Caffeine inhibits induction of endogenous C-type virus

ENDOGENOUS C-type viruses can be induced by treating the rodent cells in which they reside with halogenated pyrimiding analogues, notably 5-iodo-2'-deoxyuridine (IUdR) (refs 1, 2). Here I show that caffeine, which inhibits repair of photolesions and interacts with DNA in various ways³, inhibits the induction of the endogenous virus by IUdR.

The MLg cell line originating from a newborn ddY mouse was used; this cell line was found to release C-type virus after IUdR treatment⁴. The induced virus was detected by the XC test⁵.

The frequency of virus production was estimated as follows. MLg cells inoculated in 60 mm plastic Petri dishes (10^5 cells per dish) were treated with IUdR ($5\text{--}50\text{ }\mu\text{g ml}^{-1}$) for 24 h; the medium was removed and untreated MLg cells (10^5 cells per dish) were laid over the IUdR-treated cells. Three days later, three subcultures were made; after a further 3 d, the cultures were submitted to the XC plaque titration test. For IUdR concentrations of 5, 20 and $50\text{ }\mu\text{g ml}^{-1}$ the mean plaque number per dish was 19, 73 and 125, respectively. A proportional relation between plaque number and IUdR concentration was observed. There was some variation of induction frequency from one experiment to another, but the variation was small within one experiment, (see Fig. 1a). In the subsequent experiments, the concentration of IUdR was used at $25\text{ }\mu\text{g ml}^{-1}$ for 24 h.

The cells were treated with caffeine (0.625 to 5 mM) for 24 h before or after IUdR treatment ($25\text{ }\mu\text{g ml}^{-1}$ for 24 h). Twenty-four hours after the removal of IUdR, the cultures were overlaid with normal MLg cells (10^5 cells per dish). Three days later, each culture was divided into three, and

submitted to the XC test. Figure 1a shows that caffeine treatment after IUdR treatment significantly inhibited virus induction; a proportional relation was observed between caffeine concentration and the inhibition of induction of the C-type virus. Treatment with doses of caffeine as high as 2.5 mM before IUdR treatment was almost without effect.

In order to test the effect of caffeine on the exogenous C-type virus infection, MLg cells were treated with caffeine (1.25 to 5 mM) for 24 h before or after infection with Friend murine leukaemia virus (adsorption for 2 h). Twenty-four hours after infection, 10^5 normal MLg cells were added per dish. Three days later, the XC test was performed. Figure 1b shows that caffeine was without effect on exogenous virus infection.

Various ways of inhibiting cellular DNA synthesis, such as treatment with arabinosyl cytosine, excess thymidine and serum starvation, have been reported to inhibit virus induction⁶. The inhibition was presumably due to inhibition of IUdR incorporation. These conditions also inhibit exogenous C-type infection^{7,8}; consequently, in some cases, propagation of the induced virus may be inhibited.

Since caffeine affects neither the exogenous virus infection nor incorporation of $^{125}\text{IUdR}$ (data not shown) caffeine may interfere with events specific to virus induction.

What happens between IUdR incorporation into the cellular DNA and the subsequent release of the induced virus is obscure. Excision of the viral genome may or may not be necessary. In any event, viral induction may be brought about by some toxic modifications of DNA by IUdR. It is possible that IUdR may induce local distortion of DNA as the halogenated pyrimidines when incorporated into DNA cause fragmentation or instability of DNA⁹. Caffeine, known to interact with denatured but not with helical DNA⁹, may interact with such distorted regions of the DNA, so as to prevent viral gene activation. This last suggestion gains support from the fact that caffeine was especially effective after IUdR incorporation.

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Fig. 1 a, Effect of caffeine on endogenous C-type virus induction. Closed symbols indicate caffeine treatment after IUdR ($25\text{ }\mu\text{g ml}^{-1}$), and open symbols indicate caffeine treatment before IUdR. Four repeated experiments are shown. The mean number of plaques per dish was obtained from three cultures which were derived from one plate. In some experiments, 2 cultures were prepared for the control; the mean plaque number in this case is the mean of the two values obtained. The mean plaque number of the control was 129 for experiment 1 ($\circ$, $\bullet$), 134 and 119 for experiment 2 ($\triangle$, $\blacktriangle$), 114 and 98 for experiment 3 ($\square$, $\blacksquare$), and 95 and 85 for experiment 4 (∇ , $\blacktriangledown$). **b**, Effect of caffeine on exogenous Friend leukaemia virus infection. Open symbols indicate treatment before infection and closed symbols after infection. Duplicate experiments are shown. Three dishes were used for each point. The mean plaque number in the control dishes was 125 and 109 for experiment 1 ($\circ$, $\bullet$) and experiment 2 ($\triangle$, $\blacktriangle$), respectively.

Isolation of an inhibitor protein of *E. coli* RNA polymerase from T7 phage infected cell

BACTERIOPHAGE T7 gene expression is under a unique transcriptional control. The 'early to late' switch in T7 development involves a switch from the host *Escherichia coli* RNA polymerase to the T7-coded enzyme (T7-specific RNA polymerase¹), transcribing early mRNA and late mRNA, respectively, from two different regions of the T7 genome². In addition to the gene 1 coding for T7-specific