# Cellular gene expression altered by human cytomegalovirus: Global monitoring with oligonucleotide arrays

Hua Zhu\*, Jian-Ping Cong\*, Gargi Mamtora<sup>†</sup>, Thomas Gingeras<sup>†</sup>, and Thomas Shenk<sup>\*‡</sup>

\*Howard Hughes Medical Institute, Department of Molecular Biology, Princeton University, Princeton, NJ 08544; and <sup>†</sup>Affymetrix, 3380 Central Expressway, Santa Clara, CA 95051

Contributed by Thomas Shenk, September 11, 1998

ABSTRACT Mechanistic insights to viral replication and pathogenesis generally have come from the analysis of viral gene products, either by studying their biochemical activities and interactions individually or by creating mutant viruses and analyzing their phenotype. Now it is possible to identify and catalog the host cell genes whose mRNA levels change in response to a pathogen. We have used DNA array technology to monitor the level of ~6,600 human mRNAs in uninfected as compared with human cytomegalovirus-infected cells. The level of 258 mRNAs changed by a factor of 4 or more before the onset of viral DNA replication. Several of these mRNAs encode gene products that might play key roles in virus-induced pathogenesis, identifying them as intriguing targets for further study.

Human cytomegalovirus (HCMV) has the potential to alter cellular gene expression though multiple mechanisms. Its initial interaction with the cell surface could initiate a regulatory signal; indeed, the virion gB and gH glycoproteins induce cellular transcription factors when added to uninfected cells (1). Constituents of the virion, such as the tegument protein, pp71, migrate to the nucleus and activate transcription after infection (2), and viral proteins synthesized after infection, such as the immediate early 1 and 2 proteins, modulate transcription (3–5). The virus encodes several G protein-coupled receptors (6, 7) that likely initiate gene regulatory signal cascades in response to ligands, and HCMV infection has been shown to perturb cell cycle regulation (8–11), which leads to changes in cellular gene expression. The complex virus–host cell interaction has the potential to modulate the expression of cellular genes dramatically.

Relatively few cellular genes have been identified whose activity changes in HCMV-infected cells (12). Recently, differential display analysis was used to identify 15 interferon-inducible genes that are activated by the virus subsequent to infection (13). However, this screen identified only genes whose mRNA levels changed dramatically, and the screen was not performed under a variety of conditions, given its labor-intensive nature. In contrast to differential display, the DNA array assay is performed easily and can detect subtle changes in mRNA levels. We report the identification of 258 cellular mRNAs whose level changes by a factor of 4 or more before the onset of HCMV DNA replication.

## MATERIALS AND METHODS

**Cells and Viruses.** Primary human foreskin fibroblasts at passage 10–15 were cultured in DMEM containing 10% fetal calf serum. After the cells remained at confluence for 3 days, they were infected at a multiplicity of 3 plaque-forming units per cell with HCMV AD169 or Toledo virions that were purified as described (14).

Sample Preparation and Analysis with DNA Arrays. Biotinylated single-stranded antisense RNA samples for hybridization were prepared as described (15) with minor modifications. Total cellular RNA was prepared by using the TRIZOL Reagent (GIBCO/BRL), polyadenylated RNA was isolated, and portions (5  $\mu$ g) were used as the template for the first strand cDNA synthesis in a reaction that was primed with oligo(dT) containing a T7 RNA polymerase promoter sequence at its 5' end [5'-GG-CCAGTGAATTGTAATACGACTCACTATAGGGAGGC-GG(T)<sub>24</sub>-3']. The second cDNA strand was synthesized by using Escherichia coli DNA polymerase I and ligase. The resulting cDNA (0.5–1  $\mu$ g) was used as template to make a biotinylated RNA probe by in vitro transcription using the T7 Megascript System (Ambion, Austin, TX). Unincorporated nucleotides were removed by using a G-50 Quick Spin Column (Boehringer Mannheim). The labeled RNA was fragmented to an average size of 50-100 bases by incubating at 94°C for 30 min in buffer containing 40 mM Tris·Ac (pH 8.1), 100 mM KOAc, and 30 mM MgOAc. The hybridization (15 h), washing, and staining protocols were as described (15) and used a set of four human gene chips (HUM6000 A, B, C, and D, Affymetrix, Santa Clara, CA). The DNA arrays were scanned by using a confocal scanner manufactured for Affymetrix by Molecular Dynamics.

Data Analysis. The data collected in each hybridization experiment was processed by using the GENECHIP software supplied with the Affymetrix instrumentation system. To evaluate whether RNA corresponding to each of the 6,600 genes encoded on the array was detectable or undetectable, a number of parameters were evaluated (15, 16), including the number of probe pairs interrogating each gene in which the intensity of the perfect match hybridization reaction exceeded that of the mismatch hybridization signal and the perfect match/mismatch ratios for each set of probe pairs. To determine the quantitative amounts of RNA from each gene, the average of the differences (perfect match minus mismatch) for each probe pair in a probe set was calculated as well as the average differences across the probe sets. The cutoff thresholds were determined empirically to be conservative; that is, they minimized false positives. The change in the level of expression for any gene was considered significant if the change in the average differences across the probe sets was >3-fold.

**RNA Analysis by Northern Blot.** GENECHIP results were confirmed by Northern blot assay. Total RNA (3  $\mu$ g) from mockinfected cells or cells infected with the HCMV AD169 or Toledo strains was subjected to electrophoresis, was blotted to a membrane, and was probed with random hexanucleotide-primed <sup>32</sup>P-labeled cDNA fragments from IMAGE (Genome Systems, St. Louis).

## RESULTS

The gene chip assay used a set of four probe arrays that together include oligonucleotides corresponding to >6,600 human mRNAs (16). Each array (1.6 cm<sup>2</sup>) contains >65,000 features, and a different oligodeoxyribonucleotide (25 bases) is

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

<sup>@</sup> 1998 by The National Academy of Sciences 0027-8424/98/9514470-6\$2.00/0 PNAS is available online at www.pnas.org.

Abbreviations: HCMV, human cytomegalovirus; NK, natural killer; cPLA2, cytosolic phospholipase A2; COX-2, cyclooxygenase 2; MITF, microphthalmia-associated transcription factor.

<sup>&</sup>lt;sup>‡</sup>To whom reprint requests should be addressed. e-mail: tshenk@ princeton.edu.

synthesized on the surface of the derivatized glass wafer within the boundaries of each feature by using light-sensitive chemistry (17–21). The arrays contain 20 pairs of oligonucleotide probes corresponding to each RNA that is interrogated. Each probe pair consists of one 25-mer that is a perfect complement to the RNA (a perfect match probe) and a companion oligonucleotide that carries a single base difference in a central position (a mismatch probe). The mismatch probes serve as internal controls for hybridization specificity. Empirically derived rules used for the selection of oligonucleotide probes with the best sensitivity and specificity have been described (16).

RNA samples were prepared for analysis at 40 min, 8 h, and 24 h after mock infection or HCMV (strain AD169) infection of primary human fibroblasts. Under these conditions, HCMV DNA replication begins between 24 and 36 h after infection (10), and the complete viral replication cycle requires  $\approx$ 72 h. So, all of the time points assayed were relatively early in the HCMV replication cycle. Biotinylated RNA target samples were generated by in vitro transcription of cDNA that was prepared from cellular mRNA by using an oligo(dT) primer with a T7 polymerase promoter at its 5' end. This protocol amplifies the mRNA population in an unbiased and reproducible fashion (16). The resulting antisense RNA was fragmented to an average size of 50 to 100 bases and was hybridized to the oligonucleotide probe arrays, and then the arrays were reacted with phycoerythrinconjugated streptavidin. The intensity of the fluorescent signal within each feature then was quantified by using a confocal scanner (Affymetrix). Previous studies have demonstrated that the fluorescent signal is linearly related to the concentration of RNA target within the range of  $\approx 1$  (1 part in 300,000) to nearly  $10^3$  copies of RNA per cell (16). Above  $10^3$  copies per cell, the signal continues to increase but in a nonlinear fashion because the oligonucleotide probes begin to saturate. RNAs corresponding to 3,020-3,380 of the 6,600 genes were detected in different experiments. The range is caused in part by virus-induced changes. However, much of the variation is caused by mRNAs expressed at the level of 1-10 copies per cell, scoring as present in one assay and absent in another experiment.

The DNA arrays contain a set of 198 oligonucleotides corresponding to sequences spread across the entire length of the glyceraldehyde-3-phosphate dehydrogenase mRNA. The target RNAs prepared at 8 h after infection with HCMV (Fig. 1*A*) or after mock infection (data not shown) hybridized to the complete glyceraldehyde-3-phosphate dehydrogenase probe set. The arrays also included oligonucleotides spanning the actin mRNA and target RNAs hybridized to this complete probe set, as well (data not shown). These controls demonstrated that the target RNA preparations span the entire length of the test gene and provided confidence that the cDNA synthesis and subsequent *in vitro* transcription generated target RNAs representative of the input mRNA.

The reproducibility of hybridization signals produced by independent preparations of target RNAs also was tested. Biotinylated target RNA was prepared from mock-infected cells (Fig. 1*B*) or at 8 h after infection (Fig. 1*C*) and was hybridized to different sets of arrays. The concentration of only one cellular mRNA differed by a factor of >3 in the replicate experiments (Fig. 1*B*). This control demonstrates that the hybridization signals observed in independent experiments are highly reproducible. Further, the two preparations of infected cell target RNAs were prepared from infected primary fibroblasts derived from two different tissue samples, ruling out the possibility that changes in RNA levels might reflect genetic differences in the host cells. Differences >3-fold observed for hybridization signals in comparisons of mock-infected versus infected cells should identify genes whose mRNA levels change after infection.

When target RNA preparations were compared at 40 min after mock or virus infection, the level of 27 mRNAs had changed in response to infection by a factor of 3 or more; at 8 and 24 h after infection, the number of altered mRNAs increased to 93 and 364,



FIG. 1. Characterization of RNA target samples and reproducibility of array-based hybridization results. (*A*) Probe pairs (82, 68, and 51 pairs) were used to interrogate the 5', middle, and 3' portions of the glyceraldehyde-3-phosphate dehydrogenase mRNA, which is expressed constitutively in fibroblasts. (*B* and *C*) Plots comparing the average difference intensities in fluorescent signal (Avg. Diff. Intensities) of the 20 probe pairs interrogating each of the genes present in two independent experiments performed on the mock-infected cells (*B*) or cells at 8 h after infection (*C*). The parallel lines flanking the center diagonal line indicate 3-, 10-, and 30-fold changes in intensity. With the exception of the thombospondin 1 gene in the mock-infected control, all genes demonstrated an average difference in their fluorescent intensities of <3-fold.

respectively (Fig. 2). Applying a more stringent 4-fold change as the cut off, we generated a set of 258 mRNAs for further analysis (Table 1). Of these mRNAs, 124 increased, and 134 decreased after infection. We assume that most changes resulted from altered transcriptional regulation, but we have not yet tested this



FIG. 2. Global survey of the differences in mRNA levels after HCMV infection. The plots show the variation in fluorescent signal intensities (Avg. Diff. Intensities) between mock-infected cells and cells at 40 min, 8 h, and 24 h after infection. Changes in expression of 3-, 10-, and 30-fold are highlighted by the parallel lines flanking the center diagonal line.

Table 1.	mRNAs	that	increase	or	decrease	$\geq$ 4-fold	after
HCMV infection							

A /. A .A. / .			
Actin/tubulin/myosin X06956	a-tubulin	24 h .6×	U 3
R59199	est = $X79535 - \beta$ tubulin	24 h, 4×	U 3
Z24727	tropomyosin isoform	24 h, 4×	D 3
192451 X05276	fibroblast tropomyosin TM30 (pl)	24 n, 5× 24 h. 4×	D 3
T60155	est = $J05192 - \alpha$ -actin (ACTA)	24 h, 11×	D 3
M19283	γ-actin	24 h, 4×	D 3
A54105 H44011	myosin heavy chain	24 n, 5× 8 h 5×	D 3
T55741	est = $X85337$ – myosin light chain kinase (MLCK)	24 h, 6×	D 3
X53416	actin-binding protein (filamin) (ABP-280)	24 h, 5×	D 1
Cell cycle	neutrai caiponin	24 n, 15×	D 3
L49231	retinoblastoma susceptibility protein (RB1)	24 h, 12×	U 3
X59798	PRAD1 mRNA for cyclin	24 h, 9×	D 3
D13891 1 13698	Id-2H gas_1 (growth-arrest-specific gene)	8 h, 24 h, 4× 8 h 24 h 15× 6×	D 3
X62048	Wee1 hu	8 h, 24 h, 5×	D 3
H50438	est = M81934 - cdc25B	8 h, 4×	D 3
U09477 Coognitation	53BP1 p53-binding protein	8 h, 5×	D 3
R16659	est = L27624 and AC002076 - TFPI-2	24 h. 8×	U 1
M59499	lipoprotein-associated coagulation inhibitor	8 h, 6×	D 3
M14083	β-migrating plasminogen activator inhibitor I	24 h, 4×	D 3
J02933 Complement	blood coagulation factor VII gene	24 h, 5×	D 3
M31516	decay-accelerating factor (DAF)	8, 24 h, 4×, 5×	U 1
T54547	est = M84526 - adipsin/complement factor D	24 h, 7×	D 3
T69603	est = $M14058$ - complement c1r component	$24 \text{ h}, 4 \times$	D 3
R120/0	complement factor H	40°, 4×	D 3
Cytokinase/receptors			
M21121	rantes	8 h, 6×	U 2
X58377	interleukin 11 (II11)	8. 24 h 4× 11×	U 3 U 1
M29696	interleulin 7 (IL-7) receptor, $\alpha$ chain	24 h, 4×	Ŭ 1
U02020	pre-B cell enhancing factor (PBEF)	8 h, 24 h, 4×	U 3
161446 1 35263	A20 CSaids hinding protein (CSBP1)	8 h, 4× 8 h 7×	U 3 D 2
M58286	tumor necrosis factor receptor	8 h. 24 h. 4×. 5×	D 3
H14506	est = L36034 - pre-B cell stimulating factor	24 h, 4×	D 1
X72012	endoglin	8 h, 23×	D 3
L36531	integrin α 8 subunit	40′. 6×	U 3
M80244	E16, an integral membrane protein	40', 4×	U 3
X14787	thrombospondin 1 (TSP1)	24 h, 21×	D 1
L12350 X05231	collagenase	24 h, 13× 40′ 8 h 4× 7×	D 3
L25285	collagen α-1 type XV (COL15A1)	24 h, 13×	D 3
J03464	collagen α-2 type I	24 h, 14×	D 3
M11718	collagen $\alpha$ -2 type V	24 h, 8×	D 3
T51558	est = K01228 - procollagen $\alpha$ 1 (I) chain	24 h, 6×	D 3
X06700	pro-α1 (III) collagen	24 h, 16×	D 1
L16895	lysyl oxidase, an extracellular copper enzyme	24 h, 4×	D 3
X53743 1134976	fibulin-1 C – a secreted glycoprotein	40', 8 h, 7×, 5×	D 3
U14394	Tissue inhibitor of metalloproteinases-3 (TIMP3)	$24 \text{ h}, 6-8\times$	D 3
R32771	est = U37791 - rasi-1 matrix metalloproteinase	8 h, 24 h, 5×, 9×	D 3
U03877	extracellular protein (S1-5)	24 h, 4×	D 3
GTP binding proteins	cadherin-11	24 n, 9×	D 3
H67367	est = D38076 - RanBP1 (Ran-binding protein 1)	24 h, 5×	U 3
T93295	est:homo. of L20294-mouse GTP-binding protein	24 h, 4×	U 3
X75593 R53966	rab 13 est = $722641 - a2$ chimaerin	24 h, 9× 24 h 8×	D 3
H19201	est – Z22041 – az-chinaerin est: homo, to mouse L07924 – gua, nucl.	24 II, 8× 8 h. 9×	D 3
	disso. stim.		
POS183	est = X75821 U07550 = chaperonin 10	24 h 8 ×	11.3
T66307	heat shock 70kDa protein 1	24 h, 0× 24 h, 11×	U 3
M86752	transformation-sensitive protein	24 h, 8×	U 3
L08069	heat shock protein, E. coli DnaJ homologue	8 h, 4×	U 3
T93272	est = M14528 $\alpha$ enouse est = M62829 early growth response protein 1	$24 \text{ II}, 13 \times$ 8 24 h 5× 6×	U 5
T51856	est: homo. to stress-inducible chaperone	24 h, 4×	Ŭ 3
¥70066	mt-GrpE#1	8 h 4×	D 2
Interferon	ERI-1	o II, 4^	D 3
M24594	interferon-stimulated genes 54K (isg54K)	8 h, 24 h, 19×, 5×	U 2
M87434	71-kDa 2'5' oligoadenylate synthetase	8 h, 6×	U 2
X02875 X02874	(2'-5') oligo A synthetase E (1.8 kilobase RNA) (2'-5') oligo A synthetase E (1.6 kilobase RNA)	8 h, 24 h, 15×, 7× 8 h 8×	U 2 U 2
M87284	69-kDa 2'5' oligoadenylate synthetase	8 h, 8×	U 2
X02530	γ-interferon inducible early response gene	8 h, 34×	U 1
L05072	interferon regulatory factor 1 (IRF-1)	24 h, 7×	U 2
X67325	interferon $\alpha$ inducible gene: p27 gene	24 h. 7×	$U_2$
H05300	interferon-induced guanylate-binding protein 1	8 h, 24 h, 17×, 9×	Ŭ 2
M55542	guanylate binding protein isoform II	8 h, 9×	U 2
D31887 X82200	KIAA0062 (cig19) interferon inducible gene staf50	8 h, 24 h, $4 \times$ 24 h 14 ×	U 2 U 1
X02492	interferon-induced protein 6-16	8 h, 24 h, 4×, 5×	U 2
R34698	interferon-inducible protein 9-27	8 h, 24 h, 6×	U 2
M13755 M28622	interferon 6	8 h, 24 h, 150×	U 2
X17668	indoleamine 2,3-dioxygenase	8 h, 47×	U 1
M33882	MxA	8 h, 24 h, 47×, 5×	U 2
M30818	MxB	8 h, 30×	U 2
A30841 T50250	est: homo, to U51904 mouse IFN astreated	o 11, 24 n, 6× 24 h, 32×	U 1 U 1
	mRNA	, <i></i> .	
M60618	nuclear autoantigen Sp100	8 h, 5×	U 2
M/3//8 Kinase/phosphate	r WIL-1	о 11, 0×	$\cup 2$
R39857	est = X97630 - serine/threonine protein	24 h, 4×	U 3
1102000	kinase EMK	24 1- 957	11.2
U25994	cell death protein (RIP protein kinase)	24 II, 8× 8 h. 4×	U 3
D21209	protein tyrosine phosphatase (PTP-BAS, type 1)	24 h, 5×	D 1
X77278	HYL tyrosine kinase	24 h, 8×	D 3

# Table 1. Continued

R60908 H65441	est = X74764 - receptor protein tyrosine kinase est = U78027, L35265 - Bruton's tyrosine kinase	24 h, 21× 24 h, 7×	D 1 D 3
X16416 Lamins	proto-oncogene tyrosine-protein kinase (abl)	24 h, 4×	D 1
M55210 X79683	laminin B2 chain	24 h, 8×	D 3
R43734	$S78569 - laminin \alpha 4$ chain	24 h, 6×	D 3
T55218 Ligands and receptor	est = M13452 or M13451 $-$ lamin A or C	8 h, 26×	D 3
L13740	TR3 orphan receptor	8, 24 h, 5×, 46×	U 1
M77140	pro-galanin	8 h, 3× 24 h, 20×	U 1
M63888 T70920	heparin-binding growth factor receptor est = M88279 - immunophilin (EKBP52)	24 h, 9× 24 h 8×	U 3
M20132	androgen receptor (AR)	$8 \text{ h}, 4 \times$	D 3
M19481 T71662	follistatin est = M14118 – insulin-like growth factor	24 h, 16× 24 h, 4×	D 1 D 1
M25070	(IGF-II)	24 h 4 ×	D 2
M65062	insulin-like growth factor-binding protein-5	24 h, 5×	D 3
M35410 X04434	insulin-like growth factor-binding protein 2 insulin-like growth factor I recentor	40′, 8 h, 5×, 11× 40′ 8 h 24 h 4×	D 3 D 3
L07594	transforming growth factor $\beta$ type III receptor	8 h, 24 h, 4×, 6×	D 1
M21574 H88938	platelet-derived growth factor receptor $\alpha$ est = M60828 M25295 - keratinocyte growth	24 h, 25× 8 h, 4×	D I D 3
X62381	factor activin receptor: growth factor-like receptor	8 h 6 X	D 3
R45296	est = $U67784 - orphan G protein-coupled$	24 h, 6×	D 3
X02157	receptor fetal erythropoietin	24 h, 4×	D 3
M64497	apolipoprotein A1 regulatory protein (ARP-1)	8 h, 4–5×	D 1
L11708 L00352	$1/\beta$ hydroxysteroid dehydrogenase type 2 low density lipoprotein (LDL) receptor	24 h, 7× 8 h, 24 h, 5×, 6×	D 3 D 3
M10065	apolipoprotein E	40′, 8 h, 7×, 26×	D 3
U04636	cyclooxygenase-2 (cox2)	8 h, 7×	U 2
M72393 X05908	cystosolic phospholipase A2 (cPLA2)	8 h, 24 h, 12×, 7×	U 3
D38145	prostacyclin synthase	24 h, 9× 24 h, 4×	D 3
Protein degradation	est = D26500 = protessome subunit HsC7 I	24 h 6×	11.3
T54276	proteasome subunit LMP7 (allele LMP7C)	$8 h, 4 \times$	U 3
T92259 D00762	proteasome component IOTA chain	24 h, 5× 24 h 6×	U 3
D00760	proteasome component C3	24 h, 5×	U 3
H05893 L02426	26S proteasome subunit p97 26S protease (S4) regulatory subunit	24 h, 4× 24 h, 16×	U 3 U 3
M91670	ubiquitin carrier protein (E2-EPF)	24 h, 4×	Ŭ 1
R67921 T56256	est = D55696 - putative cysteine protease est = U20657 - ubiquitin protease proto-oncogene	8 h, 4× 8 h, 4×	U 3 D 3
J03589	ubiquitin-like protein (GdX)	24 h, 7×	D 3
150500	est = Z22658 – a placental thrombin inhibitor (PTI)	24 h, 4×	D 3
Protooncogenes X89985	BCI 7B gene	8 h 24 h 5×	<b>I</b> I 1
H48122	est = U43746, breast cancer susceptibility (BRCA2)	8 h, 4×	U 1
M83751 M27903	arginine-rich protein (ARP) pim-1 proto-oncogene	24 h, 11× 24 h, 16×	U 3 U 3
T53138	est = Y11306 - hTCF-4	$8 h, 4 \times$	D 3
D43969	AML1c protein	$^{24}$ h, $^{4\times}$ 8 h, 24 h, 4×	$D_{3}$
X82209 Splicing factors	MN1	8 h, 24 h, 7×	D 3
X13482	U2 small nuclear ribonucleoprotein A'	24 h, 4×	U 3
M15841 R41349	U2 small nuclear RNA-associated B" splicing factor, arginine/serine-rich 7 (SFRS7)	24 h, 4× 24 h, 10×	U 3 U 3
Transcription factors		211, 107	
R55041 H46624	est = J03161 - serum response factor (SRF) est: homology to mouse AB012276 - ATFx	24 h, 5× 8 h, 4×	U 3 U 3
M97676	homeobox protein (HOX7)	8 h, 4×	U 1
M69043	est = $X59268 - IFIIB$ MAD-3, encodes an I $\kappa$ B-like protein	8 h, 8× 24 h, 10×	U 1 U 1
R26146	NF-kB p105 subunit	24 h, 4×	U 3
H88261	est = $U90304$ iroquois-class homeodomain protein	8 h, 4×	U 3
Z29678 X03348	mitF & glucocorticoid receptor	24 h, 8× 8 h 24 h 4×	D 3
M83667	NF-IL6-β protein	8 h, 4×	D 3 D 1
X57435 M36711	transcription factor AP-4 sequence-specific DNA-binding protein (AP-2)	8 h, 24, 4× 8 h 6×	D 3
Y00345	polyA binding protein	24 h, 5×	D 3
R39209	est = X65644 - MBP-2 for MHC binding protein 2	24 h, 5×	D 3
L19872	aryl hydrocarbon receptor (AhR)	8 h, 24 h, 8×, 5×	D 3
R72300	est = $U17969$ – translation initiation factor	24 h, 149×	U 1
H04333	est = $U49436$ – translation initiation factor 5 (eIF5)	24 h, 4×	U 3
T69446	est = D13748 - initiation factor 4A-1 (eIF-4A1)	24 h, 36×	U 3
H01943	eukaryotic initiation factor 4E (eIF-4E)	24 h, 39× 24 h, 4×	$U_3$
X62570 M81502	IFP53, trypotophanyl-tRNA synthetase	8 h, 800×	U 1
Z12830	SSR (signal-sequence receptor) $\alpha$ subunit	24 h, 5×	U 3
R60357 D31762	alanyl tRNA synthetase KIA 40057 gene – homolog of TR A MP	8 h, 5× 24 h 8×	D 2 D 3
Miscellaneous	KIAA0057 gene homolog of TRAWI	24 11, 0	0.5
M34551	52k autoantigen in Ro/SSA ribonucleoprotein compl.	8 h, 24 h, 12×	U 3
U33286	chromosome segregation gene homolog CAS	24 h, $4\times$	U3
188721 M26697	est = $\bigcup 52100$ , X94770 - XMP mRNA nucleolar phosphoprotein B23	24 h, 4× 24 h, 12×	U 3 U 3
J05682	subunit C of V-ATPase (vat C)	24 h, 8×	U 1
M24470	glucose-6-phosphate dehydrogenase	24 n, o× 8 h, 24 h, 4×, 6×	U 3 U 3
T47964 U07681	purine nucleoside phosphorylase NAD(H)-specific isocitrate debydrogenase o	24 h, 8× 24 h 9×	U 1 U 3
507001	subunit	2.11, 20	0.5

(Table continues on the opposite page)

### Table 1. Continued

R42235	est = X56452 - CYP2C gene for cytochrome P450	24 h. 6×	U 1
H52120	ast = D12676 - lusasamal sialastwaanratain	24 h 4×	11.2
H33120	est = D12676 - iysosoniai siaiogiycoprotein	24 11, 4×	0.5
J00123	enkephalin gene	24 h, 8×	U 3
H28131	est = M64784 - platelet 6-phosphofructokinase	24 h. 20×	U 3
P 42244	ast = X57522 – antigan paptida transportar 1	24 h 6×	11.2
R42244	est = X57522 antigen peptide transporter 1	24 11, 0	
R26294	est = AF01//32 - chromosome 2 cosmids	24 h, 4×	U 3
L25270	XE169	24 h. 4×	U 3
1101833	a putative nucleotide binding protein	24 h 7×	II 1
001055	a putative indeconde-binding protein	24 11, 7 ^	
H/2850	est = X56351.5 - aminolevulinate synthase	8 h, 24 h, 4×	U 3
U29195	neuronal pentraxin II (NPTX2)	24 h. 7×	U 3
L110523	GTP cyclobydrolase I	8 h 24 h 4 ×	II 1
1102000		24.1 51	11 2
H03980	est = AB001106 - gia maturation factor	24 n, 5×	0.3
L19779	histone H2A.2	8 h, 7×	U 3
X69978	ERCC5 excision repair protein	8 h 5×	D 3
X65024	nere dame alarmantanam anna Canaral fastar	24 1 4 4	D 2
A03024	xerodernia pignentosum group C compi. factor	24 11, 4×	0.5
R60318	est = AF022813 - tetraspan (NAG-2)	24 h, 28×	D 3
H87106	est = AF043906 - T245  protein (T245)	24 h. 4×	D 3
L114650	platelet endothelial tetraspan antigen 3	24 h 23 V	D 3
014050	plateiet-endothenai tetraspan antigen 5	24 11, 25 1	D 3
L13385	Miller-Dieker lissencephaly protein (LISI)	24 h, 4×	D 3
L35263	CSaids binding protein (CSBP1)	8 h, 7×	D 3
D10522	80K-L protein – a major substrate for protein	8 h 4×	D 1
D10522	kinoso C	0 11, 473	D 1
******	Killase C		-
J02814	chondroitin sulfate proteoglycan core protein	24 h, 6×	D 3
X75090	HLA-DR associated protein I (PHAPI)	8 h. 4×	D 3
1108002	histomina N mathyltransfaraca (HNMT)	8 h 4×	D 2
D14074	instainine iv-inetrigitransierase (Trivivi)	01, 40	D 3
D14874	adrenomedullin	8 h, 24 h, $18 \times$ , $4 \times$	D 1
H80262	est = X15422 - mannose-binding protein C	24 h. 8×	D 3
K88575	est = U55017 M86521 = transketolase (TKT)	24 h 5×	D 1
K00575	$c_{st} = 0.55017 \text{ Mo0521}$ transketolase (TK1)	24 11, 5 7	DI
M13994	cytoscolic aldehyde dehydrogenase (ALDH1)	24 h, 5–/×	DI
M22324	aminopeptidase N/CD13 mRNA	24 h. 4×	D 3
M12006	glucose 6 phosphate dehydrogenase (G6PD)	24 h 5 ×	D 1
112330	glucose-o-phosphate denydrogenase (Oor D)	24 11, 3 ~	D 1
152343	est = Y00433 - glutathione peroxidase	24 h, 4×	D 3
T64167	est = dihydrodiol dehydrogenase isozyme DD2	8 h. 24 h. 4×	D 3
X58022	Corticotropin releasing factor binding protein	24 h 4×	D 3
X30022	Control of the state of the sta	24 11, 44	D 3
U03688	Dioxin-inducible cytochrome P450 (CYPIB1)	24 h, 11×	D 3
H67764	est = U55764 – estrogen sulfotransferase	24 h. 4×	D 3
D00137	class I alcohol dehydrogenase & 1 subunit	8 h 5 Y	D 3
D00137	class 1 alcohol denydrogenase p-1 subulit	01, 5	D 3
M7/836	pyrroline 5-carboxylate reductase (EC 1.5.1.2)	24, 15×	D 3
T57002	est = M29038 - stem cell protein (SCL)	24 h, 4X	D 3
D13665	est = D13665 - osteoblast specific factor 2	24 h 7×	D 3
D15005	(OSE 2n1)	24 11, 773	0.5
	(031-201)		-
M22490	bone morphogenetic protein-2B (BMP-2B)	8 h, 4×	D 3
M95787	22kDa smooth muscle protein (SM22)	24 h. 5×	D 3
711550	iron regulatory factor (IPE)	8 h 24 h 5 Y	D 3
211339	non regulatory factor (fiki)	0 11, 24 11, 3 1	0.5
163612	ferrifin heavy chain	8 h, 4×	D 3
T72863	est = M10119 - ferritin light subunit	24 h. 287×	D 3
P 56881	est - U61166 - Venopus laevis intersectin	8 h 4 Y	D 3
100001	homolog	0 11, 473	0.5
	nomolog		
163052	est: homolog of Mus musculus X52102	24 h, 5×	U 3
D14663	KIAA0107 gene	24 h. 4×	U 3
R61352	$est = D42085 \text{ KIA} \Delta 0095 \text{ gene}$	24 h 6×	II 3
1116054	A E1-	24 h, 07	11 2
010934	AFIQ	24 II, / ×	0.5
R98335	est = L13744 AF-9 (unknown function)	8 h, 5×	U 3
R01072	est: unknown	24 h. 5×	U 3
P10664	osti unknown	24 h 7×	11.2
K10004	cst. ulikilowii	24 11, 7 ^	0.5
H24245	est: unknown	24 h, 5×	U 3
H11036	est: unknown	24 h. 6×	U 3
P 42152	osti unknown	24 h 7×	11.2
R42132	cst. ulikilowii	24 11, 7 ^	0.5
K/4454	est: unknown	8 h, 24 h, 4×	U 3
R54359	est: unknown	24 h, 10×	U 1
R01124	est: unknown	24 h 15×	II 3
D56442	est unlight	24 1. 72	11 2
K30445	est: unknown	24 II, /X	0 3
170251	est: unknown	40′, 8 h, 4×	D 3
H92205	est: unknown	8 h. 4×	D 3
T80666	est: unknown	8 h 4 Y	D 2
107000	cst. unknown	0 11, 4 ×	03
H13281	est: unknown	24 h, 15×	D 3
R60741	est: unknown	8 h. 4×	D 3
H16597	est: unknown	24 h 4×	D3
D 40402	cot. unknown	241, 40	20
K40403	est: unknown	24 h, 6×	D 3
H78404	est: unknown	8 h, 4×	D 3
T48692	est: unknown	8 h 24 h 6×	D 3
1140677	est unlight	01,271,07	50
r1400//	est: unknown	o n, o×	D 3
H86071	est: homology to mouse AF003234	8 h, 5×	D 3

Identity of columns, from left to right: GenBank accession number; name of gene encoding mRNA; time(s) after infection when a change in mRNA level was observed plus fold change; increase (U) or decrease (D) in steady state level of RNA; and gene chip results confirmed in this report by Northern blot analysis (1), confirmed by another literature report (2), or not confirmed (3).

supposition. We confirmed 49 (40%) of the mRNAs predicted to be increased and 23 (17%) of the mRNAs predicted to be decreased either by Northern blot analysis of independent RNA preparations (representative results in Fig. 3) or by reference to earlier studies (12, 13) that demonstrated a change. All attempts to confirm a predicted alteration in the group of 258 mRNAs were successful.

We assayed changes in mRNA levels for a total of 58 genes in this study by Northern blot. When we performed these assays, we included RNA preparations from cells infected with HCMV strain AD169, the laboratory-adapted strain used for the DNA array analysis, and HCMV strain Toledo, a clinical isolate that has not been passaged extensively in cultured cells (22). We observed the same alteration in mRNA level for both infections (representative results in Fig. 3). Although we might find some differences as more genes are assayed, our results to date argue that the

	AD169			To	oledo	)			
М	0.7	8	24	0.7	8	24	h after infection		
				100	•	٠	52 kDa Ro/SSA ribonucleoprotein		
	-			-	-	ø	HLA-E heavy chain		
ŝ	E	10.5		1	-		FAP-1 tyrosine phosphatase		
ŝ	-					-	GAS-1		

FIG. 3. Representative Northern blot analyses confirming changes in mRNA levels predicted by DNA array assay. Cultures of primary human diploid fibroblasts were infected with HCMV strain AD169 or Toledo, and total cellular RNA was examined by Northern blot analysis at 40 min, 8 h, and 24 h after infection. Genes to which the probes correspond are identified to the right of the autoradiograms. M, mock-infected cells.

laboratory and clinical isolates of HCMV alter cellular gene expression in a similar fashion.

### DISCUSSION

HCMV replicates in many different cell types within its infected host, some of which might respond to infection differently than the primary fibroblasts we have studied here. Keeping this caveat in mind, we nevertheless can speculate that several of the cellular genes whose mRNA levels change after infection of fibroblasts might profoundly influence HCMV replication and pathogenesis.

HLA-E mRNAs. To protect infected cells from cytotoxic T lymphocytes, multiple HCMV gene products act to reduce cell surface expression of classical class I major histocompatibility complex molecules (23-28). Although these viral activities protect infected cells from cytotoxic T lymphocytes, they also have the potential to render infected cells susceptible to natural killer (NK) cells that can recognize and destroy cells that no longer express class I major histocompatibility complex molecules. HLA-E mRNA was induced by a factor of 6 at 24 h after infection (Table 1; Fig. 3) whereas HLA-A, HLA-D, and HLA-G family members that were represented in the DNA arrays were not changed (data not shown). HLA-E is a nonclassical class I molecule whose cell surface expression requires that it bind peptides derived from the signal sequences of other class I molecules (HLA-A, -B, and -C) (29). Recently, it has been shown that NK cells recognize and spare target cells expressing HLA-E on their surface (30, 31). This recognition is mediated by the NK cell CD94-NKG2 cell surface receptor. Assuming that the elevated mRNA leads to elevated cell surface expression of HLA-E, this modulation should protect virus-infected cells from NK cell killing. This would be the second mechanism by which HCMV avoids NK cell surveillance. The viral UL18 protein is a major histocompatibility complex homologue that engages another receptor (NKIR) on the NK cell to avoid attack (32).

**Ro/SSA 52-kDa mRNA.** HCMV-infected cells contain enhanced levels of the Ro/SSA 52-kDa protein mRNA (Table 1). The mRNA encoding this protein, which is a constituent of a ribonucleoprotein complex, was induced by a factor of 12 at 24 h after infection (Fig. 3). Autoantibodies to this protein are found

in a variety of connective tissue diseases: commonly in systemic lupus erythematosis, neonatal lupus erythematosis, and Sjogren's syndrome and less frequently in rheumatoid arthritis (33). There is good evidence that these autoantibodies play a direct pathogenic role in neonatal lupus erythematosis and subacute cutaneous lupus erythematosis (33, 34). However, the mechanism by which the immune system initially responds to Ro/SSA and other intracellular self-antigens is not clear. One popular hypothesis suggests that molecular mimicry is an important initiating mechanism; that is, aspects of the immune response to a microbe cross-react with self-proteins (35). Conceivably, overexpression of a commonly targeted autoantigen, such as the Ro/SSA antigen in HCMV-infected cells, also could favor an autoimmune response. Although the Ro/SSA 52-kDa antigen normally is found in the nucleus and cytoplasm, it can be detected on the surface of peripheral lymphocytes that have been stressed by heat shock or treatment with ultraviolet light (36). Perhaps stress induced by HCMV infection also leads to cell surface presentation of Ro/ SSA, facilitating an autoimmune response to the overexpressed antigen. Murine cytomegalovirus has been shown to induce autoimmune antibodies in infected mice (37-40), although Ro/ SSA antibodies were not monitored in these studies.

Lipocortin 1, Cytosolic Phospholipase A2 (cPLA2), and Cyclooxygenase 2 (COX-2) mRNAs. Multiple constituents of the pathway that produces prostaglandin E2 from arachidonic acid are modulated by HCMV (Table 1). cPLA2 mRNA increased by a factor of 12, and COX-2 mRNA was elevated by a factor of 7 at 24 h after infection. Lipocortin 1, also known as annexin I, mRNA decreased by a factor of 9 at 24 h after infection. When cPLA2 is activated by phosphorylation, it translocates to membranes, where it selectively cleaves and releases arachidonic acid; then, COX-2 converts it to prostaglandin E2. Lipocortin 1 inhibits the activation of cPLA2 (41). Thus, in HCMV-infected fibroblasts, the synthesis of prostaglandin E2 is activated by the induction of cPLA2 and COX-2 and the inhibition of the negative regulator lipocortin 1, assuming that the changes in mRNA levels translate to changes in active proteins. Further, HCMV infection has been shown to activate latent cPLA2 by inducing its phosphorylation (42). Thus, this pathway is induced strongly at both the transcriptional and posttranslational levels after infection, and this should lead to a marked increase in the production of prostaglandin E2. Prostaglandins serve as second messengers to stimulate a variety of responses, including inflammation. Perhaps the activation of this pathway is a cellular reaction to HCMV infection designed to induce a cell-mediated response that will kill the infected cell and thereby inhibit spread of the infection. Alternatively, one might speculate that the virus either induces the pathway or fails to antagonize the induction as a strategy to facilitate spread of the virus within the infected host. Inflammation might serve to lure monocytes and monocytic precursors to the vicinity of the infected cells, where they can be infected. Cells of the monocytic lineage harbor HCMV on a long-term basis in a latent state (43-45).

It is possible that the concerted changes in cPLA2, COX-2, and lipocortin 1 are an indirect effect of HCMV gene action. Interleukin 1 $\beta$  has been shown to regulate this set of genes (46) in the same manner as seen in infected cells. Although several reports have suggested that interleukin 1 $\beta$  activity is decreased in cultures of HCMV-infected monocytes (47, 48), the HCMV IE1 gene has been shown to induce the accumulation of interleukin 1 $\beta$  mRNA in transfected monocytes (49, 50). The interleukin 1 $\beta$  gene was not included in the oligonucleotide array assayed in this report, so we do not know if its mRNA is induced by infection of fibroblasts.

**Thombospondin-1 mRNA.** Thombospondin-1 is a calciumbinding protein released on platelet activation (51). It is a constituent of the extracellular matrix that regulates cell growth and differentiation, and it might potentiate tumor progression (52). Recently, thombospondin 1-deficient mice have been produced (53) whose lungs exhibit acute and chronic cell infiltrates with increased fibroblastic and epithelial cell proliferation, matrix deposition, and diffuse alveolar hemorrhage characteristic of pneumonia. Thombospondin-1 mRNA is reduced by a factor of 21 by 24 h after infection with HCMV (Table 1). Replication in the lung that leads to pneumonia is one of the principle consequences of active HCMV infection in immunosuppressed individuals (54). Given the phenotype of thombospondin 1-deficient mice, one can speculate that the reduction in this mRNA might contribute to pneumonia induced by acute HCMV infection.

Microphthalmia-Associated Transcription Factor (MITF) mRNA. MITF is the product of the microphthalmia gene. Mice have been described with a variety of mutations in this gene (55), and the most severe manifestations of the mutations include microphthalmia, oeteopetrosis, and deafness. In the human, MITF mutations were identified in two families afflicted with Waardenberg syndrome type 2, which causes hearing loss and patchy pigmentation of the eyes, hair, and skin (56). Infection of humans with HCMV early in pregnancy has been reported to cause anophthalmia (57), and congenital infection of mice with murine cytomegalovirus can cause microphthalmia (58). Modulation of MITF mRNA levels by the virus could contribute to these abnormalities. MITF mRNA is reduced by a factor of 4-8 at 24 h after HCMV infection of fibroblasts. Although the association of HCMV with eye abnormalities appears to be rare, congenital HCMV infection is a common cause of hearing loss. Conceivably, HCMV-induced hearing loss is a consequence of an inhibitory effect on MITF mRNA expression during development. This supposition is consistent with the observation that MITF mutations are associated with hearing loss in the Waardenberg syndrome. HCMV potentially could modulate MITF in cells that are eventually killed or in cells where viral gene expression does not lead to cell death.

**Conclusion.** The roles of the cellular genes discussed above in HCMV replication and pathogenesis remain highly speculative. Nevertheless, the ability to identify cellular genes whose functions provide tantalizing hints of potential mechanistic roles in infectious disease processes underscores the utility of gene array technology in the study of pathogens. The global analysis of changes in mRNA levels provides a catalog of genes that are modulated as a result of the host–pathogen interaction and therefore deserve further scrutiny. DNA array analysis provides an important new approach for the investigation of pathogenic mechanisms.

We thank Lynn Enquist and David Lockhart for critical reading of the manuscript. T. S. is an American Cancer Society Professor and an Investigator of the Howard Hughes Medical Institute.

- Yurochko, A. D., Hwang, E. S., Rasmussen, L., Keay, S., Pereira, L. & Huang, E. S. (1997) J. Virol. 71, 5051–5059.
- 2. Liu, B. & Stinski, M. F. (1992) J. Virol. 66, 4434-4444.
- Pizzorno, M. C., O'Hare, P., Sha, L., LaFemina, R. L. & Hayward, G. S. (1988) J. Virol. 62, 1167–1179.
- Malone, C. L., Vesole, D. H. & Stinski, M. F. (1990) J. Virol. 64, 1498–1506.
- Stenberg, R. M., Fortney, J., Barlow, S. W., Magrane, B. P., Nelson, J. A. & Ghazal, P. (1990) J. Virol. 64, 1556–1565.
- Chee, M. S., Satchwell, S. C., Preddie, E., Weston, K. M. & Barrell, B. G. (1990) *Nature (London)* 344, 774–777.
- Welch, A. R., McGregor, L. M. & Gibson, W. (1991) J. Virol. 65, 3915–3918.
- Jault, F. M., Jault, J. M., Ruchti, R., Fortunato, E. A., Clark, C., Corbeil, J., Richman, D. D. & Spector, D. H. (1995) *J. Virol.* 69, 6697–6704.
- Bresnahan, W. A., Boldogh, I., Thompson, E. A. & Albrecht, T. (1996) Virology 224, 150–160.
- 10. Lu, M. & Shenk, T. (1996) J. Virol. 70, 8850-8857.
- 11. Dittmer, D. & Mocarski, E. S. (1997) J. Virol. 71, 1629-1634.
- 12. Mocarski, E. S. (1996) in Fields Virology, eds. Fields, B. N., Knipe,
- D. M. & Howley, P. M. (Lippencott, Philadelphia), pp. 2447–2492.
  13. Zhu, H., Cong, J-P. & Shenk, T. (1997) *Proc. Natl. Acad. Sci. USA* 94, 13985–13990.
- 14. Baldick, C. J. & Shenk, T. (1996) J. Virol. 70, 6097-6105.

- Wodicka, L., Dong, H., Mittmann, M., Ho, M. & Lockhart, D. J. (1997) *Nat. Biotechnol.* 15, 1359–1367.
- Lockhart, D. J., Dong, H., Byrne, M. C., Follette, M. T., Gallo, M. V., Chee, M. S., Mittmann, M., Wang, C., Kobayashi, M., Horton, H., *et al.* (1996) *Nat. Biotechnol.* 14, 1675–1680.
- Fodor, S. P. A., Read, J. L., Pirrung, M. C., Stryer, L., Lu, A. T. & Solas, D. (1991) *Science* 251, 767–773.
- Fodor, S. P. A., Rava, R. P., Huang, X. C., Pease, A. C., Holmes, C. P. & Adams, C. L. (1993) *Science* 364, 555–556.
- Pease, A. C., Solas, D., Sullivan, E. J., Cronin, M. T., Holmes, C. P. & Fodor, S. P. A. (1994) Proc. Natl. Acad. Sci. USA 91, 5022–5026.
- Lipshutz, R. J., Morris, D., Chee, M., Hubbell, E., Kozal, M. J., Shah, N., Shen, N., Yang, R. & Fodor, S. P. (1995) *BioTechniques* 19, 442–447.
- Chee, M., Yang, R., Hubbell, E., Berno, A., Huang, X. C., Stern, D., Winkler, J., Lockhart, D. J., Morris, M. S. & Fodor, S. P. A. (1996) *Science* 274, 610–614.
- Quinnan, G. V., Jr., Delery, M., Rook, A. H., Frederick, W. R., Epstein, J. S., Manischewitz, J. F., Jackson, L., Ramsey, K. M., Mittal, K., Plotkin, S. A., *et al.* (1984) *Ann. Intern. Med.* 101, 478–483.
- Ahn, K., Angulo, A., Ghazal, P., Peterson, P. A., Yang, Y. & Fruh, K. (1996) Proc. Natl. Acad. Sci. USA 93, 10990–10995.
- Jones, T. R., Wiertz, E. J., Sun, L., Fish, K. N., Nelson, J. A. & Ploegh, H. L. (1996) Proc. Natl. Acad. Sci. USA 93, 11327–11333.
- Wiertz, E. J., Jones, T. R., Sun, L., Bogyo, M., Geuze, H. J. & Ploegh, H. L. (1996) *Cell* 84, 769–779.
- Ahn, K., Gruhler, A., Galocha, B., Jones, T. R., Wiertz, E. J., Ploegh, H. L., Peterson, P. A., Yang, Y. & Fruh, K. (1997) *Immunity* 6, 613–621.
- Hengel, H., Koopmann, J. O., Floh, T., Muranyi, W., Goulmy, E., Hammerling, G. J., Koszinowski, U. H. & Momburg, F. (1997) *Immunity* 6, 623–632.
- 28. Jones, T. R. & Sun, L. (1997) J. Virol. 71, 2970-2979.
- Braud, V., Jones, E. Y. & McMichael, A. (1997) *Eur. J. Immunol.* 27, 1164–1169.
- Borrego, F., Ulbrecht, M., Weiss, E. H., Coligan, J. E. & Brooks, A. G. (1998) J. Exp. Med. 187, 813–818.
- Braud, V. M., Allan, D. S. J., O'Callaghan, C. A., Soderstrom, K., D'Andrea, A., Ogg, G. S., Lazetic, S., Yound, N. T., Bell, J. I., Phillips, J. H., *et al.* (1998) *Nature (London)* **391**, 795–799.
- Rayburn, H. T., Mandelboim, O., Vales-Gomez, M., Davis, D. M., Pazmany, L. & Strominger, J. L. (1997) *Nature (London)* 386, 514–517.
- Bouffard, P., Laniel, M-A. & Boire, G. (1996) J. Rheumatol. 23, 1838–1841.
- Finkelstein, Y., Adler, Y., Harel, L., Nussinovitch, M. & Youinou, P. (1997) Ann. Med. Interne (Paris) 148, 205–208.

- Herrath, M. G. & Oldstone, M. B. A. (1996) *Curr. Opin. Immunol.* 8, 878–885.
- Igarashi, T., Itoh, Y., Fukunaga, Y. & Yamamoto, M. (1995) Autoimmunity 22, 33–42.
- O'Donoghue, H. L., Lawson, C. M. & Reed, W. D. (1990) *Immunology* 71, 20–28.
- Lawson, C. M., O'Donoghue, H. L., Farrell, H. E., Shellam, G. R. & Reed, W. D. (1991) *Immunology* 72, 426–433.
- Price, P., Olver, S. D., Gibbons, A. E. & Shellam, G. R. (1993) Immunology 78, 14–21.
- Chapman, A. J., Farrell, H. E., Thomas, J. A., Papadimitriou, J. M., Garlepp, M. J., Scalzo, A. A. & Shellam, G. R. (1994) *Immunology* 81, 435–443.
- Croxtall, J. D., Choudhury, Q., Newman, S. & Flower, R. J. (1996) Biochem. Pharmacol. 52, 351–356.
- 42. Shibutani, T., Johnson, T. M., Yu, Z. X., Ferrans, V. J., Moss, J. & Epstein, S. E. (1997) *J. Clin. Invest.* **100**, 2054–2061.
- Kondo, K., Xu, J. & Mocarski, E. S. (1996) Proc. Natl. Acad. Sci. USA 93, 11137–11142.
- 44. Sinclair, J. & Sissons, P. (1996) Intervirology 39, 293-301.
- Soderberg-Naucler, C., Fish, K. N. & Nelson, J. A. (1997) *Cell* 91, 119–126.
   Croxtall, J. D., Newman, S. P., Choudhury, Q. & Flower, R. J. (1996)
- 40. Croxtall, J. D., Newman, S. P., Choudhury, Q. & Flower, K. J. (1996) Biochem. Biophys. Res. Commun. 220, 491–495.
- Rogers, B. C., Scott, D. M., Mundin, J. & Sissons, J. G. P. (1985) J. Virol. 55, 527–532.
- 48. Kapasi, K. & Rice, G. P. A. (1998) J. Virol. 62, 3603-3607.
- Iwamoto, G. K., Monick, M. M., Clark, B. D., Auron, P. E., Stinski, M. F. & Hunninghake, G. W. (1990) J. Clin. Invest. 85, 1853–1857.
- Crump, J. W., Geist, L. J., Auron, P. E., Webb, A. C., Stinski, M. F. & Hunninghake, G. W. (1992) Am. J. Respir. Cell Mol. Biol. 6, 674–677.
- 51. Adams, J. C. (1997) Int. J. Biochem. Cell Biol. 29, 861-865.
- 52. Tuszynski, G. P. & Nicosia, R. F. (1995) BioEssays 18, 71-76.
- Lawler, J., Sunday, M., Thibert, V., Duquette, M., George, E. L., Rayburn, H. & Hynes, R. O. (1998) J. Clin. Invest. 101, 982–992.
- Britt, W. J. & Alford, C. A. (1996) in *Fields Virology*, eds. Fields, B. N., Knipe, D. M. & Howley, P. M. (Lippencott, Philadelphia), pp. 2493–2523.
- Steingrimsson, E., Moore, K. J., Lamoreux, M. L., Ferre-D'Amare, A. R., Burley, S. K., Zimring, D. C. S., Skow, L. C., Hodgkinson, C. A., Arnheiter, H., Copeland, N. G., et al. (1994) Nat. Genet. 8, 256–263.
- 56. Tassabehji, M., Newton, V. E. & Read, A. P. (1994) *Nat. Genet.* 8, 251–255.
- McCarthy, R. W., Frenkel, L. D., Kollarits, C. R. & Keys, M. P. (1980) Am. J. Ophthalmol. 90, 558–561.
- Tsutsui, Y., Kashiwai, A., Kawamura, N. & Kadota, C. (1993) *Am. J. Pathol.* 143, 804–813.