METAPHASE CHROMOSOME UPTAKE BY MAMMALIAN CELLS AND EXPRESSION OF THE GENES TRANSFERRED

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SUMMARY

Isolated metaphase chromosomes can be incorporated into mammalian cells. The genetic information thereby transferred, in the form of a chromosome, can be replicated and expressed in the recipient cell and its progeny, thus resulting in permanent genetic change. Whether this information transfer involves an intact chromosome or merely a fragment, which may be integrated into a host chromosome, has not yet been established. The possible implications of these findings for analysis of genetic linkage in somatic cells are discussed. Probable steps currently limiting the efficiency of this transfer process are also considered.

1. INTRODUCTION

Cell fusion, combined with karyotypic analysis of the resultant hybrid clones and derivatives by the quinacrine (CASPERSSON et al. 1970) and Giemsa (DRETS and SHAW 1971) banding techniques, has provided a means for the genetic mapping of mammalian chromosomes. The recent development of a number of mutant mammalian cell lines subject to chemical selection has provided another tool for genetic studies. These mutants generally fall into two classes involving either nutritional auxotrophs (KAO and PUCK 1972) or those related to enzymes of the purine and pyrimidine salvage pathways (LONG et al. 1973, MEDRAN and GREEN 1974). Genetic markers have now been located on 15 of the 22 human autosomes and X chromosome by the analysis of reduced somatic cell hybrids between human and rodent cells (RUDDLE 1973). Cell hybridization has also been used for mapping integration sites of oncogenic viruses

CROCE et al. 1973a), mapping regulatory genes (CROCE et al. 1973b), and investigating cytoplasmic inheritance (COON et al. 1973). This rapidly expanding and exciting area has been covered in recent monographs (HARRIS 1970, EPHRUSSI 1972) and reviews (HANDMAKER 1973, RAO and JOHNSON 1972), and it will not be discussed further.

Genetic analysis by somatic cell hybridization is complicated by the long delay required for segregation of chromosomes with resultant possible loss of distinctive chromosome characteristics through translocations. Preferential retention of certain combinations, during segregation in interspecies hybrids, poses another limitation of this technique. The concomitant transfer of cytoplasmic factors and nuclear genes is inherent in this method, and changes in regulatory events are also difficult to exclude. An alternative approach which might circumvent some of these problems would be to transfer only a portion of the entire genome. Several methods (Table 1) for introducing a restricted number of genes into eukaryotic cells have been considered by various investigators, and these will be considered briefly.

Table 1. Methods of genetic transfer

1. DNA transformation
2. Transformation by
a. viruses
b. pseudoviruses
c. biochemically 'altered' viruses
3. Transduction
4. Modified cell fusion
5. Isolated metaphase chromosomes

2. GENETIC TRANSFER METHODS OTHER THAN METAPHASE CHROMOSOMES

2.1 DNA TRANSFORMATION

The uptake of viral DNA or RNA by eukaryotic cells, resulting in transformation or lytic infections, is well documented (SARKAR 1968, RUECKERT 1968). Comparable evidence relating to the uptake and fate of mammalian DNA is much less clear, despite investigation in many laboratories (BHARGAVA and SHANMUGAN 1971). It seems reasonably certain that there can be uptake of DNA into the cytoplasm and perhaps nuclei. It also appears that some of this DNA can persist in cells for considerable time. However, the evidence for replication and transcription of this DNA is considerably more tenuous.

2.2 TRANSFORMATION BY VIRUSES

The viral gene for thymidine kinase is expressed in thymidine kinase-deficient mouse cells after infection with UV inactivated herpes simplex virus (MUNYON et al. 1971, 1972). The expression of the viral enzyme could be suppressed and reactivated with high efficiency shortly after infection, but on continued multiplication in nonselective medium, the proportion of cells producing the viral enzyme decayed exponentially (DAVIDSON et al. 1973). However, this decay seemed to represent a change in the expression of the viral gene for thymidine kinase rather than the loss of the gene from the cells, since the viral enzyme could apparently be reactivated in every cell with a very low probability. The mechanism for suppression and reactivation is undoubtedly interesting but still unknown.

2.3 TRANSFORMATION BY PSEUDOVIRUSES

Pseudovirions are formed when cells are infected with Polyoma (MICHEL et al. 1967) or SV 40 (TRILLING and AXELROD 1970) viruses under certain conditions. They consist of completely heterogeneous (TRILLING and AXELROD 1970) linear host DNA fragments of nearly uniform (15S) size (QASBA et al. 1972) encapsidated by the respective viral coats. An appreciable portion of this DNA is transported to the nucleus and uncoated after infection of either mouse or human embryo cells with pseudoviruses (OSTERMAN et al. 1970, QASBA and APOSHIAN 1971). Much of this DNA is degraded but a significant fraction persists in a high molecular weight form in the nucleus after 24 hours and it could presumably become intergrated into the host DNA and be functional. However efforts to demonstrate specific gene expression in a selective system were unsuccessful (discussed in QASBA et al. 1972). Since these heterologous DNA fragments are relatively small (i.e. about 10^{-6} fraction of the entire genome), it is quite possible that integration, transcription, and translation of the heterologous DNA occurred, but could not be demonstrated due to the heterogeneity of sequences and dependence on the appearance of a single specific gene product in this assay system.

2.4 BIOCHEMICALLY ALTERED VIRUSES

A very elegant procedure has been developed (JACKSON et al. 1972) for biochemically inserting defined genetic sequences into the DNA of a transforming virus. The principle involved is to covalently attach a small amount of functional genetic information to the viral DNA and thereby use the natural properties of the virus to permit integration of these covalently attached genes into the host genome. This requires that the genes of interest be preserved intact and that the viral DNA not be so extensively modified as to prevent its normal integration. The general approach used to generate circular, covalently-closed DNA molecules from two separate DNA's in-

volved initial conversion of the circular structures to linear duplexes by a double strand scission at random locations using pancreatic DNase, or at a unique site with R_I restriction endonuclease. Relatively short poly (dA) or poly (dT) extensions were added on the 3'-hydroxyl termini with terminal transferase. The linear duplexes containing poly (dA) extensions were then annealed with those containing the poly (dT) extensions at low concentrations to prevent the formation of linear or circular concatamers. Covalent closure of the hydrogen-bonded circular structure was effected with *E. coli* DNA polymerase I and DNA ligase. It was pointed out that by minor modifications of these methods, many different types of DNA molecules could be similarly joined. Circular SV40 DNA molecules of about 10^7 daltons, containing λ phage genes and the galactose operon of *E. coli*, were prepared and are currently undergoing biological testing.

2.5 TRANSDUCTION BY VIRUSES

Galactosemic fibroblasts have been infected with λ_p *gal* at high multiplicity and evidence has been presented for transcription of the viral genome as well as translation of the message and synthesis of galactose-1-phosphate uridyl transferase at a level comparable to, or higher than, that in wild-type control human fibroblasts (MERRIL et al. 1971, GEIER and MERRIL 1972). Treatment of the cells with λ_p *gal* DNA produced similar results. The state of the viral DNA in these cells is not known but the viral genome did not segregate out, as evidenced by the continued production of viral RNA and enzyme at maximal levels for at least 40 days after infection with either the bacteriophage or phage DNA. Several different types of controls were used to exclude the most serious reservation to this type of experiment, namely phage replication secondary to coexisting bacterial infection. The enzyme has not been identified as to species of origin, however.

2.6 MODIFIED CELL FUSION

It is possible to introduce very small amounts of genetic material into cells by cell fusion under the appropriate conditions (SCHWARTZ et al. 1971). Fusion of chick erythrocytes with mouse A9 cells results in activation of the quiescent erythrocyte nucleus. However, when the resultant heterokaryons enter mitosis, the erythrocyte nucleus is fragmented by a process called premature chromosome condensation (JOHNSON and RAO 1970). Some of the chick genetic material is incorporated into the mouse nucleus during post-mitotic reconstitution. The important observations were that 1) permanent expression of this genetic information occurs as demonstrated by the fact that a small fraction (about 10^{-5}) of the heterokaryons permanently express the chick *hprt* gene, 2) only a very small fraction of the total genome is present and functional as evidenced by lack of expression of foreign surface antigens and absence of any morphologically identifiable chick material, and 3) the

linkage to the host genome is relatively loose, i.e. there is loss of the hypoxanthine phosphoribosyl transferase gene (*hprt*) from 20% of the cells after 27 generations in non-selective medium.

Each of these approaches has certain advantages as well as disadvantages. Those involving transduction or transformation by viruses or biochemically altered viruses result in transfer of bacterial or viral genes and thus would not facilitate mappaing of eukaryotic genes. Conversely, in situations where it is desirable to transfer a relatively small amount of genetic information, these methods could be useful and might provide a vector for entry into the cell and integration into the host DNA. The modified cell fusion approach could be helpful for ordering genes closely linked to a selective marker.

3. METAPHASE CHROMOSOMES

Mammalian metaphase chromosomes appear eminently suitable for information transfer since a meaningful biological fractionation of genes is present in chromosomes, and numerous methods have been described for isolation of these organelles (MAIO and SCHILDKRAUT 1969, MENDELSOHN et al. 1968, HEARST and BOTCHAN 1970, WRAY et al. 1972). Chromosomal DNA might be somewhat better protected from degradation during cellular uptake than free DNA due to its compact structure and its association with proteins and RNA. Furthermore, the introduction of intact chromosomes into cells could circumvent problems of integration of DNA into the host genome; subsequent replication and expression of chromosomal genes should be analogous to the steps following cell fusion.

Several discrete steps can be considered between uptake of isolated chromosomes and permanent expression of these chromosomal genes in a recipient cell and its progeny. These include binding to the recipient cell and transfer to the cell cytoplasm, processing of the chromosome in the cytoplasm particularly by lysosomal degradative enzymes, transfer of the intact chromosome or chromosomal fragments to the cell nucleus, replication of the chromosomal DNA with, or without, integration of fragments into host chromosomes, attachment to the spindle during mitosis, and finally expression of this new genetic information by transcription and translation. Nearly all of these steps are amenable to analysis with varying degrees of difficulty.

3.1 BINDING AND UPTAKE OF CHROMOSOMES

There is considerable evidence that isolated metaphase chromosomes can penetrate into mammalian cells *in vitro* (see McBRIDE and OZER 1973a, SEKIGUCHI et al. 1973 for references). Two general methods have been employed to study this process and they involve either measurement of radioactivity associated with cells after incubation with chromosomes labeled

with ^{3}H-thymidine or assessment of uptake by photomicroscopy sometimes including autoradiography. Photomicrographs of chromosome binding and/or uptake are shown in Figures 1 and 2. Many chromosomes are observed bound to the surface of cells (Figure 1) while many others appear to be located within cells (Figure 2) but the distinction between these two alternatives is frequently extremely difficult. This poses the principal limitation to the potential use of this technique for quantitative assessment of the rate and extent of chromosome uptake. It is possible to obtain additional information from the depth of focusing when the individual chromosomes and cells are viewed, but this is not a sufficiently

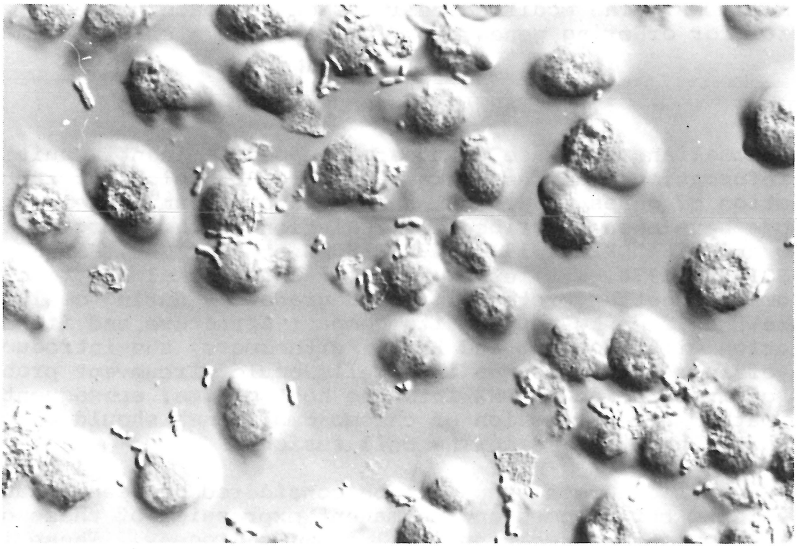

Figure 1. Hamster metaphase chromosomes were added to mouse A9 cells in a culture flask and photographed two hours later using Nomarski interference optics. The cells are rounded and they exhibited very little movement when viewed by time lapse cinematography. This effect probably resulted from a toxic level of poly-L-ornithine. Most of the chromosomes appear to be bound to cells rather than in the cytoplasm. This micrograph was provided by Dr. Cecil Fox.

rigorous criterion since it is quite subjective and it is further complicated by the irregular three-dimensional topography of cells. This problem could be resolved by either light or electron microscopic examination of serial thin-sections of cells after exposure to chromosomes. This procedure would be quite laborious and no results have been reported yet.

The uptake or binding by cells of chromosomes labeled with ^{3}H-thymidine is shown in Table 2. An equal number of

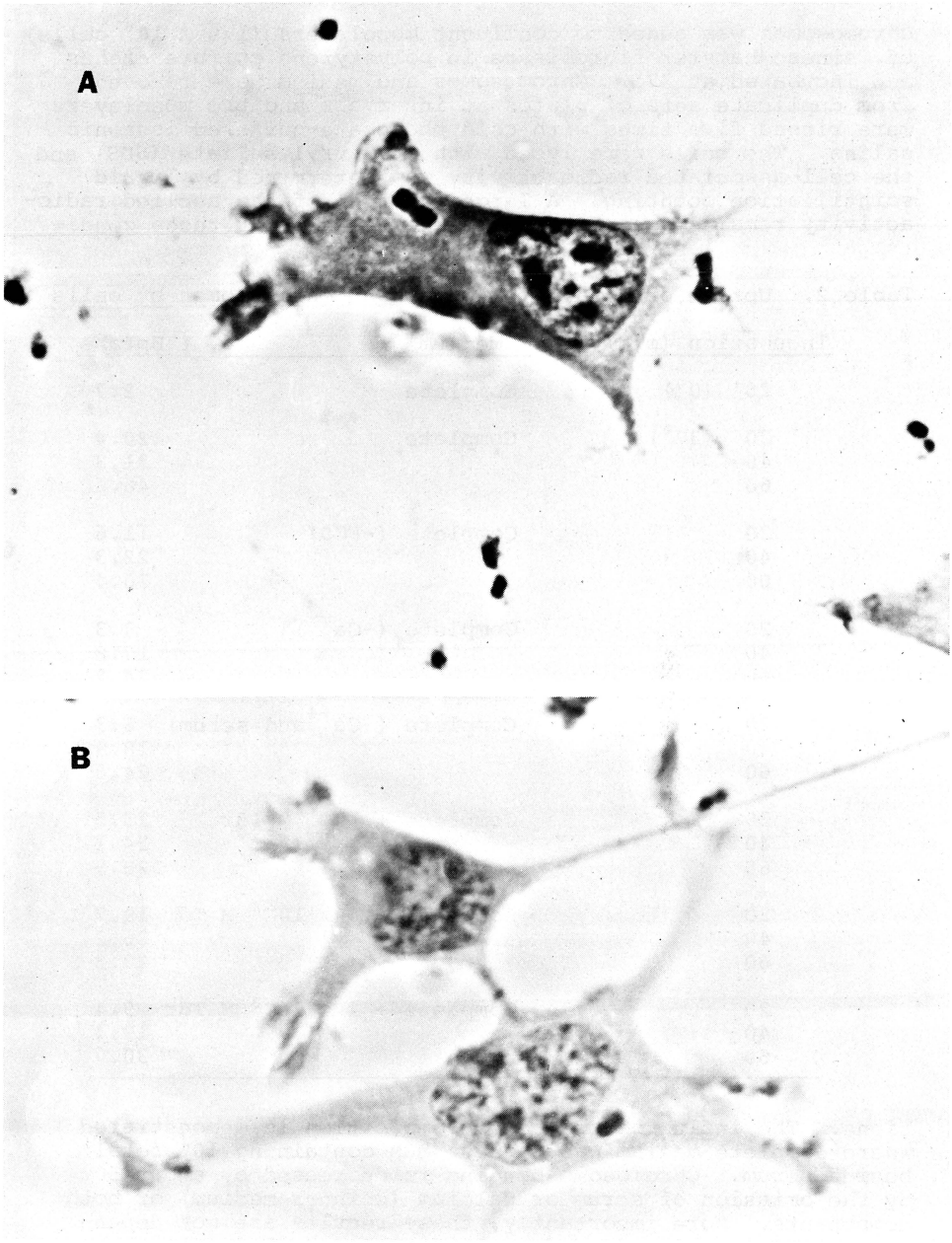

Figure 2. Mouse A9 cells were exposed to chromosomes, fixed, stained with Feulgen reagent, and counter stained with light green. There is probably a chromosome inside a cell in both A and B and both chromosomes appear to be located in vacuoles. These photomicrographs were provided by Dr. W.Bennett and Dr. C. Fox.

chromosomes was added to confluent monolayers (1.6 X 10^6 cells) of Chinese hamster fibroblasts in polystyrene culture dishes and incubated at 37^0. Chromosomes and medium were removed from duplicate sets of plates at intervals and the monolayers were rinsed five times with cold phosphate-buffered isotonic saline. The cells were lysed with 1% lauryl sulfate (SDS) and the cell-associated radioactivity was determined by liquid scintillation counting. A large fraction of the applied radioactivity remains associated with the cells under these condi-

Table 2. Uptake or binding of metaphase chromosomes by cells

Incubation (min)	Medium	% Uptake
25' (0^0)	Complete	2.7
20 (37^0)	Complete	20.4
40		31.3
60		46.6
20	Complete (-FCS)	11.6
40		22.3
60		30.4
20	Complete (-Ca^{++})	8.3
40		17.8
60		24.2
20	Complete (-$Ca^{++}$and serum)	8.3
40		18.9
60		24.0
20	Complete + 10^{-5} M Tdr	13.5
40		24.1
60		28.9
20	Complete + 6 x 10^{-5} M Tdr	19.7
40		26.2
60		29.3
20	Complete + 2 x 10^{-3} M Tdr	9.4
40		19.4
60		30.7

tions. The influence of medium composition is demonstrated where complete medium was Eagles' MEM containing 10% fetal bovine serum. Chromosome binding is decreased by about 50% by the omission of serum or calcium (spinner medium) or both components. More importantly, these results are not dependent primarily upon degradation of chromosomal DNA to the nucleoside level and subsequent uptake, since addition of unlabeled thymidine to the incubation medium, at levels varying from ten-fold that present in the chromosomal nucleic acid to a level sufficient to block cellular DNA synthesis, de-

creases the cell associated radioactivity by only about one-third. A major difficulty in this type experiment is again determining what fraction of chromosomes are actually inside cells as opposed to those which are tightly bound to the cell surface. The process is clearly time and temperature dependent. Cell-associated radioactivity is essentially invariant with time when the incubation occurs at 0^0, and it is comparable with the extent of binding after about 2 minutes at 37^0, representing less than 10% of that found after 1 hour at 37^0. Further indications that an appreciable fraction of this radioactivity may represent uptake, rather than binding to the cell surface, is provided by the fact that we have found in other experiments that well over half is not removed by treatment with pancreatic DNase. The cell-associated radioactivity after 1 hour incubation is reduced by about 1/3 to 2/3 when the cells are removed from the culture dish by dissociation with 5 mM EDTA and centrifuged for 5 minutes at 500 g prior to assay.

Chromosomes are also bound by cells when incubated in suspension at 37^0, although the rate is only about 1/3 to 1/10 that found for monolayers (McBRIDE and OZER 1973b). The total number of bound chromosomes increases with chromosome input, although at a decreasing rate at high input. There is binding of about 2 chromosomes per cell per hour when the input ratio of chromosomes to cells is approximately 5-10, and binding is nearly linear with time for approximately 1 hour. Others have reported uptakes of 1-20 (ITTENSOHN and HUTCHISON 1969) to 7-40 (KATO et al. 1971) chromosomes/cell after several hours incubation. We have compared the rate of binding of chromosomes by mitotic and interphase cells in suspension, and there appears to be little, if any, binding by mitotic cells. Very similar rates of binding have been observed with both Chinese hamster and mouse (A9) fibroblasts. The polycations, DEAE-dextran and poly-L-ornithine, did not markedly influence the rate of binding but we have not systematically studied binding under a wide range of conditions.

The general consensus from all of the studies cited is that isolated chromosomes can probably be incorporated into cells without prior extensive degradation in the medium, although the precise fraction which is actually inside the cells remains open to some question in all cases. The uptake most probably occurs by phagocytosis. This interpretation is supported by reports that engulfed chromosomes were often located in cytoplasmic vacuoles (CHORAZY et al. 1963, ITTENSOHN and HUTCHISON 1969, KATO et al. 1971).

3.2 PROCESSING OF CHROMOSOMES AFTER UPTAKE

Most of the ingested chromosomes are degraded in the cytoplasm of the recipient cells and the degradation products are incorporated into their DNA (McBRIDE and OZER 1973b, CHORAZY et al. 1963, WHANG-PENG et al. 1967, ITTENSOHN and HUTCHISON 1969, KATO et al. 1971, BURKHOLDER and MUKHERJEE

1970). It has been suggested that integration of extracellular chromosomal DNA into the recipient cell DNA may involve DNA macromolecules rather than free nucleotides (BURKHOLDER and MUKHERJEE 1970). Others have found that unlabeled thymidine successfully competes with the ^{3}H-thymidine labeled chromosome digestion products for incorporation into nuclear DNA (WHANG-PENG et al. 1967, KATO et al. 1971). This is obviously a very important distinction since functional genes could conceivably be incorporated in the former process, whereas the ingested chromosomes simply form part of the nucleotide pool in the latter case. One of our studies indicating that the majority of chromosomes associated with cells are rapidly destroyed and unavailable for gene expression is shown in Table 3. Inclusion of protamine sulfate in the incubation

Table 3 Stability of cell-associated chromosomes[a]

Duration of chase (hours)	Percent cell-associated radioactivity[b]		
	treatment:		
	A (-TdR)	B (+TdR)	C* (+TdR)
0	(100)	(100)	(100)
5	94	84	78
10	138	53	58
20	122	30	32

[a] Purified ^{3}H-TdR Chinese hamster chromosomes were incubated with CH 386 cells (37° C, 1 hr) without (A) or with (B,C) cold thymidine (5×10^{-5} M); non-bound chromosomes were then removed, the culture reincubated ('chase'). Residual counts were obtained at intervals after washing and lysing the cells.

[b] (cpm cell-associated during chase/cpm cell-associated following initial incubation) x 100. Percent input bound at start of chase designated as 100% (A=39.2%, B=24.8%, C=27.9%).

* At the end of the treatment the monolayer was fixed with 4% formaldehyde in phosphate buffered saline (PBS) (20 minutes at room temperature), followed by two extractions (3 ml) with 5% trichloroacetic acid (TCA) at 0°C for 10 minutes, and one wash with H_2O. The monolayer was detached with 1 mg pronase in sodium dodecyl sulfate (SDS) and radioactivity determined. (From McBride and Ozer 1973b).

medium at a concentration of 10 µg/ml, or pretreatment of the chromosomes alone with higher concentrations, protected the chromosomes to a considerable extent against the action of degradative enzymes in the recipient cells in one study (KATO et al. 1971). Incorporation of chromosomes increased linearly for 6 hours before reaching a plateau if protamine was present, whereas uptake was linear for only 4 hours in the absence of the polycation. Then it declined rapidly as the rate of intracellular digestion of chromosomes reached, and exceeded, the rate of incorporation.

3.3 REPLICATION OF INGESTED CHROMOSOMES IN RECIPIENT CELLS

An interesting study (SEKIGUCHI et al. 1973) suggests that foreign chromosomes may replicate in recipient cells, albeit at a very low frequency. When metaphase spreads were examined at intervals after incubation of mouse embryo cells, containing only acrocentric and teleocentric chromosomes, with chromosomes isolated from rat ascites cells, chromosomes identified morphologically as rat species were detected with a frequency of 0.9% at 2 days and 0.3% at 4 days. This decreasing frequency was compensated by the appearance of cells containing a "denatured" rat chromosome. Unlabeled rat chromosomes and ^{3}H-thymidine were then added simultaneously to mouse embryo cells, and metaphase spreads containing a labeled rat chromosome were detected with a frequency of 3×10^{-6} after 2 days. None were found after 4 days, but there was a similar frequency of cells containing a labeled denatured rat chromosome which was similar in appearance to chromosomes that have undergone premature chromosome condensation (JOHNSON and RAO 1970). Chinese hamster chromosomes uniformly labeled with ^{3}H-thymidine were added to unlabeled Chinese hamster cells (SEKIGUCHI et al. 1973) and two cells containing a semi-conservatively labeled chromosome were detected among $1\text{-}2 \times 10^{6}$ cells after 2 days but none were found among $2\text{-}3 \times 10^{6}$ cells after 4 days. These combined results suggest that intact ingested chromosomes may replicate in the recipient cell with a very low frequency, but that they frequently undergo dissolution within a few cell divisions perhaps as a result of asynchromy between replication of the donor chromosomal DNA and the host DNA.

3.4 DETECTION OF GENETIC TRANSFER EMPLOYING *hprt*$^{-}$ RECIPIENT CELLS

A simple and sensitive system (Figure 3) was employed to detect the permanent transfer of genetic information from isolated metaphase chromosomes to recipient cells. The methods have been described in detail elsewhere (McBRIDE and OZER 1973a, 1973b). Basically ^{3}H-thymidine-labeled chromosomes were isolated from wild type Chinese hamster fibroblasts (*hprt*$^{+}$) at pH 3 (MENDELSOHN et al. 1968) or pH 7 (MAIO and SCHILDKRAUT 1969) and subsequently separated from debris and intact cells by differential and isopycnic centrifugation.

Nuclei were removed by unit-gravity sedimentation at pH 7. A typical preparation of chromosomes after isolation and separation from intact cells, nuclei, and debris is shown in Figure 4.

Purified metaphase chromosomes (1 cell equivalent per recipient cell) were dispersed with A9 mouse fibroblasts (6 X 10^6/ml) in complete Eagle's MEM spinner medium containing 12 µg/ml of poly-L-Ornithine (mol. wt. 70,000) and 10% fetal bovine serum in a sterile culture tube and incubated for 2 hours at 37^0 while rolling. Aliquots were plated in culture dishes and the medium was replaced with HAT (10^{-4}M hypoxanthine, 4 X 10^{-7}M amethopterin, 1.6 X 10^{-5}M TdR, 3 X 10^{-6}M glycine) medium after 3 days. The plates were refed with HAT selective medium twice weekly for 6 weeks and colonies which appeared were cloned. The results of a series of experiments are shown in Table 4.

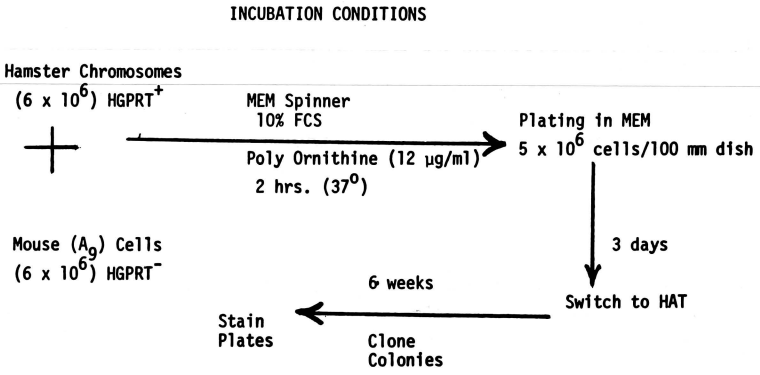

Figure 3. The principle of the method of detection of information transfer by isolated chromosomes. (HGPRT: hypoxanthine phosphoribosyl transferase, MEM: Eagle's minimal essential medium, HAT: hypoxanthine-amethopterine-thymidine medium).

The A9 cells are a line derived from wild-type mouse L cells by LITTLEFIELD (1966). Since they are deficient in the enzyme hypoxanthine phosphoribosyl transferase (HPRT), unaltered parental cells fail to survive in the selective medium. Colonies appeared at a relatively low frequency of about 10^{-7}, and they were detected in experiments employing chromosomes which had been isolated at either pH 3 or pH 7. Colonies also appeared in one experiment employing chromosomes isolated from human HeLa cells.

No colonies were found in a control experiment which was otherwise identical to experiment 1 (Table 4) except for the omission of chromosomes during the incubation.

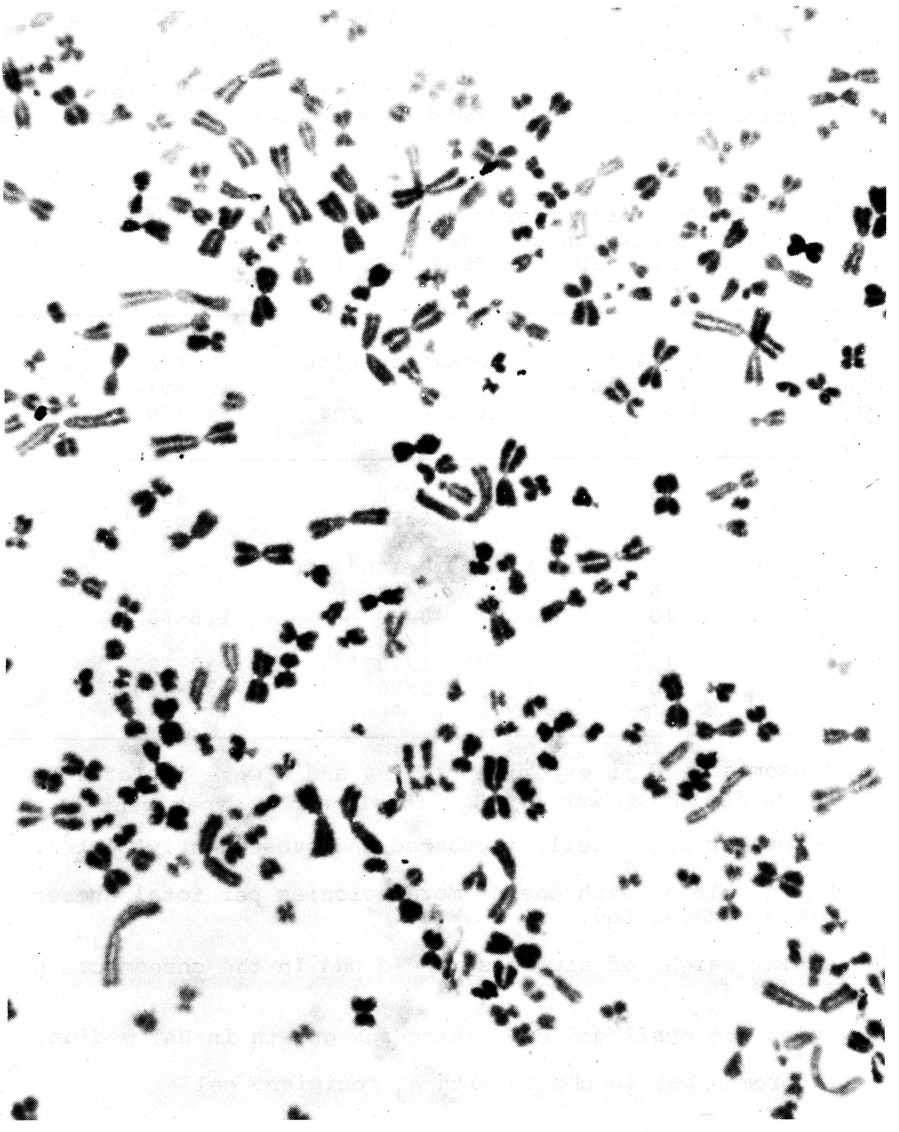

Figure 4. Chinese hamster chromosomes were isolated at pH 3 and purified by differential, isopycnic, and unit-gravity sedimentation before fixation and staining with crystal violet.

Larger numbers of cells (1.4 X 10^9) have been plated in selective HAT medium and only 2 revertant colonies were found. Thus, the A9 cells have a very low reversion frequency that appears to be lower than the frequency of gene transfer, even considering that the cells would have doubled about three times

Table 4. HPRT-positive colonies[a] after incubation of A_9 cells with chromosomes (from McBRIDE and OZER 1973a)

Experiment	Total no. of cells[b] X10^{-6}	Positive plates per total plates[c]	DNA[d] mol. wt. X10^{-6}
1	6	5/12[e]	30
2	25	2/50[f]	3
3	25	2/49[g]	30
4	50	0/100[g]	20
5	6	1/12[g]	25
6	10	0/20[g]	1.5-30[h]
7	9	1/18[i,j]	30
8A	10	1/20[i]	30-130
8B	10	3/20[i]	30-130

[a]Chromosomes used in experiments 2, 4 and 8 were isolated at pH 7; the isolation was at pH 3 in all other experiments.

[b]Total number of A_9 cells incubated and subsequently plated.

[c]Number of plates with one or more colonies per total number of plates inoculated.

[d]Molecular weight of single-stranded DNA in the chromosome preparations.

[e]Colonies not confirmed by cloning and growth in HAT medium.

[f]HeLa chromosomes incubated with A_9 recipient cells.

[g]Incubation medium contained 2 mM $CaCl_2$ (monolayer medium).

[h]Very heterogeneous molecular weight.

[i]Ratio of cell equivalents of chromosomes to recipient cells was 10:1 in experiment 8B; the ratio was 1:1 in 8A and all other experiments.

[j]This colony was a revertant.

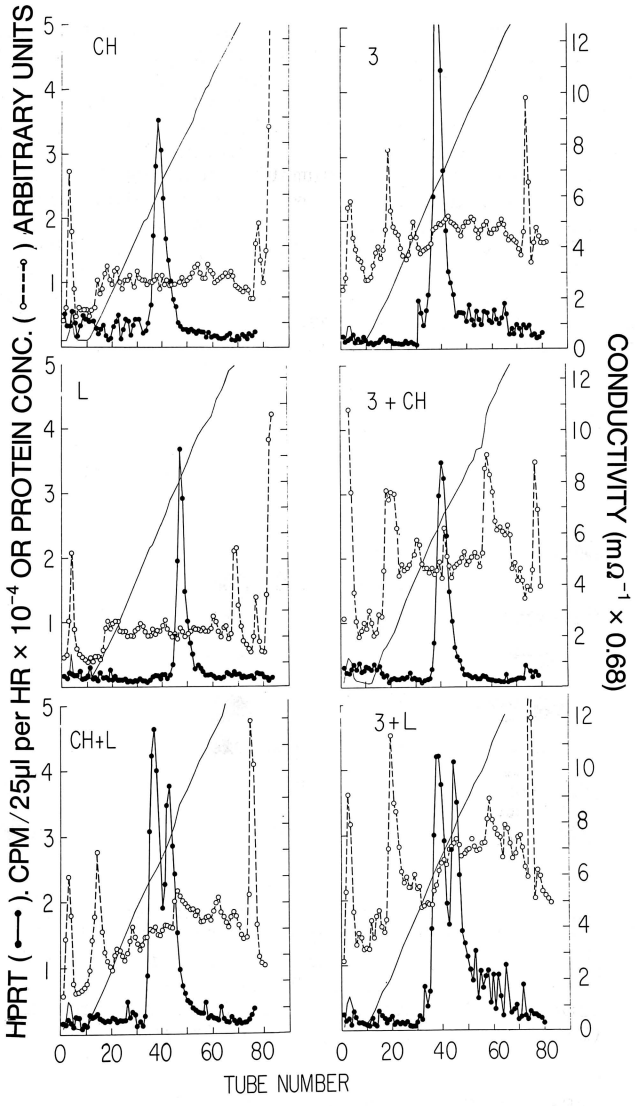

Figure 5. Chromatography at 5° C of crude HPRT extracts isolated from Chinese hamster (CH), mouse (L), clone 3 cells, and artificial mixtures of these solutions. Extracts (3-15 mg of protein) were applied to DEAE-cellulose columns and eluted with a gradient of NaCl in 0.01 M Tris.HCl (pH 8.7). Fractions of 1 ml were collected and assayed for HPRT activity (●—●), protein (o---o) and conductivity (—). Enzyme activity is plotted at 0.5 the normal scale for L and 3+CH. (from McBride and Ozer 1973a).

before HAT selection began under the experimental conditions. Furthermore, incubation of chromosomes isolated from A9 cells with A9 recipient cells did not result in colonies (o/6 X 10^6 total). Chromosomes isolated from *hprt*$^-$ Chinese hamster cells were also incubated with A9 cells in 2 separate experiments and only one colony was found (0/6 X 10^6 and 1/6 X 10^6 cells). This colony presumably represented a revertant but it was lost due to bacterial contamination prior to biochemical characterization. It has been pointed out in other studies involving DNA uptake by cells (SHIN et al. 1973) that statistics of the type presented here are not reliable indicators of gene transfer, and additional studies were required to elimate the possibility that all of these experimental clones might represent revertants.

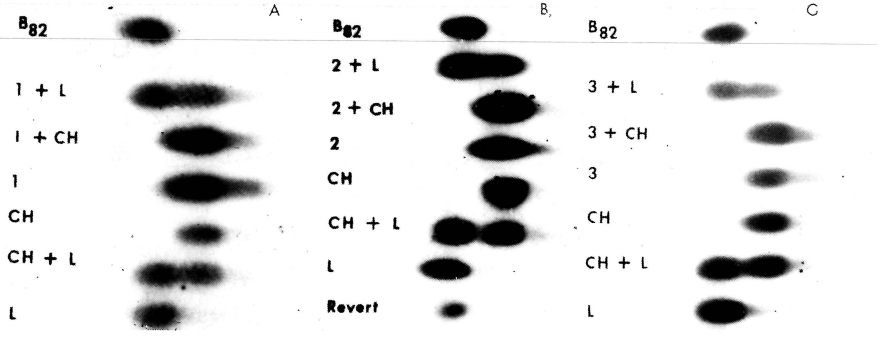

Figure 6. Gel electrophoresis of HPRT extracts on vertical slabs of polyacrylamide at 5^0. The individual extracts and artificial mixtures are identified as in Figure 5 where 1, 2, and 3 represent separate clones. An extract of an A_9 revertant is also shown (B). (from McBride, and Ozer 1973a).

3.4.1 CHARACTERIZATION OF THE GENE PRODUCT *IN VITRO*

The clones (Table 4) were propagated in suspension culture in selective HAT medium and crude enzyme extracts were prepared and examined for HPRT activity. The specific activities of these extracts were very similar to those obtained with Chinese hamster fibroblast or wild-type (L_{929}) mouse fibroblast extracts (McBRIDE and OZER 1973a).

The HPRT gene products of the two parental species (i.e. Chinese hamster and mouse) are distinguishable by both ion-ex-

change chromatography on DEAE-cellulose columns and electrophoresis in polyacrylamide gels. A typical chromatographic profile of the extract of one clone (Figure 5) and electrophoretic pattern of 3 clones (Figure 6) are shown. Extracts from 11 of the clones from Table 4 were similarly examined by both methods. The HPRT was indistinguishable from the hamster donor parental species in all cases except one which was identical to the mouse recipient species and therefore represented a revertant. The extracts from an authentic revertant and from thymidine kinase mutant L cells (B82) were both indistinguishable from wild type mouse HPRT by both criteria. These results all support the contention that 10 of the 11 colonies examined resulted from transfer of a gene from the ingested chromosome and its expression in the recipient cell rather than reversion.

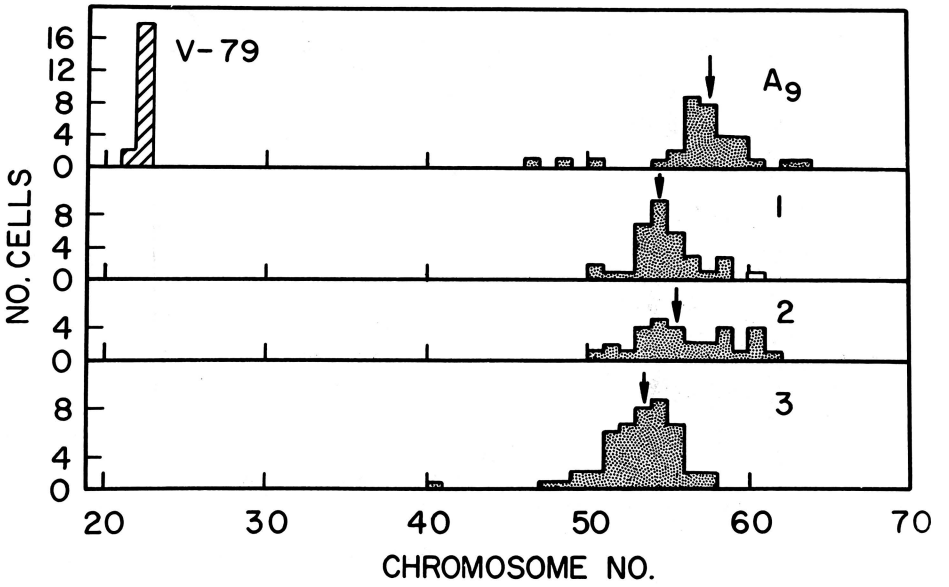

Figure 7. Histogram of chromosomes in parental cell lines (V-79 and A_9) and in 3 clonal lines. Arrows indicate the median number of chromosomes in each line. (from McBride, and Ozer 1973a).

3.4.2 *KARYOTYPES*

The karyotypes of all clones closely resembled the parental A9 cells as expected and the histograms of total chromosome numbers for 3 clones and the parental lines are shown in Figure 7. Clearly none of the clones could have arisen through contamination of the cultures with hamster cells.

3.4.3 *STABILITY OF THE GENOTYPE AFTER CHROMOSOME TRANSFER*

The stability over a two month interval of the *hprt*+ genotype after removing selective pressure was studied in 3 clones, and they fell into two different groups (Figure 8). When clone 3 was cultured in non-selective medium, there was a rapid accumulation of HPRT deficient cells. The results are compatible with the loss of the *hprt* gene by about 10-20% of the cells at each division. Similar reversion behavior was reported by SCHWARTZ et al. (1971) for cells probably containing the *hprt* gene on a chromosome fragment. Conversely, the other two clones were quite stable under non-selective conditions.

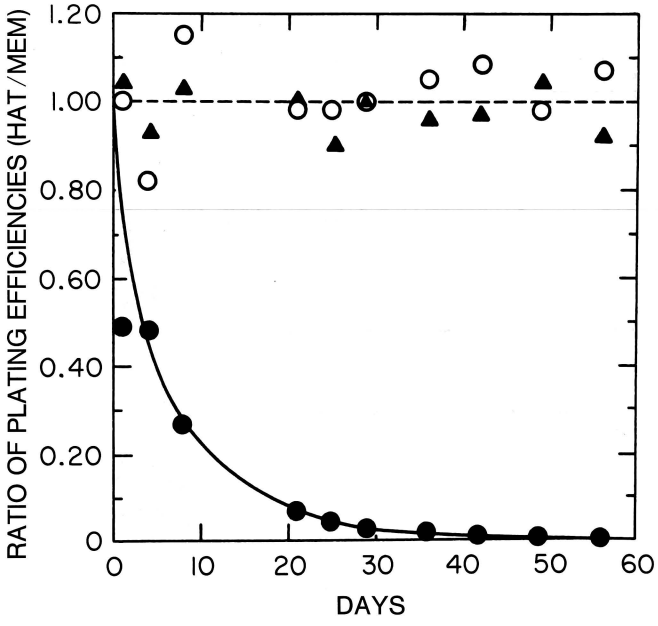

Figure 8. Stability of the $hprt^+$ genotype in colonies after removing selective pressure. The plating efficiency in selective HAT medium compared to that in non selective medium is plotted as a function of the time interval after the cells were removed from HAT medium. The cell lines depicted are separate clones 1 (o), 2 (Δ), and 3 (●—●) (from McBRIDE and OZER 1973a).

3.4.4 *NUMBER OF GENES TRANSFERRED TO A RECIPIENT CELL*

It is of general interest, and of crucial importance for consideration of this procedure as a method for gene mapping, to understand the size of the incorporated chromosome segment which is expressed by the recipient cell. One might question

whether an intact chromosome is transferred or only a chromosome fragment. If the transfer does involve only a fragment, does this fragment replicate independently as an episome or is it integrated into the host DNA? It is equally important to understand whether multiple chromosome fragments might be incorporated into the genetic apparatus of a single cell and whether these fragments originate from a single chromosome or several chromosomes. We have very little information on these points currently but more definitive information should be forthcoming in the near future. The genes from cell surface antigens are thought to be reasonably widely distributed throughout the entire chromosomes set (WEISS and GREEN 1967). We examined the cells from 3 clones for hamster surface antigens in collaboration with Dr. M. Mage, and none were detected. Of course, antibodies against all surface antigens may not be present in the antiserum which was used. Drs. Martin Bobrow of Oxford and Jean P. Thirion of Sherbrook examined cells from these same 3 clones by quinacrine mustard and Giemsa banding techniques, and they were unable to detect any hamster chromosomes. As expected, we have not detected adenyl phosphoribosyl transferase (APRT) in any of the clones tested, since *aprt* and *hprt* are located on different chromosomes in man (RUDDLE 1973).

More favorable systems for investigating this question are now being used. The colonies resulting from incubation of human chromosomes with A9 cells (Table 4) were not characterized biochemically. However, more recently one colony has been obtained in which chromosomes isolated from human cells were incubated with A9 cells (BURCH and McBRIDE, unpublished), and the HPRT product has been identified as human species. It should be possible to obtain a number of $hprt^+$ colonies in experiments (in progress) employing chromosomes isolated from diploid human cells with rodent recipient cells. All of these colonies will be analyzed for the presence of the other X-linked biochemical markers and for co-segregation of these genes on back-selection for $hprt^-$ cells. Recipient cells other than A9 could be more favorable for karyotypic analysis in some of these studies.

3.4.5 *APPLICABILITY TO STUDY OF OTHER GENETIC MARKERS*

Fortunately, A9 cells are deficient in APRT as well as HPRT (COX et al. 1972) so that it is possible to simultaneously test for the transfer of either, or both, of these non-linked markers. Hypoxanthine and adenine (5×10^{-5} M) are added to the previously described selective medium. Some $aprt^+$ colonies have been obtained and extracts are being analyzed to determine whether their presence resulted from gene transfer or merely reversion. No colonies have yet been obtained which are both $hprt^+$ and $aprt^+$ as might occur if a very limited fraction of the cells are very efficient in gene transfer. It is obviously important to demonstrate that transfer can occur for a variety of markers employing various recipient cells as well as differing chromosome donor cell types.

CONCLUSION

It appears that permanent genetic transfer can occur through incorporation of a chromosome and expression of its genes by recipient cells, although confirmation of these results in other laboratories is awaited. It would be highly desirable to increase the efficiency of this process for most applications, and the possible approaches to this problem are numerous. Degradation of chromosomes by lysosomal enzymes may well be the major limitation, but other subsequent steps, including asynchromy of DNA replication between the incorporated chromosome and those of the recipient cell or defects in karyokinesis, may also be of fundamental importance in limiting permanent expression. It is also not clear whether those foreign chromosomal genes which are expressed represent statistically rare escapes from the phagosomal pathway before complete degradation, or whether they represent rare instances in which the pathway is totally circumvented.

The low efficiency of transfer currently restricts the use of this process to systems where some type selective system or amplification mechanism can be applied, although this can vary from selective medium to rescue of a virus. This relative inefficiency also presently requires that the gene product be characterized as chromosomal donor species in order to exclude confusion with gene mutations (reversion) in the recipient cells. This method has potential advantages for analysis of gene linkage in somatic cells, and future advances in this area could broaden its field of applicability. Methods for fractionating metaphase chromosomes are currently limited to density gradient velocity sedimentation (see references in HEARST and BOTCHAN 1970, McBRIDE and OZER 1973b) and isoelectric focusing (LANDEL et al. 1972). Future development of additional techniques for metaphase chromosome fractionation should enhance the usefulness of this method.

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LITERATURE CITED

BHARGAVA, P.M. and G. SHANMUGAN 1971. Uptake of nonviral nucleic acids by mammalian cells. Progr. Nucleic Acid Res. and Mol. Biol. (eds. J. N. Davidson and W. E. Cohn, Acad. Press, N.4, 11:103-192.

BURKHOLDER, G.D. and B.B. MUKHERJEE 1970. Uptake of isolated

metaphase chromosomes by mammalian cells in vitro. Exptl. Cell Res. 61:413-422.

CASPERSSON, T., L. ZECH and C. JOHANSSON 1970. Differential binding of alkylating fluorochromes in human chromosomes. Exptl. Cell Res. 60:315-319.

CHORAZY, M., A. BENDICH, E. BORENFREUND, O. L. ITTENSOHN and D. J. HUTCHISON 1963. Uptake of Mammalian chromosomes by mammalian cells. J. Cell Biol. 19:71-77.

COON, H. G., I. HORAK and I. B. DAWID 1973. Propagation of both parental mitochondrial DNA's in rat-human and mouse-human hybrid cells. J. Mol. Biol. 81:285-298.

COX, R. P., M. R. KRAUSS, M. E. BALIS and J. DANCIS 1972. Communication between normal and enzyme deficient cells in tissue culture. Exptl. Cell Res. 74:251-268.

CROCE, C. M., A. J. GIRARDI and H. KOPROWSKI 1973a. Assignment of the T-antigen of Simian virus 40 to human chromosome C-7. Proc. Nat. Acad. Sci. U.S. 70:3617-3620.

CROCE, C. M., G. LITWACK and H. KOPROWSKI 1973b. Human regulatory gene for inducible tyrosine aminotransferase in rat-human hybrids. Proc. Nat. Acad. Sci. U.S. 70:1268-1272.

DAVIDSON, R. L., S. J. ADELSTEIN and M. N. OXMAN 1973. Herpes simplex virus as a source of thymidine kinase for thymidine kinase-deficient mouse cells: suppression and reactivation of the viral enzyme. Proc. Nat. Acad. Sci. U.S. 70:1912-1916.

DRETS, M. E. and M. W. SHAW 1971. Specific banding patterns of human chromosomes. Proc. Nat. Acad. Sci. U.S. 68: 2073-2077.

EPHRUSSI, B. 1972. Hybridization of somatic cells. Princeton Univ. Press. Princeton, N. J.

GEIER, M. R. and C. R. MERRIL 1972. Lambda phage transcription in human fibroblasts. Virology 47:638-643.

HANDMAKER, S. D. 1973. Hybridization of eukaryotic cells. Ann. Rev. Microbiol. 27:189-204.

HARRIS, H. 1970. Cell Fusion. Harvard Univ. Press, Cambridge, Mass.

HEARST, J. E. and M. BOTCHAN 1970. The eukaryotic chromosome. Ann. Rev. Biochem. 39:151-182.

ITTENSOHN, O. L. and D. J. HUTCHISON 1969. Cytologic manifestations of the phagocytosis of L 1210 chromosomes by L 1210 cells in culture. Exptl. Cell Res. 55:149-154.

JACKSON, D. A., R. H. SYMONS and P. BERG 1972. Biochemical method for inserting new genetic information into DNA of Simian virus 40: Circular SV40 DNA molecules containing lambda phage genes and the galactose operon of Escherichia coli. Proc. Nat. Acad. Sci. U.S. 69:2904-2909.

JOHNSON, R. T. and P. N. RAO 1970. Mammalian cell fusion: Induction of premature chromosome condensation in interphase nuclei. Nature 226:717-722.

KAO, F. T. and T. T. PUCK 1972. Genetics of somatic mammalian cells XIV. Genetic analysis in vitro of auxotrophic mutants. J. Cell Physiol. 80:41-50.

KATO, H., K. SEKIYA and T. H. YOSIDA 1971. Uptake of isolated chromosomes by mammalian cells and protective effect of protamine sulfate. Exptl. Cell Res. 65:454-462.

LANDEL, A. M., Y. ALONI, M. A. RAFTERY and G. ATTARDI 1972. Electrofocusing analysis of HeLa cell metaphase chromosomes. Biochemistry 11:1654-1663.

LITTLEFIELD, J. W. 1966. The use of drug-resistant markers to study the hybridization of mouse fibroblasts. Exptl. Cell Res. 41:190-196.

LONG, C., T. CHAN, V. LEVYTSKA, T. KUSANO and H. GREEN 1973. Absence of demonstrable linkage of human genes for enzymes of the purine and pyrimidine salvage pathways in human mouse somatic cell hybrids. Biochem. Genet. 9: 283-297.

MAIO, J. J. and C. L. SCHILDKRAUT 1969. Isolated mammalian metaphase chromosomes II. Fractionated chromosomes of mouse and Chinese hamster cells. J. Mol. Biol. 40:203-216.

McBRIDE, O. W. and H. L. OZER 1973a. Transfer of genetic information by purified metaphase chromosomes. Proc. Nat. Acad. Sci. U.S. 70:1258-1262.

McBRIDE, O. W. and H. L. OZER 1973b. Gene transfer with purified mammalian chromosomes. In: Possible Episomes in Eukaryotes:Le Petit Colloquia on Biology and Medicine. 4:255-267 (North-Holland, Amsterdam).

MEDRANO, L. and H. GREEN 1974. A uridine kinase-deficient mutant of 3T3 and a selective method for cells containing the enzyme. Cell 1:23-26.

MENDELSOHN, J., D. E. MOORE, and N. P. SALZMAN 1968. Separation of isolated Chinese hamster metaphase chromosomes into three size-groups. J. Mol. Biol. 32:101-112.

MERRIL, C. R., M. R. GEIER, and J. C. PETRICCIANI 1971. Bacterial virus gene expression in human cells. Nature

233:398-400.

MICHEL, M. R., B. HIRT, and R. WEIL 1967. Mouse cellular DNA enclosed in polyoma viral capsids (pseudovirions). Proc. Nat. Acad. Sci. U.S. 58:1381-1388.

MUNYON, W., E. KRAISELBUD, D. DAVIS, and J. MANN 1971. Transfer of thymidine kinase to thymidine kinaseless L cells by infection with ultraviolet-irradiated herpes simplex virus. J. Virol. 7:813-820.

MUNYON, W., R. BUCHSBAUM, E. PAOLETTI, J. MANN, E. KRAISELBURD and D. DAVIS 1972. Electrophoreseis of thymidine kinase activity synthesized by cells transformed by herpes simplex virus. Virology 49:683-689.

OSTERMAN, J. V., A. WADDELL, and H. V. APOSHIAN 1970. DNA and gene therapy: Uncoating of polyoma pseudovirus in mouse embryo cells. Proc. Nat. Acad. Sci. U.S. 67:37-40.

QASBA, P. K., and H. V. APOSHIAN 1971. DNA and gene therapy: Transfer of mouse DNA to human and mouse embryonic cells by polyoma pseudovirions. Proc. Nat. Acad. Sci. U.S. 68:2345-2349.

QASBA, P., D. B. YELTON, Q. A. PLETSCH and H. V. APOSHIAN 1972. Properties of polyoma pseudovirions in mouse and human cells. Pp. 169-89. 25th Symp. Fundamental Cancer Res. Univ. Texas and M. D. Anderson Hosp. and Tumor Inst. Williams and Wilkins, Baltimore, Md. (1974).

RAO, P. N. and R. T. JOHNSON 1972. Cell fusion and its application to studies on the regulation of the cell cycle. in: Methods in Cell Physiology, ed. D.M. Prescott, Vol. 5, Acad. Press, New York pp. 75-126.

RUDDLE, F. H. 1973. Linkage analysis in man by somatic cell genetics. Nature 242:165-169.

RUECKERT, R. R. 1968. In: Methods in Cancer Research, H. Busch, ed. Vol. 4 Acad. Press, New York. p. 455.

SARKAR, S. 1968. IN: Methods in Virology K. Maramorosch and H. Koprowski, ed., Acad. Press, New York, Vol. 2 p. 607.

SCHWARTZ, A. G., P. R. COOK and H. HARRIS 1971. Correction of a genetic defect in a mammalian cell. Nature New Biol. 230:5-8.

SEKIGUCHI, T., F. SEKIGUCHI and M. YAMADA 1973. Incorporation and replication of foreign metaphase chromosomes in cultured mammalian cells. Exptl. Cell Res. 80:223-236.

SHIN, S., R. CANEVA, C. L. SCHILDKRAUT, H. P. KLINGER and M. SINISCALCO 1973. Cells with phosphoribosyl transferase activity recovered from mouse cells resistant to 8-azaguanine. Nature New Biol. 241:194-196.

TRILLING, D. M. and D. AXELROD 1970. Encapsidation of free host DNA by simian virus 40: A simian virus 40 pseudovirus. Science 168:268-271.

WEISS, M. C. and H. GREEN 1967. Human-mouse hybrid cell lines containing partial complements of human chromosomes and functioning human genes. Proc. Nat. Acad. Sci. U.S. 58:1104-1111.

WHANG-PENG, J., J. H. TJIO and J. C. CASON 1967. Reutilization of presumably degraded radioactive label from a chromosomal fraction of homologous and heterologous cells. Proc. Soc. Exp. Biol. Med. 125:260-263.

WRAY, W., E. STUBBLEFIELD and R. HUMPHREY 1972. Mammalian metaphase chromosomes with high molecular weight DNA isolated at pH 10.5 Nature New Biol. 238:237-238.

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