, 680–685.

Mezêncio, J. M. S., Souza, W., Fonseca, M. E. F., and Rebello, M. A. (1989): Replication of Mayaro virus in *Aedes albopictus* cells: An electron microscopic study. (Manuscript in preparation).

Nevins, J. R. (1982): Induction of the synthesis of a 70 000 dalton mammalian heat shock protein by the adenovirus EIA gene product. *Cell* **29**, 913–919.

Notarianni, E. L., and Preston, C. M. (1982): Activation of cellular stress protein genes by herpes simplex temperature-sensitive mutants which overproduce immediate early polypeptides. *Virology* **123**, 113–122.

Peluso, R., Lamb, R. A., and Choppin, P. W. (1978): Infection with paramyxoviruses stimulates synthesis of cellular peptides that are also stimulated in cells transformed by Rous sarcoma virus or deprived of glucose. *Proc. natn. Acad. Sci. U.S.A.* **75**, 6120–6124.

Schlesinger, M. J., Ashburner, M., and Tissières, A. (1982): *Heat Shock from Bacteria to Man.* Cold Spring Harbor Laboratory, New York.

Volkmer, N., and Rebello, M. A. (1981): A plaque assay method for Marituba virus in mouse L-A9 cells. *IRCS med. Sci.* **9**, 157–158.

Legends to Figures 1, 2 and 5 (pages 241—245)

Fig. 1. Effect of continuous heat shock on virus protein synthesis in *Aedes albopictus* cells infected with Mayaro virus at an input of 100 PFU/cell. Cells were labelled with ^{35}S-methionine and protein synthesis analysed by SDS-PAGE and autoradiography as described in Materials and Methods.

A — Mock infected cell (lane 1) and infected with Mayaro virus for 16 hrs at 28 °C (lane 2);

B — Cells infected with Mayaro virus for 16 hrs at 28 °C and incubated at 37 °C for 15, 30, 45 and 60 min, respectively (lanes 2, 4, 6, and 8). Lanes 1, 3, 5, and 7 are mock infected cells submitted to same heat treatment;

C — Cells infected with Mayaro virus for 16 hrs at 28 °C and incubated at 37 °C for 2, 3, 4, 5, and 6 hrs at 37 °C (lanes 2, 4, 6, 8, and 10, respectively). Lanes 1, 3, 5, 7, and 9 are mock infected cells submitted to same heat treatment. (▲) heat shock proteins, (△) viral proteins.

Fig. 2. Effect of continuous heat shock on viral protein synthesis in *Aedes albopictus* cells infected with Mayaro virus at an input multiplicity of 5 PFU/cell. The cells were submitted to heat treatment in the same conditions as described in the legend to Fig. 1. A, B, and C represent the cells analysed in the same order as in Fig. 1.

Fig. 5. Modifications in the pattern of protein synthesis after viral adsorption. Cells were infected with Mayaro virus at a multiplicity of 100 PFU/cell (A) or 5 PFU/cell (B) and labelled with ^{35}S-methionine during 1 hr, 30 min after viral adsorption lanes 2 and 3 or 5 hrs (lanes 4 and 5). The lanes 3 and 5 (A) the cells were treated with AMD (1 μg/ml) 30 min before and during labelling. Lanes 3, 4, and 5 (B) the cells were pulse labelled 1, 3, and 5 hrs p.i. Lane 1 (A and B) are mock infected cells.

(△) viral proteins.

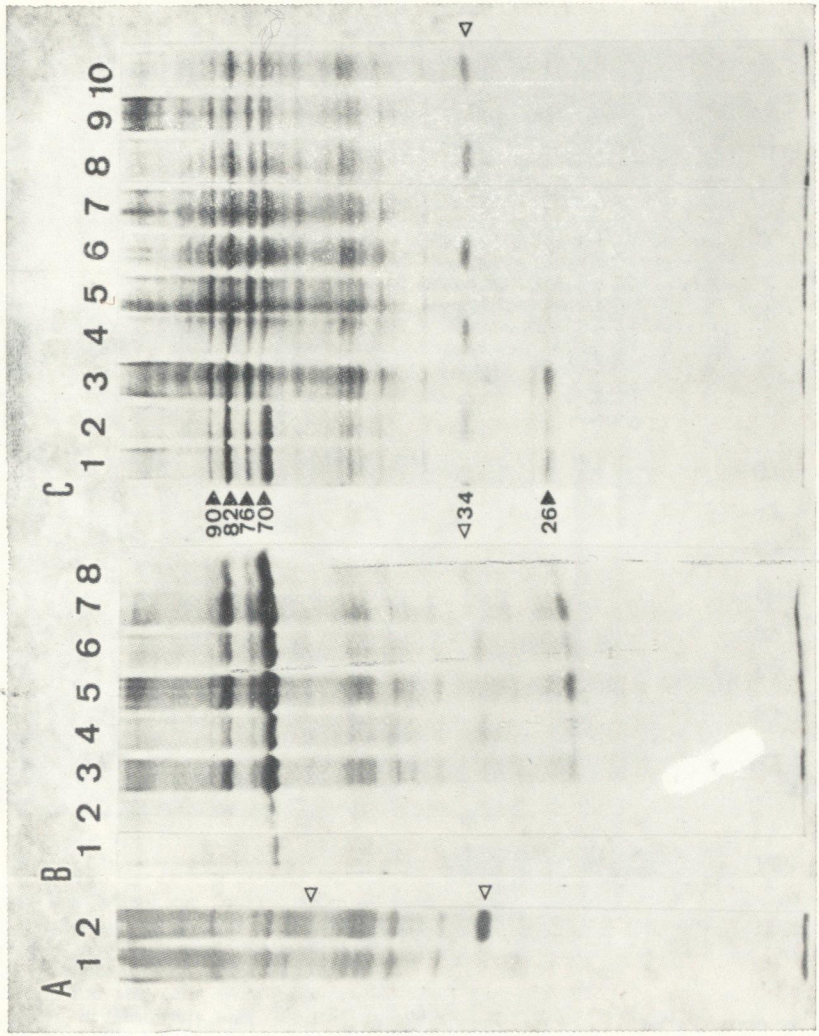

Fig. 1

For legend see page 240

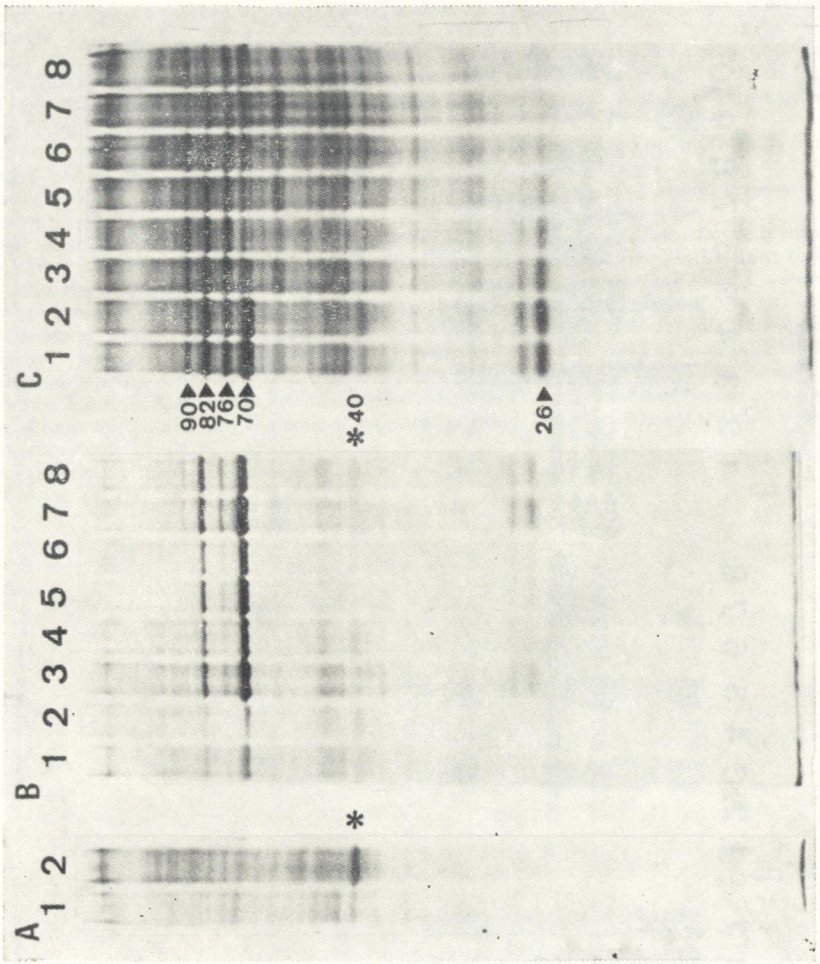

Fig. 2

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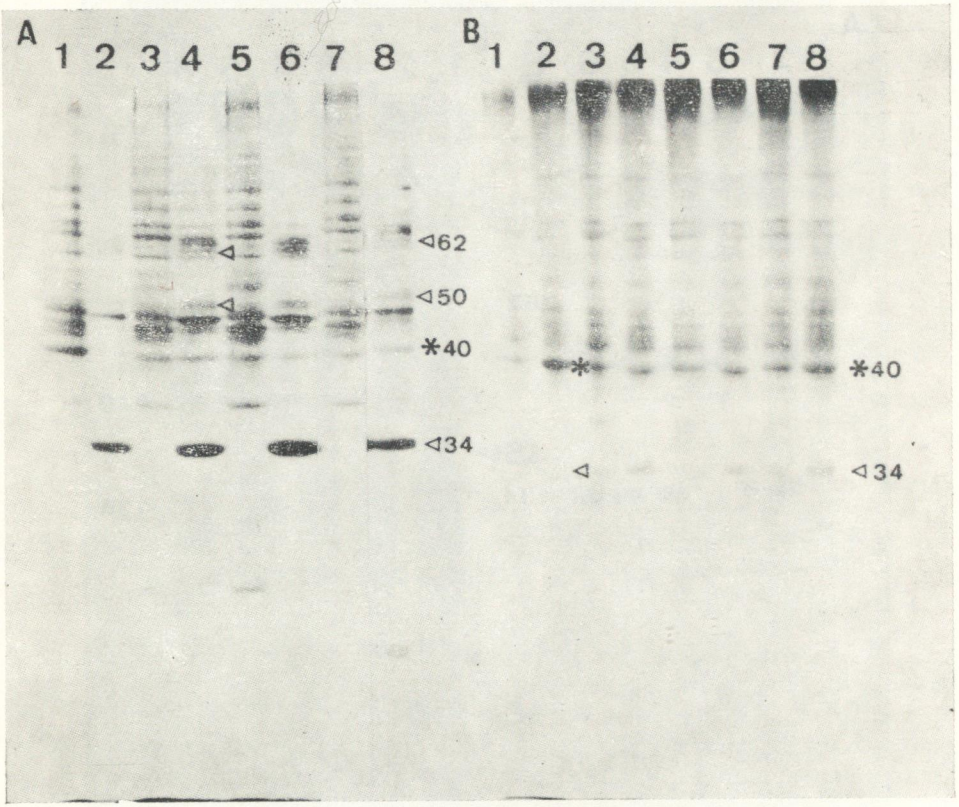

Fig. 3

Effect of heat shock on the recovery of viral protein synthesis. Cells were infected with Mayaro virus at an input multiplicity of 100 PFU/cell (A) or 5 PFU/cell (B). After 16 hrs p.i. at 28 °C the cells were incubated at 37 °C for 1 hr (lane 4), 3 hrs (lane 6) or 6 hrs (lane 8), followed incubation at 28 °C for 24 hrs.
After this time the cells were pulse-labelled with ^{35}S-methionine and proteins were analysed as described in Materials and Methods. Lane 3, 5, and 7 are mock infected cells submitted to the same heat treatment. Lane 1 represent mock infected cell not submitted to heat shock. Lane 2 represent cells infected with Mayaro virus during 48 hrs at 28 °C. (△) Viral protein.

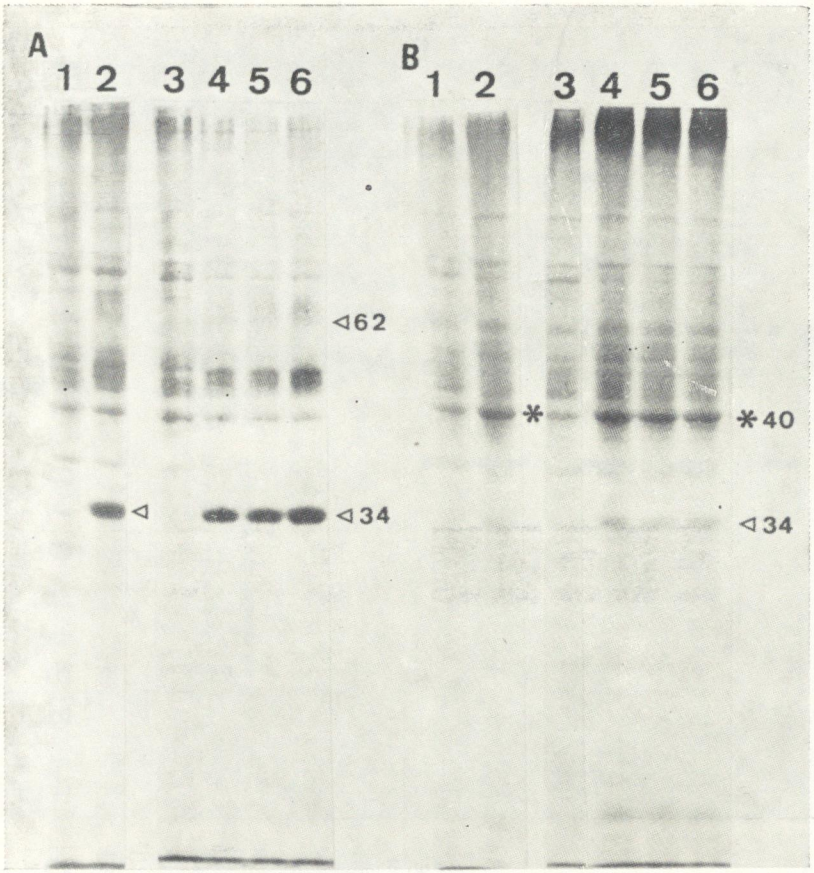

Fig. 4

Effect of heat shock on the recovery of viral protein synthesis. Cells were infected with Mayaro virus at an input multiplicity of 100 PFU/cell (A) or 5 PFU/cell (B). After viral adsorption, the cells were incubated at 37 °C in the period of zero to 2 hrs (lane 4), 2 to 4 hrs (lane 5) and 4 to 6 hrs p.i. (lane 6). After heat shock, the cells were returned to 28 °C and incubated for 24 hrs followed labelling and analysis as described in Materials and Methods. Lane 1 and 3 represent mock infected cells and lane 2 represent cells infected for 16 hrs at 28 °C.
(△) Viral proteins.

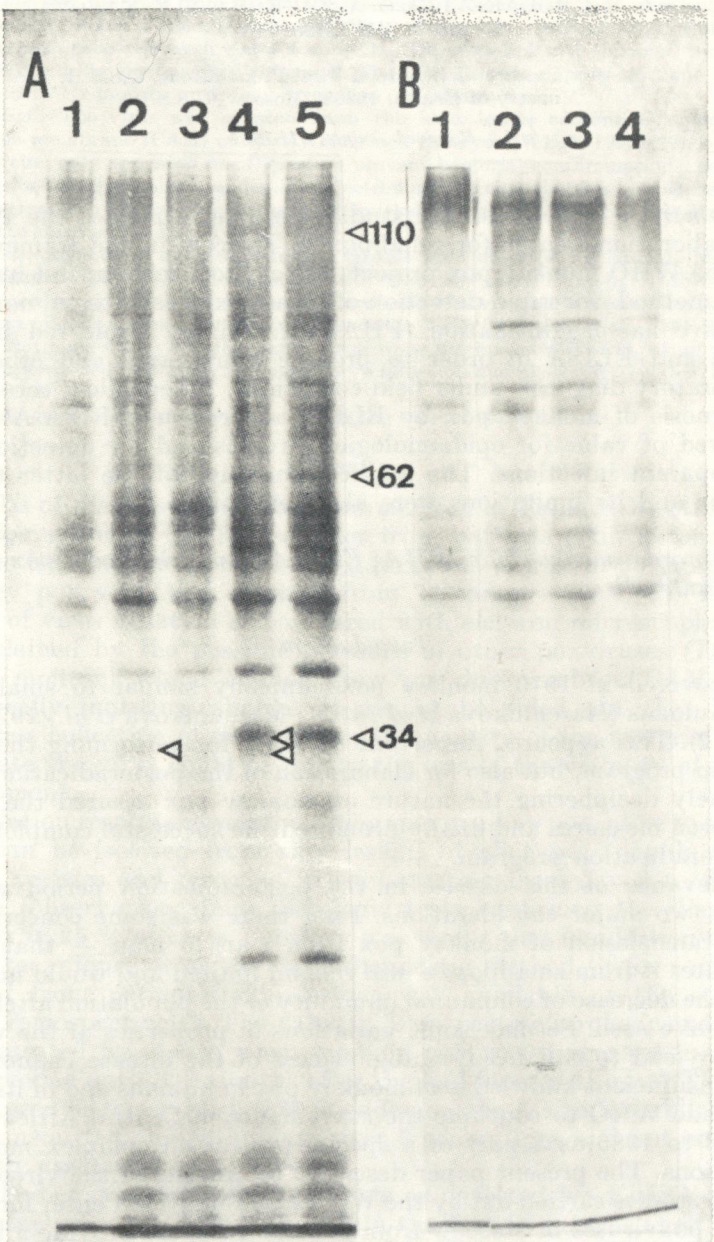

Fig. 5

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