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Mitotic Segregation of Mitochondrial DNAs in Human Cell Hybrids and Expression of Chloramphenicol Resistance

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Abstract—*The relationship between the chloramphenicol (CAP) -resistant phenotype and the mtDNA genotype was investigated in segregating human, HeLa × HT1080, somatic cell hybrids. The parental mtDNAs were quantitated in heteroplasmic cells by using restriction fragment length polymorphisms (RFLPs) detected in Southern blots. CAP-resistant (R) × CAP-sensitive (S) hybrids selected and grown in CAP for brief periods had as little as 25% CAP-R mtDNA. With prolonged selection, the CAP-R mtDNA increased to 90–95%. Hybrids selected and passaged without CAP either retained both mtDNAs or progressively lost one mtDNA (mitotic segregation). The CAP-resistance phenotype of these hybrids changed abruptly when the proportion of CAP-R mtDNAs fluctuated around approximately 10% (threshold effect). Hybrids with greater than 25% HT1080 mtDNA had an additional characteristic. They cloned better with CAP than without. The cloning efficiency in CAP of hybrids having 90% HT1080 mtDNA was more than fivefold greater than the control.*

INTRODUCTION

The human cell contains several thousand mtDNAs (1, 2). Each 16.6 kilobase (kb) mtDNA codes for 13 polypeptides, 12 coded on the H-strand and involved in oxidative phosphorylation and one coded on the L-strand and of unknown function (3–5). The large copy number and cyloplasmic location of mtDNAs create unique problems for the inheritance of mtDNAs and the coordination of mtDNA and nuclear DNA replication.

Mitotically replicating cells of certain mammalian somatic cell hybrids have been observed to rapidly lose the cytoplasmic CAP-resistance phenotype (6, 7). Since CAP resistance results from a mutation in the large rRNA gene of the mtDNA (8), this "mitotic segregation" probably reflects changes in the proportion of cellular CAP-R and CAP-S mtDNAs. Genetic studies suggest that CAP resistance may only be expressed after a certain "threshold" of CAP-R mtDNAs has been achieved (9). However, previous attempts to understand the kinetics of mtDNA segregation and the nature of the "threshold" effect have been unsuccessful due to the insensitivity of previous assays for cellular mtDNAs (10–12).

In somatic cell hybrids and cybrids between human HeLa and HT1080 cells, it is possible to determine the precise proportion of parental mtDNAs by exploiting naturally occurring RFLPs. Previous studies in this system have revealed that less than 40% of the cellular mtDNA must be inherited from the CAP-R parent for the cell to express CAP resistance (11). Recent advances in the analysis of RFLPs have greatly increased the sensi-

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tivity of these assays. This has made it possible to directly examine the molecular basis of mitotic segregation and mitochondrial gene expression and led to the discovery that HeLa and HT1080 mtDNAs differ in a genetic factor which affects cell growth.

MATERIALS AND METHODS

Cell Lines and Cell Fusion. Somatic cell hybrids were prepared between human HeLa S3 cells and HT1080 cells. Derivatives of HeLa included BU25, a thymidine kinase-deficient variant; 296-1, a CAP-R mutant; and HEB7A, a CAP-R cybrid from the fusion of 296-1 cytoplasts to BU25 cells (en296-1 × BU25). Derivatives of HT1080 included the HT1080C clone; HT1080-6TG1, a hypoxanthine phosphoribosyltransferase-deficient clone; HT102W, a CAP-R mutant; and WER1A, a CAP-R cybrid (enHT102W × HT1080-6TG1 (11, 13).

Cell Culture and Analysis. All cells were cultured in Eagle's minimal essential medium, Earl's salts (MEME, GIBCO Laboratories, Grand Island, New York) supplemented with 10% fetal calf serum or, in certain cases, pretested newborn calf or calf serum. All serum lots were checked for their ability to support maximum cloning efficiency of HT1080C, about 40%. All conditions for culture maintenance, cloning efficiency tests, cell fusions, drug selection and cytogenetics have been described (11, 13). Tests for CAP resistance were performed at 50 μg/ml of the drug.

MtDNA Analysis. Confluent cultures in 150-cm^2 flasks were harvested by mild EDTA treatment, washed once in saline, and the pellets frozen at −80°C. The cell pellets were suspended in HEPES-buffered saline and gently lysed with 0.5% SDS. The suspension was brought to 1 M NaCl, incubated at 4°C overnight, and the chromatin removed by centrifugation (14). The supernantant was brought to 0.5% SDS, digested with 100 μg/ml protease K (Boehringer Mannheim, Indianapolis, Indiana), extracted with phenol, 1:1 phenol–chloroform, and chloroform, and precipitated with ethanol. The pellet was resuspended in TE (10 mM Tris and 1 mM EDTA, pH 8.0), digested 1 h with 100 μg/ml RNAase, extracted and precipitated as before, and resuspended in TE for digestion. DNAs were digested with Hae II (Bethesda Research Laboratories, Rockville, Maryland), the fragments separated on 1% agarose gels, and the DNAs depurinated, denatured, and transferred to cellulose nitrate (15, 16). Filters were probed with nick-translated closed circular HeLa mtDNA (16, 17). Autoradiographs of the filters were made by exposing X-OMAT film in the presence of Dupont Cronex Lighting-Plus intensification screens. Autoradiographs of ^{32}P-labeled filters are roughly linear when screens are used (16).

The mtDNA in each band was determined by densitometric analysis. (E.C. Densitometer and Hewlett-Packard 3309A integrator, E.C. Apparatus Corp., St. Petersburg, Florida) subtracting the background taken at a point below the 1.4- and 1.3-kilobase (kb) bands. The relative number of molecules in each band was calculated by dividing the area under the peak by its molecular weight:

$$N_{8.6} = A_{8.6}/8.6$$

$$N_{2.2+1.9} = (A_{2.2} + A_{1.9})/4.1$$

The percentage of parent mtDNA molecules were calculated from the HT1080-specific 8.6-kb and HeLa-specific 2.2- and 1.9-kb bands by:

$$\% \text{ HT1080 mtDNAs} = N_{8.6}/(N_{8.6} + N_{2.2+1.9}) \times 100$$

and

$$\% \text{ HeLa mtDNAs} = N_{2.2+1.9}/(N_{8.6} + N_{2.2+1.9}) \times 100$$

The accuracy of this mtDNA quantitation method was estimated by analyzing known mixtures of purified HeLa and HT1080 mtDNAs. Thirteen mixtures were examined, each containing 0.1 μg mtDNA. Optimal autoradiographic exposures were quantitated for each. The calculated percentages of the mtDNAs differed from the prepared mixtures with an overall standard deviation of 4.0 (units of %). The minority mtDNA tended to be slightly overestimated in mixtures containing less than 10% of one of the mtDNAs. This was more apparent when the minority mtDNA was from HT1080.

RESULTS

To study the relationship between the CAP-resistance phenotype and the mtDNA genotype, a series of somatic cell hybrids and cybrids were isolated between CAP-R and CAP-S HeLa and HT1080 cells. At various times after fusion, these were examined for the origin of their mtDNAs and their resistance to CAP.

Cybrid and Hybrid Crosses Involving HeLa and HT1080 Cells. In the cybrid crosses, the CAP-R HeLa mitochondria were transferred to HT1080 cells (HEH cybrids, enHEB7A × HT1080C selected in HAT + CAP) and the CAP-R HT1080 mitochondria were transferred to HeLa cells (WEH cybrids, enHT102W × BU25 selected in BrdU + CAP). Both fusions resulted in an initially high frequency of CAP-R cybrid colonies, but by 20 days after fusion there was a marked difference. The HEH cybrids continued to grow and developed into established cybrid lines while the numerous WEH cybrids stopped growing and the vast majority disintegrated. Only about 10% of the WEH colonies subsequently grew into established lines (11).

Hybrid fusions were designed such that all of the hybrid nuclei would be isogenic and differ only in their CAP-R mtDNAs. The HEB7A (CAP-R BU25) × HT1080-6TG1 fusion yielded the TIR hybrids, while the WER1A (CAP-R HT1080-6TG1) × BU25 fusion gave the RIB hybrids. Each cross was divided in two parts, half was inoculated into HAT medium (TIR1 or RIB1) and half into HAT + CAP medium (TIR2 or RIB2). Surprisingly, in both hybrid crosses more colonies appeared in HAT + CAP than in HAT. The colony frequencies in HAT and HAT + CAP for the TIR fusion were 4.5×10^{-5} and 5.7×10^{-5} and for the RIB fusion were 2.3×10^{-5} and 4.2×10^{-5}.

Several HAT-selected hybrids were isolated and passaged in HAT medium. Of these, TIR1M was derived from 31 colonies; RIB1M from 127 colonies; and TIR11, RIB11, and RIB12 were from single colonies.

Chromosome counts confirmed that the HEH cybrids had HT1080 nuclei (46 ± 2 chromosomes) while the WEH cybrids had BU25 nuclei (about 58 ± 3 chromosomes) (18). All hybrids had approximately 100 chromosomes (range 90–114), the sum of the two parents (Table 1). Some chromosome loss was observed after prolonged hybrid passage.

MtDNA Analysis. To study the kinetics of mtDNA segregation, a procedure was developed for quantitating the proportion of parental mtDNAs from small numbers of cells. This was accomplished by combining the Hirt procedure for enriching for circular mtDNAs with the Southern blotting and mtDNA hybridization procedure for detecting small amounts of polymorphic restriction fragments. With these procedures, it was possible to determine the proportion of the parental mtDNAs from a single flask of cells. Hence mtDNA determinations could be made concurrently with cloning efficiency tests. Figure 1 shows one of the autoradiographs used in this analysis. The HT1080C control channel shows the 8.6-kb HT1080-specific band but not the 2.2- and 1.9-kb HeLa-specific bands. HeLa S3 controls gave the reciprocal result. Several hybrids and cybrids with differing proportions of HeLa and HT1080 mtDNAs are shown.

Table 1. CAP Resistance and mtDNA Proportions of Hybrids, Cybrids and Clones[a]

Cell line	Origin	Doublings post-fusion	(CE + CAP)/(CE − CAP)	mtDNA (%)	
				HT1080	HeLa
HeLa S3	parent	—	0.052	0	100
HT1080C	parent	—	0.22	100	0
HEH71	enHEB7A × HT1080	79	1.4	6	94
WEH12	enHT102W × BU25	>25	0.76	91	9
TIR2M	HEB7A × HT1080-6TG	23	NT	62	38
TIR21	HEB7A × HT1080-6TG	29	1.4	45	55
TIR22	HEB7A × HT1080-6TG	24	NT	65	35
RIB2M	WER1A × BU25	19	NT	24	76
TIR1MA	HEB7A × HT1080	84	2.7	73	27
TIR1MB	HEB7A × HT1080	84	1.1	59	41
TIR1MC	HEB7A × HT1080	84	1.1	NT	NT
TIR11A	HEB7A × HT1080	94	2.8	44	56
TIR11B	HEB7A × HT1080	89	1.6	18	82
TIR11C	HEB7A × HT1080	88	2.7	NT	NT
RIB1MA	WER1A × BU25	79	0.87	24	76
RIB1MB	WER1A × BU25	79	0.73	17	83
RIB1MC	WER1A × BU25	79	0.87	NT	NT
RIB11C	WER1A × BU25	88	0.42	19	81
RIB11F	WER1A × BU25	92	0.60	22	78
RIB11G	WER1A × BU25	91	0.60	NT	NT
RIB12A	WER1A × BU25	84	1.3	16	84
RIB12B	WER1A × BU25	84	1.5	70	30
RIB12C	WER1A × BU25	84	1.1	NT	NT

[a]CE = cloning efficiency. NT = not determined. During the 79 doublings prior to its testing, HEH 71 was cloned in the absence of CAP (25.5 doublings). This did not affect its CAP resistance. The exact length of the WEH12 passage in CAP is unknown. The CE of WEH12 (91% HT1080 mtDNA) was not stimulated by CAP. This trait may have been lost when this cell line survived the massive cybrid colony death that occurred shortly after fusion. Mean chromosome counts were HEH7 (46.5 chromosomes), WEH1A (60.6), TIR21 (87), TIR22 (100), TIR1M (113 at 51 doublings and 96 at 74 doublings), TIR1MA (107), TIR1MB (93), TIR11 (112), TIR11A (114), TIR11B (107), TIR11C (95), RIB1MA (99), RIB1MB (99), RIB1MC (85), RIB12A (91), RIB12B (93) and RIB12C (90). For mean parental chromosome numbers, see reference 9.

The proportion of the parental mtDNAs in CAP selected hybrids and cybrids and in the clones of the hybrids from the free segregation experiment are reported in Fig. 1 and Table 1. The time after fusion and the level of CAP resistance of the line [(CE + CAP)/(CE − CAP)] (CE = cloning efficiency) are also given. The WEH1A cybrid (HeLa nucleus) contained 91% CAP-R HT1080 mtDNA and 9% CAP-S HeLa mtDNA. By contrast, the HEH71 cybrid (HT1080 nucleus) contained 94% CAP-R HeLa mtDNA and 4% CAP-S HT1080 mtDNA. Both lines were assayed after prolonged selection and confirm that selection enriches for the CAP-R mtDNA but does not eliminate the CAP-S mtDNAs (11).

Several of the CAP-selected TIR2 and RIB2 hybrids were analyzed shortly after fusion. All of these cell lines were selected and grown in CAP. Those that were tested also cloned in the drug. However, at this early stage, the proportion of parental mtDNAs did not necessarily favor the mtDNA of the CAP-R parent. Only 1/3 of the mtDNAs of hybrids TIR2M and TIR22 (examined about 24 doublings after fusion) were from the CAP-R HeLa parent while only 1/4 of the mtDNAs of the RIB2M hybrid (examined at 19 doublings) were inherited from the CAP-R HT1080 parent. After 29 doublings growth in CAP, the TIR21 hybrid had acquired a slight excess of the CAP-R HeLa mtDNA (55%). These TIR2 and RIB2 results confirm that

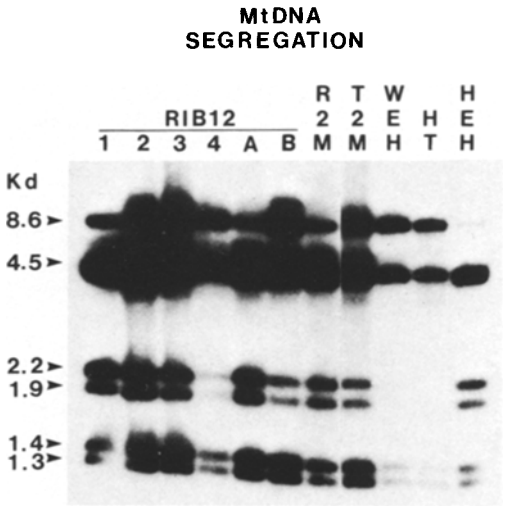

Fig. 1. Representative autoradiograph of Hae II digested hybrid and cybrid mtDNAs. Cell lines are described in the text and Table 1. RIB12 determinations 1–4 were done at 26, 44, 62, and 114 doublings postfusion. A and B are RIB12 clones. R2M = RIB2M, T2M = TIR2M; WEH = WEH1A, HT = HT1080C, and HEH = HEH7.

CAP resistance is expressed in cells with mixed mtDNA populations (6–8, 18) and reveal that less than 25% of the cellular mtDNA must be CAP-R for the cells to grow in the drug.

Segregation of mtDNAs in HeLa-HT1080 Hybrids. Five HT1080-HeLa hybrid cell lines were selected and passaged in HAT. Approximately every 20 doublings, they were tested for their mtDNA content and CAP resistance. All lines were cloned at 60 doublings.

The variations in the hybrid mtDNAs are shown in Fig. 2, while the mtDNAs of the clones isolated at 60 doublings are presented in Table 1. Each cell line was assumed to have started from a hybrid with an equal mixture of HeLa and HT1080 mtDNAs (Fig. 2), although the exact input ratio of parental mtDNAs is unknown.

The direction of mtDNA segregation differed in the various hybrids, indicating that neither mtDNA was preferentially lost. The proportion of HeLa and HT1080 mtDNAs remained relatively constant for the RIB1M and RIB11 hybrids, with the HT1080 mtDNA oscillating between 12 and 25%. For TIR11, the HT1080 mtDNA declined from 47% to 17%, for TIR1M it increased from 71% to 89% and for RIB12 it increased from 20% to 66%.

The direction of mtDNA segregation was unrelated to the mtDNA carrying the CAP-R mutation. In TIR1M and RIB12 hybrids, the CAP-R markers were on different mtDNAs, yet the proportion of HT1080 mtDNAs increased in both.

For those hybrids in which the mtDNA ratios changed, the rate of change was variable. For TIR11, the maximum rate of change was 0.7% per generation, for RIB12 it was 0.5% per generation, and for TIR1M it was between 0.2 and 1.7% per generation.

None of the hybrids were observed to lose all of the mtDNA from either parent. Indeed, three hybrid lines (TIR1M, RIB11, and RIB1M) approached a 90% predominance of one mtDNA, although none went beyond. This asymptotic limit of mtDNA segregation appears to reflect the intracellular mixture of mtDNAs. Clones from all hybrids also had mixtures of mtDNAs, most of them similar to those of the parental lines (Table 1). Further, TIR1M retained only 10% CAP-R mtDNAs, yet all TIR1M cells cloned in CAP (see Fig. 3). Hence all TIR1M cells must have retained some CAP-R HeLa mtDNA.

Segregation of CAP Resistance in HeLa-HT1080 Hybrids. The five TIR1 and RIB1 hybrids were also examined for their CAP resistance during passage (Fig. 3, Table 1). Hybrids RIB1M and TIR11 retained their CAP resistance throughout the experiment, cloning roughly equally well with and without CAP [(CE + CAP)/(CE − CAP) = 1)]. RIB11, on the other hand, had become CAP sensitive at the first determination following fusion (20 doublings) and remained sensitive until 40 doublings. Unexpectedly, by 50 doublings, RIB11 switched back to CAP-R,

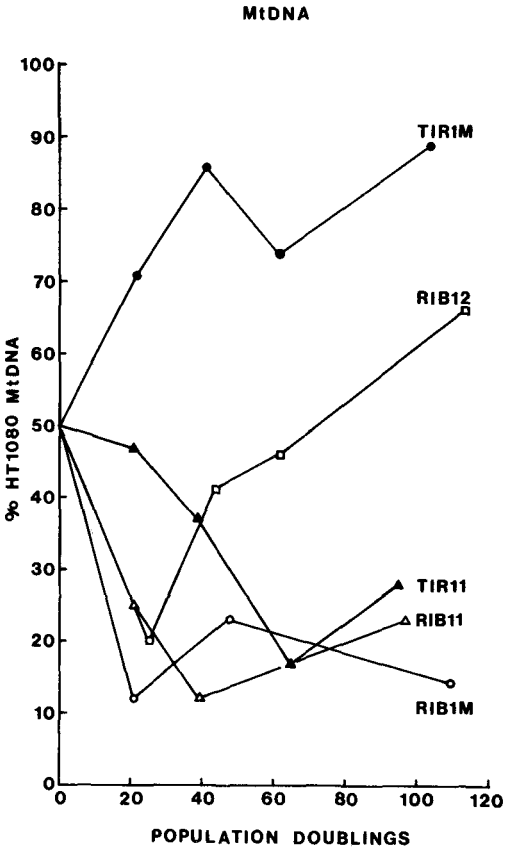

Fig. 2. Mitotic segregation of hybrid HT1080 and HeLa mtDNAs. Percent HT1080 mtDNA is the proportion of the cellular mtDNA derived from the HT1080 parent. The percent HeLa mtDNA is 100 − (% HT1080 mtDNA). Initial hybrids were assumed to have equal proportion of parental mtDNAs. Population doublings were calculated from the initial and final cell numbers at each flask passage.

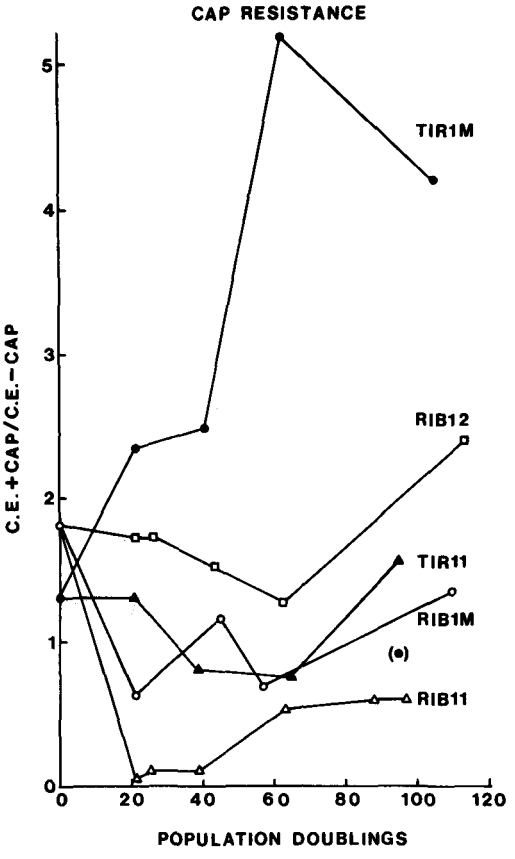

Fig. 3. Mitotic segregation of hybrid CAP resistance. (CE + CAP)/(CE − CAP) is the ratio of cloning efficiencies in HAT + CAP versus HAT medium. One TIR1M point at 97 doublings was inconsistent with other data and is shown in parenthesis. Population doublings were calculated as in Fig. 2.

although the colony size remained small. This abrupt loss and reacquisition of CAP resistance shows that the loss of the CAP-R phenotype is not equivalent to the loss of all of the CAP-R genetic determinants.

Analysis of the CAP resistance of the TIR1M and RIB12 hybrids revealed a startling new phenomenon (Fig. 3, Table 1). Consistently, these hybrids cloned better with CAP than without. This was most dramatically shown for TIR1M were the HAT + CAP cloning efficiency increased progressively to a peak value 5.2-fold above that of the HAT control. Similarly, RIB12 cloned consistently better in HAT + CAP with a maximum stimulation of 2.4-fold. This CAP stimulation of cloning is in marked contrast to our previous studies in which the cloning efficiency in HAT + CAP was always less than or equal to that in HAT (6, 7). Three clones from each hybrid were tested for their CAP resistance and generally found to be similar to each other and to their parental line (Table 1). Differences in CAP resistance and

mtDNA genotype probably reflect divergence which occurred during the 20–30 doublings required for clone isolation.

Relationship of mtDNA Segregation to CAP Resistance. Comparison of the proportion of CAP-R and CAP-S mtDNAs in these hybrids with their resistance to CAP suggests that slightly more than 10% CAP-R mtDNAs are both necessary and sufficient for a cell line to express CAP resistance as defined by the cloning assay. At 104 doublings, the TIR1M hybrid cloned better with CAP than without, yet only 11% of its mtDNA was derived from the CAP-R HeLa parent. Similarly, RIB1M cloned nearly equally well with or without CAP, yet only 12–23% of its mtDNAs were from the CAP-R HT1080 parent.

On the other hand, at 40 doublings RIB11 had about 12% CAP-R mtDNAs, but was unable to clone in CAP. CAP resistance was reexpressed when the CAP-R mtDNAs increased to 22%.

Although the ability of a cell to clone in CAP required a minimum of about 10% CAP-R mtDNA, the ability of a cell line to be stimulated by CAP correlated with the intracellular proportion of HT1080 mtDNA, whether or not this mtDNA carried the CAP-resistance locus. The dramatic rise in CAP stimulation of TIR1M cloning efficiency from 2.4- to 5.2-fold paralleled the progressive enrichment of the CAP-S HT1080 mtDNAs from 71% to 89%. Similarly, the final rise in CAP stimulation of RIB12 to 2.4-fold corresponded to the rise in the CAP-R HT1080 mtDNA to 66% (Figs. 2 and 3). For virtually all of the hybrids (Table 2), stimulations in cloning efficiency greater than two-fold were found in hybrids with more than 50% HT1080 mtDNA. Stimulations between one- and two-fold primarily occurred in hybrids with 25–50% HT1080 mtDNA, while the lack of CAP stimulation or CAP inhibition was found in cells with less than 25% HT1080 mtDNA. Chi-square analysis showed that this correlation (Table 2) was highly significant ($P < 0.001$).

DISCUSSION

CAP Resistance and mtDNA Genotype. Analysis of changes in the mtDNA population of heteroplasmic cells (10, 11, 19, 20) have revealed that the mtDNA proportions can shift during mitotic growth (mitotic segregation). In the present study this drift of parental mtDNAs was gradual and appeared to be random. Such random drift might result from two factors, a slight disproportionate distribution of mtDNAs into daughter cells at each mitosis and the random enrichment of deviant cells during subculturing. Because of the high mtDNA copy number (1, 2), unequal partitioning would be expected to result in only slight differences between cells. However, during weekly subculturing, the cell population was reduced from approximately 3×10^6 cells per flask to 5×10^4 cells per flask. These repeated cell population bottlenecks may have accentuated the genetic drift occurring at the cellular level.

Table 2. Correlation between CAP Stimulation and HT1080 mtDNA in Segregating Hybrids[a]

(CE + CAP)/ (CE − CAP)	HT1080 mtDNA (%)			
	90–51	50–26	25–10	Totals
>2.0	6	1	0	7
1.0–2.0	2	4	4	10
<1.0	0	1	10	11
Totals	8	6	14	28

[a]The chi square for 28 determinations = 21.3. At 4 degrees of freedom, $P < 0.001$.

Changes in the mtDNA genotype were found to correlate only partially with changes in the cellular phenotype (11, 19, 20). CAP resistance was expressed in cells with over 11% CAP-R mtDNA. Yet one cell with an estimated 12% CAP-R HT1080 mtDNA was CAP sensitive, reverting abruptly to CAP-R as the percentage of CAP-R mtDNAs increased to 22%. Since the mtDNA quantitation method used here may have slightly overestimated the proportion of HT1080 mtDNA in this hybrid (see Materials and Methods), the actual proportion of CAP-R HT1080 mtDNAs may have been somewhat less. These results would imply that the CAP-resistance phenotype is expressed in cells with 10% or more CAP-R mtDNAs, but that resistance is abruptly lost when the proportion of CAP-R mtDNAs falls much below this "threshold" value.

The fact that mammalian cells can express CAP resistance when only 11% of their mtDNAs are CAP-R indicates that mtDNA gene expression follows fundamentally different rules from nuclear gene expression. The cellular expression of a small proportion of CAP-R mtDNAs may result from the observation that the CAP-R mtDNA allele can act in *trans* within heteroplasmic cells to permit expression of CAP-S mtDNA genes in the presence of CAP (18). This observation implies that mitochondria fuse, mtDNAs mix, and CAP-R ribosomes translate the available mRNAs. Since, each mtDNA generates 20–60 times more rRNA than mRNA (21) and each mitochondrion contains at least four mtDNAs (18), only a small percentage of CAP-R mtDNAs might be required to provide sufficient CAP-R ribosomes to provide adequate mtDNA gene expression.

A Mitochondrial DNA Factor Affecting Growth? A totally unexpected discovery was that CAP increased the cloning efficiency of hybrids with 25% or more HT1080 mtDNA, whether or not this mtDNA carried the *CAP-R* locus. This phenomenon was not observed in cells with predominantly HeLa mtDNA and suggests that HT1080 mtDNA may be genetically different from HeLa mtDNA.

CAP is known to inhibit mitochondrial protein synthesis (8). Hence, one possibility is that CAP partially inhibits the synthesis of an HT1080 mtDNA gene product which inhibits hybrid growth. By contrast, the comparable HeLa mtDNA gene product must have no such adverse effect. The presence of such an inhibitory or deleterious gene in HT1080 mtDNA might explain the massive death of the enHT102W × BU25 cybrids shortly after their appearance (a phenomenon not seen for the enHEB7A × HT1080C cybrids) and the CAP stimulation of HeLa–HT1080 hybrid formation and cloning. Hybrid formation has also been reported to be stimulated by the elimination of one of the parental mtDNAs using rhodamine-6-G (22).

If the CAP stimulation is the result of a genetic difference between HeLa and HT1080 mtDNAs, it is unclear which gene is responsible. HeLa and HT1080 mtDNAs do differ in a 15,000-dalton mitochondrially synthesized protein which we have assigned to the *URF3* gene (18, 23, 24). However, this polypeptide has been proposed to be a component of respiratory complex I (4). The biochemical basis of the CAP-stimulation phenomenon is also unknown. It could reflect a biochemical incompatibility between the genes of the HT1080 mtDNA and the HeLa cell nucleus or it could result from an HT1080 mtDNA gene product which acts as a negative regulator of cell growth.

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