GENETIC CONTROL OF MITOCHONDRIAL THYMIDINE KINASE IN HUMAN-MOUSE AND MONKEY-MOUSE SOMATIC CELL HYBRIDS

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ABSTRACT
Distinctive thymidine (dT) kinase molecular forms are present in mouse, human, and monkey mitochondria. Disk polyacrylamide gel electrophoresis (disk PAGE) analyses have shown that the mitochondrial-specific dT kinases differ from cytosol dT kinases in relative electrophoretic mobilities (Rm). Furthermore, the mouse mitochondrial dT kinase differs in Rm value from primate mitochondrial dT kinases. The mouse and primate cytosol dT kinases can also be distinguished.

Disk PAGE analyses have been carried out on the cytosol and mitochondrial dT kinases of human-mouse (WIL-8) and monkey-mouse (mK.CV 111) somatic cell hybrids in order to learn whether the mitochondria of the hybrid cells contained murine mitochondrial-specific, primate mitochondrial-specific, or both dT kinases. WIL-8 cells were derived from cytosol dT kinase-negative, mitochondrial dT kinase-positive mouse fibroblasts and from cytosol dT kinase-positive, mitochondrial dT kinase-positive human embryonic lung cells; they contained mostly mouse chromosomes and a few human chromosomes, including the determinant for human cytosol dT kinase. The mK.CV 111 cells were derived from cytosol dT kinase-negative, mitochondrial dT kinase-positive mouse kidney cells and from cytosol dT kinase-positive, mitochondrial dT kinase-positive monkey kidney cells; they contained mostly mouse chromosomes and a few monkey chromosomes, including the determinant for monkey cytosol dT kinase. Disk PAGE analyses demonstrated that the mitochondria of human-mouse and monkey-mouse somatic cell hybrids contained the mouse-specific mitochondrial dT kinase but not the human- or monkey-specific mitochondrial dT kinase. These findings suggest that primate cytosol and mitochondrial thymidine kinase genes are coded on different chromosomes.

INTRODUCTION
Although bromodeoxyuridine (dBU)-resistant human and mouse lines are deficient in cytosol thymidine (dT) kinase activity, the mitochondria of the mutant cells contain relatively normal levels of an organelle-specific dT kinase (Berk and Clayton, 1973; Kit et al., 1973 a-d). The mitochondrial dT kinases of the mutant cells differ from the major cytosol enzymes of parental cells in disk polyacrylamide gel electrophoretic (disk PAGE) mobilities, isoelectric points, ribonucleoside 5'-triphosphate donor specificities, sensitivity to dCTP inhibition, and in sedimentation coefficients. Mitochondria from parental cells contain the distinctive dT kinase activities found in
the mitochondria of mutant cells, but in addition, a cytosol-like dT kinase activity (Kit et al., 1973 a–d).

Using human-mouse and monkey-mouse somatic cell hybrids, we have investigated the question of whether the genetic determinants for the mitochondrial and cytosol dT kinases are genetically linked. The human-mouse and monkey-mouse somatic cell hybrids were selected so as to retain the chromosomal determinants, respectively, of human and monkey cytosol dT kinases. They also contain a substantial complement of mouse chromosomes, but only a few human or monkey chromosomes. In this study, disk PAGE analyses of the dT kinases present in human-mouse and monkey-mouse somatic cell hybrids were carried out. Previous studies had shown that the relative electrophoretic mobilities (Rm) of mouse cytosol, mouse mitochondrial, human or monkey cytosol, and human or monkey mitochondrial dT kinases are distinguishable (Kit et al., 1973 a–c). Thus, it was possible to ascertain whether the mitochondria of the somatic cell hybrids contained the characteristic mouse mitochondrial dT kinase, the primate mitochondrial dT kinase, or both. The data to be presented demonstrate that the distinctive mouse mitochondrial dT kinase, but not the primate mitochondrial dT kinase, was present.

MATERIALS AND METHODS

Cell Lines

The properties of the cell lines used in this study are shown in Table I. The monkey-mouse (mK-CVIII clone 1) somatic hybrid cell line contained a modal chromosome number of 74, including 9.1 small biarmed chromosomes which were definitely of monkey origin and a great majority of acrocentric chromosomes of mouse origin. An average of 1.72 mouse marker chromosomes were present but no monkey marker chromosomes (Kit et al., 1970). The human-mouse (WIL-8) somatic hybrid cell line was obtained through the courtesy of T. B. Shows, Roswell Park Memorial Institute, Buffalo, N. Y. WIL-8 cells possess a modal chromosome number of about 100, with 12–18 human chromosomes including the human thymidine kinase (El7) and the hypoxanthine-guanine phosphoribosyl transferase (X) chromosomes (Shows, 1972).

| Table I |
|---|
| Properties of Cell Lines |

| Cell line | Description | Reference |
|---|---|---|
| mKS-A | SV40-transformed, dT kinase-positive mouse kidney | Dubbs et al., 1967 |
| mKS(BU100) | SV40-transformed, dT kinase-deficient, and dBU-resistant mouse kidney cells derived from mKS-A | Dubbs et al., 1967 |
| LM | dT kinase-positive mouse fibroblasts | Kit et al., 1963 |
| LM(TK−) | dT kinase-deficient, dBU-resistant mouse fibroblasts derived from LM | Kit et al., 1963 |
| WI38 Val3A | dT kinase-positive, SV40-transformed human embryonic lung (WI38) cells | Dubbs and Kit, 1973; Girardi et al., 1965 |
| CV-1 | dT kinase-positive African green monkey kidney | Jensen et al., 1964 |
| WIL-8 | Human-mouse somatic cell hybrid derived from normal human embryonic lung (WI38) and dT kinase-deficient, hypoxanthine-guanine phosphoribosyl transferase-deficient mouse fibroblast (LM(TK−)[PRT−]) cells | Shows, 1972 |
| mK-CVIII, clone 1 | Monkey-mouse somatic hybrid derived from mKS-(BU100) and CV-1 cells | Cassingena et al., 1971; Kit et al., 1970 |
| HeLaS3 | dT kinase-positive human cervical carcinoma cells | Gey et al., 1952 |
Cell lines were grown in 8-ounce prescription bottles in Eagle's minimal essential medium (Auto Pow; Flow Laboratories, Inc., Rockville, Md.) supplemented with 10% calf serum (Kit et al., 1973). The medium for LM(TK- and mK-S (BU100) cells also contained 25 μg/ml dBU, except for the passage immediately preceding an experiment. The medium for the somatic cell hybrids, WIL-8 and mK-CV III, was supplemented with HATG (hypoxanthine-aminopterin-thymidine-glycine) (Littlefield, 1965). Cells were harvested by trypsinization after they reached confluence.

**Preparation of Cell Fractions and Analysis of dT Kinase Activity**

For the preparation of cytosol and mitochondrial fractions, trypsinized cells were washed with Hank's balanced salt solution and with RSB (0.01 M KCl, 0.0015 M MgCl₂, 0.01 M Tris-HCl buffer, pH 7.4 at 25°C), resuspended in RSB buffer and allowed to swell for 10 min at 4°C. Epsilon aminocaproic acid (EACA), a protease inhibitor, was added to a concentration of 0.05 M, and the cells were disrupted with a Dounce homogenizer. Cytosol and mitochondrial cell fractions were prepared as described previously (Kit et al., 1973).

For cytosol preparations, one-ninth volume of 1.5 M KCl, 0.03 M 2-mercaptoethanol and 2 mM dT at 84,000 g for 1 h at 4°C to obtain a high-speed supernatant fraction. Nonidet P-40 was added to the supernatant to a final concentration of 0.5% (vol/vol) (Shell Chemical Co., New York).

The mitochondrial preparations depicted in Figs. 1–4 were purified as described by Kit and co-workers (1973 c). Washed mitochondrial fractions were layered over 4 ml of an 0.8 M sucrose solution containing 0.01 M NaCl, 0.001 M EDTA, and 0.01 M Tris-HCl, pH 7.4 (at 25°C), and centrifuged for 1 h at 84,000 g. The pellet was washed with TENS buffer solution (0.01 M Tris-HCl buffer, pH 7.4 at 25°C; 0.001 M EDTA; 0.01 M NaCl; 0.25 M sucrose) and with enzyme buffer solution (0.15 M KCl; 0.003 M 2-mercaptoethanol; 0.01 M Tris-HCl, pH 8.0 at 25°C), resuspended in enzyme buffer solution containing 0.05 M EACA, 0.2 mM dT, and 0.5% (vol/vol) Nonidet P-40, frozen and thawed twice, sonicated for 3 min at 10 kcycles, and centrifuged at 105,000 g for 1 h at 4°C to obtain a mitochondrial supernatant.

**Disk Polyacrylamide Gel Electrophoresis (Disk PAGE)**

Disk PAGE analyses were carried out using 5% acrylamide gels at pH 8.6 as described previously (Kit et al., 1973 c). At the end of the disk PAGE runs, the gels were sliced in the cold room with a razor blade into 1-mm sections and the slices were immediately incubated with shaking for 1 or 2 h at 38°C in small vials containing 150 μl of dT kinase reaction mixture.

The dT kinase reaction mixture contained the following constituents at the indicated final concentrations: 0.07 M KCl, 1.3 mM 2-mercaptoethanol, 8 mM MgCl₂, 12 mM ATP, 4.8 mM potassium phosphoenolpyruvate, 1.5 μg rabbit muscle pyruvate kinase (150 U/mg) (Boehringer Mannheim Corp., New York), 4.5 μCi [³H]thymidine (50.7 Ci/mmol; New England Nuclear, Boston, Mass.), and 0.1 M Tris-HCl buffer, pH 8.0 at 25°C. The reaction was terminated by the addition of 25 μl of 50% trichloroacetic acid (wt/vol) and 20-μl aliquots were chromatographed on Whatman DE-81 paper to separate the nucleoside acceptor, [³H]dT, from the products, [³H]dTMP. To determine the amount of [³H]dTMP formed in the reaction, the eluted spots were counted.
in a Packard Tri-Carb liquid scintillation spectrometer.

It is to be emphasized that during electrophoresis, the upper buffer solution contained 2.5 mM ATP and 0.2 mM dT to protect dT kinase. When disk PAGE analyses of dT kinase were carried out in the presence of ATP and dT, essentially all of the dT kinase activity applied to the gels was recovered, and the disk PAGE patterns were very consistent. If ATP was omitted from the upper buffer solution, recovery of cytosol enzyme activity was very poor (Kit et al., 1974).

In one of the experiments shown in Fig. 1 b, 2.5 mM UTP was substituted for ATP in the upper buffer solution during electrophoresis. In this experiment, UTP was also substituted for ATP as phosphate donor in the dT kinase reaction mixture.

RESULTS

dT Kinase Activities of Cytosol and Mitochondrial Fractions

Table II shows the relative distribution of dT kinase activities in the cytosol and mitochondrial fractions of the dT kinase-positive WI38 Val3A (human), CV-1 (monkey), and of the human-mouse (WIL-8) and monkey-mouse (mK-CVIII) somatic cell hybrids. About 96–97% of the total cytosol plus mitochondrial dT kinase activity was found in the cytosol fraction of WI38 Val3A and CV-1 cells. Also, about 84–91% of the total cytosol plus mitochondrial dT kinase activity was found in the cytosol fraction of the somatic cell hybrids. The specific activities (picomoles nucleoside phosphorylated per microgram protein) of the cytosol dT kinases were about 2–10 times greater than those of the corresponding mitochondrial fractions. These experiments demonstrate that the human-mouse and monkey-mouse somatic hybrids contained active cytosol dT kinase activities which permit the hybrid cell lines to grow in selective HATG medium (Littlefield, 1965). In contrast, the dBU-resistant LM(TK−) and mKS(BU-100) cells lack cytosol dT kinase activity and fail to grow in HATG medium (Kit et al., 1973 b).

Disk PAGE Analyses of Cytosol and Mitochondrial dT Kinases

The disk PAGE patterns of cytosol and mitochondrial dT kinases from WI38 Val3A cells are shown in Fig. 1; those of cytosol and mitochondrial dT kinases from mKS(BU100), mKS-A, and CV-1 cells are shown in Fig. 2. The numbers above the peaks indicate the electrophoretic mobilities relative to the tracking dye (Rm) of each of the dT kinase molecular forms. Cytosol dT kinase of WI38 Val3A cells had an Rm value of about 0.27. The WI38 Val3A mitochondrial fraction contained two dT kinase molecular forms; the first resembled the cytosol enzyme (Rm = 0.26), and the second migrated with an Rm value of about 0.66. Only the 0.66 Rm dT kinase efficiently utilized UTP in place of ATP as a phosphate donor in the dT kinase reaction (Fig. 1 b) (Kit et al., 1973 c). The ability to utilize UTP, CTP, or GTP in place of ATP is characteristic of the

Table II

| Cell line | Cell fraction | dT kinase activity per 10⁶ cells | % of total cytosol plus mitochondrial dT kinase activity | dT kinase activity per µg protein |
|-----------|---------------|---------------------------------|--------------------------------------------------------|---------------------------------|
| WIL-8     | Cytosol       | 68*                             | 90.5*                                                  | 1.2*                            |
|           | Mitochondria  | 7*                              | 9.5*                                                   | 0.5*                            |
| mK-CVIII  | Cytosol       | 116*                            | 84.3*                                                  | 5.2*                            |
|           | Mitochondria  | 22*                             | 15.7*                                                  | 2.6*                            |
| WI38 Val3A| Cytosol       | 1110†                           | 97.3†                                                  | 23.0†                           |
|           | Mitochondria  | 32†                             | 2.7†                                                   | 2.3†                            |
| CV-1      | Cytosol       | 232†                            | 95.8†                                                  | 4.0†                            |
|           | Mitochondria  | 10†                             | 4.2†                                                   | 0.8†                            |

* Picomoles dTMP formed at 38°C in 20 min.
† Picomoles dUMP formed at 38°C in 10 min.
FIGURE 1 Disk PAGE analyses of dT kinase from (a) SV40-transformed human embryonic lung (WI38 Val3A) cytosol (60 μg protein) and (b) WI38 Val3A mitochondrial (125 μg protein) fractions. (see Materials and Methods.) The upper buffer solution (Fig. 1 a) contained 2.5 mM ATP; ATP was also used as the phosphate donor in the dT kinase reaction mixture for the enzyme assay with each of the gel slices. In the experiments of Fig. 1 b, either 2.5 mM ATP or 2.5 mM UTP was present in the upper buffer solution. When UTP was substituted for ATP in the upper buffer solution, UTP was also substituted for ATP in the dT kinase reaction mixture. For enzyme assays, gel slices were incubated for 1 h at 38°C. Numbers above the peaks indicate Rm values.

FIGURE 2 Disk PAGE analyses of dT kinase from (a) SV40-transformed mouse kidney (mKS(BU100)) cytosol (90 μg protein) and mKS(BU100) mitochondrial (256 μg protein) fractions, (b) SV40-transformed mouse kidney (mKS-A) cytosol (60 μg protein) and mKS-A mitochondrial (290 μg protein) fractions, and (c) African green monkey kidney (CV-1) cytosol (90 μg protein) and CV-1 mitochondrial (150 μg protein) fractions. Incubation time for enzyme assays was 1 h at 38°C for all samples except mKS(BU100) and mKS-A mitochondria (2 h). Numbers above the peaks indicate Rm values.

Genetically distinct mitochondrial isozymes of dT kinase. In contrast, the cytosol dT kinases efficiently utilize only ATP (dATP) as phosphate donors in the dT kinase reaction (Kit et al. 1973 a-c; 1974).

Fig. 2 a shows that the cytosol fraction of mKS(BU100) cells lacks detectable dT kinase activity and that the mitochondrial fraction contains a dT kinase with a disk PAGE Rm of about 0.8. The disk PAGE patterns of dT kinase activity in cytosol and mitochondrial fractions of dT kinase-deficient LM(TK−) mouse fibroblasts are the same as those of mKS(BU100) mouse kidney cells (Fig. 3 a) (Kit et al., 1973 b).

mKS(BU100) cells were isolated after prolonged cultivation of mKS-A cells in dBU-containing media. The disk PAGE patterns of mKS-A cytosol and mitochondrial fractions are shown in Fig. 2 b. mKS-A cytosol dT kinase exhibited an Rm value of about 0.31. mKS-A mitochondria contained two dT kinase molecular forms with Rm values of about 0.8 and 0.4. The disk PAGE patterns of cytosol and mitochondrial fractions of LM mouse fibroblast cells are identical to those of mKS-A cells (Kit et al., 1973 b, d). The LM cell line is the parental dT kinase-positive line from which the mutant LM(TK−) cells were derived (Kit et al., 1963).

The disk PAGE patterns of CV-1 (monkey kidney) cytosol and mitochondrial dT kinases are shown in Fig. 2 c. The CV-1 cytosol dT kinase...
had an Rm of about 0.24. The CV-1 mitochondria contained two dT kinase molecular forms; the first resembled the cytosol dT kinase (Rm = 0.27), and the second migrated more rapidly with an Rm of about 0.60.

The following points may be noted: (a) human and monkey mitochondria contain a distinctive dT kinase molecular form (Rm = 0.6–0.7) which migrates more slowly than the mitochondrial dT kinase of mouse cells (Rm = 0.8); (b) human and monkey cytosol dT kinase (Rm about 0.25) also migrate more slowly than mouse cytosol dT kinase (Rm = 0.31); (c) dT kinase-positive human and monkey mitochondria contain a second dT kinase molecular form resembling the cytosol dT kinase; and (d) the dT kinase-positive mouse mitochondria contain a second dT kinase molecular form which migrates faster than the cytosol dT kinase and may represent a modified cytosol enzyme (Kit et al. 1973 b, d).

**Disk PAGE Analyses of Mixture of Primate and Mouse Mitochondrial dT Kinases**

Fig. 3 a illustrates the disk PAGE patterns of mitochondrial dT kinases from mouse fibroblasts (LM(TK−)) and human embryonic lung (WI38 Val3A) cells and the disk PAGE pattern obtained by mixing the mouse and human mitochondrial extracts. Fig. 4 a shows the disk PAGE patterns of mouse kidney (mKS(BU100)) and monkey kidney (CV-1) mitochondrial dT kinases and the disk PAGE pattern obtained after mixing the mouse and monkey mitochondrial extracts. It may be seen that the characteristic mouse mitochondrial dT kinase peak is clearly resolved from the mitochondrial dT kinase molecular forms of human and monkey cells.

**Disk PAGE Analyses of Mixtures of Human and Mouse Cytosol dT Kinases**

Fig. 5 illustrates the disk PAGE patterns of cytosol dT kinases from mouse fibroblasts (LM) and human carcinoma (HeLaS3) cells and the disk PAGE patterns obtained by mixing the mouse and human cytosol extracts. It may be seen that the characteristic mouse cytosol dT kinase peak is

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**Figure 3** Disk PAGE analyses of dT kinases from (a) WI38 Val3A mitochondria (125 μg protein), LM(TK−) mouse fibroblast mitochondria (180 μg protein) or a mixture of WI38 Val3A LM(TK−) mitochondria, and (b) WIL-8 (human-mouse somatic hybrid) cytosol (238 μg protein) or WIL-8 mitochondrial (214 μg protein) fractions. Incubation time for enzyme assays: 2 h at 38°C. Numbers above the peaks indicate Rm values.

**Figure 4** Disk PAGE analyses of dT kinases from (a) CV-1 mitochondria (204 μg protein), mKS(BU100) mitochondria (204 μg protein), or a mixture of CV-1 and mKS(BU100) mitochondria, and (b) monkey-mouse somatic hybrid (mK-CVT11) cytosol (99 μg protein) and mK-CVT11 mitochondrial (241 μg protein) fractions. Gel slices were incubated for 1 h at 38°C. Numbers above the peaks indicate Rm values.
readily distinguished from the human cytosol dT kinase.

**Disk PAGE Analyses of dT Kinases from Somatic Cell Hybrids**

Disk PAGE patterns of dT kinases from cytosol and mitochondrial fractions of human-mouse and monkey-mouse somatic cell hybrids are shown in Fig. 3 b and Fig. 4 b. As expected, cytosol fractions of the human-mouse (WIL-8) and monkey-mouse (mK-CVIII) cells contained only the primate cytosol enzymes. WIL-8 mitochondria contained a dT kinase activity with an Rm similar to that of the human cytosol enzyme (Rm = 0.21) and also a dT kinase activity with an Rm similar to that of LM(TK−) mitochondrial dT kinase (Rm = 0.70). A dT kinase molecular form with an Rm corresponding to that of the distinctive human mitochondrial dT kinase (Rm = 0.6–0.7) was not detected (Fig. 3 b). The mK-CVIII mitochondria contained a disk PAGE molecular form with an Rm similar to that of the monkey cytosol enzyme (Rm = 0.23) and a second molecular form with an Rm similar to that of the mKS (BU100) mitochondrial enzyme (Rm = 0.79). A disk PAGE molecular form with an Rm resembling the distinctive monkey mitochondrial dT kinase (Rm = 0.6) was not detected.

The finding that mitochondria from the hybrid cells contain a primate cytosol-like dT kinase could signify that mitochondria can package a heterologous cytosol-like enzyme or that the predominant primate cytosol enzyme contaminates the mitochondrial fraction. To investigate the latter possibility, the mitochondria from WIL-8 cells were further purified by banding in a discontinuous sucrose gradient. Fig. 6 shows that WIL-8 mitochondria purified by banding in a discontinuous sucrose gradient still contained two dT kinase molecular forms, the human cytosol-like dT kinase (Rm = 0.27) and the characteristic mouse mitochondrial isozyme (Rm = 0.82) (compare with Fig. 3 b). This experiment adds further assurance that the human cytosol-like dT kinase is intrinsic to WIL-8 mitochondria.
DISCUSSION

Interspecific somatic cell hybrids have been useful for the study of gene linkage, the control of gene expression, and the rescue of infectious virus from nonproducer, transformed cells (see, for example, Davidson, 1971; Kao and Puck, 1970; Kit et al., 1970; Shows, 1972). Recently, hybrid cells have also been used to investigate genetic control of mitochondrial enzymes. Thus, Van Heyningen and co-workers (1973) have demonstrated with human-mouse somatic cell hybrids the nuclear gene specification of two mitochondrial enzymes, NAD-malate dehydrogenase and isocitrate synthase, and the possible linkage between them and with the enzymes, lactic dehydrogenase-B and peptidase B, on human chromosome 12. The genetic independence of the mitochondrial and supernatant forms of malic enzyme and malate dehydrogenase have been confirmed in population and hybrid studies (Cohen and Ornstein, 1972; Van Heyningen et al., 1973). Furthermore, Attardi and Attardi (1972) and Clayton et al. (1971) have shown that human-mouse somatic cell hybrids with a reduced complement of human chromosomes contain mouse mitochondrial DNA but not human mitochondrial DNA.

In the present study, disk PAGE analyses of mitochondrial dT kinases were carried out on human-mouse and monkey-mouse somatic cell hybrids. Mouse mitochondria contain a distinctive dT kinase molecular form that differs in electrophoretic mobility from the enzyme found in the cytosol of parental cells and from mitochondrial-specific human and monkey dT kinases.

The somatic cell hybrids contained the distinctive murine mitochondrial dT kinase, but not the dT kinase molecular form characteristic of human and monkey mitochondria. However, the human-mouse and monkey-mouse somatic hybrids did contain, respectively, the human and monkey cytosol-like dT kinases. Since the somatic hybrids contained mostly mouse chromosomes and a reduced complement of human or monkey chromosomes, the results strongly suggest that mitochondrial and cytosol dT kinase determinants are on different genetic linkage groups. An alternative possibility is that the genetic determinants for the primate mitochondrial dT kinases are present in the hybrids but that their synthesis is repressed. In this connection, however, it is to be noted that the primate cytosol dT kinases are not repressed in the hybrids.

Previous studies (Kit et al., 1973 a-d) and the experiments shown in Figs. 1 b, 2 b, and 2 c demonstrate that some of the predominant cytosol dT kinase of dT kinase-positive cell lines can be packaged by mitochondria. It is therefore interesting that human and monkey cytosol-like enzymes are found in the mitochondrial fraction of the somatic cell hybrids (Figs. 3 b, 4 b, 6). Furthermore, we have observed that mouse and human mitochondria of dT kinase-deficient cells contain the high specific activity virus-specific enzymes induced by herpes simplex and vaccinia viruses (Kit et al., 1974). The predominant localization of the viral-induced dT kinases is also in the cytosol fraction. These findings suggest that mouse mitochondria can package some of the cytosol dT kinase of a heterologous species, provided that the cytosol enzyme is present in excess in the cytosol fraction.

It is difficult to rule out rigorously an alternative hypothesis, namely, that the cytosol-like enzyme is a contaminant of the mitochondrial fraction. It is to be acknowledged that some contamination is likely to occur. However, the following experiments contradict the hypothesis that contamination, alone, accounts for the cytosol-like enzymes in mitochondrial fractions. First, even after extensive washing and careful purification of mitochondria, cytosol-like enzymes are found in mitochondrial extracts (Fig. 6). Second, reconstruction experiments have been performed in which a large excess of HeLaS3 cytosol was deliberately mixed with mitochondria from HeLa(BU25) cells. The HeLa(BU25) mitochondria were then repurified and the HeLa(BU25) mitochondrial dT kinases analyzed by disk PAGE (Kit et al., 1973 a). The results of these experiments demonstrated that the mitochondrial purification procedure removed about 99.2% of the added cytosol enzyme. Only a very small 0.24-Rm HeLaS3 cytosol dT kinase was detected in the HeLa(BU25) mitochondria (6.7% of the total dT kinase recovered from the gel). In contrast, the 0.24-Rm enzyme peak of a HeLaS3 mitochondrial fraction prepared and analyzed on the same day represented 33.2% of the total dT kinase activity recovered from the gel (Kit et al., 1973 a). Third, mitochondrial subfractionation studies indicate that 84% of the cytosol-like dT kinase is present in the matrix mitochondrial fraction, 4% is associated with the inner membrane, and only 12% with outer membrane plus intermembrane space fractions. In contrast, essentially all of the dis-
distinctive 0.6 to 0.7-Rm human mitochondrial dT kinase was detected in the mitochondrial matrix fraction of HeLaS3 and HeLa(BU25) cells (Kit and Leung, 1974). It is therefore plausible to conclude that a cytosol-like dT kinase may be intrinsic to mitochondria of dT kinase-positive cells.

Recent experiments with ethidium bromide and chloramphenicol support the hypothesis that the distinctive mitochondrial-specific dT kinase isozymes (Rm = 0.6 to 0.7 in primates and 0.8 in mice) are determined by nuclear genes, synthesized on cytoplasmic polyribosomes, and subsequently translocated to and packaged in the mitochondria (Kit et al., 1973 d and unpublished experiments). Ethidium bromide and chloramphenicol selectively inhibit mitochondrial nucleic acid and protein syntheses, respectively. Nevertheless, after growth for 3-6 generations in the presence of 0.3 μg/ml ethidium bromide, or after growth for 2-3 generations in the presence of 100 μg/ml chloramphenicol, or after growth for 2-3 generations in the presence of ethidium bromide plus chloramphenicol, the mitochondria of dT kinase-deficient HeLa(BU25) cells contained a higher specific activity 0.6 to 0.7–Rm dT kinase than did the mitochondria of untreated cells. During the growth (4-12 days) in the presence of the drugs, the HeLa(BU25) cells exhibited a net increase in mitochondrial dT kinase comparable to that observed in untreated cells. Moreover, cell fractionation studies revealed that there was little, if any, 0.6-0.7-Rm dT kinase in the cytosol fraction of untreated HeLa(BU25) cells. In contrast, the 0.6 to 0.7-Rm activity was readily detected in the cytosol fraction of ethidium bromide-treated cells (unpublished experiments). The findings are best explained by the hypothesis that the 0.6 to 0.7-Rm dT kinase activity exists only transiently and at very low concentrations in the cytosol fraction before being translocated to and packaged in the mitochondria. By disrupting mitochondrial biogenesis, ethidium bromide treatment might induce a derepression of mitochondrial dT kinase synthesis and an accumulation of the mitochondrial enzyme in the cytosol fraction. It is known that mitochondrial RNA polymerase accumulates in the postribosomal supernatant fraction after specific inhibition of mitochondrial RNA polymerase in Neurospora crassa. Nat. New Biol. 240:195.

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