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Linkage of Markers Controlling Consecutive Biochemical Steps in CHO Cells as Demonstrated by Chromosome Transfer

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Summary

Using the technology of metaphase chromosome transfer, evidence has been obtained in CHO cells that genes controlling enzymes in a common pathway in folate metabolism are closely linked. Mtx^{RI} and Mtx^{RIII} are co-dominant mutations which affect the structure and level of dihydrofolate reductase. Gat^- is a glycine-, adenosine- and thymidine-requiring auxotrophic mutant with a lesion in folylpolyglutamate synthetase, an enzyme responsible for addition of glutamates to folate residues. $GlyB^-$ is an auxotrophic glycine-requiring mutant whose phenotype may be reversed by folinic acid. Using purified metaphase chromosomes, the Mtx^R genes were co-transferred into recipient cells with the auxotrophic markers, as demonstrated by the isolation of transferents when two of the phenotypes, either Mtx and Gat, or Mtx and GlyB, were selected at the same time. When recipient cells were selected for Gat^+ or $GlyB^+$ alone, the transferents carried the Mtx^R markers. The GlyA mutation, another glycine-requiring auxotrophic change, is not co-transferred with methotrexate resistance.

Because of previous evidence that only a small fragment is involved in chromosomal transfer experiments, these results seem to provide the first indication that some genes which control enzymes on a common metabolic pathway in eucaryotes are closely linked or are at least syntenic.

Introduction

The feasibility of transfer of genetic properties in somatic cells by means of metaphase chromosomes has now been demonstrated in several systems and with a variety of markers (McBride and Ozer, 1973; Burch and McBride, 1975; Willecke and Ruddle, 1975; Wullems, van der Horst and Boostma, 1975; Degnen et al., 1976; Spandidos and Siminovitch, 1977). The transferred genetic properties tend to be unstable in the selected recipient cells, although stable transferents can be isolated. Although one exceptional case has been reported (Wullems, van der Horst and Boostma, 1976), there is good evidence that in general, the stable transferent only contains a small segment of the donor chromosomal material of the order of, or less than, 0.2% of the haploid genome (Willecke et al., 1976).

The ability to transfer a small segment of chromosomal material in somatic cells theoretically offers the opportunity to test for genetic linkage or synteny of appropriate markers in such systems. For example, there is little or no information in mammalian cells on whether genes controlling steps in a common enzymatic pathway are linked, or whether operons can be distinguished in such cells. Whereas there is considerable evidence in bacteria for clustering of functionally related genes, in the form of operons, present information on fungi indicates that such genes are not contiguous to each other and that they are located widely throughout the genome (Fink, 1971). In fact, information which is available at present on the organization of functionally related enzymes in fungi and animal cells indicates that they are often part of a single polypeptide unit and not part of an operon (Brutlag et al., 1969; Creighton, 1970; Veron, Falcoz-Kelly and Cohen, 1972; Kempe et al., 1976; Paukert, D'Azi Straus and Rabinowitz, 1976).

In our own laboratory, we have shown that the dominant markers controlling resistance to methotrexate (Mtx^{RI} and Mtx^{RIII}) can be transferred and selected with an efficiency of about 4×10^{-6} in recipient CHO cells, and that stable lines bearing Mtx resistance can be isolated from such transferents (Spandidos and Siminovitch, 1977). The target enzyme for both these mutations is dihydrofolate reductase. Mtx^{RI} cells contain altered enzyme with about 10 fold increased resistance to the drug in vitro, and Mtx^{RIII} cells contain 10 fold increased activities of the altered enzyme (Flintoff, Davidson and Siminovitch, 1976a). Fortunately, another mutation is available in CHO cells called Gat^- in which the target enzymatic step lies in the folate metabolic pathway (McBurney and Whitmore, 1974) (see Figure 1). Gat^- is an auxotrophic mutant (called Aux B1 originally) which carries a requirement for glycine, adenosine and thymidine, and in which the deficiency was identified as an inability to add glutamate residues to tetrahydrofolate residues (McBurney and Whitmore, 1974). This enzyme has been recently characterized as formyl polyglutamate synthetase (Taylor and Hanna, 1977).

GlyB, an auxotrophic mutant, may carry a lesion on this same pathway (Kao, Chasin and Puck, 1969). The phenotype of glycine B can be reversed by folinic acid, and a possible site(s) for this lesion is shown in Figure 1.

The availability of these particular mutants has allowed us to use chromosome transfer techniques to examine whether there is any genetic linkage between markers affecting a common biochemical pathway in somatic cells. In this paper, we show

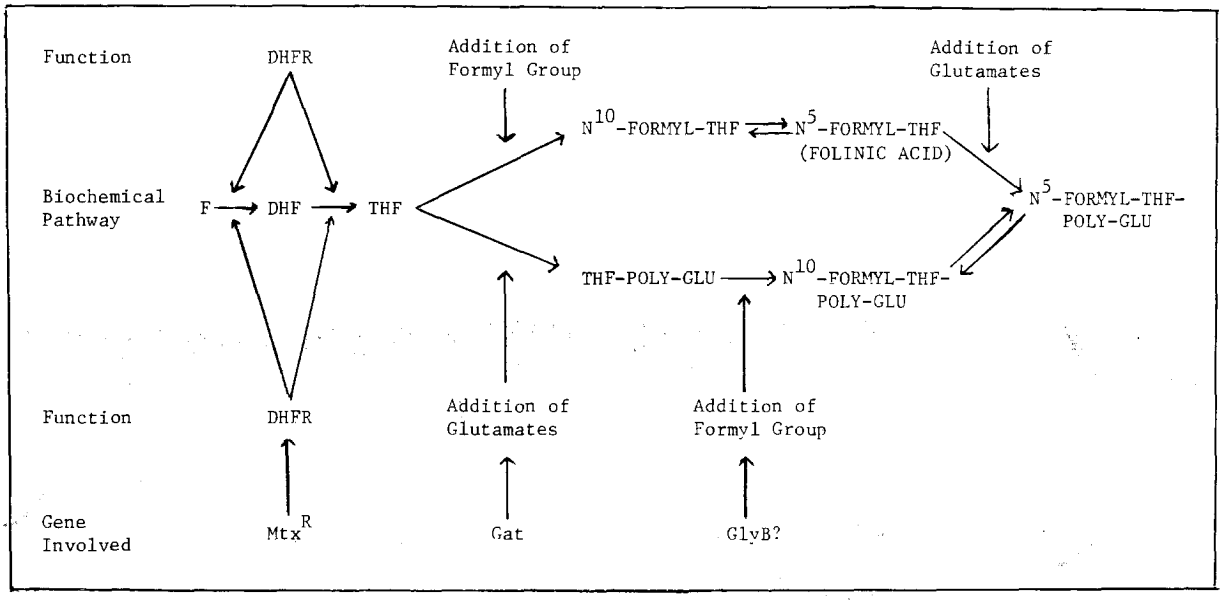

Figure 1. Pathway for the Formation of N5-Formyl-THF-Poly-GLU from Folic Acid

F, folate; DHF, dihydrofolate; THF, tetrahydrofolate; DHFR, dihydrofolate reductase; GLU, glutamate.

that the markers for the Gat$^+$ and GlyB$^+$ phenotypes are indeed co-transferred with that of Mtx^{RIII}. The marker for the GlyA$^+$ phenotype which involves the enzyme serine hydroxymethylase (Kao, et al., 1969) is not co-transferred.

Results

Co-Transfer of Mtx^{RIII} and Gat$^+$ Markers

As indicated in the Introduction and in Figure 1, dihydrofolate reductase—the enzyme responsible for reduction of folates—and folylpolyglutamate synthetase—the enzyme responsible for addition of glutamate residues to tetrahydrofolates—are involved in a common metabolic pathway. To test for linkage of the genes which code for these enzymes, metaphase chromosomes were prepared from Mtx^{RIII} cells and added to Gat$^-$ cells, and after allowing 2 days for gene expression, the recipient cells were plated in a medium containing Mtx and missing glycine, adenosine and thymidine. In order for cells to produce colonies under these conditions, the genes for both Mtx resistance (Mtx^{RIII}) and Gat prototrophy (Gat$^+$) must be present.

As can be seen in Table 1, transferents were obtained in four separate experiments using double selection (line 1), with about the same frequency found in our earlier work when only Mtx resistance was selected (Spandidos and Siminovitch, 1977). Thus the additional selection for the Gat marker did not reduce the number of transferents obtained, indicating that the two genetic characteristics were co-transferred. When only the Gat marker was selected, the number of colonies obtained when chromosomes were used (line 2) was much higher than in the absence of chromosomes (line 4), indicating that many of the former were transferents. Of special interest, however, was the fact that 4 out of 7 of the colonies obtained in the experiment described in line 2 were resistant to methotrexate.

It is probable that only a small chromosomal fragment is transferred in experiments of this kind (Willecke et al., 1976), and we could find no additional chromosomal material in the transferent cell on karyotypic examination. These results provide strong evidence that the Mtx and Gat markers are closely linked.

Behavior of Transferents in Selective and Nonselective Medium

In our previous studies, we showed that transferents carrying the Mtx marker tend to lose their resistance when grown in nonselective medium, a property shared by similar isolates examined by other investigators (McBride and Ozer, 1973; Willecke and Ruddle, 1975; Degnen et al., 1976; Spandidos and Siminovitch, 1977). Stable transferents can be obtained, however, either as a small fraction of the original isolates or after growth in selective medium. To obtain further evidence on the linkage of Mtx^{RIII} and Gat$^+$, we picked three subclones (tran711A, tran711B and tran711C) of the Mtx^{RIII}-tran711 clone, and grew aliquots for 30 days in selective and nonselective media. At various times thereafter, the plating efficiencies were measured

Table 1. Transfer of Methotrexate Resistance (Mtx^{RIII}) and Gat^+ Markers by Metaphase Chromosomes in CHO Cells

Donor Markers	Genotype of Recipient Cells	Selection[a] Medium	Colonies per 10^6 Cells Experiment Number 1	2	3	4	Total Colonies per 4×10^6 Cells
(1)[b] $Mtx^{RIII}Gat^+$	Mtx^SGat^-	α-special + Mtx	8	6	5	10	29
(2) $Mtx^{RIII}Gat^+$	Mtx^SGat^-	α-special	12	15	9	20	56
(3)[c]	Mtx^SGat^-	α-special + Mtx	0	0	0	0	0
(4)	Mtx^SGat^-	α-special	4	2	1	5	12
(5)[d] $Mtx^{RIII}Gat^+$		α-complete	0	0	0	0	0

[a] Mtx = 5×10^{-7} M methotrexate; α-special = α medium missing glycine, adenosine and thymidine, as well as several other constituents (McBurney and Whitmore, 1974).
[b] For lines 1 and 2, a total number of 5×10^6 cell equivalents of chromosomes was added to each 2×10^6 cells. After 2 days, 1×10^6 cells were plated (2×10^5 cells per plate) under the selection conditions.
[c] For lines 3 and 4, a total number of 1×10^6 cells was plated (2×10^5 cells per plate).
[d] A total of 5×10^6 cell equivalents of chromosomes was plated directly (1×10^6 cell equivalent chromosomes per plate).

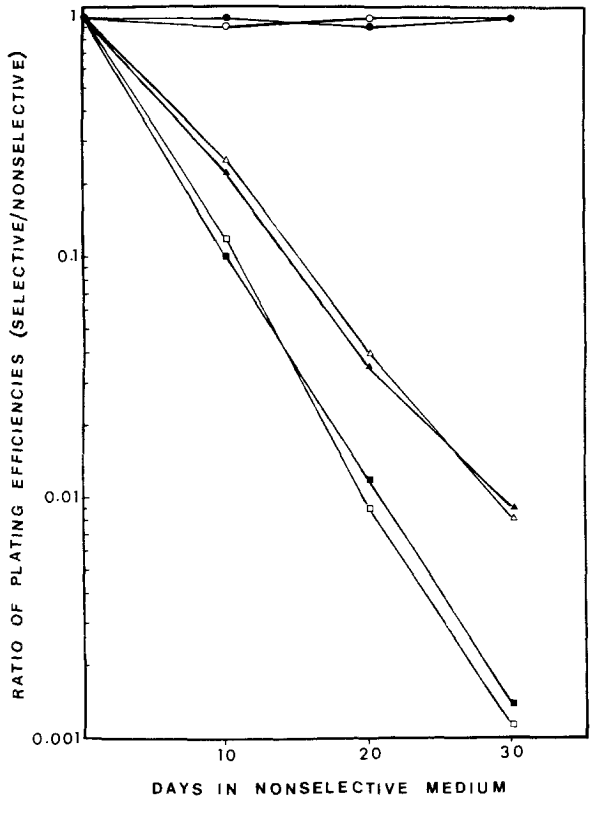

Figure 2. Stability of the Mtx^{RIII} and Gat^+ Markers under Nonselective Conditions in Three Subclones (A, B and C) of Mtx^{RIII}tran711

At time 0, all three cell lines that had been maintained for 10 days in selective medium (α-special containing 5×10^{-7} M Mtx and 10% DFCS) were transferred to nonselective medium (complete α medium containing 10% FCS). Aliquots of the cell lines were removed at the indicated times and tested for plating efficiencies in selective and nonselective media. Two types of selective media were now used: first, to test the Mtx resistance, the α-special medium contained 5×10^{-7} M Mtx and 10% DFCS (open symbols), and second, to test the expression of the Gat^+ marker, α-special containing 10% DFCS (closed symbols) was used. tran711A (○, ●); tran711B (△, ▲); and tran711C (□, ■).

in selective medium. The clones kept in selective medium maintained their Mtx^{RIII} and Gat^+ phenotype (data not shown), while those grown in nonselective medium showed the unstable behavior depicted in Figure 2. One clone maintained both markers, whereas in the other two, both Mtx resistance and Gat prototrophy were lost simultaneously at a rate observed previously (Spandidos and Siminovitch, 1977). The most significant conclusion from these results is the concordant behavior of the two markers, again suggesting strong linkage between them.

Phenotype of Stable Transferents

A further indication that the transferents carried both markers was obtained by comparing the phenotype of cells which had been grown in nonselective and selective media.

As indicated earlier, Mtx^{RIII} cells contain an altered dihydrofolate reductase, and Gat^- cells have lost the ability to add glutamate residues to folates. As can be seen in Figure 3, the resistance to Mtx of dihydrofolate reductase in extracts of a stable transferent which was grown in selective medium and which had maintained its cellular methotrexate resistance was similar to that of the Mtx^{RIII} donor, whereas that of the transferent grown in nonselective medium and which was no longer Mtx^R was similar to that of the recipient methotrexate-sensitive cell. Not shown here is the fact that the transferents grown in selective medium also contained increased levels of dihydrofolate reductase, again confirming their Mtx^{RIII} character. Similarly, as seen in Figure 4, the folate pattern of the stable transferent grown in selective medium was similar to that of the donor Gat^+ cells, whereas that of the unstable transferent which had lost its chromosomal markers was similar to that of the recipient Gat^- cells.

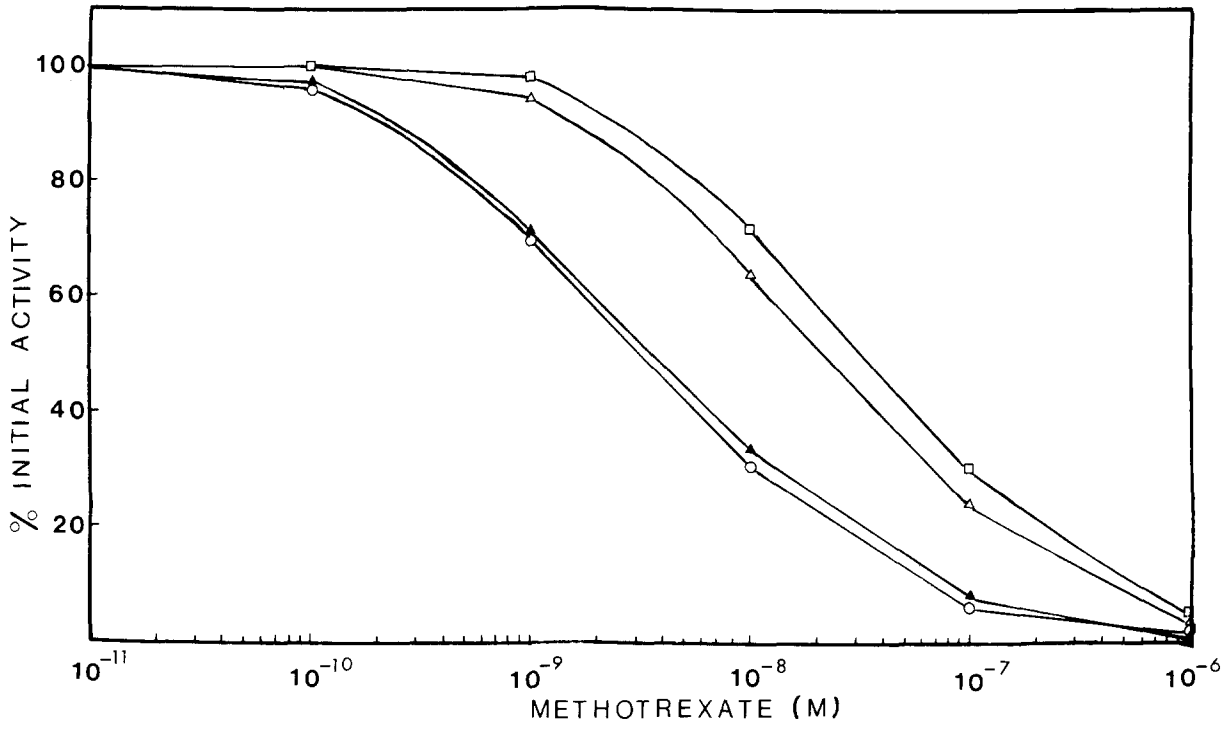

Figure 3. Titration of Dihydrofolate Reductase with Mtx in Extracts of Donor, Recipient and Transferent Cells

Dihydrofolate reductase in $Mtx^{RIII}Gat^{+}$ (□—□), $Mtx^{S}Gat^{-}$ (○—○), and Mtx^{RIII}tran711C cells grown for 30 days in selective medium (α-special containing 5×10^{-7} M Mtx and 10% DFCS) (△—△) and nonselective medium (α-complete containing 10% FCS) (▲—▲). Reactions were carried out with equivalent amounts of initial activity in cell extracts and titrated with Mtx.

Chromosomal Location of Mtx^{RIII} and Gat^{+} Markers

In our earlier experiments, we showed that CHO chromosomes could be fractionated into three size classes, and that the Mtx^{RIII} marker was located exclusively in the middle size class (Spandidos and Siminovitch, 1977). On the basis of the results described so far, it would thus be expected that the Gat marker would also be found in this size class.

Chromosomes from the Mtx^{RIII} donor were fractionated into three size classes as before (A, large; B, medium; C, small), and each chromosome fraction was tested for its ability to transfer the Mtx^{RIII} and Gat markers. As shown in Table 2, lines 1–3, both the Mtx^{RIII} and Gat markers were found in the middle size class of chromosomes (fraction B). The appropriate controls for colonies in the absence of either chromosomes or recipient cells were again negative (lines 7 and 9).

A further confirmation of this assignment is provided from the data obtained when only the Gat^{+} marker was selected. The results show that the number of colonies found when the B class chromosomes were used (line 5) was much higher than when either the A or C fractions (lines 4 and 6) were tested or when recipient cells were plated in selective medium without previous addition of chromosomes (line 8).

The results of this experiment are again consistent with the linkage of the Mtx^{RIII} and Gat markers.

Co-Transfer of Mtx^{RI} and Gat Markers

One possible explanation for the co-transfer of Gat^{+} with Mtx^{RIII} was that the increased levels of dihydrofolate reductase in the Mtx transferent interfered with the expression of the Gat^{-} phenotype. Although there was no biochemical indication that this was so, as a control we carried out experiments similar to those described in Table 1 using the Mtx^{RI} rather than the Mtx^{RIII} mutant. As indicated earlier, this mutant carries normal levels of dihydrofolate reductase which shows increased resistance to Mtx in vitro. As shown in Table 3, transferents selected for both Mtx resistance and Gat prototrophy were obtained in three separate experiments (line 1), and the numbers were again similar to those obtained when Mtx^{RIII} was selected alone (Spandidos and Siminovitch, 1977). Appropriate controls were negative as before (lines 2 and 3).

Tests for Co-Transfer of Mtx^{RIII} and $GlyA^{+}$ and $GlyB^{+}$ Markers

Our success in showing probable linkage of Mtx^{RIII} and Gat^{+} encouraged us to examine linkage to other genetic loci which might be involved in folate metabolism. Both the GlyA and GlyB mutants origi-

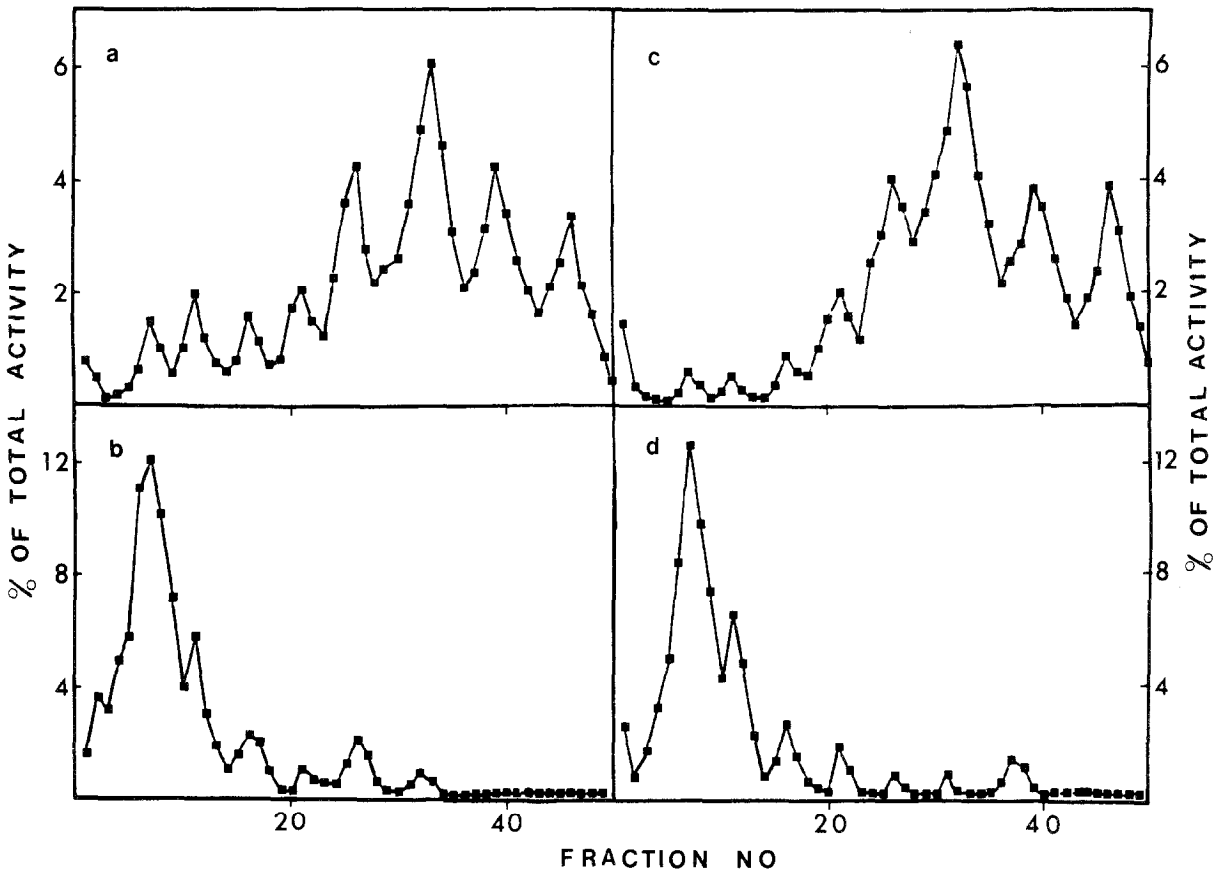

Figure 4. Chromatographic Elution Profile of Intracellular Folates Extracted from Donor, Recipient and Transferent Cells

Folates in (a) $Mtx^{RIII}Gat^{+}$, (b) $Mtx^{S}Gat^{-}$, (c) Mtx^{RIII}tran711C cells grown for 30 days in selective medium (α-special containing 5×10^{-7} M Mtx and 10% DFCS) and (d) nonselective medium (α-complete containing 10% FCS). The data are presented as percentage of total radioactivity per fraction. The extracts in (b, c and d) contained 1, 82 and 1.2%, respectively, of the total radioactivity obtained from either wild-type or $Mtx^{RIII}Gat^{+}$ cells.

nally isolated by Kao and Puck (1968) seemed possible candidates, since the lesion in GlyA has been identified as a serine hydroxymethylase which participates in the conversion of tetrahydrofolate to methylene tetrahydrofolate, and the phenotype of the GlyB mutant is reversed by folinic acid (Kao et al., 1969) (see Figure 1).

Chromosomes were therefore prepared from the Mtx^{RIII} donor cells and transferred separately to GlyA and GlyB cells under two conditions of selection. Two selections were made, one involving both markers at the same time (medium containing methotrexate and minus glycine) and one for glycine prototrophy alone.

As can be seen from Table 4 (lines 1 and 3), both $GlyA^{+}$ and $GlyB^{+}$ can be successfully transferred by metaphase chromosomes. When additional selection was imposed using Mtx, the number of transferents in the GlyA case was reduced to zero (line 2), whereas there was no reduction with GlyB (line 4). No colonies were observed when chromosomes were not added to the recipient cells (lines 5–8) or when the chromosome preparation was plated in the absence of recipient cells (line 9).

These results provide strong evidence that GlyB is syntenic and probably closely linked to Mtx^{RIII} and by inference to Gat, whereas GlyA is not so linked.

A further confirmation of this conclusion was obtained by examining the growth of some of the transferents described in Table 4 in media minus glycine with and without methotrexate. The results are shown in the same table. As expected, transferents isolated in Mtx grew in methotrexate (line 4). Five transferents selected only for GlyB prototrophy were tested and all grew in Mtx (line 3), whereas similarly selected GlyA transferents did not do so (line 1). Thus the chromosome fragment carrying the GlyB marker carries Mtx resistance with it, again indicating close linkage.

Discussion

The fact that the phenotype of $GlyB^{-}$ cells can be

Table 2. Co-Transfer of Mtx^{RIII} and Gat^+ Markers into Recipient CHO Cells Using Fractionated Metaphase CHO Chromosomes

Size Class of Donor Chromosomes	Genotype of Recipient Cells	Selection[a] Medium	Colonies per 10^6 Cells Experiment Number		
			1	2	3
(1)[b] Large (A)	Mtx^SGat^-	α-special + Mtx	0	0	0
(2) Medium (B)	Mtx^SGat^-	α-special + Mtx	7	14	11
(3) Small (C)	Mtx^SGat^-	α-special + Mtx	0	0	1
(4) Large (A)	Mtx^SGat^-	α-special		2	1
(5) Medium (B)	Mtx^SGat^-	α-special		18	21
(6) Small (C)	Mtx^SGat^-	α-special		3	4
(7)[c]	Mtx^SGat^-	α-special + Mtx	0	0	0
(8)	Mtx^SGat^-	α-special		5	3
(9)[d] A + B + C		α-complete	0	0	0

[a, b] (lines 1–6), [c](7 and 8) and [d](9) are as described in the legend to Table 1.

Table 3. Co-Transfer of Mtx^{RI} and Gat^+ Markers by Metaphase Chromosomes in CHO Cells

Donor Chromosome Markers	Genotype of Recipient Cells	Selection[a] Medium	Colonies per 10^6 Cells Experiment Number			Total Colonies per 3×10^6 Cells
			1	2	3	
(1)[b] $Mtx^{RI}Gat^+$	Mtx^SGat^-	α-special + Mtx	7	10	6	23
(2)[c]	Mtx^SGat^-	α-special + Mtx	0	0	0	0
(3)[d] $Mtx^{RI}Gat^+$		α-complete	0	0	0	0

[a] Mtx = 1×10^{-7} M methotrexate.
[b,c,d] are as described in the legend to Table 1.

reversed with folinic acid indicates that this auxotrophic mutant carries a defect in some enzyme involved in folate metabolism. One possible site of the enzymatic defect is at the formylation steps shown in Figure 1. However, other sites in the folate pathway for this defect are not excluded. Thus together with the results found with the Gat marker, the data described in this paper provide strong evidence that genes controlling enzymes in a common pathway in folate metabolism are closely linked. This conclusion is based on the co-transfer of the markers in experiments using metaphase chromosomes, the evidence from other work that only small chromosomal fragments are transferred in such manipulations (Willecke et al., 1976) and, in the case of the Gat^+ and Mtx^{RIII} transfer, the concordant behavior of the phenotypes during stabilization or loss of the chromosomal material.

Our negative results with the $GlyA^-$ mutant are not surprising, since the enzyme involved in this mutation could form part of several other biochemical pathways in the cell.

The close linkage of the Mtx^{RIII}, Gat and GlyB markers cannot, of course, be taken to indicate the presence of an operon. Nevertheless, to our knowledge, it is the first evidence that has been obtained for the possible contiguity of markers governing a common metabolic pathway, and the possible existence of operons, in somatic cells.

It must be recognized, however, that present evidence indicates that the size of the transferred genetic material is at least 5×10^6 nucleotide pairs, enough to code for a very large number of genes. Thus at the moment, the possible contiguity of the markers is just inferred.

As indicated earlier, until this time, studies in eucaryotes on enzymes involved in common metabolic pathways have shown that such enzymes often form part of a single polypeptide unit consisting of two or three enzymatic activities (Brutlag et al., 1969; Creighton, 1970; Veron et al., 1972; Kempe et al., 1976; Paukert et al., 1976). It seems improbable that this is true in our case, since dihydrofolate reductase is a well characterized enzyme of relatively small molecular weight (~23,000 daltons) (Gupta, Flintoff and Siminovitch, 1977), and since we have found that the increase in levels of this enzyme in Mtx^{RIII} cells does not engender any similar alterations in the folylglutamate synthetase (D. A. Spandidos and L. Siminovitch, unpublished results).

The data also illustrate the powerful potential of

Table 4. Transfer of Methotrexate Resistance (Mtx^{RIII}), $GlyA^+$ and $GlyB^+$ Markers by Metaphase Chromosomes in CHO Cells

Donor Chromosome Markers	Genotype of Recipient Cells	Selection[a] Medium	Colonies per 10^6 Cells Experiment Number		Tested for Growth[e] in:	
			1	2	α-Special	α-Special + Mtx
(1)[b] $Mtx^{RIII}GlyA^+GlyB^+$	Mtx^SGlyA^-	α-special	6	7	+	−
(2) $Mtx^{RIII}GlyA^+GlyB^+$	Mtx^SGlyA^-	α-special + Mtx	0	0		
(3) $Mtx^{RIII}GlyA^+GlyB^+$	Mtx^SGlyB^-	α-special	8	4	+	+
(4) $Mtx^{RIII}GlyA^+GlyB^+$	Mtx^SGlyB^-	α-special + Mtx	6	5	+	+
(5)[c]	Mtx^SGlyA^-	α-special	0	0		
(6)	Mtx^SGlyA^-	α-special + Mtx	0	0		
(7)	Mtx^SGlyB^-	α-special	0	0		
(8)	Mtx^SGlyB^-	α-special + Mtx	0	0		
(9)[d] $Mtx^{RIII}GlyA^+GlyB^+$		α-complete	0	0		

[a,b] (lines 1–4), [c](5–8) and [d](9) are as described in the legend to Table 1.
[e] 1×10^5 cells of each transferent clone were plated per 100 mm plate in medium containing dialyzed fetal calf serum at 37°C. After 4 days, the cells were trypsinized and counted. (+) indicates $>1 \times 10^6$ cells per plate and (−) indicates $<2 \times 10^5$ cells per plate. The number of transferents tested for the experiments in lines 1, 3 and 4 were 5, 6 and 4, respectively.

this technology for the analysis of gene organization in somatic cells.

Experimental Procedures

Cell Lines

Three auxorophic recipient CHO cell lines were used. Pro^+Gat^- is a revertant for proline carrying a triple requirement for glycine, adenosine and thymidine. This line was originally called AUX B1 (McBurney and Whitmore, 1974). GlyA and GlyB are cell lines isolated by Kao and Puck (1968) which require glycine for growth. Both these lines were obtained from Dr. L. Chasin.

Metaphase chromosomes were obtained from two different CHO cell lines. Most of the experiments were performed with $Pro^-3Mtx^{RIII}Oua^R3$–5, a line previously described (Flintoff, Spindler and Siminovitch, 1976b) which is auxotrophic for proline and resistant to methotrexate and ouabain.

The second donor line was Pro^-Mtx^{RI}, a line generated in our own laboratory (Flintoff et al., 1976a). Mtx^{RI} carries the first-step methotrexate resistance marker involving an altered dihydrofolate reductase. Since the presence of the Pro and Oua markers did not have any role in the experiments described in this paper, the Pro or Oua designations are not indicated in the text for any of the lines.

Media and Methods

The methods for growing and cloning cells, as well as for isolation and transfer of chromosomes, and for karyotype analysis have been described elsewhere (Thompson and Baker, 1973; Spandidos and Siminovitch, 1977). The assay procedures for dihydrofolate reductase have also been outlined previously (Flintoff, 1976a).

Chromatography of cellular folates was performed as described (McBurney and Whitmore, 1974). Briefly, cells were cultivated for 48 hr in a medium lacking folic acid (FA) and supplemented with ^{3}H-labeled FA (1 μCi/ml, 30 Ci/mmole). The cells were then centrifuged and resuspended in a medium containing 1 μg/ml of unlabeled FA for an additional 24 hr of growth. The cells were centrifuged, washed with PBS and resuspended in freshly prepared 1% w/v sodium ascorbate in 0.005 M phosphate buffer (pH 7.0). This suspension was placed in a boiling water bath for 30 min, cooled on ice and recentrifuged, and the supernatant was subjected to column chromatography.

Chromatography of folates was carried out on a DEAE-cellulose column (0.9 x 5.0 cm). The column was equilibrated with 1% v/v β-mercaptoethanol in 0.005 M phosphate buffer and eluted with a 0.005–0.5 M linear gradient of phosphate buffer (pH 7.0) containing 1% v/v β-mercaptoethanol. Approximately 50 fractions of 1 ml each were collected. Radioactivity in each fraction was determined in a liquid scintillation counter after the addition of 5 ml of Aquasol (Amersham-Searle). The chromatography data was presented as percentage of total radioactivity per fraction. However, the extracts made from Pro^+Gat^- parental cells and the Mtx^{RIII} transferents grown in selective and nonselective medium contained only 1, 82 and 1.2%, respectively, of the folate concentration of an equivalent number of wild-type cells.

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References

Brutlag, D., Atkinson, M. R., Setlow, P. and Kornberg, A. (1969). An active fragment of DNA polymerase produced by proteolytic cleavage. Biochem. Biophys. Res. Commun. *37*, 982–989.

Burch, J. W. and McBride, O. W. (1975). Human gene expression in rodent cells after uptake of isolated metaphase chromosomes. Proc. Nat. Acad. Sci. USA *72*, 1797–1801.

Creighton, T. E. (1970). N-(5′-phosphoribosyl) anthranilate isomerase-indolyl-3-glycerol phosphate synthetase of tryptophan biosynthesis. Relationship between the two activities of the enzyme from Escherichia coli. Biochem. J. *120*, 699–707.

Degnen, G. E., Miller I. L., Eisenstadt, J. M. and Adelberg, E. A.

(1976). Chromosome-mediated gene transfer between closely related strains of cultured mouse cells. Proc. Nat. Acad. Sci. USA *73,* 2838–2842.

Fink, G. R. (1971). Gene clusters and the regulation of biosynthetic pathways in Fungi. In Metabolic Pathways, *5,* third edition, H. J. Vogel, ed. (New York: Academic Press), pp. 199–223.

Flintoff, W. F., Davidson, S. V. and Siminovitch, L. (1976a). Isolation and partial characterization of three methotrexate-resistant phenotypes from Chinese hamster ovary cells. Somatic Cell Genet. *2,* 245–261.

Flintoff, W. F., Spindler, S. M. and Siminovitch, L. (1976b). Genetic characterization of methotrexate-resistant Chinese hamster ovary cells. In Vitro *12,* 749–757.

Gupta, R. S., Flintoff, W. F. and Siminovitch, L. (1977). Purification and properties of dihydrofolate reductase from methotrexate-sensitive and -resistant Chinese hamster ovary cells. Can. J. Biochem. *55,* 445–452.

Kao, F.-T. and Puck, T. T. (1968). Genetics of somatic mammalian cells: VII. Induction and isolation of nutritional mutants in Chinese hamster cells. Proc. Nat. Acad. Sci. USA *60,* 1275–1281.

Kao, F.-T., Chasin, L. and Puck, T. T. (1969). Genetics of somatic mammalian cells: X. complementation analysis of glycine-requiring mutants. Proc. Nat. Acad. Sci. USA *64,* 1284–1291.

Kempe, T. D., Swyryd, E. A., Bruist, M. and Stark, G. R. (1976). Stable mutants of mammalian cells that overproduce the first three enzymes of pyrimidine nucleotide biosynthesis. Cell *9,* 541–550.

McBride, O. W. and Ozer, H. L. (1973). Transfer of genetic information by purified metaphase chromosomes. Proc. Nat. Acad. Sci. USA *70,* 1258–1262.

McBurney, M. W. and Whitmore, G. F. (1974). Isolation and biochemical characterization of folate deficient mutants of Chinese hamster cells. Cell *2,* 173–182.

Paukert, J. L., D'Azi Straus, L. and Rabinowitz, J. C. (1976). Formyl-methenyl-methylenetetrahydrofolate synthetase-(combined). J. Biol. Chem. *251,* 5104–5111.

Spandidos, D. A. and Siminovitch, L. (1977). Transfer of codominant markers by isolated metaphase chromosomes in Chinese hamster ovary cells. Proc. Nat. Acad. Sci. USA, in press.

Taylor, R. T. and Hanna, M. L. (1977). Folate dependent enzymes in cultured Chinese hamster cells: folyl polyglutamate synthetase and its absence in mutants auxotrophic for glycine + adenosine + thymidine. Arch. Biochem. Biophys. *181,* 331–334.

Thompson, L. H. and Baker, L. M. (1973). Isolation of mutants of cultured mammalian cells. In Methods in Cell Biology, *6,* D. M. Prescott, ed. (New York: Academic Press), pp. 209–281.

Veron, M., Falcoz-Kelly, F. and Cohen, G. N. (1972). The threonine-sensitive homoserine dehydrogenase and aspartokinase activities of Escherichia coli K12. The two catalytic activities are carried by two independent regions of the polypeptide chain. Eur. J. Biochem. *28,* 520–527.

Willecke, K. and Ruddle, F. H. (1975). Transfer of the human gene for hypoxanthine-guanine phosphoribosyltransferase via isolated human metaphase chromosomes into mouse L-cells. Proc. Nat. Acad. Sci. USA *72,* 1792–1794.

Willecke, K., Lange, R., Kruger, A. and Reber, T. (1976). Cotransfer of two linked human genes into cultured mouse cells. Proc. Nat. Acad. Sci. USA *73,* 1274–1278.

Wullems, G. J., van der Horst, J. and Boostma, D. (1975). Incorporation of isolated chromosomes and induction of hypoxanthine phosphoribosyltransferase in Chinese hamster cells. Somatic Cell Genet. *1,* 137–152.

Wullems, G. J., van der Horst, J. and Boostma, D. (1976). Transfer of the human X chromosome to human-Chinese hamster cell hybrids via isolated Hela metaphase chromosomes. Somatic Cell Genet. *2,* 359–371.