ACQUISITION OF CHICK CYTOSOL THYMIDINE KINASE ACTIVITY BY THYMIDINE KINASE-DEFICIENT MOUSE FIBROBLAST CELLS AFTER FUSION WITH CHICK ERYTHROCYTES

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ABSTRACT

Chick-mouse heterokaryons were obtained by UV-Sendai virus-induced fusion of chick erythrocytes with thymidine (dT) kinase-deficient mouse fibroblast [LM(TK$^-$)] cells. Autoradiographic studies demonstrated that 1 day after fusion, [^{3}H]dT was incorporated into both red blood cell and LM(TK$^-$) nuclei of 23% of the heterokaryons. Self-fused LM(TK$^-$) cells failed to incorporate [^{3}H]dT into nuclear DNA. 15 clonal lines of chick-mouse somatic cell hybrids [LM(TK$^-$)/CRB] were isolated from the heterokaryons by cultivating them in selective hypoxanthine-aminopterin-thymidine-glycine medium. LM(TK$^-$) and chick erythrocytes exhibited little, if any, cytosol dT kinase activity. In contrast, all 15 LM(TK$^-$)/CRB lines contained levels of cytosol dT kinase activity comparable to that found in chick embryo cells. Disk polyacrylamide gel electrophoresis and isoelectric focusing analyses demonstrated that the LM(TK$^-$)/CRB cells contained chick cytosol, but not mouse cytosol dT kinase. The LM(TK$^-$)/CRB cells also contained mouse mitochondrial, but not chick mitochondrial dT kinase. Hence, the clonal lines were somatic cell hybrids and not LM(TK$^-$) cell revertants. The experiments demonstrate that chick erythrocyte cytosol dT kinase can be activated in heterokaryons and in hybrid cells, most likely as a result of functions supplied by mouse fibroblast cells.

When chick erythrocyte nuclei are introduced into the cytoplasm of tissue culture cells of the same or a different species, they are activated to resume DNA synthesis (Cook, 1970; Harris, 1970; Harris and Cook, 1969). Numerous studies have shown that initiation of DNA synthesis is associated with an enhanced activity of the salvage pathway enzyme, thymidine (dT) kinase, in synchronized cell cultures, regenerating rat liver, compensatory hypertrophy of rat kidney, hormone-stimulated tissues, phytohemagglutinin-stimulated lymphocytes, and in virus-infected cells [see Kit et al., 1974 *b* for review]. It was therefore of interest to learn whether activation of chick erythrocyte dT kinase occurs after chick erythrocyte nuclei are introduced into the cytoplasm of mouse fibroblasts. Chick erythrocytes have little, if any, dT kinase activity. The chick erythrocytes were fused in the presence of UV-inactivated Sendai virus with LM(TK$^-$) cells, a mutant mouse fibroblast line

deficient in dT kinase activity (Kit et al., 1963). The dT kinase activity of the heterokaryons was measured at various times after fusion. In addition, fusion mixtures were cultivated in selective medium containing hypoxanthine, aminopterin, thymidine, and glycine (HATG). 15 clonal lines of chick-mouse somatic cell hybrids were isolated and were capable of growth in HATG medium. The experiments to be described reveal that all 15 clonal lines contain chick, but not mouse, cytosol dT kinase and that all 7 lines investigated for mitochondrial dT kinase contained mouse mitochondrial, but not chick mitochondrial, dT kinase activity.

MATERIALS AND METHODS

Cells

LM(TK$^-$) mouse fibroblasts (Kit et al., 1963) were grown in 8-oz prescription bottles in Eagle's minimal essential medium (APMEM) (Auto Pow, Flow Laboratories, Inc., Rockville, Md.) supplemented with 10% calf serum. The medium also contained 25 μg/ml bromodeoxyuridine, except for the passage immediately preceding an experiment. LM(TK$^-$) cells contain a mitochondrial specific dT kinase but lack the cytosol dT kinase and are unable to grow in HATG medium (Kit et al., 1973 *a*). No revertants of LM(TK$^-$) cells have been observed during selection in HATG medium. The reversion frequency is probably less than 10^{-8}.

Chick Embryos

White leghorn chick embryos (Rich-glo Rabbitry, Houston, Tex.) were used after 8-12 days of incubation. Primary cultures of chick embryo fibroblasts were prepared from 10-day old decapitated embryos. The embryos were minced and trypsinized for 3 h at 23°C in 0.1% trypsin. Cultures were grown in APMEM supplemented with 10% fetal calf serum and 0.1% yeast extract (Difco Laboratories, Detroit, Mich.).

Chick embryo blood was obtained from 12-day old embryos as described by Bolund et al. (1969). Allantoic vessels were cut and allowed to bleed into the allantoic fluid. The allantoic fluid was centrifuged and the buffy coat removed. The packed red blood cells were washed three times in phosphate-buffered saline (PBS) and were resuspended in PBS at a concentration of about 10^7 cells per ml.

Formation of Heterokaryons

Exponentially growing LM(TK$^-$) cells were trypsinized and mixed with chick erythrocytes in a ratio of 1:10. The cells were pelleted and resuspended in UV-irradiated Sendai virus. About 100,000 hemagglutinating units were used for 10^7 LM(TK$^-$) cells. The cells were allowed to agglutinate at 4°C for 10 min and were then shaken at 38°C for 20 min (Dubbs and Kit, 1968). Many of the chick erythrocytes lysed during this process. The UV-irradiated Sendai virus was removed by washing and the cells were resuspended in growth medium. The fusion mixture was then used to seed 8-oz bottles in growth medium so that each culture received the equivalent of $1-3 \times 10^6$ LM(TK$^-$) cells. To select LM(TK$^-$)/CRB somatic cell hybrids, the culture medium was supplemented with HATG to prevent the growth of unfused or self-fused LM(TK$^-$) cells. Surviving colonies were observed in 10-14 days and were first subcultured about 21 days after fusion. Thereafter, the LM(TK$^-$)/CRB lines were subcultured at 5-7-day intervals.

Preparation of Cell Fractions and Analysis of dT Kinase Activity

Trypsinized cells were washed with Hanks' balanced salt solution and with RSB (0.01 M KCl, 0.001 M $MgCl_2$, 0.01 M Tris-HCl buffer, pH 7.4, at 25°C), resuspended in RSB, and allowed to swell for 10 min at 4°C. Thymidine and epsilon aminocaproic acid (EACA), a protease inhibitor, were added to give concentrations of 0.2 mM and 0.05 M, respectively, and the cells were disrupted with a Dounce homogenizer. Cytosol and mitochondrial fractions were prepared and purified as described previously (Kit and Leung, 1974 *a*, *b;* Kit et al., 1974 *c*). Portions of the cytosol and mitochondrial supernatant fluids were analyzed for protein content and for dT kinase activity. For dT kinase assays, [^{3}H]thymidine was utilized as nucleoside acceptor and ATP as phosphate donor (Kit and Leung, 1974 *a*, *b;* Kit et al., 1974 *c*).

Disk PAGE and Isoelectric Focusing Experiments

Disk PAGE analyses in 5% acrylamide gels at pH 8.6 (at 25°C) and isoelectric focusing experiments in polyacrylamide gels were carried out as described previously (Kit and Leung, 1974 *a*, *b;* Kit et al., 1974 *c*). It is to be emphasized that the gels and the upper buffer solution during disk PAGE contained 0.2 mM dT and 2.5 mM ATP. The ampholyte solution used in isoelectric focusing was a mixture of 2/5 ampholyte in the pH range of 10-8 and 3/5 ampholyte in the pH range of 8-5. At the end of the disk PAGE and isoelectric focusing runs, gels were sliced at 4°C with a razor blade and immediately incubated with shaking for 1 or 2 h at 38°C with 150 μl of dT kinase reaction mixture. The reaction was terminated by the addition of 25 μl of 50% (wt/vol) trichloroacetic acid, and 20-μl aliquots were chromatographed on Whatman DE-81 paper to separate the nucleoside acceptor, [^{3}H]dT, from the product, [^{3}H]dTMP. The amount of [^{3}H]dTMP formed in the reaction was determined using a Packard Tri-Carb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.).

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Glycerol Gradient Centrifugation of Enzyme Extracts

The sedimentation coefficient of dT kinase, relative to that of horse liver alcohol dehydrogenase (ADH) (once crystallized, Worthington Biochemical Corp., Freehold, N. J.), was determined by centrifuging enzyme extracts in linear 10–30% (vol/vol) glycerol gradients (Kit and Leung, 1974 *a*, *b*). Buffer solutions for gradient centrifugation contained 2.5 mM ATP and 0.2 mM dT.

RESULTS

dT Kinase Activity of Cytosol and Mitochondrial Extracts from LM(TK⁻), Chick Embryo, and Chick Red Blood Cells

Mutant LM(TK⁻) mouse fibroblast cells contain little, if any, cytosol dT kinase activity (0.02 pmol [^{3}H]dTMP formed from [^{3}H]dT in 20 min at 38°C per microgram protein). Despite the loss of the cytosol dT kinase activity, however, the LM(TK⁻) cells do retain a mitochondrial-specific isozyme of dT kinase, which differs from the cytosol dT kinase of parental LM mouse fibroblast cells in electrophoretic mobility, isoelectric point, sedimentation coefficient, phosphate donor specificity, and pH optimum (Table I) (Kit et al., 1972; 1973 *a;* 1974 *b*). In contrast, primary cultures of chick embryo fibroblasts exhibit a major cytosol dT kinase activity (about two-thirds of the total cellular enzyme) and a mitochondrial-specific isozyme. Cytosol and mitochondrial dT kinase activities are also present in extracts prepared directly from 8-day old embryos (Table I) and in extracts prepared from the chorioallantoic membrane of chick eggs (unpublished experiments).

Extracts prepared from the red blood cells of 12-day old chick embryos and from adult hens exhibited negligible dT kinase activity (about 0.02 and 0.01 pmol [^{3}H]dTMP formed in 20 min at 38°C per microgram protein, respectively). For control purposes, extracts from adult New Zealand rabbit erythrocytes were also analyzed and they exhibited about 0.002 units of enzyme activity.

Cytosol dT Kinase Activity of Heterokaryons of LM(TK⁻) and Chick Red Blood Cells

At various times from 2 to 12 days after fusion, cytosol extracts of the heterokaryon cultures were prepared and assayed for dT kinase activity. In all instances, the dT kinase activities of the cultures containing heterokaryons were not significantly greater than that of control LM(TK⁻) cells or of self-fused LM(TK⁻) cells.

Autoradiographic Studies on Heterokaryons

The fact that a definitive increase in cytosol dT kinase activity was not detected in heterokaryons of chick erythrocytes and LM(TK⁻) cells suggested (*a*) that too few heterokaryons were formed, (*b*) that too few heterokaryons in the culture were activated, or (*c*) that the level of activated chick cytosol dT kinase was too low. Therefore, autoradiographic experiments were carried out to assess dT kinase activity by the incorporation of exogenous [^{3}H]dT into the nuclei of heterokaryons.

Control LM(TK⁻) cells and self-fused LM(TK⁻) cells fail to incorporate [^{3}H]dT into nuclear DNA (Dubbs and Kit, 1964). Incorporation of exogenous [^{3}H]dT into nuclear DNA not only requires an active cytosol dT kinase, but also requires that the cell be synthesizing DNA, i.e. be in the S phase. To assess the percentage of cells in the S phase, the incorporation of [^{3}H]deoxycytidine ([^{3}H]dC) into DNA of LM(TK⁻) cells and heterokaryons was measured by autoradiography. During a 1-h pulse with [^{3}H]dC, about 50% of exponentially growing LM(TK⁻) cells incorporated [^{3}H]dC into nuclear DNA. At 24 h after fusion about 0.5% of the heterokaryons also exhibited labeling with [^{3}H]dC in both the LM(TK⁻) and chick erythrocyte nuclei, indicating that DNA synthesis had been turned on in the chick nuclei. In a small percentage (less than 10%) of heterokaryons, DNA was being synthesized in the nuclei of one species but not the other.

To learn whether dT kinase activity was activated in heterokaryons, incorporation of [^{3}H]dT into nuclear DNA was studied. At 24 h after fusion about 23% of heterokaryons incorporated [^{3}H]dT into the DNA of both LM(TK⁻) and chick erythrocyte nuclei. Only about 6% of heterokaryons displayed [^{3}H]dT labeling in chick or LM(TK⁻) nuclei but not in both. These experiments demonstrated that (*a*) LM(TK⁻) cells can activate DNA synthesis of chick erythrocytes in heterokaryons and (*b*) dT kinase was activated in about 50% of the heterokaryons in the S phase.

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TABLE I

*Cytosol and Mitochondrial Thymidine (dT) Kinase Activities of Chick Embryo Cells, LM(TK⁻) Mouse Fibroblast Cells, and Cell Lines Derived from the Fusion of Chick Erythrocytes with LM(TK⁻) Cells**

Source of enzyme extracts	Subcellular fraction	dT kinase activity per 10^6 cells	Total dT kinase activity per culture	% of total dT kinase activity	dT kinase activity per μg protein
LM(TK⁻)	Cytosol	0.6	14	6.8	0.02
	Mitochondria	8	191	93.2	1.01
Chick embryo fibroblasts (primary culture)	Cytosol	8	74	63.6	0.59
	Mitochondria	4	38	36.4	0.98
Chick embryo (8 days old)	Cytosol	ND‡	ND‡	ND‡	0.37
	Mitochondria	ND‡	ND‡	ND‡	2.38
LM(TK⁻)/CRB Cl 1	Cytosol	27	358	82.1	0.56
	Mitochondria	6	78	17.9	0.70
LM(TK⁻)/CRB Cl 2	Cytosol	18	214	79.9	0.44
	Mitochondria	5	54	20.1	0.66
LM(TK⁻)/CRB Cl 3	Cytosol	6	117	46.5	0.13
	Mitochondria	7	134	53.5	1.06
LM(TK⁻)/CRB Cl 4	Cytosol	13	172	67.0	0.31
	Mitochondria	6	84	33.0	1.06
LM(TK⁻)/CRB Cl 7	Cytosol	ND‡	ND‡	ND‡	0.27
	Mitochondria	ND‡	ND‡	ND‡	0.79
LM(TK⁻)/CRB Cl 9	Cytosol	9	161	66.4	0.23
	Mitochondria	5	70	33.6	0.70
LM(TK⁻)/CRB Cl 10	Cytosol	6	137	58.0	0.20
	Mitochondria	4	99	42.0	0.84

* Activity expressed as picomoles [^{3}H]dT converted to [^{3}H]dTMP in 20 min at 38°C.
‡ Not done.

dT Kinase Activity of Chick-Mouse Somatic Cell Hybrids

Cultivation in HATG medium resulted in the death of control LM(TK⁻) and self-fused LM(TK⁻) cells. However, after cultivation of fusion mixtures of chick erythrocytes and LM(TK⁻) cells in HATG medium, numerous surviving colonies grew out. 15 clones were selected from the survivors and passed several times in HATG medium. Cytosol extracts were prepared from the third to the seventh passages of the clonal lines, designated LM(TK⁻)/CRB. All clonal lines contained levels of dT kinase activity comparable to that of cytosol extracts from chick embryo fibroblasts. Table I shows the distribution of dT kinase activity in the cytosol and mitochondrial fractions of seven of the clonal lines. From 47 to 82% of the total dT kinase activity of the clonal lines was found in the cytosol fraction. The specific activities of the cytosol dT kinases of the clonal lines (picomoles dTMP formed in 20 min at 38°C per microgram protein) varied from 0.13 to 0.56; that is, about 6.5–28 times greater than that of chick red blood cell extracts. The specific activities of the mitochondrial dT kinases of LM (TK⁻)/CRB cells were of the same order of magnitude as that of LM(TK⁻) mitochondria.

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Disk PAGE Analyses of the Cytosol and Mitochondrial dT Kinase Activities of Chick-Mouse Somatic Cell Hybrids

The disk PAGE mobilities relative to the tracking dye (Rm) of mouse cytosol and mitochondrial dT kinases are about 0.30 and 0.80, respectively (Kit et al., 1973 *a*, 1974 *b*). In contrast, the disk PAGE Rm value of chick embryo cytosol dT kinase is about 0.2 (Fig. 1 *f*). Chick embryo mitochondria contain a cytosol-like dT kinase (Rm = 0.2) and, in addition, a mitochondrial-specific isozyme (Rm = 0.6) (Fig. 2 *c*, *e*). Because of their different Rm values, mixtures of chick and mouse cytosol dT kinases and chick and mouse mitochondrial dT kinases can easily be resolved by disk PAGE (Figs. 1 *c* and 2 *d*).

Disk PAGE analyses of cytosol extracts from all of the clonal lines were carried out to learn whether the chick-mouse hybrids contained chick-specific or mouse-specific dT kinase. The disk PAGE patterns of five of the clonal lines are illustrated in Fig. 1. All of the chick-mouse clonal lines contained the chick-specific cytosol dT kinase and none contained the mouse cytosol dT kinase.

Similar experiments were carried out on mitochondrial extracts from seven of the chick-mouse clonal lines (clones 1, 2, 3, 4, 7, 9, and 10). Analyses of clones 7 and 4 are illustrated in Fig. 2 *a* and *b*. The mitochondrial extracts from the clonal lines contained a minor dT kinase activity similar in Rm to the chick cytosol enzyme and a major dT kinase activity with an Rm similar to that of the mouse mitochondrial-specific dT kinase. A dT kinase activity with an Rm similar to that of the chick mitochondrial-specific dT kinase was not detected.

Isoelectric Focusing Experiments

Fig. 3 *a* and *d* show that the isoelectric points of LM mouse cytosol and LM(TK^-) mouse mitochondrial dT kinases are about 9.1 and 5.1,

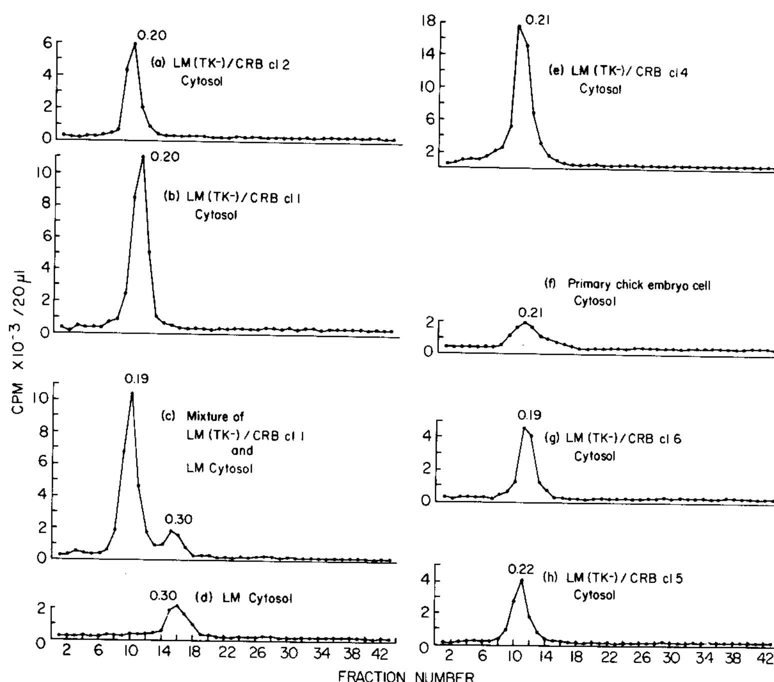

FIGURE 1 Disk PAGE analyses of cytosol dT kinase activities from chick embryo cells, LM mouse fibroblast cells, and from chick-mouse somatic cell hybrids. (*a*) LM(TK^-)/CRB clone 2 (254 μg protein); (*b*)LM(TK^-)/CRB clone 1 (205 μg protein); (*c*) a mixture of cytosol extracts from LM(TK^-)/CRB clone 1 and LM cells (203 and 15 μg protein, respectively); (*d*) LM (15 μg protein); (*e*) LM(TK^-)/CRB clone 4 (296 μg protein); (*f*) primary chick embryo cultures (176 μg protein); (*g*) LM(TK^-)/CRB clone 6 (210 μg protein); and (*h*) LM(TK^-)/CRB clone 5 (216 μg protein). Values above the peaks signify electrophoretic mobilities relative to the tracking dye (Rm).

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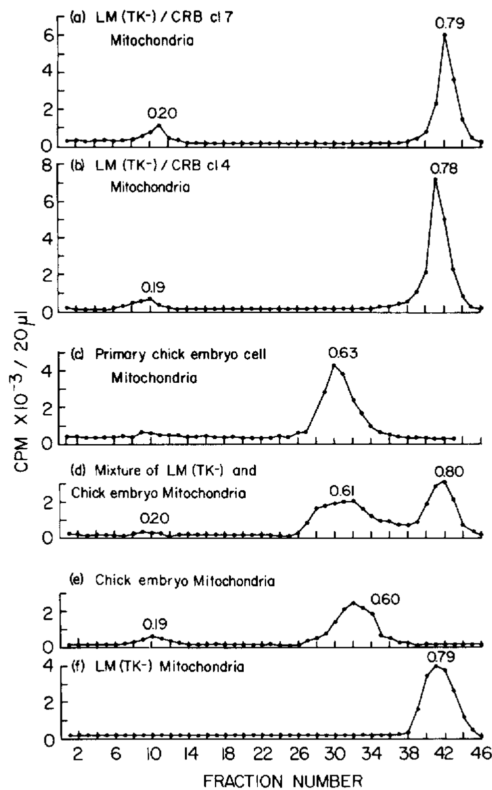

FIGURE 2 Disk PAGE analyses of mitochondrial dT kinases from chick embryo cells, LM(TK⁻) mouse fibroblast cells, and chick-mouse somatic cell hybrids. (*a*) LM(TK⁻)/CRB clone 7 (150 µg protein); (*b*) LM(TK⁻)/CRB clone 4 (156 µg protein); (*c*) primary chick embryo cell cultures (152 µg protein); (*d*) a mixture of mitochondrial extracts from LM(TK⁻) and chick embryos (105 and 117 µg protein, respectively); (*e*) 8-day old chick embryos (147 µg protein); and (*f*) LM (TK⁻) (105 µg protein). Values above the peaks signify electrophoretic mobilities relative to the tracking dye (Rm).

respectively (Kit et al., 1974 *c*). In contrast, the isoelectric points of chick embryo cytosol and mitochondrial dT kinases are about 9.8 and 6.0, respectively (Fig. 3 *c*, *f*). Cytosol extracts from four of the chick-mouse hybrids (clones 3, 4, 9, and 10) and mitochondrial extracts from clone 9 were analyzed by isoelectric focusing. As illustrated by clone 9, the chick-mouse hybrids contained the typical chick cytosol dT kinase and the typical mouse mitochondrial dT kinase (Fig. 3 *b*, *e*).

Glycerol Gradient Centrifugation Experiments

Glycerol gradient centrifugation analyses were carried out to determine the sedimentation coefficients of the cytosol and mitochondrial dT kinases from mouse cells, chick cells, and chick-mouse somatic cell hybrids. The sedimentation coefficients of the dT kinases of the chick-mouse hybrids were in the same range as those of other vertebrate cytosol dT kinases (Kit et al., 1973 *a*, *b;* 1974 *b*, *c*) (Table II). The sedimentation coefficients of mouse and chick mitochondrial dT kinases were smaller than those of mouse and chick cytosol dT kinases. Sedimentation coefficients of human and monkey mitochondrial dT kinases were also smaller than those of human and monkey cytosol dT kinases (Kit et al., 1973 *b;* 1973/1974). Assuming that the enzymes are all globular proteins and that the partial specific volumes are the same as that of horse liver alcohol dehydrogenase, the molecular weights of the dT kinases can be estimated by the method of Martin and Ames (1961). The molecular weights of the cytosol dT kinases of LM, chick embryo, and chick-mouse hybrids were calculated to be 85,000, 77,900, and 82,000, respectively. The estimated molecular weights of LM(TK⁻) and chick embryo mitochondrial dT kinases were about 69,000 (Table II).

DISCUSSION

The experiments that have been presented: (*a*) provide the first characterizations of chick dT kinase isozymes; (*b*) illustrate another example of the association between the initiation of DNA synthesis and the enhancement of cytosol dT kinase activity; and (*c*) show that chick erythrocytes can be switched on for the synthesis of the dT kinase isozyme, which has been referred to by Stafford and Jones (1972) as the fetal form of the enzyme.

Autoradiographic experiments demonstrated that after chick erythrocyte nuclei are introduced into the cytoplasm of LM(TK⁻) cells, exogenous[^{3}H]dT can be incorporated into the DNA of both the LM(TK⁻) nuclei and the chick erythrocyte nuclei. These observations indicate that chick cytosol dT kinase and chick nuclear DNA synthesis had been activated in the heterokaryons. Furthermore, cultivation of the heterokaryons in selective HATG medium permitted the isolation of 15 clonal lines capable of growth in HATG medium. Growth in HATG medium requires the presence of a cytosol dT kinase activity (Kit et al., 1973 *a*, *b*).

The species of origin of the activated cytosol dT kinase was identified by electrophoretic and isoelectric focusing experiments. The latter experi-

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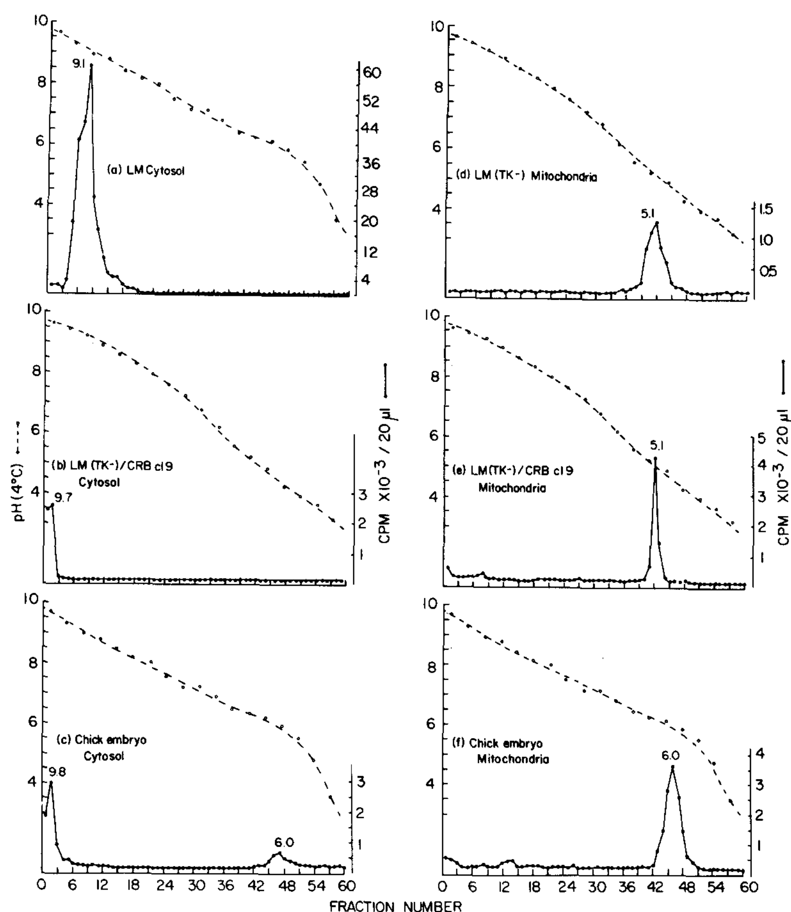

FIGURE 3 Isoelectric focusing analyses of cytosol and mitochondrial dT kinases from LM and LM(TK^-) mouse fibroblasts, chick embryos, and chick-mouse hybrids. (*a*) LM cytosol (160 μg protein); (*b*) LM(TK^-)/CRB clone 9 cytosol (224 μg protein); (*c*) chick embryo cytosol (185 μg protein); (*d*) LM(TK^-) mitochondria (162 μg protein); (*e*) LM(TK^-)/CRB clone 9 mitochondria (100 μg protein); and (*f*) chick embryo mitochondria (117 μg protein). Values above the peaks signify isoelectric points.

TABLE II

Sedimentation Coefficients in 10–30% Glycerol Gradients and Estimated Molecular Weights of dT Kinases from Parental Cells and Chick-Mouse Somatic Cell Hybrids

Source of enzyme	Cell fraction	S*‡	Molecular weights*
LM (mouse)	cytosol	5.19 ± 0.04 (5)	85,000
LM(TK^-) mouse	mitochondria	4.50 ± 0.06 (3)	69,000
Chick embryo	cytosol	4.90 ± 0.07 (11)	77,900
Chick embryo	mitochondria	4.52 ± 0.05 (4)	69,100
Chick-mouse hybrids	cytosol	5.07 ± 0.09 (8)	82,000

* Relative to horse liver alcohol dehydrogenase (S = 5.1 and mol wt = 83,000).

‡ Arithmetic mean ± standard error of the mean. Numbers in parentheses signify number of determinations.

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ments demonstrated that the clonal lines all contained a *chick* cytosol dT kinase, but not a mouse cytosol dT kinase activity. Also, they contained a mouse mitochondrial, but not a chick mitochondrial dT kinase activity. The latter observations lead to the further conclusions that: (*a*) the clonal lines were chick-mouse somatic cell hybrids and not LM(TK$^-$) cell revertants to the dT kinase-positive phenotype; and (*b*) the mutation in LM(TK$^-$) cells is in a structural, and not a regulatory gene for dT kinase.

Experiments in which chick erythrocytes and chick embryo fibroblasts, respectively, have been fused with mouse fibroblast cells deficient in hypoxanthine phosphoribosyl transferase activity have previously been reported by Schwartz et al. (1971) and by Bakay et al. (1973). Also, Boyd and Harris (1973) have fused chick erythrocytes with Chinese hamster cells deficient in hypoxanthine phosphoribosyl transferase activity. It should be emphasized, however, that chick erythrocytes contain appreciable hypoxanthine phosphoribosyl transferase activity before fusion (Cook, 1970; Harris, 1970; Harris and Cook, 1969). This chick enzyme activity is low immediately after fusion of chick erythrocytes with mouse fibroblast cells, but increases markedly subsequent to the development of nucleoli by the chick nuclei. Electrophoretic analyses of the chick erythrocyte-mouse fibroblast hybrids and of the chick erythrocyte-Chinese hamster hybrids revealed that the cells contained a chick-specific hypoxanthine phosphoribosyl transferase (Schwartz et al., 1971; Boyd and Harris, 1973). In contrast, electrophoretic studies of the chick embryo fibroblast-mouse fibroblast "hybrids" indicated that the latter cells contained a mouse-specific, but not a chick enzyme (Bakay et al., 1973). The dT kinase system used in the present study differs from the hypoxanthine phosphoribosyl transferase system in that chick red blood cells contain negligible levels of cytosol dT kinase activity before fusion, and unequivocal detection of enzyme activity occurred only after fusion. Emergence of cytosol dT kinase activity could be demonstrated by autoradiographic methods in the heterokaryons, but significant enzyme activity by direct assay was only demonstrable in the somatic cell hybrids.

The karyotypes of the HAT-resistant "hybrid" clones derived from chick-mouse and chick-Chinese hamster heterokaryons have been examined (Bakay et al., 1973; Boyd and Harris, 1973; Schwartz, et al., 1971). Chick chromosomes were not detected in the hybrid clones; nor could any chick-specific antigens be detected on the surface of the cells. However, Kao (1973) has recently shown that hybrids resulting from the fusion of chick erythrocytes with different adenine-requiring mutants of Chinese hamster cells do retain at least one identifiable chick chromosome. Indeed, Kao (1973) was able to assign two chick genes for the endogenous synthesis of adenine to chick chromosomes C1 and C2, respectively. Karyological analyses of the chick erythrocyte-mouse fibroblast hybrids described in the present study have not as yet been performed. From the studies cited above, however, it may be anticipated that the LM(TK$^-$)/CRB clones contain few, if any, intact chick chromosomes. If karyological analyses should reveal that the LM(TK$^-$)/CRB clones do contain chick chromosomes, the chick determinant for dT kinase can be identified.

After the present study was completed, we learned that Boyd and Harris (1973) had studied the dT kinase activity of HAT-resistant hybrid clones obtained by fusing chick erythrocytes with dT kinase-deficient mouse 3T3(TK$^-$) cells. Their chick erythrocyte-3T3(TK$^-$) clonal lines exhibited mouse dT kinase, but not chick dT kinase activity. Hence, the 3T3(TK$^-$)-chick erythrocyte hybrids differ from the LM(TK$^-$)/CRB hybrids described in the present study. Although it is difficult to compare analyses made in different laboratories, the following points may be pertinent: (*a*) the 3T3(TK$^-$) cells contained appreciable dT kinase activity (possibly eight times greater than the cytosol dT kinase activity of LM(TK$^-$) cells); (*b*) 3T3(TK$^-$)-chick erythrocyte hybrids exhibited only two to three times the dT kinase activity of 3T3(TK$^-$) cells, but LM(TK$^-$)/CRB hybrids contained 6.5- to 28-fold greater cytosol dT kinase activity than LM(TK$^-$) cells; and (*c*) karyological analyses of 3T3(TK$^-$)-chick erythrocyte hybrids failed to reveal any chick chromosomes or chick-specific surface antigens, suggesting that the introduction of foreign genetic material into 3T3(TK$^-$) cells had somehow enhanced the effective residual dT kinase activity of 3T3(TK$^-$) cells (Boyd and Harris, 1973). Also, the extracts from 3T3(TK$^-$) cells and from 3T3(TK$^-$)-chick erythrocyte hybrids were not fractionated into cytosol and mitochondrial dT kinase isozymes, as was done in the present study. Thus, the contribution of 3T3(TK$^-$) mitochondrial dT kinase to the analyses of Boyd and Harris (1973) cannot be assessed.

To explain their findings, Boyd and Harris

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(1973) suggested that a heritable chick factor might have corrected the dT kinase deficiency of 3T3(TK⁻) cells by restoring normal thymidine transport across the cell membrane, thereby increasing the stability of the residual dT kinase activity of the 3T3(TK⁻) cells. Since the restoration of the mouse enzyme enabled the corrected cells to survive in HAT medium, selective pressure to retain the structural genes for chick dT kinase was removed and the superfluous chick genetic determinants were eliminated. In contrast, the cytosol dT kinase activity, required for survival in HATG medium, is very low in LM(TK⁻) cells and does not appear to be reactivatable. Hence selective pressure did exist for retaining the reactivated chick cytosol dT kinase activity in the LM(TK⁻)/CRB hybrids cultivated in HATG medium. It should be emphasized, however, that HATG medium is not required to reactivate chick cytosol dT kinase activity, since autoradiographic experiments demonstrated that $[^3H]$dT is incorporated into chick and mouse cells in heterokaryons incubated in the absence of HATG.

Recent experiments have shown that LM(TK⁻) cells can readily be converted to stable dT kinase-positive clonal lines by infection with UV-irradiated herpes simplex virus and selection in HAT medium (Davidson et al., 1973; Kit et al., 1974 *a*, Munyon et al., 1972). Since the herpes virus-converted LM(TK⁻) cells contain a herpes virus-specific dT kinase, and not a mouse cytosol dT kinase, the transformation experiments attest to the lack of reactivation of LM(TK⁻) cytosol dT kinase and the feasibility of incorporating a foreign dT kinase determinant into the LM(TK⁻) cells.

In the present study, somatic cell hybrids were derived by fusing LM(TK⁻) cells with red blood cells from 12-day old chick embryos. The red blood cell populations contain small numbers of erythroblasts as well as terminally differentiated cells (Attardi et al., 1970). The observation that about 23% of the heterokaryons incorporated exogenous $[^3H]$dT into both LM(TK⁻) and chick nuclei strongly suggests, however, that differentiated chick erythrocytes were among the cells induced to form chick cytosol dT kinase activity. Additional studies have since demonstrated that numerous chick-mouse hybrids containing chick cytosol dT kinase can be isolated after LM(TK⁻) cells and adult hen erythrocytes are cultivated in HATG medium (unpublished experiments).

The failure to detect chick mitochondrial dT kinase in LM(TK⁻)/CRB clonal lines suggests that the chick cytosol, but not the chick mitochondrial, dT kinase was activated. Alternatively, the chick mitochondrial dT kinase determinant may have been activated by the LM(TK⁻) cells, but lost from the hybrid cells. The lack of chick mitochondrial dT kinase activity in the somatic cell hybrids supports the conclusion from studies on human-mouse and monkey-mouse somatic cell hybrids that cytosol and mitochondrial dT kinase genes are determined by different linkage groups (Kit and Leung, 1974 *a*).

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