Transcription and Translation of the Herpes Simplex Virus Type 1 Thymidine Kinase Gene after Microinjection into Xenopus laevis Oocytes

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SUMMARY

The hybrid plasmid pTK1 consists of the herpes simplex virus type 1 (HSV-1) BamHI p fragment, which contains the thymidine kinase (TK) gene, inserted into the vector pAT 153. When pTK1 DNA was microinjected into nuclei of Xenopus laevis oocytes, functional HSV-1-specific TK was produced, showing that transcription and translation of the gene occurred. Investigation of pTK1-specific RNA by 'Southern' blot hybridization revealed that all regions of the hybrid plasmid were transcribed by RNA polymerase II, but sequences present in TK mRNA were most highly represented in stable transcripts.

It has recently become clear that eukaryotic and animal virus genes are expressed when microinjected into nuclei of Xenopus laevis oocytes as purified DNA (Brown & Gurdon, 1977; Cortese et al., 1978; De Robertis & Mertz, 1977; De Robertis & Olson, 1979; Kressmann et al., 1978; Mertz & Gurdon, 1977; Probst et al., 1979; Rungger & Turler, 1978; Wickens et al., 1980). The most extensively studied are those specifying 5S rRNA and tRNA, which are transcribed by RNA polymerase III, and in these cases it has been possible to define the nucleotide sequences necessary for initiation of RNA synthesis (Federoff, 1979; Grosschedl & Birnstiel, 1980; Sakonju et al., 1980). When genes normally transcribed by RNA polymerase II are introduced by microinjection, it is possible to detect the synthesis of functional mRNAs which are subsequently translated by the oocyte into specific polypeptides (De Robertis & Mertz, 1977; Probst et al., 1979; Rungger & Turler, 1978; Wickens et al., 1980).

As an initial approach towards investigating transcription of cloned herpesvirus DNA fragments in X. laevis oocytes, we have examined expression of the extensively studied HSV-1 thymidine (pyrimidine deoxyribonucleoside) kinase (TK) gene. Thymidine kinase presents an attractive system for many reasons. The enzyme can be detected in sensitive and specific assays (Preston, 1977; Summers & Summers, 1977), and its mol. wt. is known to be 40000 to 45000 using SDS-polyacrylamide gel electrophoresis (Honess & Watson, 1974; Summers et al., 1975; Kit et al., 1978). Recent work has determined the orientation of transcription (Smiley et al., 1980), located coding sequences to fine limits, and indicated that introns of significant size do not exist (Colbère-Garapin et al., 1979; Wilkie et al., 1979; C. M. Preston & D. J. McGeoch, unpublished results; J. B. Clements & N. M. Wilkie, personal communication), a property which may improve expression in oocytes (Wickens et al., 1980). Information regarding the control of TK expression is equivocal. In productive infection of BHK cells, the 'immediate early' (IE) polypeptide Vmw175 is required in a functional form for synthesis of TK mRNA (Preston, 1979a, b). Nevertheless, tissue culture cells can be 'biochemically transformed' from a TK- to a TK+ phenotype by a cloned 3.5 kilobase pair (kbp) HSV-1 DNA fragment, BamHI p, containing the TK gene, showing that expression is possible in the absence of IE polypeptides under appropriate conditions (Colbère-Garapin et al., 1979; Wigler et al., 1977; Wilkie et al., 1979). Thymidine kinase activity in such transformed cells can be increased by superinfection with TK- HSV-1 mutants, suggesting that the incorporated gene still responds to regulating virus polypeptides (Kit & Dubbs, 1977;
Table 1. RNA- and DNA-directed synthesis of HSV TK*

<table>
<thead>
<tr>
<th>Source of extract</th>
<th>Antiserum</th>
<th>TTP (50 µM)</th>
<th>TK activity (ct/min × 10⁻³)</th>
</tr>
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<tr>
<td>HSV-1-infected BHK cells</td>
<td>None</td>
<td>+</td>
<td>232.0</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>−</td>
<td>249.1</td>
</tr>
<tr>
<td></td>
<td>Anti-HSV</td>
<td>+</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>Preimmune</td>
<td>+</td>
<td>161.6</td>
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<tr>
<td>Mock-infected BHK cells</td>
<td>None</td>
<td>+</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>−</td>
<td>76.0</td>
</tr>
<tr>
<td></td>
<td>Anti-HSV</td>
<td>−</td>
<td>55.8</td>
</tr>
<tr>
<td></td>
<td>Preimmune</td>
<td>−</td>
<td>56.9</td>
</tr>
<tr>
<td>Mock-injected oocytes</td>
<td>None</td>
<td>+</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>−</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>Anti-HSV</td>
<td>−</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>Preimmune</td>
<td>−</td>
<td>4.2</td>
</tr>
<tr>
<td>Polyadenylated RNA-injected oocytes</td>
<td>None</td>
<td>+</td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>−</td>
<td>41.0</td>
</tr>
<tr>
<td></td>
<td>Anti-HSV</td>
<td>+</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Preimmune</td>
<td>+</td>
<td>46.2</td>
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<td>None</td>
<td>+</td>
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<td></td>
<td>None</td>
<td>−</td>
<td>156.0</td>
</tr>
<tr>
<td></td>
<td>Anti-HSV</td>
<td>+</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Preimmune</td>
<td>+</td>
<td>88.6</td>
</tr>
</tbody>
</table>

* Groups of 20 oocytes (stage 5) were microinjected in the cytoplasm with 50 nl HSV-1-infected cell polyadenylated RNA (500 µg/ml) or in the nucleus with 20 nl pTK1 DNA (800 µg/ml). Oocytes were collected after 1 day (RNA microinjections) or 3 days (DNA microinjections) and homogenized in lysis buffer (20 mM-tris-HCl pH 7.5, 50 mM-NaCl, 5 mM-MgCl₂, 0.5% Nonidet P40). After standing on ice for 10 min, lysates were centrifuged at 13 000 g for 2 min and supernatants assayed for thymidine kinase as described previously (Preston, 1977) using an amount of extract equivalent to one oocyte. Infected or uninfected BHK cell extracts were prepared as described by Jamieson & Subak-Sharpe (1974). Antiserum, where present, were preincubated for 30 min at 4 °C with extracts before assay.

Leiden et al., 1976; Wilkie et al., 1979). Microinjection of cloned BamHI p DNA into X. laevis oocytes might provide a suitable system to study the role of specific nucleotide sequences and virus or cellular proteins in transcription of this gene.

Initial experiments showed that microinjection of polyadenylated RNA extracted from HSV-1-infected cells resulted in increased TK activity of oocytes (Table 1). The activity was inhibited by anti-HSV-1 serum but not by preimmune rabbit serum, and was insensitive to inhibition by 50 µM-TTP, a characteristic property of HSV TK (Jamieson & Subak-Sharpe, 1974). These results confirm that HSV-1 TK mRNA can be translated accurately in oocytes and that addition of 50 µM-TTP provides specific assay conditions for the virus enzyme in this system.

Plasmid pTK1 consists of the HSV-1 BamHI p fragment, known to contain the coding and regulating sequences of the TK gene (Wilkie et al., 1979), inserted into the vector pAT 153 (Twigg & Sherratt, 1980). When oocytes were analysed after microinjection of pTK1 DNA into their nuclei by the technique of Mertz & Gurdon (1977), increased levels of TK were found (Table 1). The new activity was inhibited by anti-HSV serum but not by preimmune rabbit serum or 50 µM-TTP, and we therefore conclude that pTK1 DNA directed the synthesis of functional TK mRNA, which was translated in the oocyte to yield active enzyme.

It is clear, therefore, that HSV IE polypeptides are not essential for production of active TK mRNA in X. laevis oocytes. To investigate further the extent of transcriptional specificity, α-³²P-GTP was injected together with pTK1 DNA, and radiolabelled RNA extracted for analysis by 'blot' hybridization (Southern, 1975). Blots were prepared from pTK1 DNA cleaved with restriction enzymes EcoRI plus SstI, a combination which yielded four bands. The orientation and location of BamHI p sequences and TK mRNA on these fragments is shown in Fig. 1 (a). Hybridization of ³²P-pTK1 DNA, labelled in vitro by 'nick-translation' with all four radioactive deoxyribonucleoside triphosphates, showed that the transfer
Fig. 1. Hybridization of pTK1-specific transcripts to 'Southern' blots. Plasmid pTK1 DNA was cleaved with EcoRI plus SstI to yield four fragments, as shown in Fig. 1(a) (broken lines represent pAT 153 sequences). The position and orientation of TK mRNA-coding regions is also shown. Fig. 1(b) shows blot strips which were hybridized with 32P-nick-translated pTK1 DNA (track A), RNA extracted from oocytes 1 day after microinjection with pTK1 DNA plus α-32P-GTP (10 mCi/ml) (track B), or RNA extracted from oocytes preinjected with 50 nl α-amanitin (α-A) (1 μg/ml) 12 h before microinjection of pTK1 DNA plus α-32P-GTP (track C). Hybridization and treatment of blot strips was as described by Clements et al. (1977). Microdensitometer tracings of tracks A and B are shown in Fig. 1(c). The results of three experiments, in which radiolabelled RNA hybridized to pTK1 bands was calculated as described in the text, are tabulated in Fig. 1(d). Cleavage sites of restriction enzymes EcoRI, BamHI and SstI are abbreviated to RI, BI and SI respectively.
efficiency and availability for hybridization was similar for all fragments on the blot strips (Fig. 1b, track A). When radiolabelled RNA from pTK1-injected oocytes was used, radioactivity was associated with all fragments, but bands 2 and 4 were relatively more highly represented (Fig. 1b, track B). No hybridization of mock-injected oocyte RNA was found (results not shown). Fig. 1(b) (track C) also shows blot hybridization with RNA synthesized when 1 µg/ml α-amanitin (α-A) was microinjected before pTK1 DNA and α-32P-GTP. At this concentration the inhibitor inactivates RNA polymerase II but does not affect either endogenous or microinjected genes transcribed by RNA polymerase III (Melton & Cortese, 1979; M. G. Cordingley, unpublished observations). The absence of radioactivity associated with pTK1 bands indicates that production of stable transcripts of this plasmid DNA is mediated by RNA polymerase II.

Fig. 1 includes a summary of three hybridization experiments in which a quantitative estimate of radioactive RNA associated with each pTK1 band was made. The weights of peaks produced by microdensitometer tracings (Fig. 1c), representing hybridized DNA or RNA, were measured and corrected in the following three ways. First, the size of fragment was accounted for, to assess the accumulation of stable transcripts per unit length of DNA. As reported above, nick-translated DNA hybridized in proportion to fragment size. Second, the GC contents of individual fragments were calculated, since RNA was labelled with α-32P-GTP. Values for GC content obtained from the nucleotide sequences of BamHI p determined by D. McGeoch & M. Wagner, and J. Sharp & W. C. Summers (personal communications) and pAT 153 (Sutcliffe, 1978; Twigg & Sherratt, 1980) were used to correct the data. This adjustment did not significantly affect the interpretation of the results, since the values obtained were: band 1, 56-5% GC; band 2, 66-5% GC; band 3, 60% GC; and band 4, 62% GC. Finally, the relative accumulation of stable transcripts hybridized to band 1 was arbitrarily given a value of unity, in order to assess the degree of preferential expression of the other regions. The results, summarized in Fig. 1(d), confirm that bands 2 and 4, which contain TK mRNA sequences were more highly represented then bands 1 and 3.

The ovalbumin gene is the only other system in which RNA polymerase II-mediated transcription of microinjected plasmid DNA has been analysed in the way we describe here (Wickens et al., 1980). It was found that a clone containing genomic ovalbumin DNA, which contains at least six introns, directed the accumulation mainly of RNA specific to the vector, pBR322, whereas a clone containing cDNA, with no introns, gave rise to RNA specific to vector and insert DNA in approximately equal amounts. Furthermore, it appeared that ovalbumin mRNA could be produced from ‘readthrough’ transcripts initiated within pBR322 sequences, since it was synthesized after microinjection of a plasmid containing genomic DNA without the TATATAT sequence or the cap site.

The results we report here, by contrast, show transcription of all pTK1 regions but an enhanced level of RNA complementary to sequences within the insert, BamHI p. The enhanced level of hybridization could be due either to preferential transcription of BamHI p sequences or greater stability of the RNA product. In either case, the closer resemblance to transcription of ovalbumin cDNA clones, rather than genomic DNA clones, is interesting since the HSV-1 TK gene appears to be unspliced through most of the coding sequences (J. B. Clements & N. M. Wilkie, personal communication). This observation raises the possibility that ovalbumin-specific RNA is lost during splicing in oocytes. A crucial difference between the two examples, however, is the synthesis of TK protein from pTK1 DNA, as opposed to the failure of plasmids containing cDNA to produce ovalbumin.

The results presented here show that a HSV gene can be expressed in X. laevis oocytes, and describe a system in which authentic gene product can be detected in sensitive assays. It should be suitable for analysis of transcription of BamHI p or other cloned HSV DNA fragments by identification and manipulation of promoter sequences. The role of proteins which modulate TK gene expression in infected or transformed cells can also be investigated.
Short communications

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