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21. The reaction mechanism used for evaluating the effects of reactions of NO_3 radicals with monoterpenes at night is as follows:

$$NO + O_3 \rightarrow NO_2 + O_2 \quad (1)$$
$$NO_2 + O_3 \rightarrow NO_3 + O_2 \quad (2)$$
$$NO_2 + NO_3 \rightarrow N_2O_5 \quad (3)$$
$$N_2O_5 \rightarrow NO_2 + NO_3 \quad (4)$$
$$NO_2 + NO_3 \rightarrow NO + NO_2 + O_2 \quad (5)$$
$$NO + NO_3 \rightarrow 2\ NO_2 \quad (6)$$
$$N_2O_5 + H_2O \rightarrow 2\ HNO_3 \quad (7)$$
$$NO_3 + \text{terpene} \rightarrow \text{products} \quad (8)$$
$$O_3 + \text{terpene} \rightarrow \text{products} \quad (9)$$

Rate constants for reactions 1 through 6 were taken from recent evaluations [R. Atkinson and A. C. Lloyd, *J. Phys. Chem. Ref. Data*, in press; *Chemical Kinetics and Photochemical Data for Use in Stratospheric Modeling* (NASA Evaluation 5, Jet Propulsion Laboratory publication 82-57, Pasadena, 1982)]. The rate constant for reaction 7 of 1.3×10^{-21} cm^3 sec^{-1} per molecule was taken from E. C. Tuazon, R. Atkinson, C. N. Plum, A. M. Winer, J. N. Pitts, Jr., *Geophys. Res. Lett.* **10**, 953 (1983). A rate constant of 5×10^{-12} cm^3 sec^{-1} per molecule was chosen for reaction 8 (*18*). The rate constant for reaction 9 was taken to be 1×10^{-16} cm^3 sec^{-1} per molecule, independent of temperature over a small temperature range around 298 K, a value consistent with available kinetic data (*22*). Sunset was assumed to occur instantaneously, an approximation that had no detectable effect on the model predictions. We derived the initial monoterpene concentrations at sunset by solving the steady-state equation involving the monoterpene emission rates and the daylight monoterpene loss processes by reaction with OH radicals (assumed to be at a constant concentration of 2×10^6 cm^{-3}) and O_3.
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23. The NO_2, O_3, and H_2O concentrations and temperature, respectively, chosen for these locations were as follows: Death Valley, 0.3 ppb, 30 ppb, 4 torr, and 303 K; Whitewater and Phelan, 3 ppb, 30 ppb, 6 torr, and 290 K: Edwards Air Force Base, 1 ppb, 30 ppb, 9 torr, and 300 K; Riverside and Claremont, 40 ppb, 40 ppb, 15 torr, and 303 K.
24. A monoterpene emission rate of 300 μg m^{-2} hour^{-1} for Riverside and Claremont was based on the upper limits for average nighttime monoterpene emissions determined for the Los Angeles area by Winer *et al.* (*5*). Monoterpene emission rates for Whitewater and Phelan of 150 μg m^{-2} hour^{-1} were based on the value for nonconifer, nonisoprene species given for desert areas by Zimmerman (*25*). An emission rate of 30 μg m^{-2} hour^{-1} for Death Valley and Edwards Air Force Base was arbitrarily chosen as a conservative upper limit for these largely nonvegetated areas.
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26. For these calculations, the O_3 and H_2O concentrations were held constant at 30 ppb and 12 torr (50 percent relative humidity at 298 K), respectively, and the temperature was held at 298 K. The NO_2 concentrations were assumed to be constant during given calculations and varied from 0.05 to 25.6 ppb. Monoterpene emission rates varied from 15 μg m^{-2} hour^{-1} (chosen as a possible lower limit for largely nonvegetated areas such as Death Valley and Edwards Air Force Base) to 7680 μg m^{-2} hour^{-1} [a value somewhat above the value of 6000 μg m^{-2} hour^{-1} reported by Zimmerman (*25*) for deciduous forested areas]. A box model was used, with a constant inversion height of 1000 m.
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29. Constant NO_2, O_3, and H_2O concentrations of 10 ppt, 30 ppb, and 15 torr, respectively, were assumed, together with a DMS emission rate of 24 μg m^{-2} hour^{-1} (*10*) and a constant inversion height of 1000 m. The reactions consuming DMS were as follows:

$$OH + DMS \rightarrow \text{products} \quad (10)$$

and

$$NO_3 + DMS \rightarrow \text{products} \quad (11)$$

with a rate constant of 1×10^{-11} cm^3 sec^{-1} per molecule for reaction 10 and a rate constant of 5.4×10^{-13} cm^3 sec^{-1} per molecule for reaction 11 (*17*).
30. We carried out calculations as described above (*29*) but assumed constant NO_3 radical concentrations of 0, 2, 5, and 10 ppt.
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33. This work was supported in part by National Science Foundation Atmospheric Chemistry Branch grant ATM-8209028 and Environmental Protection Agency grant R-810917. We thank B. J. Finlayson-Pitts for helpful discussions and M. Poe for generating Fig. 2.

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Cytomegalovirus Replicates in Differentiated but Not in Undifferentiated Human Embryonal Carcinoma Cells

Abstract. *To study the mode of action of human cytomegalovirus, an important teratogenic agent in human populations, the susceptibility of a pluripotent human embryonal carcinoma cell line to the virus was investigated. Viral antigens were not expressed nor was infectious virus produced by human embryonal carcinoma cells after infection, although the virus was able to penetrate these cells. In contrast, retinoic acid–induced differentiated derivatives of embryonal carcinoma cells were permissive for antigen expression and infectious virus production. Replication of human cytomegalovirus in human teratocarcinoma cells may therefore depend on cellular functions associated with differentiation.*

Intrauterine infection by human cytomegalovirus (HCMV) is a common cause of abnormal embryogenesis and fetal death (*1*). In the United States HCMV has been implicated in 2700 to 7600 cases of congenital birth defects annually (*2*). The mechanism and timing of fetal infection are obscure, since maternal infections are not usually clinically apparent, but HCMV transmission and severe damage to the fetus may occur very early in pregnancy (*3*). Also, only human fibroblasts are normally susceptible to productive infection by HCMV in vitro, and little is known about the control of cellular susceptibility to HCMV infection, particularly in the human embryo. Since embryonal carcinoma (EC) cells, the stem cells of teratocarcinomas, are thought to resemble cells of the early embryo in their biochemical and developmental properties (*4*), the recent characterization of a pluripotent human EC cell line now permits experimental study of the growth of HCMV in a cell akin to early human embryonic cells.

Line NT2/B9, a clonal human EC cell line isolated from TERA-2 (*5*), exhibits a

Table 1. Susceptibility of NT2/B9 EC and RA-induced differentiated cells to HCMV infection, as determined from the expression of viral antigens and the production of infectious virus. Cells were grown, infected, and tested for the presence of viral antigens by immunofluorescence as described in the legend to Fig. 1. The cells (3×10^3 to 5×10^3) were counted in each preparation to determine the percentage of cells positive for viral antigen. The number of cells containing infectious virus was determined by an infectious center assay. Infected NT2/B9 cells were washed five times in phosphate-buffered saline (PBS), trypsinized, and counted. Cells (3×10^2) were incubated with an excess of antiserum to HCMV (1 ml; 1:8 dilution of the antiserum and 1:24 dilution of guinea pig complement) for 1 hour, washed three times with PBS, and seeded on three parallel cultures of confluent MRC-5 monolayers at 1×10^2 NT2/B9 cells per petri dish. Cultures were overlaid with agar and the plaques were counted after 2 weeks. The mean number of plaques in the three parallel cultures are given. The immune serum fully neutralized approximately 3000 infectious particles at a dilution of 1:32 in separate experiments. The yield of virus in the culture medium was determined by the method of Wentworth and French (*19*). Expression of SSEA-3 was detected by reactivity of the cells with a specific monoclonal antibody assayed by flow cytofluorometry (*5*). Abbreviation: PFU, plaque-forming units.

Cells	Cells containing viral antigen (percent)			Infectious virus 7 days after infection in		Cells expressing SSEA-3 at time of infection (percent)
	Days after infection			Intact cells (PFU per 10^2 cells)	Culture medium (PFU/ml)	
	2	4	7			
NT2/B9	1.2	1.4	1.8	0.03	12	94.5
NT2/B9 + RA	68	76	85	45	$.5 \times 10^3$	3.2

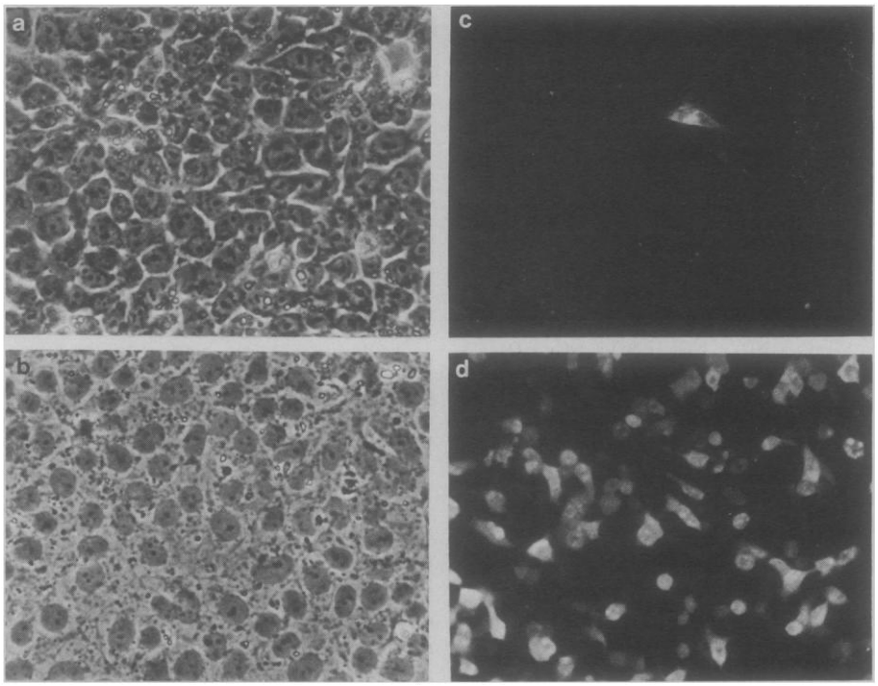

Fig. 1. Susceptibility of NT2/B9 EC cells and their RA-induced differentiated derivatives to HCMV replication. Stock cultures of NT2/B9 cells exhibiting an EC morphology (a) and expressing the cell surface antigen SSEA-3 (not shown) were maintained as described previously (*6*). RA-induced differentiated cells were derived by growing cultures in medium containing $10^{-5}M$ RA for 7 days, after which most cells were morphologically distinct from the parental EC cells (b). Few cells (< 5 percent) retain an EC phenotype, such as the expression of SSEA-3, when grown under these conditions, although they are fully viable (*6*). To study susceptibility to HCMV, experimental cultures were established in the absence of RA on cover slips by seeding cells from EC stock cultures or 7-day RA-induced cultures. One day later the cells were infected with the recently plaque-purified Towne strain of HCMV, propagated in MRC-5 human fibroblasts (*17*) at a multiplicity of infection of 5 to 10. The percentage of cells containing viral antigen was determined by indirect immunofluorescence by using human antiserum to HCMV at a dilution of 1:20 and fluorescein isothiocyanate–labeled antiserum to human immunoglobulin G at a dilution of 1:30 (Cappel Laboratories). The immune serum reacted with early and late HCMV antigens in infected human fibroblast cells (*18*). (c) Field of EC cells infected with HCMV and fixed and stained with an antiserum to HCMV 7 days later. Note the single HCMV antigen–positive cell, the remaining cells being HCMV antigen–negative. (d) Field of RA-induced differentiated cells that were similarly infected and tested for HCMV antigens.

typical EC phenotype characterized by a high nucleus to cytoplasm ratio, few prominent nucleoli (Fig. 1a), and the expression of the cell surface antigen SSEA-3 (*5*). When inoculated with HCMV, only a few cells in these cultures were susceptible to the induction of viral antigens or the replication of infectious virus (Fig. 1c and Table 1). These permissive cells probably represent some of the spontaneously differentiated cells (10 to 15 percent) that are usually found in these cultures (*5*).

Table 2. Adsorption and penetration of HCMV into NT2/B9 EC cells and their RA-induced differentiated derivatives. Cultures seeded on the previous day from NT2/B9 EC stock cultures or from NT2/B9 cultures treated with RA for 7 days were infected at a multiplicity of infection of 5 to 10; 2 hours later they were harvested by trypsinization and the number of cells containing infectious virus was determined by infectious center assay as described in the legend to Table 1. Percentage of penetration was calculated as the ratio of the number of plaques formed after treatment of cells with excess HCMV antiserum for neutralization to the number of plaques found after treatment with nonimmune human serum.

Cells	Number of plaques after treatment of cells with		Penetration (percent)
	Normal serum	Immune serum	
NT2/B9	75	32	42.6
NT2/B9 + RA	95	48	50.5

Although only limited spontaneous differentiation occurs in cultures of NT2/B9 cells, extensive and irreversible differentiation occurs following exposure to $10^{-5}M$ retinoic acid (RA) (*6*): after 7 days, almost all the cells lose their EC morphology (Fig. 1b) and their cell surface expression of SSEA-3 (Table 1). Further culture of the RA-treated cells, which are fully viable, leads to the appearance of various morphologically distinct somatic cell types that include neurons expressing neurofilaments and tetanus toxin receptors (*6*). Many of the differentiated cells obtained by a 7-day exposure of NT2/B9 cells to RA were susceptible to viral infection, as shown by their expression of HCMV antigens and the production of infectious virus (Fig. 1d and Table 1). The susceptibility to infection was not due to a direct potentiating action of the RA, since the differentiated cells were harvested, washed, and reseeded in the absence of RA 1 day before infection. Similarly, the differentiated cells in cultures maintained without RA for up to 2 months also retained their susceptibility to HCMV infection. Treatment of another line of human EC cells (2102 Ep) with RA induces neither their differentiation nor their susceptibility to HCMV infection (*7*).

To determine whether the resistance of the EC cells to HCMV was due to a failure of the virus to penetrate the cells, we measured virus adsorption and penetration with an infectious center assay. As indicated by the protection of the virus from inactivation by antiserum to HCMV and complement, HCMV does penetrate NT2/B9 EC cells (Table 2).

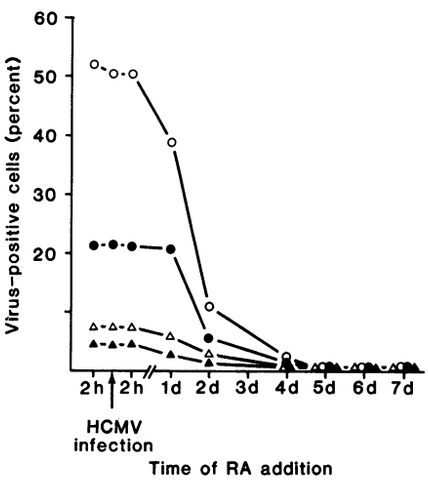

Fig. 2. Activation of HCMV replication by RA-induced differentiation. NT2/B9 EC cells were seeded on cover slips and infected 1 day after seeding at a multiplicity of infection of 5 to 10. Cultures were washed extensively 2 hours after infection to remove unadsorbed virus. Medium containing $10^{-5}M$ RA was added to the NT2/B9 cells 2 hours before infection, at the time of infection, and at various times after infection. The cells were fixed and tested for their expression of viral antigen by immunofluorescence (see legend to Fig. 1) 4 (▲), 5 (△), 6 (●), and 7 (○) days after RA was added. Between 3×10^3 and 5×10^3 cells were counted to determine the percentage of viral antigen–positive cells.

Similar penetration of the virus into differentiated cells induced by RA was also observed. These results indicate that the block to HCMV replication in the EC cells occurs after penetration of the virus.

Since HCMV is able to penetrate NT2/B9 EC cells, we tried to determine whether the virus could be activated by inducing differentiation after infection. Whereas less than 2 percent of the EC cells were positive for viral antigen 7 days after infection, 50 percent of the cells in cultures to which RA was added 2 hours before infection were positive 7 days later (Fig. 2). The same level of viral expression was found in cultures to which RA was added simultaneously with virus or 2 hours after infection. However, if RA was added 5 days after infection, no more cells expressing viral antigen than in untreated EC cultures were found 7 days later, even though the RA still induced cellular differentiation (*8*). Thus either the undifferentiated cells gradually eliminate the viral genome or the viral genome persists in a modified form that cannot be activated by later cellular differentiation.

The replication of HCMV in differentiated but not undifferentiated NT2/B9 cells is, to our knowledge, the first observation of a change in susceptibility to a virus upon differentiation in human teratocarcinomas. On the other hand, it has been known for some time that murine EC cells are resistant to several DNA (*9*) and RNA viruses (*10*), whereas their differentiated derivatives are not (*9, 10*). For example, murine cytomegalovirus (MCMV) penetrates PCC4 murine EC cells without subsequent transcription of viral messenger RNA, although cells differentiating in PCC4 cultures under the influence of dimethylacetamide do permit MCMV replication (*11*). The mechanism by which expression of the viral genome is restricted by murine EC cells may involve blocks to transcription, perhaps attributable to viral enhancer sequences (*12*) or host EC cells methylating newly acquired DNA (*13*), as well as blocks to viral messenger RNA processing (*14*) and to translation (*15*). Nevertheless, the biology of murine and human EC cells differs in many respects (*5, 16*), so it is not correct to extrapolate findings from one to the other.

Since HCMV penetrates undifferentiated human EC cells but replicates only in their differentiated derivatives, this system provides an opportunity to identify the cellular factors that enable HCMV to replicate. It should also now be possible to study the effect of HCMV infection on the cellular events that occur during differentiation of cells resembling those of the early human embryo. This information might, in turn, bear on the mechanism of latent infection and reactivation by the virus and ultimately on its teratogenic effects.

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Single-Copy Inverted Repeats Associated with Regional Genetic Duplications in γ Fibrinogen and Immunoglobulin Genes

Abstract. *We have found that a portion (150 base pairs) of the seventh exon of the human γ fibrinogen gene is duplicated in the preceding intron. This duplicated sequence, termed a "pseudoexon," is flanked on each side by a single-copy inverted repeat sequence consisting of 102 base pairs. Frequencies of point substitutions indicate that both the pseudoexon and the inverted repeat sequence arose approximately 10 to 20 million years ago. The generality of this type of duplication is suggested by the occurrence of a similiar duplication in the mouse immunoglobulin μ-δ region. As in the fibrinogen pseudoexon, the portion of the immunoglobulin μ-δ region containing the duplication and the inverted repeat was reported to be single-copy in the mouse genome. Since both of the first two single-copy inverted repeats to be sequenced are associated with regional duplications, it is likely that many of the single-copy inverted repeat sequences, which make up 1 to 2 percent of the genome, are also associated with regional duplications.*

Long inverted repeat (IR) sequences, which consist of self-complementary sequences nearby on the same strand, are reported to represent approximately 5 percent of the human and other eukaryotic genomes (*1*). The significance of IR's in eukaryotes is uncertain, although several examples of repetitive genetic elements with terminal IR's, which function as transposable elements, have been identified in *Drosophila* (*2*), sea urchin (*3*), and *Dictyostelium* (*4*). The IR from the FB transposable elements of *Drosophila* and the TU1 of sea urchin have a partial subunit structure consisting of short direct repeats. Inverted repeat sequences consist of both repetitive and single-copy IR sequences in the same ratio, as is found in total cell DNA (*1*). The function and significance of single-copy IR's is unknown even though they represent 1 to 2 percent of most eukaryotic genomes (*1*).

While analyzing the human γ fibrinogen gene, we found that a portion of the seventh exon was duplicated in an adjacent intron. This duplicated sequence or "pseudoexon" is flanked on both sides