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Use of Monkey–Mouse Hybrid Cells for the Study of the Cellular Regulation of Interferon Production and Action

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ABSTRACT **Four clones of a somatic monkey–mouse hybrid cell line were studied. One of these clones produced both mouse and monkey interferon, while the three others produced only mouse interferon. All four were, however, sensitive to both mouse and monkey (or human) interferon. The karyotype analysis of these four hybrid clones and the parental cell lines enabled us to locate the possible genetic site governing the synthesis of monkey interferon on a small subtelocentric monkey chromosome. The genetic site responsible for the synthesis of the antiviral protein is located on a different (monkey or mouse) chromosome.**

The objective of these investigations is the study of the cellular regulation of interferon production and action and its relationship with the karyotype. Two considerations enabled us to carry out these studies: (*a*) the known cell species specificity of interferon (1); and (*b*) the availability of a somatic hybrid cell line (MKCV^{III}) obtained after the fusion of monkey (CV$_1$) and mouse (MKS-BU 100) cells (2). From this hybrid cell line (6th passage), six clones were isolated and four of them were selected for this study. The morphology of monkey chromosomes was markedly different from that of mouse chromosomes. In addition, the small number of monkey chromosomes present in the hybrid cells facilitated the interpretation of the karyotype analysis. The most relevant observation of this study is that the site responsible for the interferon synthesis is located on a segment of the cellular genome different from that involved in the synthesis of the protein responsible for the antiviral state. These data were summarized in a preliminary publication (3).

MATERIALS AND METHODS

Cells

Parental Cell Lines. (*a*) CV$_1$ cell line, stemmed from green monkey kidney cells (4), and (*b*) MKS-BU 100 cells, which are SV40-transformed mouse kidney cells, highly resistant to 5-bromodeoxyuridine and lacking thymidine kinase activity (5).

Hybrid Cell Lines. MKCV^{III} is a somatic hybrid monkey–mouse cell line (2) obtained in our laboratory from a cell colony isolated from a mixed culture of CV$_1$ and MKS-BU 100 cells constantly grown in the selective HATG medium (see *Medium*, below) of Littlefield (6). From this selected hybrid population, cell clones were subsequently isolated in soft agar in the presence of HATG, by a technique described by Montagnier and Macpherson (7). Four clones (*1*, *2*, *3*, and *4*) were studied for their sensitivity and for the production of monkey and mouse interferon. Their karyotype was also analyzed.

Other Cell Lines. (*a*) The mouse L cells employed were routinely carried in the laboratory. (*b*) The BSC-1 cells were obtained from Microbiological Associates; they were derived from Cercopithecus monkey kidney cells. (*c*) The MSV-IF$^+$ cells were Balb/C embryonic fibroblasts transformed by the Moloney strain of mouse sarcoma virus and grown *in vitro* for more than 200 passages in the presence of mouse interferon (8).

Medium

All the cells were cultured in Eagle's medium containing 10% calf serum. The MKS-BU 100 cells were grown in the presence of 5-bromodeoxyuridine (25 μg/ml) and the somatic hybrid cells were grown in the presence of HATG (10^{-4} M hypoxanthine, 10^{-5} M aminopterin, 4×10^{-5} M thymidine, and 10^{-5} M glycine) (6).

Viruses

(*a*) Newcastle disease virus (NDV), Hertfordshire strain, employed at a multiplicity of infection (MOI) of 100 plaque-forming units, was used as the inducer of interferon. (*b*) Vesicular stomatitis virus (VSV), Indiana strain, was routinely passaged in L cells in the laboratory.

Chromosomal preparations

Cell cultures, growing exponentially on 12 × 32 mm coverslips in Leighton tubes, were first treated for 5 hr at 37°C with colchicine (1 μg/ml), and then maintained for 15 min at room temperature in a hypotonic solution (one part phosphate-buffered saline and three parts distilled water). After fixation for 1 hr in acetic acid–methanol 1:4, the cultures were dried, stained with 1% orcein in acetic acid, and mounted without squashing. A Zeiss Ultraphot II microscope was used for microscopic and photographic observations. Karyotype analysis was performed on 25 mitoses per cell line. The parental monkey cells (CV$_1$) and mouse cells (MKS-BU 100) were studied at the 47th and 151st *in vitro* passages, respectively. The hybrid cells MKCV^{III} clone *1* were studied at the

Abbreviations: NDV, Newcastle disease virus; MOI, multiplicity of infection; HATG, 10^{-4} M hypoxanthine–10^{-5} M aminopterin–4×10^{-5} M thymidine–10^{-5} M glycine.

31st passage, $MKCV^{III}$ clone *2* and clone *3* at the 25th, and $MKCV^{III}$ clone *4* at the 27th and 64th passages.

Interferon preparations

Sources of Interferon. Human interferon was used in this study because of the known cross protection observed between interferons produced in monkey and human cells (9). This interferon was obtained in human leukocytes as described by Gresser (10) and modified by Falcoff *et al.* (11).

Mouse interferon was obtained in MSV-IF$^+$ cells, which yield high quantities of interferon (8).

Procedures for Interferon Production. Human white blood cells, MSV-IF$^+$ cells, and the different hybrid cells were infected with NDV (MOI 200 plaque-forming units). After an absorption period of 30 min, the unabsorbed virus was discarded, fresh medium was added, and the preparations were incubated at 37°C for 24 hr. The supernatant fraction was used as the interferon preparation. Residual virus was destroyed by lowering the pH to 2 with 1 N HCl and keeping the preparation at 4°C for 4–5 days. The pH was then readjusted to pH 7 with 1 N NaOH. Appropriate controls showed that no residual infectious virus was present in the preparation.

Interferon assay

Interferon was assayed (*a*) in terms of a 50% inhibition of the cytopathogenic effect and (*b*) by inhibition of viral yield, as follows. Serial twofold dilutions of interferon were incubated either with BSC-1 cells (for human interferon) or with L cells. The cells were incubated for 18–20 hr at 37°C, washed, challenged with VSV (MOI 10 plaque-forming units), and incubated for an additional 16 hr at 37°C. The total viral yield was established by plaque titration using L cells, and compared with the yield in suitable control cultures.

Statistical analysis

Student's *t*-test (12) was used for the comparison of mean values.

Glossary. Abbreviations used to designate the different groups of chromosomes:

VLM = very large metacentric
LM = large metacentric
MM = medium metacentric
SM = small metacentric
VSM = very small metacentric
VLSM = very large submetacentric
LSM = large submetacentric
MSM = medium submetacentric
SSM = small submetacentric
VSSM = very small submetacentric
VLST = very large subtelocentric
LST = large subtelocentric
MST = medium subtelocentric
SST = small subtelocentric
VSST = very small subtelocentric
VVSST = very very small subtelocentric
T = telocentric
mini = minute

RESULTS

Chromosomal studies

The parental CV_1 monkey cells contained only biarmed chromosomes. These chromosomes were classified into different groups according to their size and to the position of the centromere (Fig. 1).

The parental MKS-BU 100 mouse cells contained essentially telocentric chromosomes. In addition, a few mini and biarmed chromosomes were present. The latter could not be distinguished from monkey chromosomes (Fig. 2).

The $MKCV^{III}$ hybrid cell clones (*1*, *2*, *3*, and *4*) contained a substantial number of mouse telocentric chromosomes, mouse mini chromosomes, and biarmed chromosomes that could be considered as of either mouse or monkey origin, since they were observed in both CV_1 and MKS-BU 100 cells. Moreover, they contained biarmed chromosomes that could be considered as monkey chromosomes since they were observed in CV_1 cells but not in MKS-BU 100 cells. An example of karyotype of the hybrid cell is shown in Fig. 3.

In Table 1 are summarized the results of the karyotype analysis of 25 mitoses per cell line. The T and mini chromosomes were never observed in the parental CV_1 monkey cells, but characterized the parental mouse MKS-BU 100 cells. They were found in all the hybrid cell clones; thus, in the hybrid cells, they could be considered as being of murine origin. On the other hand, since VSSM, LST, MST, SST, VSST, and VVSST chromosomes were never observed in the mouse MKS-BU 100 cells, but were present in the monkey CV_1 cells and in all hybrid cell clones, they were considered as being of simian origin. In the hybrid cell lines, the biarmed chromosomes VLM, LM, MM, SM, VSM, VLSM, LSM, MSM, SSM, VLST were considered as being either of simian or of murine origin.

When the karyotypes of the different hybrid cell clones were closely analyzed, the VLM and SST were the chromosomal groups most frequently found in the $MKCV^{III}$ clone *4* (27th passage). However, in this clone, at the 64th passage, a decrease in the average number of SST was observed, while that of VLM remained unchanged. Another observation was that the VLSM chromosomes were found in greater numbers in $MKCV^{III}$ clone *3* when compared with $MKCV^{III}$ clone *4*. On the contrary, for the VLM chromosomes, the count was reversed: they were present in almost every cell in $MKCV^{III}$ clone *4* and absent in $MKCV^{III}$ clone *3* and all the other hybrid cells.

Interferon production in the hybrid and parental cells

Table 2 shows the amounts of interferon produced in the different hybrid cell clones. In clones *1*, *2*, and *3*, a significant and reproducible amount of murine interferon was produced. This was especially studied in $MKCV^{III}$ clone *3* between the 19th and 44th passages. In all clones, mouse interferon was produced again when reinduced 48 hr after the first induction with NDV. Consequently, for mouse interferon, no refractory state to re-induction was observed. Clones *1*, *2*, and especially *3*, which were analyzed after several different numbers of passages, never produced interferon detectable in monkey cells.

In clone *4* (Table 3), mouse interferon was also constantly produced; however, this was the only hybrid cell line that also produced a significant amount of monkey interferon. The quantity of monkey interferon produced varied from one

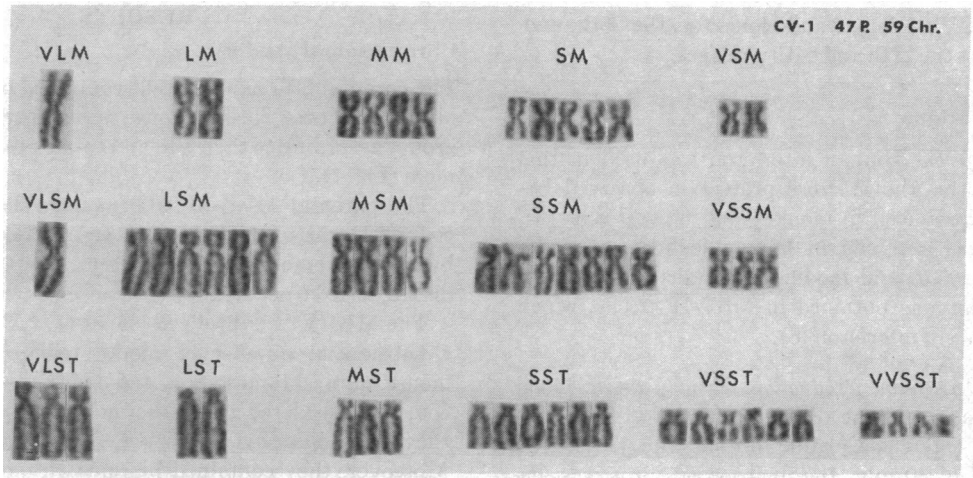

FIG. 1. Karyotype of parental CV_1 monkey cell line. Passage: 47. Total number of chromosomes: 59. (For abbreviations, see glossary in *Methods*.)

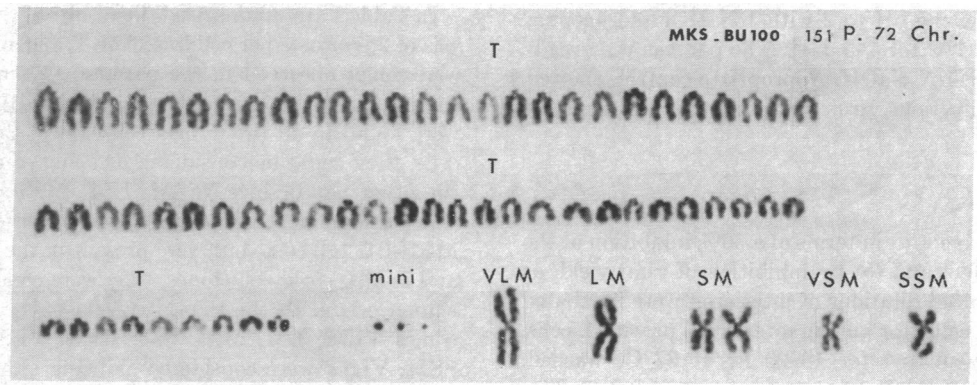

FIG. 2. Karyotype of parental MKS-BU 100 mouse cell line. Passage: 151. Total number of chromosomes: 72.

passage to another and was no longer detected after the 59th passage. Special care was taken in several experiments to check the possibility of the persistence of residual NDV (which could obscure the results), by detection of NDV in embryonated eggs, and by titration of the monkey interferon in the presence of NDV antiserum. In early passages of hybrid clone *4*, monkey interferon was also produced after secondary induction. In late passages, a small amount of such virus-inhibitory material was found only after the second induction.

Sensitivity of the hybrid cells to human and mouse interferon

As shown in Table 4, all four hybrid cell lines were sensitive to both human and mouse interferons in all experiments. In the parental cell lines, the antiviral state was only induced with interferon of homologous species. The interesting finding was the greater sensitivity of clone *2* to human interferon since, as previously shown, the mean number of biarmed chromosomes present in these hybrid cells was the lowest (6.36 ± 0.66).

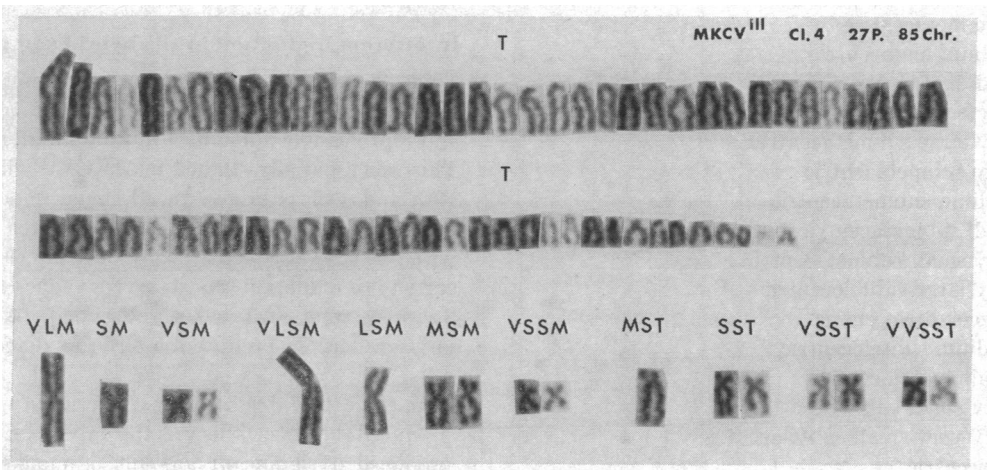

FIG. 3. Karyotype of hybrid cell line $MKCV^{III}$ clone *4*. Passage: 27. Total number of chromosomes: 85.

TABLE 1. *Mean values and 0.95 confidence intervals of the different kinds of chromosomes*

Chromosomal group	CV_1	MKS-BU	MKCV^{III} hybrid, clone 1	2	3	4*	4†
VLM‡	1.04 ± .08	0.28 ± .22	0.00 ± .00	0.00 ± .00	0.08 ± .08	0.80 ± .24	1.00 ± .29
LM	1.96 ± .08	0.52 ± .27	0.12 ± .12	0.08 ± .08	0.20 ± .24	0.20 ± .17	0.84 ± .31
MM	4.24 ± .22	0.52 ± .40	0.24 ± .22	0.00 ± .00	0.08 ± .08	0.60 ± .32	0.80 ± .46
SM	4.24 ± .30	0.48 ± .34	0.40 ± .24	0.04 ± .04	1.04 ± .39	1.52 ± .42	1.60 ± .48
VSM	2.32 ± .39	0.64 ± .31	2.40 ± .45	0.36 ± .31	2.64 ± .55	2.56 ± .51	1.68 ± .41
VLSM	1.44 ± .21	0.32 ± .26	1.24 ± .18	1.72 ± .19	1.76 ± .22	0.84 ± .20	0.88 ± .28
LSM	5.60 ± .29	0.28 ± .22	0.20 ± .20	0.08 ± .08	0.32 ± .31	0.52 ± .34	0.80 ± .40
MSM	4.00 ± .12	0.24 ± .22	0.92 ± .38	0.12 ± .12	1.08 ± .56	0.68 ± .33	1.28 ± .48
SSM	6.00 ± .27	0.48 ± .27	1.40 ± .34	0.32 ± .26	1.56 ± .49	2.08 ± .36	1.92 ± .38
VSSM	3.80 ± .34	0.00 ± .00	1.16 ± .41	0.48 ± .30	2.32 ± .53	2.68 ± .60	2.80 ± .48
VLST	4.00 ± .27	0.04 ± .04	0.00 ± .00	0.00 ± .00	0.12 ± .12	0.16 ± .15	0.16 ± .16
LST	1.88 ± .14	0.00 ± .00	0.16 ± .15	0.04 ± .04	0.36 ± .29	0.12 ± .12	0.32 ± .29
MST	3.84 ± .20	0.00 ± .00	0.84 ± .35	0.92 ± .26	1.08 ± .43	0.68 ± .31	0.64 ± .34
SST	5.56 ± .45	0.00 ± .00	0.32 ± .26	0.04 ± .06	1.08 ± .53	2.04 ± .31	0.40 ± .29
VSST	5.72 ± .40	0.00 ± .00	2.84 ± .50	0.48 ± .30	2.00 ± .45	2.32 ± .43	0.92 ± .38
VVSST	2.92 ± .26	0.00 ± .00	0.96 ± .45	1.68 ± .46	1.08 ± .55	2.04 ± .61	1.04 ± .50
Total	58.56 ± .63	3.80 ± .53	13.20 ± 1.11	6.36 ± .66	16.80 ± 1.24	19.84 ± 1.12	17.08 ± 1.34
T	0.00 ± .00	64.04 ± .86	62.72 ± 1.18	62.28 ± .96	67.64 ± 1.23	67.88 ± 1.38	67.40 ± 1.88
Mini	0.00 ± .00	3.16 ± .65	0.84 ± .28	0.96 ± .28	1.56 ± .40	1.40 ± .40	1.40 ± .49

CV_1, monkey; MKS-BU, mouse cell lines.
* 27th passage.
† 64th passage.
‡ for abbreviation, see glossary in *Methods*.

DISCUSSION AND CONCLUSION

The results presented show (*a*) that the cistrons governing the production of interferon are located on the cellular genome on a different site (even on a different chromosome) from that of the cistrons responsible for the antiviral protein; and (*b*) the absence of a state refractory to the induction of mouse interferon, observed in parental mouse cells, was found in the two cell hybrid clones studied (clones *3* and *4*). Probably, only two groups of biarmed chromosomes could carry the genetic information responsible for monkey interferon synthesis in the cell: VLM and SST. The comparison of the 27th and 64th passages of MKCV^{III} clone *4* showed that the hybrid cell seemed to have lost the capacity to produce monkey interferon at late passages. Only after re-induction, a small amount of monkey interferon-like material could be recovered. Moreover, a decrease of the number of SST chromosomes was also observed, while the VLM chromosomes persisted at comparable numbers. In addition, preliminary experiments using VERO monkey cells, which do not produce monkey interferon after induction, showed the constant presence of the VLM chromosomes (unpublished experiments). All these data suggest that the SST chromosomal group could carry the genetic information for monkey interferon synthesis. This hypothesis is currently under investigation.

A striking observation was the consistent high sensitivity of all the hybrid cell clones to both human and murine interferons. This finding could be related to that described by Guggenheim (13) showing that heterokaryons produced by the fusion of human cells and nucleated chick red cells are sensitive to both human and chick interferons.

The genome governing the synthesis of the interferon-induced antiviral protein (responsible for the antiviral state) could be located on a VVSST chromosome (of simian origin) or on a VLSM chromosome (which could be of either simian or murine origin). An alternative hypothesis could be that none of these biarmed chromosomes are involved. If so, since the data shown in Table 1 indicate that none of the other biarmed

TABLE 2. *Production of mouse and monkey interferons in clones 1–3 of hybrid cells MKCV^{III} after single and repeated inductions with NDV*

	Passage	Induction	Interferon: Monkey	Interferon: Mouse
Clone 1	26th	1	<2	1280/2560
		2	<2	1280
	40th	1	<2	640/1280
		2	<2	1280/2560
Clone 2	20th	1	<2	1280/2560
		2	<2	320/640
	33rd	1	<2	1280
		2	<2	320
Clone 3	19th	1*	<10	5120
	22nd	1	<10	2560/5120
	24th	1*	<5	1280
		2*	<5	160/320
	36th	1†	<2	640/1280
		2†	<2	>1280
	37th	1	<10	5120/10240
	41st	1	<2	>2560
		2	<2	1280/2560
	44th	1*	<5	5120
		2*	<5	1280

* Study of residual NDV by passage in eggs: negative.
† Titration in the presence of NDV antiserum.

TABLE 3. *Production of monkey and mouse interferons in hybrid cells MKCV^{III} clone 4 after single and repeated induction with NDV*

Passage	Induction	Monkey interferon	Mouse interferon
19th	1†	2/4	1280/2560
	2†	2/4	320/640
21st	1*	160	2560
23rd	1†	16/32	ND
	2†	32	ND
24th	1	40/80	2560
26th	1†	10/20	640
	2*	5/10	160
32nd	1†	16	ND
	2†	16/32	ND
36th	1†	8/16	ND
	2†	16/32	ND
38th	1†	2	1280/2560
	2†	16	1280/2560
39th	1†	16/32	ND
	2†	16/32	ND
43rd	1	<2	⩾2560
	2	2/4	⩽2560
46th	1*	<5	1280/2560
	2*	<5	640/1280
59th	1†	<2	ND
	2†	2/4	ND
64th	1†	<2	ND
	2†	8/16	ND

ND: Not done.
* Study of residual NDV by passage in eggs: negative.
† Titration in the presence of NDV antiserum.

chromosomes could be considered, the antiviral protein could originate from a mouse telocentric or mini chromosome. In this case, unlike interferon, the antiviral protein would not be species-specific. The presence of monkey chromosomes would modify the cell constitution in such a manner that both the monkey and the mouse interferons could induce the antiviral state.

In summary, analysis of the data here reported confirms that the production of interferon and the production of the protein responsible for the antiviral state in the cell are two separate cellular functions.

TABLE 4. *Sensitivity of hybrid cells MKCV^{III} clones 1–4 and the parental cells to human and mouse interferons*

Cell line	Passage	Human interferon	Mouse interferon
MKCV^{III} Cl$_1$	23rd	1280	1024
MKCV^{III} Cl$_2$	17th	5120	8192
MKCV^{III} Cl$_3$	25th	400	640/1280
	30th	80	320
	37th	40	640/1280
	42nd	640	2560
MKCV^{III} Cl$_4$	20th	640	640/1280
	27th	200	640
	32nd	80	320
	39th	320	1280
	44th	1280	2560
MKS/BU	189th	<2	10240
	193rd	"	2560
	199th	"	2560
	205th	"	640/1280
CV$_1$	73rd	800	<2
	98th	1280	"
	101st	640/1280	"
	105th	1280	"

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