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Transfer of chloramphenicol-resistant mitochondrial DNA into the chimeric mouse

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Abstract

The mitochondrial DNA (mtDNA) chloramphenicol (CAP)-resistance (CAP^R) mutation has been introduced into the tissues of adult mice via female embryonic stem (ES) cells. The endogenous CAP-sensitive (CAP^S) mtDNAs were eliminated by treatment of the ES cells with the lipophilic dye Rhodamine-6-G (R-6-G). The ES cells were then fused to enucleated cell cytoplasts prepared from the CAP^R mouse cell line 501-1. This procedure converted the ES cell mtDNA from 100% wild-type to 100% mutant. The CAP^R ES cells were then injected into blastocysts and viable chimeric mice were isolated. Molecular testing for the CAP^R mutant mtDNAs revealed that the percentage of mutant mtDNAs varied from zero to approximately 50% in the tissues analyzed. The highest percentage of mutant mtDNA was found in the kidney in three of the chimeric animals tested. These data suggest that, with improved efficiency, it may be possible to transmit exogenous mtDNA mutants through the mouse germ-line.

Keywords

mouse; mitochondria; Rhodamine-6-G; cybrid; chloramphenicol; embryonic stem cell

Introduction

A variety of degenerative diseases affecting the central nervous system (CNS), heart, skeletal muscle, renal and endocrine systems can result from mutations in the mtDNA. While the importance of these diseases has been defined, there are currently no animal model systems available to investigate their pathophysiology or to perfect new therapeutic regimes.

The mammalian mtDNA is a 16 kb circular genome that encodes 13 polypeptides involved in oxidative phosphorylation (OXPHOS) as well as the small (12S) and large (16S) ribosomal RNAs and the 22 transfer RNAs required for mitochondrial protein synthesis. The first pathogenic mtDNA mutations were described in 1988 and the number of reported pathogenic mutations has since rapidly expanded (Wallace et al., 1988a; Wallace, 1992). Although the biochemical defect of several of these reported mutations has been studied in *in vitro* cell systems, there is still no clear understanding of how a systemic defect caused by a mtDNA mutation can result in tissue-specific degenerative disease. Hence animal models are required to understand the pathophysiology of mitochondrial disease.

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Several experimental approaches have been employed for importing defined mtDNA mutations into the mouse. Isolated mitochondria have been microinjected into fertilized ova, but the resulting progeny have lacked any detectable foreign mtDNA (Ebert et al., 1989). When mitochondria were microinjected into zygotes of related species of mice, foreign mitochondria were detected in the cultured embryos up to day 4.5 of embryonic development. However, no viable mice with detectable levels of transferred mtDNA were observed (Pinkert et al., 1997). Mice heteroplasmic for mtDNAs with different naturally occurring polymorphisms have been created by fusion of oocyte cytoplasms as well as transplantation of embryonic karyoplasts and the segregation of the mtDNAs monitored along the maternal lineages (Jenuth et al., 1996, 1997; Meirelles and Smith, 1997, 1998). However, none of these experiments permitted introduction of potentially pathogenic mutations into the adult mouse tissues.

There was one previous report of an attempt to introduce the potentially deleterious CAP^R mtDNA mutant into the mouse. This used the cytoplasmic hybrid (cybrid) transfer technique (Bunn et al., 1974; Wallace et al., 1975) to import the CAP^R marker from B16 melanoma cells into the embryonal teratocarcinoma cell line OTT6050 (Watanabe et al., 1978). The CAP^R B16 cells were enucleated, the cytoplasts fused to the CAP^S OTT6050 cells, and the cybrids selected in CAP. The resulting CAP^R cybrids were then injected into blastocysts and chimeric animals obtained, as confirmed by nuclear genome encoded isoenzyme analysis. However, no evidence was obtained that the CAP^R mtDNAs were introduced into the mouse tissues.

One of the limitations of the cybrid transfer technique is that the resulting cybrid cells acquire a mixture of the resident CAP^S and the transferred CAP^R mtDNAs. Subsequent propagation of these heteroplasmic cells can result in the loss (segregation) of one or the other mitochondrial genomes. Therefore, to remove the resident CAP^S mtDNAs in cybrid fusions, we pretreat the recipient cells with R-6-G prior to fusion with the CAP^R donor cytoplasts. In cultured cell studies, this has resulted in the virtual complete replacement of the CAP^S mtDNAs by the CAP^R mtDNAs (Trounce and Wallace, 1996). The mechanism by which R-6-G inactivates the resident mitochondria is unknown, though R-6-G is a potent inhibitor of mitochondrial respiration. It was first thought that R-6-G inhibited the adenine nucleotide translocator, but other studies have suggested that R-6-G inhibits the ATPase and possibly inhibits the proton transfer of other respiratory complexes (Gear, 1974; Higuti et al., 1980; Mai and Allison, 1983; Wieker et al., 1987; Bullough et al., 1989).

In contrast to the toxic R-6-G, the related compound Rhodamine-123 (R-123) is non-toxic and can be used to stain active mitochondria (Lampidis et al., 1985; Chen, 1988). Both of these dyes have a de-localized positive charge at physiological pH which attracts them to the negatively charged mitochondrial matrix. Furthermore, they are able to freely transverse the outer mitochondrial membrane (Johnson et al., 1980; Chen, 1988; Mannella and Wang, 1989). Hence, they can be used to selectively stain actively respiring mitochondria.

With this background, we have attempted to transfer the CAP^R mtDNAs from cultured mouse cells into adult mouse tissues using R-6-G treated female ES cells. We report here the first successful identification of mice harboring CAP^R mtDNAs.

Materials and methods

Cell lines

The mouse 501–1 cell line was derived from the mouse HPRT-, LA9 cell line by isolation of a cytoplasmic CAP^R mutation (Bunn et al., 1974). The CAP resistance of the 501–1 cell line is due to a mtDNA T-C transition at position 2433 in the 16S ribosomal RNA gene which

creates a new *MaeII* restriction site (Blanc et al., 1981). Using this as a molecular marker, our 501-1 cell line has been found to be homoplasmic (100%) CAP^R mutant. Clone 501-1 was grown in Gibco-BRL DMEM (Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS) (Gibco-BRL) and 50 μg/ml CAP.

The female (40, XX by karyotype) mouse ES cell line AK11.1, derived from 129/SvJae@Sor mice, was provided by Akira Imamoto of the University of Chicago. These ES cells were grown on a mitotically inactivated feeder layer of SNL 76/7 cells, a leukemia inhibitory factor (LIF) producing STO cell line (McMahon and Bradley, 1990), in DMEM media (4.5 g/ml glucose, GIBCO) supplemented with 15% HyClone FBS (Logan, UT), 100 U/ml penicillin, 100 U/ml steptomycin, 2 mM L-glutamine, 100 μM non-essential amino acids, and 100 μM β-mercaptoethanol. When selecting CAP^R ES cells, CAP^R STO feeder cells were used. These were prepared by fusion of the CAP^S STO feeder cells with enucleated 501-1 cell cytoplasts and selection in HAT medium plus 50 μg/ml CAP.

Biochemical analysis of CAP-R cell line 501-1

To determine if the CAP^R mutation in the mtDNA 16S rRNA gene had an effect on mitochondrial OXPHOS, biochemical analysis was performed on 501-1 mitochondria (Trounce et al., 1996). Mitochondria were isolated from 501-1 cells and assayed for Complex I (NADH:ubiquinone oxidoreductase), Complex II+III (succinate:cytochrome c oxidoreductase), Complex III (ubiquinol:cytochrome c oxidoreductase), Complex IV (cytochrome c oxidase) and citrate synthase as previously described (Wallace et al., 1988b; Zheng et al., 1990; Trounce et al., 1996).

Rhodamine-6-G treatments

AK11.1 cells in the log phase of growth on CAP^R feeder cells were transferred to 35 mm, 6-well plates (also with CAP^R feeder cells) at a density of 5×10^5 cells. After a 48-h recovery period, six 6-well plates were treated with R-6-G (250 μg/ml stock in 3% ethanol) for 69 h. Three plates were treated at a concentration of 0.5 μg/ml and three at 0.75 μg/ml R-6-G, all in ES cell media supplemented with 1 mM pyruvate and 50 μg/ml uridine. The media was changed every 24–28 h. The R-6-G treatment was stopped by washing the cells three times with phosphate buffered saline (PBS) and adding ES cell media without R-6-G.

Enucleation and electrofusion

Enucleation of the 501-1 cells was carried out by Ficoll step gradients (Stocco, 1983). Ficoll was prepared as a 50% (w/w) stock solution by adding equal weights of Ficoll 400 (Sigma, St Louis, MO) and water and stirring at 37°C overnight. The resulting solution was filter sterilized and diluted to the appropriate concentration with DMEM media (no serum). Cytochalasin B was added to a final concentration of 20 μg/ml in all Ficoll solutions. Gradients were poured 12–16 h prior to enucleation by layering 25%, 17%, 16%, 15% and 12.5% Ficoll into sterile gradient tubes. Gradients were covered with a sterile cap and incubated at 37°C in a humid atmosphere until used. 4×10^7 trypsinized and washed 501-1 cells were resuspended in 12.5% Ficoll plus 20 μg/ml cytochalasin B and incubated 15 min at 37°C. The cell solution was then layered on the prepared gradients and spun at 77, 000 × *G* for 1 h at 31°C. The resulting cytoplast band was recovered and washed in 10 ml of media to remove any remaining Ficoll. Cytoplasts were then washed with 3 ml of 0.3 M mannitol electrofusion media, pH 7.2.

R-6-G treated ES cells were trypsinized, counted and washed with 0.3 M mannitol electrofusion medium, pH 7.2, 2–3 h after the R-6-G treatment. Aliquots of 2.5×10^6 and 5×10^5 cells R-6-G treated were then mixed with approximately 1×10^7 cytoplasts and fused by electric shock (Trounce et al., 1994). The electrofusion conditions used were: 50 V AC

alignment field for 20 s followed by two 20 μs 800 V DC pulses (ECM200, BTX-Genetronics, San Diego, CA). No post-fusion AC field was used. After a 2 min recovery period, the cells were plated onto 35 mm plates containing CAP^R feeder cells.

Selection of cybrids

After fusion, cells were immediately plated onto 35 mm dishes prepared with CAP^R feeders using ES cell media supplemented with HAT (0.1 mM hypoxanthine, 0.4 μM aminopterin, 16 μM thymidine, 3 μM glycine). After a 24 h recovery period, the media was changed and the ES cells were placed under HAT selection with 50 μg/ml CAP. Media was changed every 24–48 h and clones were picked 7–9 days after fusion.

PCR and restriction analysis of mtDNA

ES cell clones were picked to 96-well feeder plates and expanded. At the first passage, cells were plated onto a second feeder cell plate for freezing as well as on gelatinized 96-well plates to expand the ES cells in the absence of CAP^R feeder cells. After the cells were grown to confluence, total DNA was prepared by overnight digestion with proteinase K followed by phenol extraction and ethanol precipitation. A 600 bp fragment of the mtDNA was amplified by polymerase chain reaction (PCR) with the following primers flanking the CAP^R mutation: (5′-3′) TTAACGGCCGCGGTATCCTG, representing heavy strand nucleotide pair (np) 1999–2018 (forward primer) and TTGTAAGGCTCTATTTC, representing np 2599–2580 (reverse primer). Amplification was achieved using 30 cycles of 94°C denaturation for 15 s followed by annealing at 51°C for 35 s and extension at 72°C for 45 s in a mixture containing 200 ng of genomic DNA, 200 μM dNTPs, and 0.5 U Taq polymerase (Boehringer Mannheim, Indianapolis, IN).

The *MaeII* site resulting from the CAP^R T to C transition at np 2433 was detected by digesting 10 μl of PCR product with 1.5 U of *MaeII* enzyme (Boehringer Mannheim) in the manufacturer's supplied buffer. Fragments were visualized by electrophoresis in a 2.5% agarose gel containing 0.5 μg/ml ethidium bromide. This PCR fragment contains one common *MaeII* site present at position np 2501 in the mtDNA, which can be compared to the presence or absence of the second *MaeII* site created by the CAP^R mutation. Upon *MaeII* digestion of the PCR fragment, the common fragment is 98 np, while the polymorphic fragment is 502 np for wild-type and 434 np plus 68 np for CAP^R mtDNAs.

Southern analysis of mouse tissue DNA

Adult mouse tissues were removed from euthanized animals, frozen in liquid nitrogen and ground to a powder with mortar and pestle. The samples were then digested with proteinase K overnight at 55°C, followed by phenol extraction and ethanol precipitation.

Three micrograms of total DNA was digested with *MaeII* and electrophoresed through a 1.5% agarose gel. The gel was transferred to positively charged nylon membrane (Hybond-N^+, Amersham, Arlington Heights, IL) with 0.4 M NaOH and probed with a 600 np PCR fragment amplified from mtDNA positions 1999–2599. After washing, the blot was visualized by phosphorimager (Molecular Dynamics, Sunnyvale, CA) and exposed to autoradiography film. A diagnostic fragment of 1757 np is observed for wild-type mtDNA and a mutant fragment of 1688 np is observed for CAP^R mutant mtDNAs. The relative levels of the mutant and normal mtDNAs were determined by quantitation of these bands in the Southern blot by phosphorimager in conjunction with the Image-Quant software supplied by the manufacturer.

ES cell Rhodamine-123 staining

Both treated and control ES cells were stained with R-123 to detect active mitochondria. Cells were washed three times with PBS and placed under fresh ES cell media containing 2.5 μg/ml R-123. After incubation at 37°C for 30 min, the cells were washed three times with PBS and destained for 15 min in ES media without R-123. Cells were then trypsinized and washed with PBS and observed under fluorescent microscopy. Images were captured directly to computer disk through the use of a charge-coupled device (CCD) camera and Adobe Photoshop software (San Jose, CA).

Results

The CAP^R mutation is a well defined mtDNA mutation that also imparts a biochemical defect. To establish the nature of this defect, the OXPHOS enzyme activities for Complexes I–IV were determined for mitochondria of the homoplasmic mutant CAP^R 501-1 cell line and compared to those of the parental wild-type CAP^S LA9 cell line. The mitochondrial matrix enzyme citrate synthase was also assayed and the respiratory complex activities were normalized to both total mitochondrial protein and citrate synthase (Wallace et al., 1988b; Zheng et al., 1990; Trounce et al., 1996). The CAP^R mitochondria were found to have a 50% reduction in Complexes I and IV specific activities and a 40% reduction in the Complexes II+III combined specific activities, as compared to wild-type CAP^S mitochondria (Table 1). This biochemical signature is comparable to that seen in human cells which harbor mtDNA protein synthesis mutants. Hence, introduction of the CAP^R mtDNA into the mouse has the potential for approximating the biochemical defects seen in humans.

We then attempted to introduce the 501-1 CAP^R mtDNA into adult mouse tissues. The 501-1 cells were enucleated and the CAP^R cytoplasts fused to AK11.1 female ES cells (Figure 1). CAP^R ES cells were then injected into C57BL/6 (B6) mouse blastocysts.

The fusion of enucleated 501-1 cells to AK11.1 ES cells resulted in the efficient transfer of the CAP^R mtDNAs (Figure 2A). All of the CAP^R ES clones isolated contained mutant mtDNA levels of approximately 50%. No clones were either homoplasmic mutant or wild-type.

Injection of these heteroplasmic CAP^R ES cells into B6 mouse blastocyts gave chimeric mice as assayed by the presence of hair pigmentation. However, none of the tissues examined from these mice had detectable levels of CAP^R mtDNA.

To reduce the level of CAP^S mtDNAs in the recipient ES cells, we pretreated them with R-6-G prior to fusion to the enucleated 501-1 cells. ES cells were treated with R-6-G in media supplemented with pyruvate and uridine to minimize the toxicity associated with respiratory deficiency (Trounce et al., 1996). In this medium, the growth rate of cells treated with 0.75 μg/ml R-6-G was much slower than untreated controls, with a doubling time of 48–50 h versus 18–24 h. Typically, cells double no more than twice during the 69 h treatment time. The morphology of the treated cells was more rounded, though otherwise similar to control cells.

AK11.1 ES cells, treated with 0.75 μg/ml R-6-G for 69 h, had cloning efficiencies of less than 4×10^{-7} after 9 days of culture, with no colonies being observed after plating 2.5×10^6 treated cells. When the ES cells were fused to 501-1 cytoplasts, the R-6-G treated ES cells were rescued and all ES cell cybrid clones genotyped had a high proportion of CAP^R mutant mtDNAs (Figure 2B), even in fusions where CAP selection was not used (data not shown).

To better understand the physiological effect of R-6-G on ES cells, R-123 was used to stain the mitochondria of ES cells prior to R-6-G treatment, after R-6-G treatment but prior to fusion, and after fusion to enucleated 501-1 and cybrid selection (Figure 3). Prior to R-6-G treatment, the ES cells have multiple highly fluorescent and metabolically active mitochondria. Following R-6-G treatment, nearly all active mitochondria were eliminated from the ES cells, as shown by the large reduction in punctate staining. Following fusion with 501-1 cytoplasts, the ES cells were again fully repopulated with active mitochondria.

Fusion of 501-1 cytoplasts with R-6-G treated ES cells resulted in colonies after 7–9 days of post-fusion culture. The cybrid frequency was 1.2×10^{-3} and 6×10^{-4} for fusion of 2.5×10^{6} and 5×10^{5} ES cells to 1×10^{7} cytoplasts, respectively. Forty-eight individual clones were isolated, expanded and genotyped. Forty-six of the forty-eight were homoplasmic for the CAP^{R} mutation (data not shown). Twelve of the fastest growing clones with the best morphologies were expanded and genotyped (Figure 2B). Two of these clones (clones 1 and 3) were heteroplasmic with a low level of wild-type mtDNA.

To prove that the transfer of the CAP^{R} mtDNA also imparted resistance to chloramphenicol, the CAP resistance of one clone was assessed by testing for its ability to grow in increasing concentrations of CAP for 7 days (Figure 4). The growth of the CAP^{S} AK11.1 ES cells was strongly inhibited by 25 μg/ml CAP while the CAP^{R} AK11.1 ES cell cybrids were resistant to drug concentrations up to 150 μg/ml CAP, the same range of resistance seen for the CAP^{R} 501-1 cells. All ES cell cybrids grew well in 50 μg/ml CAP, with the growth of heteroplasmic and homoplasmic clones being comparable.

Blastocysts were then injected with the homoplasmic CAP^{R} ES cell clones 2 and 5 and the heteroplasmic clone 1 shown in Figure 2B. A total of 77 pups were born, 20 of which were chimeric by coat color assessment (12 male and eight female). The chimeras appeared healthy without any overt phenotype.

Of the original chimeric animals, the levels of chimerism observed by coat color was low. The highest was approximately 30% in two of the females with the majority of the mice being between 10% and 20%. One female and two males had chimerism levels of less than 5%. Five representative 8–10 week male mice with 5–20% chimerism were analyzed for the presence of CAP^{R} mtDNA by *MaeII* digestion of PCR amplified mtDNA or mtDNA Southern analysis. The CAP^{R} mutation was detectable in several tissues of three of the five analyzed mice by PCR, but the accuracy of quantitating mouse mtDNA heteroplasmy by PCR has been shown to be potentially inaccurate (Jenuth et al., 1996; Jenuth et al., 1997). To more accurately assess the levels of CAP^{R} mtDNA present in the mouse tissues, further quantitative assessments were made by Southern blot analysis (Figure 5). The ratio of mutant to wild-type mtDNAs was quantitated in the Southern blots by densitometry. The highest percentage of mutant mtDNA was found in the kidneys of the three positive mice, with percentages of mutant ranging from 20 to 50. Detectable levels of mutant mtDNA were found in the hearts of two of the mice with mutant levels of less than 15%. Finally, one sample each of brain and liver also had detectable levels of CAP^{R} mtDNA.

All of the viable female chimeras were bred with B6 males and each was allowed to litter three times. However, none showed genetic evidence that the CAP^{R} ES cells formed the germ cells.

Discussion

We report here the first successful confirmation of introduction of mtDNAs harboring a deleterious mutation into adult mouse tissues via female ES cells. The resulting chimeric animals were found to harbor both mutant and wild-type mtDNAs, with the levels of mutant

mtDNAs being highest in the kidney. While heteroplasmic mice harboring naturally occurring polymorphic mtDNAs have been constructed by embryo fusion technologies or the use of microsurgical procedures (Jenuth et al., 1996, 1997; Meirelles and Smith, 1997, 1998), and mitochondria from *M. spretus* have been microinjected into the fertilized ova of *M. m. domesticus* (Pinkert et al., 1997), no mtDNA mutants which alter mitochondrial function have been introduced into the mouse. Therefore, the current introduction of CAP^R mitochondria into mouse tissues is the first report of the successful transfer of a potentially pathogenic mtDNA mutation into the adult mouse.

Unfortunately, we were not able to achieve germ-line transmission of the CAP^R mutation with any of the eight females that resulted from blastocyst injection of the CAP^R ES cells. There are at least three possible reasons for this lack of success: the CAP^R biochemical defect may be too severe, the CAP^R ES cell sublines derived from the AK11.1 female ES cell line may have been developmentally impaired, or the R-6-G may have been too toxic. The biochemical defect of the CAP^R mutant might be a problem since the percentage of chimerism seen in mice derived from the CAP^R ES cells was low. Possibly, the inhibition of growth of the CAP^R ES cells in the blastocyst limited contribution of the ES cells to the chimeric animals. On the other hand, control injections with untreated AK11.1 also yielded mice with low levels of chimerism, giving only two out of 10 mice with higher percentages of chimerism than found in the mice from CAP^R ES cells. Alternatively, the AK11.1 ES cells might be developmentally impaired. Control injections using the AK11.1 ES cells have yet to yield a germ-line transmitting female mouse. Finally, the R-6-G treatment might have impaired the developmental potential of the AK11.1 ES cells. This possibility seems unlikely since the R-6-G treatment has not been found to impair function in cultured cell studies and the treated cells do not exhibit any gross morphological changes.

In conclusion, we have successfully introduced a deleterious mtDNA mutation into the tissues of adult mice. This protocol offers great promise relative to other methods for creating mouse models of mitochondrial disease since a variety of deleterious mutations have been isolated in cultured cells and can now be transferred directly into the mouse through ES cells. With the development of female ES cell lines that can provide high percentage chimerism, it is likely that this method will ultimately result in the germ-line transmission of a variety of pathogenic mtDNA mutations in the mouse. Development of mouse model systems of mitochondrial dysfunction will permit much more detailed studies of the pathophysiology of mitochondrial disease as well as provide model systems for analyzing the transmission and therapeutics of mtDNA disease.

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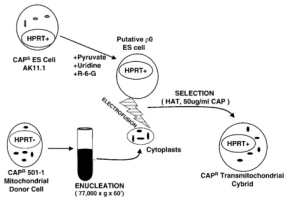

Figure 1.
Schematic representation of the protocol for generating CAP^R AK11.1 ES cells.

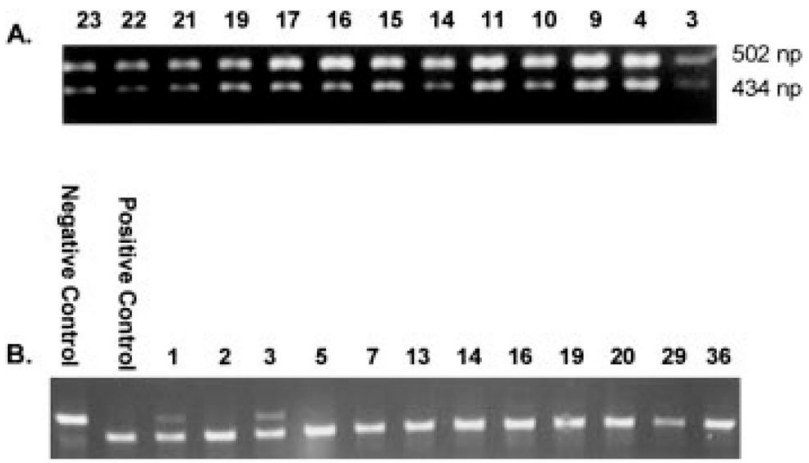

Figure 2.

PCR genotyping of CAP^R ES cell clones. Lane numbers indicate the clone analyzed. Panel 2A: The PCR genotype of 13 ES cell clones derived from the fusion of AK11.1 to enucleated 501-1 without the use of the R-6-G protocol. Panel 2B: The PCR genotype of 12 of the 48 CAP^R ES clones that were isolated using the R-6-G treatment protocol. Homoplasmic ES cell clones 2 and 5 and heteroplasmic ES cell clone 1 were used in blastocyst injections. The controls are PCR amplifications from the wild-type cell line LA9 (negative control) and the CAP^R cell line 501-1 (positive control).

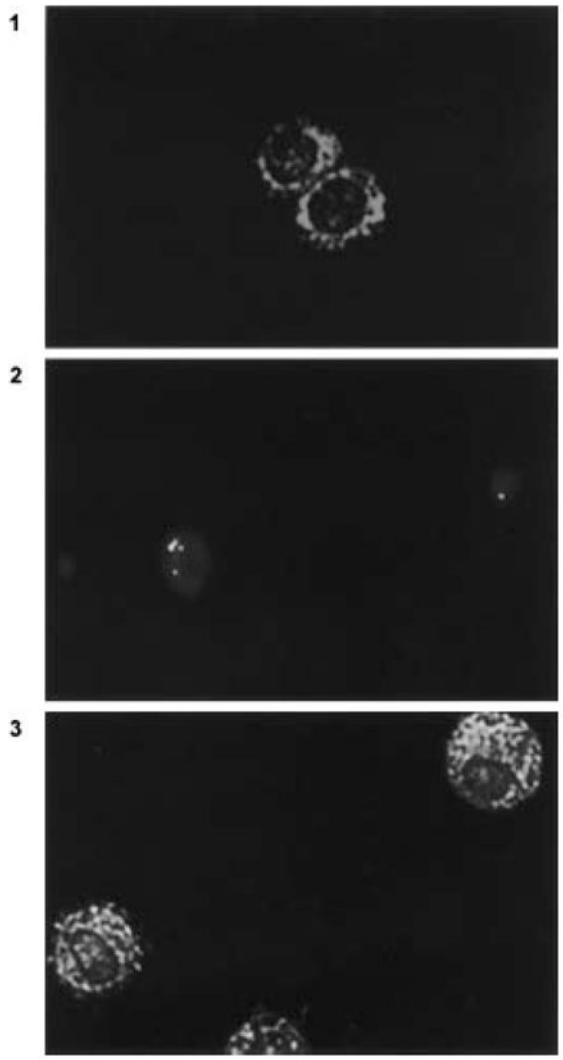

Figure 3.
Mitochondrial changes in AK11.1 ES cells during the cybrid fusion protocol as revealed by R-123 staining. Panel 1: Staining prior to R-6-G treatment. Panel 2: Staining after 69 h treatment with 0.75 μg/ml R-6-G. Panel 3: Staining after fusion of R-6-G treated ES cells with enucleated 501-1 and cybrid isolation. All images were taken by CCD camera under 400 × magnification.

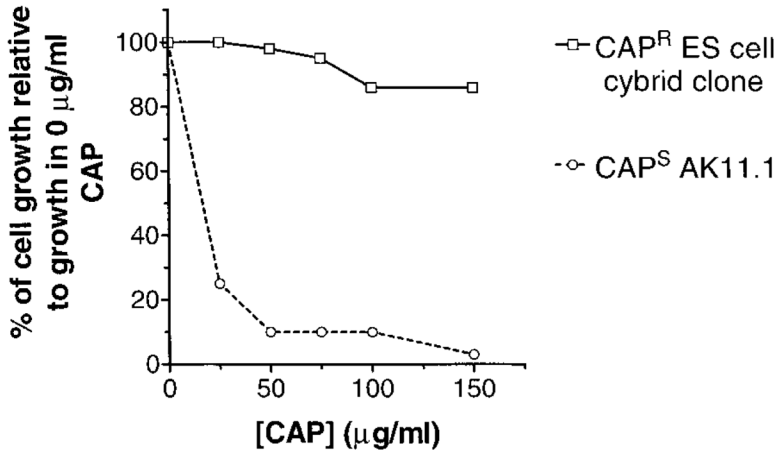

Figure 4.

CAP toxicity on $CAP^S$and CAP^R ES cells as assessed by single point growth curve. 1×10^5 cells were plated into replicate 35 mm wells containing CAP^R feeder cells. The ES cells were cultured for 7 days at CAP concentrations of 0, 25, 50, 75, 100, and 150 μg/ml. The cells were then removed and counted. Results are graphed as the concentration of CAP versus the percentage of cell growth relative to growth without CAP selection.

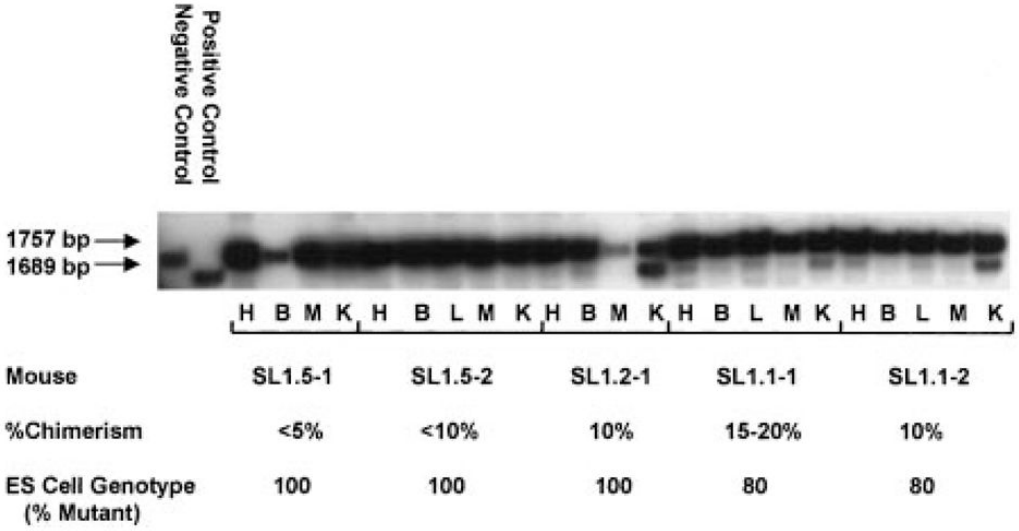

Figure 5.

Molecular evidence for introduction of CAP^R mtDNA into adult mouse tissues through CAP^R ES cells by Southern blot. The gel shows the diagnostic CAP^S (1757 np) and CAP^R (1689 np) mtDNA bands detailed by Southern blot of mouse tissues. The controls are DNA from wild-type LA9 cells (negative control) and CAP^R 501-1 cells (positive control). H = heart, B = brain, M = skeletal muscle, L = liver, and K = kidney.

Table 1

OXPHOS enzymology of CAP^S and CAP^R cell mitochondria. Normalized values are specific activities/citrate synthase.

			Normalized values	
Oxidative phosphor. complex	**CAP^R cells (501-1)**	**CAP^S cells (LA9)**	**501-1**	**LA9**
I	17 ± 4	28 ± 9	0.04	0.10
II+III	356 ± 54	440 ± 34	0.87	1.59
III	673 ± 86	754 ± 51	1.66	2.75
IV	795 ± 193	1196 ± 190	1.89	4.27
Cit. Syntase	432 ± 142	282 ± 56		