ARGININE REQUIREMENT FOR LATE mRNA TRANSCRIPTION OF VACCINIA VIRUS IN KB CELLS

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SUMMARY

Investigations have been made of the virus specified mRNA transcribed in KB cells infected with vaccinia virus in the absence of arginine. Sedimentation studies have shown that, although early messengers RNA synthesis occurs, late mRNA synthesis is completely inhibited. Annealing have shown that all mRNA sequences transcribed in the absence of arginine are identical with early mRNA sequences transcribed in the presence of arginine.

Recently it has been shown that the growth of vaccinia virus in KB cells requires arginine (1). In the absence of arginine the production of virions was completely inhibited. However, synthesis of viral DNA reached 70 % of that in cells incubated in the presence of arginine. These results indicate that arginine is required for some late events of the vaccinia growth cycle. The events involved could be either the transcription of late mRNA or the translation of arginine rich proteins. The present report describes the effect of arginine deprivation on the transcription of early and late mRNA in vaccinia infected KB cells.

METHODS

The Lister strain of vaccinia virus was grown in KB cells and purified by sucrose gradient centrifugation (2-3). KB cells were cultivated in monolayers (4). Virus infectivity was determined by a plaque assay on KB cells monolayers (5). In order to reduce the intracellular pool of arginine the cells were incubated 16 hours before infection in Eagle's minimum essential medium without serum and arginine. The monolayers were infected with 40 plaque forming units per cell. After adsorption for 30 minutes, the cells were incubated in medium without ar-

ginine. The controls were maintained in complete medium (with 0,6 mM arginine). The time course of vaccinia mRNA synthesis was measured by pulse labelling for 10 minutes of 5.10^6 cells with 0,1 µCi/ml of ^{14}C uridine (30 mCi/mM C.E.A. France). The cells were washed, disrupted and radioactivity was determined by scintillation spectrophotometry (6). The size of viral mRNA was determined by pulse labelling of 2×10^7 cells with 4 µCi/ml of ^{3}H uridine (24 Ci/mM C.E.A. France). The cytoplasmic fraction of the cells was made 1 % with SDS and sedimented through a 37 ml linear 15 % to 30 % w/w sucrose density gradient (rotor SW 27, 24.000 rpm, 16 hours) and collected in 1 ml fractions (6). Optical density at 260 nm and radioactivity of the fractions were determined. Vaccinia DNA was extracted from purified virus according to the method of Oda and Joklik (7). Preparation of cytoplasmic RNA for hybridization has been described (8). Hybridization experiments were performed according the method of Gillepsie and Spiegelman (9) modified by Stevenin et al. (8).

RESULTS

The pattern of ^{14}C uridine incorporation into cytoplasmic vaccinia virus specified RNA in the presence of arginine showed two clearly defined peaks (Fig. 1A). The first represents mRNA transcribed from the parental genome, the second represents predominantly late mRNA and is dependent on DNA synthesis. Only one peak can be shown in the absence of arginine (Fig. 1B). Three and a half hour post infection the incorporation of the label into arginine deprived cells was similar to that observed in uninfected cells. Since the synthesis of the mRNA transcribed in the absence of arginine was not affected by the inhibition of DNA synthesis, this mRNA was transcribed from the parental genome. Further experiments were made to determine the nature of mRNA synthesized under these conditions. The size of vaccinia mRNA was measured in the cytoplasm of pulse labelled infected cells. In these conditions labelled cellular RNA except for the 4S transfer RNA is not released into the cytoplasm (6). Early viral mRNA has a lower sedimentation rate (8S - 12S) than late mRNA (16 - 22S) (6-7). Whereas in the presence of arginine late mRNA appeared at 2 hours, in the absence of arginine no 16 - 22S mRNA was transcribed (Fig. II).

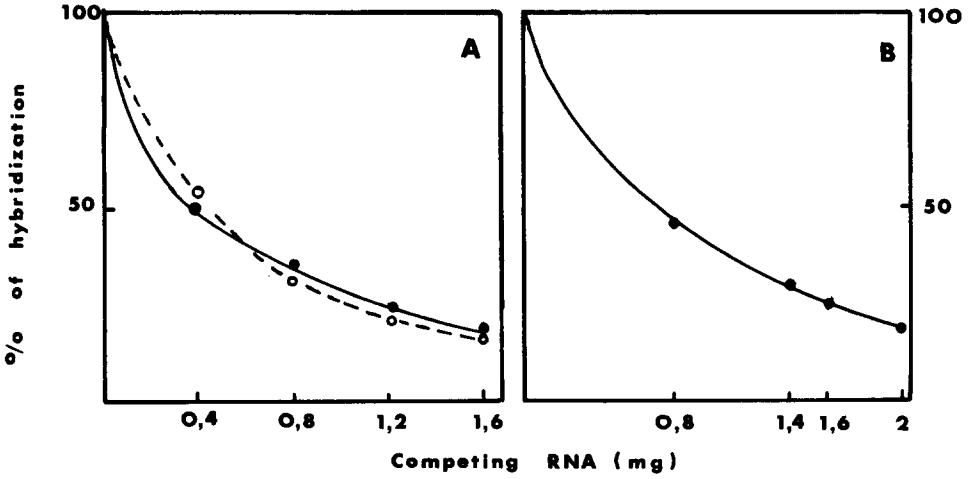

Fig. IA : Incorporation of ^{14}C uridine into cytoplasm of vaccinia virus infected and non infected cells in the presence of arginine : ●—●—● infected without 5 Fluorodeoxyuridine (FUdr) ; -O-O-O- infected with 5.10^{-5}M FUdr to block DNA synthesis ; ×—×—× uninfected cells.

Fig. IB : Incorporation of ^{14}C uridine into cytoplasm of vaccinia virus infected and non infected KB cells in the absence of arginine : ●—●—● infected without FUdr ; -O—O—O- infected with FUdr ; ×—×—× uninfected cells.

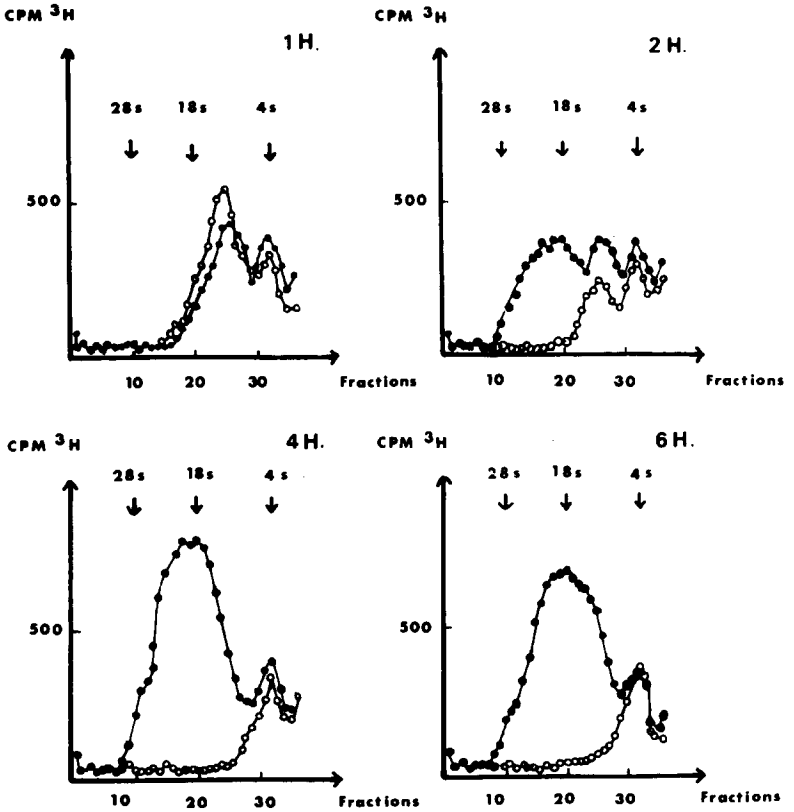

Fig. II : Determination of the size of the mRNA transcribed : ●—●—● in the presence of arginine ; -O—O—O- in the absence of arginine at 1 hr, 2 hr, 4 hr, 6 hr post infection.

These results, indicating that only early mRNA was transcribed in the absence of arginine were confirmed by hybridization experiments. Viral DNA was annealed in the presence of increasing amounts of unlabelled early mRNA (synthesized in the presence of FUDR) with ^{14}C labelled mRNA transcribed in the absence of arginine (A^- mRNA) and with ^{14}C labelled early mRNA (Fig. IIIA). Unlabelled early RNA competed with equal efficiency with both the labelled RNA preparation. Thus in the absence of arginine, no late nucleotide sequences were transcribed. In further experiments labelled early mRNA was annealed with vaccinia virus RNA in the presence of increasing amounts of cold RNA transcribed in the absence of arginine (Fig. IIIB). These A^- RNA preparations completely inhibited further annealing with early mRNA. Therefore, there was complete transcription of early mRNA in the absence of arginine.

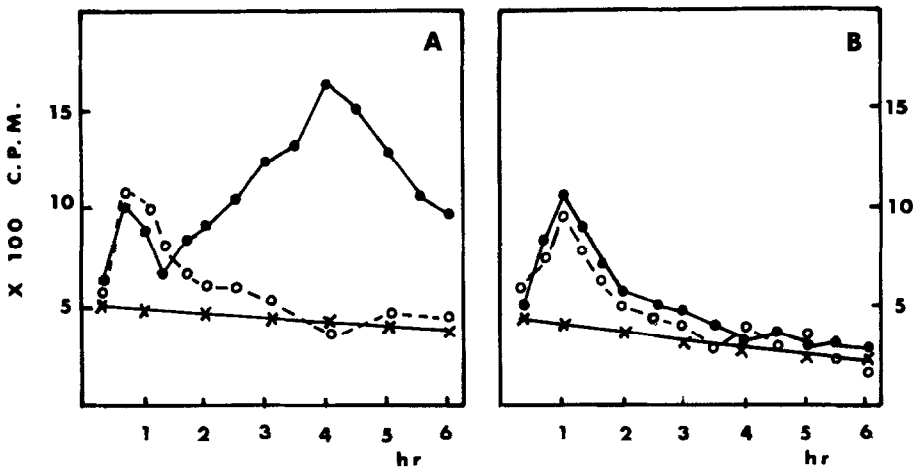

Fig. IIIA : Competition against cold early RNA of —●—●—●— ^{14}C labelled RNA synthesized in the absence of arginine (A^- RNA) and of -O--O--O-- ^{14}C labelled early RNA. Early RNA was synthesized in the presence of FUdr and was labelled between 0 and 2 hr. A^- RNA was labelled between 0 and 3 hr.

Fig. IIIB : Competition between early labelled RNA and unlabelled A^- RNA.

DISCUSSION

The transcription of vaccine specific late mRNA was completely

inhibited in the absence of arginine ; only early mRNA was synthesized. This mRNA was translated into proteins involved in DNA synthesis and into early structural proteins (1). Thus the arginine dependent step of vaccinia virus grown in KB cells appears to be the transcription of late mRNA. This requirement is different for that of nuclear replicating DNA viruses. The arginine dependent event in adenovirus and herpes virus replication is in the synthesis of certain structural or maturation proteins (10-13). In vaccinia virus infected HeLa cells, virus specified DNA synthesis, was inhibited in the absence of arginine (14). However in KB cells, the availability of arginine determined the transcription of late mRNA without significantly affecting DNA replication. Thus in KB cells the intracellular level of arginine controls the transcription of vaccinia virus late mRNA without significantly affecting viral DNA replication. These results clearly demonstrate although if DNA synthesis is necessary for the transcription of late mRNA, it is not the only requirement. Similar results have been only obtained with certain DNA phages (15-18) : other genes than those controlling DNA replication are involved in late mRNA transcription. Work is in progress to elucidate the mechanism by which arginine controls the late mRNA transcription.

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