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Partial outlet obstruction of the rabbit bladder results in changes in the mitochondrial genetic system

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Abstract

In the rabbit, partial outlet obstruction of the urinary bladder results in significant changes in the physiology, cellular structure, and cellular metabolism of that organ. One of the most striking changes observed is a 50% decrease in oxidative metabolism. Here we investigate whether the function of the mitochondrial (mt) genetic system is altered in rabbit bladder tissue following partial outlet obstruction. Southern analyses of total DNA prepared from bladder tissue excised as a function of time after initiation of partial outlet obstruction showed that the relative number of copies of the mt genome decreases as much as 10-fold during the first 7 d after obstruction, and that this attenuated mt genome copy number is maintained until at least 14 d post-obstruction. Northern analyses, in contrast, showed that mt *COII* and cytochrome *b* transcript levels initially decrease but recover to control levels by about 5 d after obstruction; that level is maintained through 14 d post-obstruction. Enzymatic analysis of cytochrome oxidase and NADH cytochrome *c* reductase activities in obstructed bladder tissue gave results which paralleled the pattern in the mt RNA analyses. Surprisingly, transcript levels for the mt-related nuclear *COIV* gene rapidly decreased to about 50% of control levels following obstruction and remained there until 14 d post-obstruction. These results indicate that partial outlet obstruction of the rabbit bladder leads to significant changes in the status and expression of the mt genetic system in bladder tissue. The maintenance of mt transcription under such circumstances may be an attempt to keep mt protein products at control levels, and this transcriptional adjustment may be responsible in part for the observed maintenance of bladder function during the initial (compensated) period which follows partial outlet obstruction. (Mol Cell Biochem **141:** 47–55, 1994)

Key words: bladder, outlet obstruction, mitochondria, genetic function, transcription

Introduction

Partial outlet obstruction of the bladder secondary to benign prostatic hyperplasia is known to be associated with an increase in bladder mass, an alteration in bladder capacity, a severely decreased level of compliance, and significantly decreased *in vitro* responses to field stimulation and pharmacological stimulation [1–3]. Animal models have been useful in defining and characterizing physiological and pharmacological responses of the bladder to partial outlet obstruction, and the rabbit has been widely studied in this respect [1–8]. The temporal response of the rabbit bladder to partial outlet obstruction is initiated by acute overdistention of the organ, followed by a rapid increase in bladder mass, which has been shown to be correlated with a marked decrease in the ability of the *in vitro* bladder to empty [1, 2]. Bladder mass and func-

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tion stablilize at about 14 d after the initiation of outlet obstruction in the rabbit model, and the increase in mass and associated dysfunction stabilize for a prolonged, variable period of time termed the period of compensated bladder function [1, 2]. Following this stable period of function is a period of progressive destabilization characterized by further increase in bladder mass, replacement of smooth muscle with connective tissue, and a progressive decrease in the ability of the bladder to generate pressure and empty; this has been termed the period of decompensated bladder function [1, 2]. Reversal of the obstruction up to the point of decompensation results in rapid decrease in mass and return of bladder function to normal, or near normal, levels [9, 10].

Although many studies in both humans and animal models have been concerned with the physiological and pharmacological responses of the bladder and urethra to outlet obstruction, relatively few detailed investigations have been made of the biochemical and molecular biological mechanisms which form the basis for these major functional changes. One study, however, has shown that lactic acid formation is increased, and CO_2 production decreased, in rabbit bladder tissue following partial outlet obstruction [11]. This same group has demonstrated a 50% decrease in activity for several mitochondrial (mt) enzymes associated with the oxidative metabolism of glucose and pyruvate, including citrate synthase (CS) and malate dehydrogenase (MDH), in bladder tissue under the same conditions (12). In contrast, the activities of several cytosolic enzymes, including creatine kinase, myosin ATPase, and phosphoribosyl transferase, were not affected [13, 14]. These data clearly suggest that decreased mt enzyme activity may be directly associated with the marked decrease in the ability of the obstructed bladder to sustain a contractile response to neuronal stimulation and empty. Importantly, recent work has shown that partial outlet obstruction of the rabbit bladder results in significant changes in the expression pattern of a number of well-described nuclear genes, including *ras*, in bladder tissue [15]. Transcript levels for the *hsp70* gene, whose product is thought to be important in mediating movement of proteins into mitochondria, are substantially increased in bladder tissue following partial outlet obstruction [15, 16]. Taken together, all these observations indicate that mt function is significantly altered in bladder tissue following the initiation of partial outlet obstruction.

Proper mt function is, of course, absolutely critical in supporting the ability of the normal bladder to contract and empty. In turn, maintenance of proper mt function requires adequate expression of not only a large number of nuclear genes, but also that of a small set of genes encoded by mt DNA. The mt genome in mammalian cells is extremely compact, and the overall structure of this organellar genome among vetebrates has been highly conserved evolutionarily (reviewed in 17). In all mammalian mt genetic systems so far studied, organellar DNA has been shown to include genes which specify apocytochrome *b* for the bc_1 complex (Complex III), several subunits of cytochrome oxidase (Complex IV), subunits of the F1 ATPase, subunits of NADH dehydrogenase (Complex I), and the rRNAs and tRNAs required for assembly and function of the organellar translation system [17, 18]. All mt genes are single copy on the mt genome in essentially all organisms studied, and those genes are not functionally duplicated in the nuclear genome; each cell contains multiple copies of the mt genome, and normal cells and tissues are homoplasmic for mt DNA [18, 19]. Importantly, no respiratory complex to which the mt genome contributes polypeptides is entirely encoded by mt DNA; rather, such complexes are mosaic, requiring proteins specified by both nuclear and mt genes [17–19]. For example, cytochrome oxidase (Complex IV) is a complex of at least ten polypeptides; subunits I–III are encoded by mt genes (*COI-COIII*), while the rest are encoded in the nucleus [17–19].

Recent evidence has demonstrated that lesions, either point mutations or larger scale deletions, in mt DNA can result in severe metabolic problems in the cells and tissues harboring such altered organellar genomes [20–22]; in one case, aberrant expression of mt genes has also been shown to cause serious metabolic difficulties [23]. We reasoned that if partial outlet obstruction of the rabbit bladder leads to altered patterns of nuclear gene expression and to significantly altered mt function, then some portion of that altered mt function might be traceable to changes in the structure or copy number of the mt genome, or to alterations in the rate or pattern of expression of mt genes. We therefore undertook an investigation of the status and expression of mt DNA in rabbit bladder tissue following initiation of partial outlet obstruction. The data presented here show that the overall number of copies of the mt genome decreases precipitously in bladder tissue over time after partial outlet obstruction, and that transcription of the remaining copies of that genome is increased to maintain the production of components specified by mt genes. These observations may provide insight into the molecular mechanisms responsible for compensated and decompensated bladder function following partial outlet obstruction [1, 2].

Materials and methods

Animals, surgical induction of partial outlet obstruction

Partial outlet obstruction of the rabbit bladder was surgically induced as previously described [1–3]. Briefly, mature male New Zealand White rabbits (10 kg) were anesthetized with injection (0.7 ml/kg) of a ketamine/xylazine mixture (30 mg/ml ketamine, 9 mg/ml xylazine). Anesthesia was maintained

with i.v. pentobarbital (50 mg/ml) as necessary. Bladders were catheterized through the urethra with an 8 Fr. Foley catheter, the bladder exposed through a midline incision, and the bladder neck and urethra cleared of fat and connective tissue. Moderate partial bladder outlet obstruction was created by placing an 0 silk tie snugly around the catheterized urethra. The catheter was then removed, creating a partial outlet obstruction. The wound was closed in two layers, using chromic catgut for the muscle and 3–0 silk for the skin. Each rabbit was placed in a recovery room and observed for several hr postoperatively until the animal recovered from anesthesia. Food and water intake, and urine excretion, were monitored daily, and each rabbit was observed for signs of pain and discomfort. For analgesia, Nubain (0.1 mg/kg, i.m.) was given immediately following surgery.

Preparation of RNA and DNA from bladder tissue

Pure preparations of whole-cell RNA and whole-cell DNA were made from rabbit bladder tissue excised as a function of time after the initiation of partial outlet obstruction as given above. Nucleic acids were prepared for analysis from bladder tissue excised from control rabbits and at 1, 3, 5, 7, and 14 d after obstruction, using a derivative of a method described by us for other cells [24]. Briefly, excised bladder tissue was frozen immediately upon excision in liquid nitrogen and then pulverized with a mortar and pestle under liquid mitrogen. Tissue samples were stored at –80°C until use. 0.5 g of tissue powder was thoroughly homogenized at 40°C in a Dounce homogenizer containing 2 ml buffered phenol and 1 ml of a buffer containing 25 mM Tris (pH 8), 100 mM EDTA, 200 mM NaCl, 0.5% SDS. The homogenizer, phenol, and buffer were equilibrated to 40°C prior to tissue addition. Organic and aqueous phases were separated by centrifugation, and the aqueous phase containing the nucleic acids was removed, extensively treated with Proteinase K, and extracted with phenol and chloroform: isoamyl alcohol (24:1); following extraction, the total nucleic acids were recovered by precipitation and redissolved in 10 mM Tris (pH 8), 1 mM EDTA. RNA was prepared from such solutions by extensive treatment with DNase I, followed by extraction and reprecipitation. Similarly, DNA was prepared by treatment with a mixture of RNases A and T1, followed by extraction and reprecipitation. The quality of RNA and DNA preparations was assessed before analysis via visualization on ethidium bromide-stained agarose gels (DNA) or formaldehyde-containing agarose gels (RNA) [25].

Southern and Northern blot analyses

For Southern analyses, 10 μg whole-cell DNA were digested to completion with the restriction endonuclease HaeIII, and the digested DNA was displayed on agarose gels run in Tris-borate buffer [25]. Nucleic acids were quantitated via absorbance in aqueous solution at 260 nm. Transfer of DNA from gels to nitrocellulose membranes was done by the standard method, and all Southern hybridizations were done at high stringency [25], using the cloned probes described below. For studies of RNA, mt and nuclear transcript levels were analyzed from Northern slot blots with 10 μg whole-cell RNA per slot, and hybridization signal was quantitated by scanning densitometry using the actin transcript as normalization control [26, 27]; Northern hybridizations were done at high stringency in a 50% formamide-containing buffer [26]. All hybridization probes employed in Southern and Northern analyses were DNA fragments cloned from the rabbit mt genome; cloned probes and some rabbit mt DNA sequences were generously supplied by Dr J.-C. Mounolou, Gif-sur-Yvette, France. Clone $pOCCI_6H$ is a plasmid whose insert is comprised of a HpaI-EcoRI mt DNA restriction fragment containing the rabbit apocytochrome *b* coding sequence; clone pOCCH1200 is a plasmid whose insert is comprised of a HindIII mt DNA restriction fragment encoding a portion of each of the rabbit large and small mt rRNA genes. In one set of experiments, Northern slot blots were probed with labeled PCR products from the rabbit mt *COII* gene and the nuclear *COIV* gene [28]. All probes were labeled by nick translation using [α-^{32}P]dCTP (3000 Ci/mmole) [ref. 25].

Enzyme analyses

CS is present exclusively in mitochondria in mammalian systems and was measured by a previously described method [29], using an 0.5 cuvette. The final reaction mixture contained 38 mM Tris (pH 7.6), 0.38 mM oxaloacetate, 0.28 mM acetyl-CoA, 0.08 mM 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB). Free coenzyme A formed in the assay reacts with DTNB to produce a yellow product measured spectrophotometrically at 412 nm. Cytochrome oxidase and NADH cytochrome *c* reductase were also measured as described previously [30]. E.g., for cytochrome oxidase, aliquots of mitochondria were incubated with 90 mM TRIS (pH 7.6) and 0.03 mM reduced cytochrome *c*; assay volume was 2.7 ml and decrease in absorbance was measured spectrophotometrically at 550 nm. Reduced cytochrome *c* was prepared from the oxidized form by reduction with ascorbate; ascorbate was removed via a Bio-Gel P4 column. For all assays, protein was quantitated by the method of Lowry *et al.* [31].

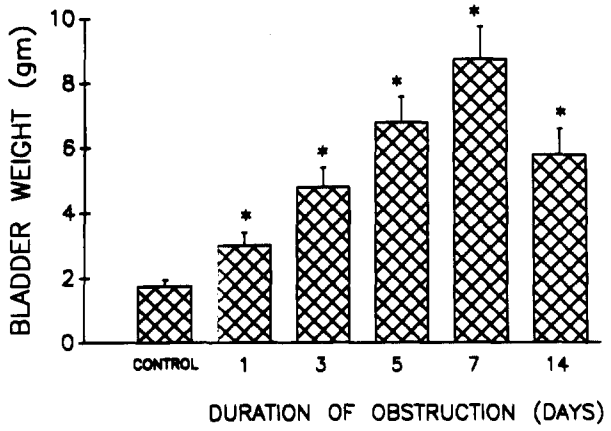
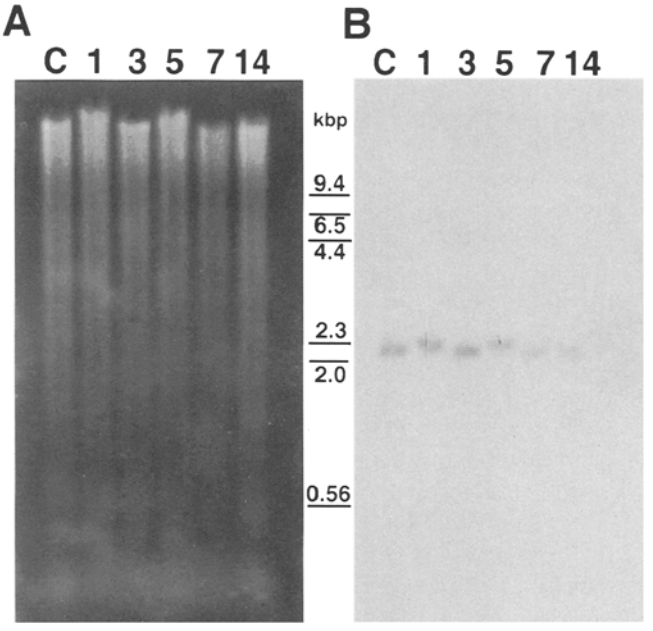


Fig. 1. Effect of partial outlet obstruction on bladder mass. Partial outlet obstruction was induced surgically in rabbits as described in Materials and methods, and bladders were excised from control animals and from experimental animals as a function of time following the initiation of obstruction. The increase in bladder mass over time after obstruction is consistent with results from similar studies [1, 2].



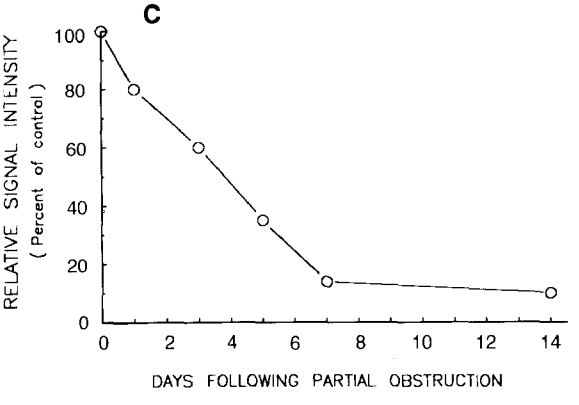


Fig. 2. Effect of partial outlet obstruction on the relative level of mt DNA in rabbit bladder tissue. Total DNA was prepared from control rabbit bladders and from bladders of rabbits which had been subjected to partial outlet obstruction, as described in Materials and methods. 10 µg DNA from each sample was restricted with HaeIII, displayed on an agarose gel run in Tris-borate buffer, transferred to nitrocellulose, and probed at high stringency with a cloned DNA fragment containing portions of the rabbit mt 12S and 16S rRNA genes. Visualization of hybridization results was via standard autoradiography. Panel A: Ethidium bromide-stained agarose gel showing restricted DNA preparations prior to Southern transfer. Panel B: Autoradiograph showing the single 2.1 kbp band hybridizing with the rabbit mt rRNA probe. Panel C: Quantitative analysis of hybridization signal as a function of time after partial outlet obstruction, given by the mt rRNA probe in the blot shown in Panel B. DNA load in the gel lanes is reasonably equal, but hybridization signal visually and quantitatively shows a decrease in the relative copy number of the mt genome/cell over time after partial outlet obstruction. The slight apparent difference in band size in Panel C is a result of slightly anomalous running of the DNA electrophoretic gel, as confirmed by inspection of the ethidium bromide stain in Panel A. Autoradiographic signal from Panel B was quantitated by scanning densitometer. DNA size standards are given between Panels A and B.

Results

Bladder mass increases and mt enzyme activity decreases following partial outlet obstruction

To be certain that the responses of bladder tissue to partial outlet obstruction in the present study conformed to those of previous studies, we assessed bladder mass as a function of time after initiation of obstruction. The data given in Fig. 1 show that, as in published experiments [1, 2], bladder mass progressively increased with time following outlet obstruction from a control value of 1.75 g to a maximum of 8.78 g at 7 d. Bladder mass then declined to a stable volume of 5.80 g by 14 d post-obstruction. We also assayed CS activity of the bladders from which we prepared DNA and RNA for the analyses described below. In agreement with published results (12), CS activity was reduced by outlet obstruction, i.e., CS activity was 59 ± 3 µmoles/min/µg protein (n=9) in bladder tissue 7 d post-obstruction, compared to 93 ± 10 µmoles/min/µg protein (n=9) in bladder tissue from control rabbits.

The relative amount of mt DNA in bladder tissue decreases following partial outlet obstruction

As an initial approach to defining the effects of partial outlet obstruction on the mt genetic system, and to provide a framework for mt transcript studies, we assessed relative changes in the overall amount of mt DNA in bladder tissue as a function of time following initiation of obstruction. Total DNA prepared from control rabbit bladders, and from bladders

excised at various times post-obstruction, was subjected to Southern blot analysis using probes for the rabbit large and small mt rRNA and apocytochrome *b* genes. Fig. 2 presents the results of a typical such hybridization analysis. When equal aliquots of whole-cell DNA from each time point examined were restricted with HaeIII (Fig. 2A) and probed with a cloned DNA fragment which includes a portion of both the 16S and 12S mt rRNA coding sequences, a single 2.1 kbp band appeared in all gel lanes (Fig. 2B). The intensity of the hybridization signal remained at control level at d 1 and d 3 after obstruction but declined thereafter until d 7. Assuming no substantial change in the relative level of nuclear DNA/cell during this period (see Discussion), these data indicate that the number of copies of mt DNA per cell decreased by about 10-fold during this 7 d period (Fig. 2C). The decreased hybridization signal over the 7 d following obstruction cannot be due to increased cell volume or tissue hypertrophy, since the experiment normalizes the signal to the constant amount of nuclear DNA loaded on the gel; in most cells, mt DNA comprises less than 1% of total cellular DNA [18, 19]. The hybridization signal given by the d 14 sample is about equal to that given by the d 7 DNA preparation, indicating that the relative level of mt DNA remains steady at the attenuated value between those two times. Repeats of this experiment using a cloned probe for the mt apocytochome *b* gene gave results identical to those of Fig. 2; in addition, both sets of probings were repeated using bladder DNA prepared from a second set of rabbits, and the results of such repeats were identical to those given here (data not shown). Thus, the relative number of copies of the mt genome in bladder tissue declines over time for the first 7 d after partial outlet obstruction and remains at that low level for at least another 7 d.

Mt transcript levels are maintained during the 14 d following outlet obstruction

Adequate transcript levels from the mt genome are absolutely required for maintenance of the electron transport system. Because the relative number of copies of the mt genome decreases significantly during the initial 7 d following partial outlet obstruction, we assayed transcript levels for the mt COII and apocytochrome *b* genes as a function of time during the first 14 d after obstruction; total RNA analyzed in these studies was prepared from the same tissues as was the total DNA analyzed above. Quantitative Northern slot blot analyses showed that *COII* transcript levels declined to about 40% of control values during the first 3 d after initiation of obstruction, but that they steadily recovered to control levels during the period from 3 to 7 d post-obstruction and remained at control level until d 14 (Fig. 3). In similar experiments not shown here, analyses of transcript levels from the mt apocytochrome *b* gene gave results which

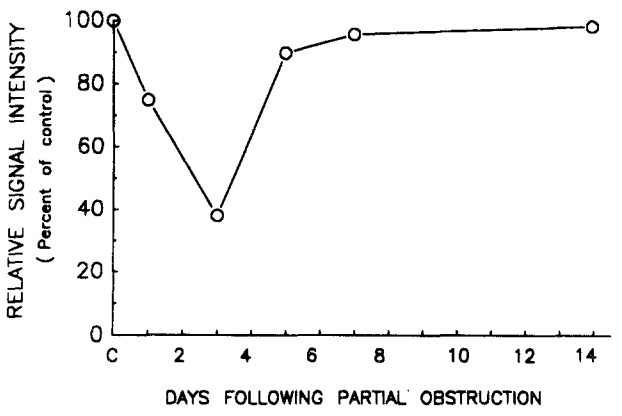


Fig. 3. Relative transcript levels for the rabbit mt *COII* gene in bladder tissue as a function of time after initiation of partial outlet obstruction. Total RNA was prepared, treated, slot-blotted, and hybridized as described in Materials and methods. Hybridization signal was visualized via standard autoradiography. In Fig. 3, relative hybridization intensity given by each RNA preparation analyzed via slot blot was quantitated by scanning densitometry, normalized to signal given by transcripts from the constitutively-expressed actin gene, and then plotted as percent of control value as a function of time after outlet obstruction. *COII* message levels decline for the first 3 d after obstruction but recover to control level by about d 7. Similar experiments using a probe for transcripts from the mt apocytochrome *b* gene gave results identical to those shown here for *COII* transcripts.

reflect those shown in Fig. 3 for *COII* transcripts. In the Northern analyses for both apocytochrome *b* and *COII* transcripts, data obtained were normalized to transcript levels for the constitutively-expressed actin gene. Thus, despite the significant decline in the relative number of copies of the mt genome in bladder tissue, and therefore the overall number of copies of each individual mt gene, the mt transcription system is able to maintain message levels at control values for 14 d following obstruction. While some other explanations are possible, these data suggest that the rate of transcriptional initiation on mt DNA is significantly increased in rabbit bladder tissue following partial outlet obstruction (see Discussion).

Cytochome oxidase and NADH cytochrome c reductase activity are maintained post-obstruction

We reasoned that if overall mt transcript levels are increased to accommodate for the attenuated number of mt genome copies per cell, then the activity of the various respiratory complexes to which the mt genome contributes subunits should reflect that accommodation. The data given in Fig. 4 show that this is indeed the case. Over the first day post-obstruction, overall activities for both cytochrome oxidase and NADH cytochrome *c* reductase decreased by 40–50%, reflecting roughly the pattern for mt transcripts specifying components of these complexes, but between d 1 and d 7 those levels recovered, rising nearly to control values. As

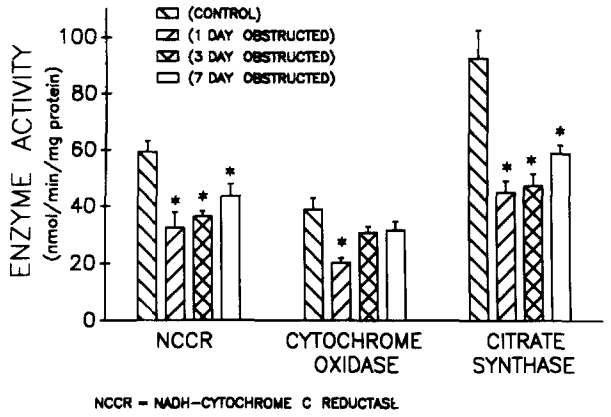


Fig. 4. Relative activities of cytochrome oxidase, NADH cytochrome *c* reductase (NCCR), and citrate synthase in homogenates of whole control rabbit bladders and in bladders excised at various times following partial outlet obstruction. Outlet obstruction was induced surgically, as described in Materials and methods; tissues were prepared and enzyme assays were also performed as given in Materials and methods. Number of determinations for each value shown is 6–9; *indicates values significantly different from those of control. As in previous studies, CS activity is attenuated at 7 d after obstruction, but cytochrome oxidase and NADH cytochrome c reductase activities recover to essentially control values by 7 d post-obstruction, despite a significant initial decrease in those activities.

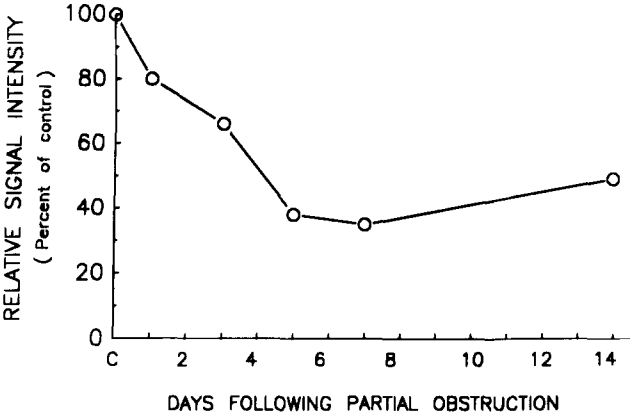


Fig. 5. Effect of partial outlet obstruction on *COIV* transcript levels in rabbit bladder tissue as a function of time after initiation of partial outlet obstruction. Control rabbit bladders and bladders from obstructed animals were excised at various times following obstruction, and RNA was prepared and analyzed from bladder tissues as given in Materials and methods. Northern analyses for *COIV* RNA were performed as given in Materials and methods and in Fig. 4, and data are expressed relative to values for the constitutively-expressed actin control, after quantitation of hybridization signals by scanning densitometry. Unlike the results for *COII*, a functionally related mt gene (Fig. 3), *COIV* transcript levels steadily decline for the first 5 d after obstruction and remain at the low level through d 14 post-obstruction.

in previous studies, however, the same bladder tissue samples showed activity levels for CS which were significantly attenuated over the 7 d following outlet obstruction, although those levels did recover somewhat from an initial low value 1 d post-obstruction. Thus, cytochrome oxidase (Complex IV) and NADH cytochrome *c* reductase activities (Complex I + Complex III) reflect the relative transcript levels for their mt-specified components as a function of time following outlet obstruction. Unlike CS, which is entirely nucleus-encoded, components of these mt respiratory complexes are specified by both nuclear and mt genes; in combination with data to be presented next, the results shown in Figs. 3 and 4 suggest that one limiting factor in the assembly of mt electron transport complexes is availability of transcription/translation products from the mt genetic system.

Transcript levels for a mt-related nuclear gene are attenuated following partial outlet obstruction

A number of recent studies have indicated that transcription of functionally-related nuclear and mt genes in vertebrate systems is coordinately controlled (see 32 for review). That is, expression of both nuclear and mt genes which contribute gene products to individual mt respiratory complexes may be subject to a common regulatory system, so as to insure adequate levels of all required gene products from each separate genome. As mentioned above, cytochrome oxidase is comprised of several subunits, only three of which are specified on mt DNA. Because our data concerning expression of *COII* following partial outlet obstruction indicated that transcription is significantly increased, we went on to assay relative transcript levels for the nuclear gene *COIV*, which specifies subunit IV of the cytochrome oxidase complex. Surprisingly, the results of such Northern analyses showed that *COIV* transcript levels are not maintained in parallel with those of the mt *COII* gene following partial outlet obstruction (Fig. 5). Rather, *COIV* message levels declined to about 40% of control values by 5 d post-obstruction, and they remained at about this same low level until at least 14 d after obstruction was initiated. While more study will be required, these data seem to suggest that the coordinate nuclear-mt transcriptional regulatory system which normally operates in mammalian cells is abrogated after partial outlet obstruction, and that expression of functionally-related nuclear and mt genes in bladder tissue is relatively independent of one another in this situation (see Discussion).

Discussion

Proper mt function is required to support metabolism in virtually all mammalian cells, and published work has shown that after partial outlet obstruction of the rabbit bladder, at least some mt functions are significantly altered in bladder tissue [11, 12]. In the present study, we investigated function of the mt genetic system in rabbit bladder tissue, and

our results indicate that the relative copy number of the mt genome decreases sharply over time following obstruction. Transcript analyses show, in contrast, that while mt message levels decline at a rate concomitant with that of mt DNA immediately following obstruction, those levels recover to control values by about 5 d after obstruction, despite the attenuated number of templates from which those messages are produced; the activity for two respiratory complexes to which the mt genome contributes polypeptides reflects mt transcript levels over time after obstruction. We were therefore surprised to find that transcripts for a nuclear gene, one which is functionally related to a mt mRNA we measured, declined permanently in bladder tissue after outlet obstruction. As developed below, we understand all these observations to mean that, while obstruction leads to problems in replication or stability of mt DNA, bladder cells compensate for the lack of mt DNA templates by increasing expression of mt genes. Our data further suggest that availability of mt transcripts, and probably mt translation products, may be a limiting step in assembly of electron transport complexes, at least under conditions of stress.

In all mammalian systems so far examined, mt genes are single-copy on the organellar genome, and the hundreds of copies of that genome within each cell are normally identical to one another [17–19]. In our experiments, we did not assess the presence of every mt gene individually by Southern analysis as a function of time after outlet obstruction. However, our independent analyses for the mt rRNA genes and the mt apocytochrome *b* gene showed identical decreases in copy number for these genes over the first 7 d post-obstruction, and a maintenance of the low copy number through d 14 post-obstruction; the kinetics of loss of the mt rRNA and cytochrome *b* genes were identical. In each analysis, restriction fragment size for each of the genes analyzed remained constant as band intensity declined with time after obstruction. While other explanations are possible, we understand these results to mean that mt genome copy number per cell declines after obstruction, rather than that portions of each mt genome are being deleted after initiation of that obstruction.

We do not understand the mechanism(s) responsible for this sharp, and apparently long-lasting, decline in relative mt genome copy number in bladder tissue. Previous studies have suggested that DNA synthesis is slightly increased in bladder 5 d after obstruction, but that by d 14 the rate has returned to control level [33]; total DNA/mg protein in bladder tissue does not change significantly or permanently following obstruction. In all eukaryotes, replication of mt DNA is accomplished by DNA polymerase γ, an enzyme which functions exclusively in the organelle and which is poorly related to the various nuclear DNA polymerases [19]; all subunits of DNA polymerase γ are encoded by nuclear genes, produced on cytoplasmic ribosomes, and imported into the organelle (e.g., 34). Our analysis for transcript levels from the nuclear *COIV* gene indicates that such levels decline in bladder tissue following outlet obstruction; this may suggest that some nuclear genes specifying mt-related proteins are transcriptionally repressed after obstruction. If this is the case for genes encoding polymerase γ subunits, then protein levels for this enzyme would eventually become limiting after obstruction, leading to a depression in the replication rate of mt DNA. Alternatively, outlet obstruction may cause decreased stability of mt DNA for some reason. We favor the former explanation and are assessing transcripts for other mt-related nuclear genes after outlet obstruction.

Despite the decline in relative mt genome copy number over time following outlet obstruction, some cellular control mechanism clearly responds to the need for maintenance of mt transcript levels. Steady-state transcript levels for any gene can be altered by one or more of several means, including modulation of the rate of transcriptional initiation, changes in the rate of elongation, and alteration of the RNA turnover rate. Our data show that mt transcript levels initially fall with the decline in mt genome copy number, and then recover to control levels by 5–7 d post-obstruction. Thus, at 7 d after obstruction and beyond, a 10-fold increase in transcriptional initiation or elongation rates, or a similar decrease in RNA turnover rate, would be required to yield control mt transcript levels. While decreased turnover and increased initiation and elongation rates together may be involved, we suspect that the primary factor allowing control mt transcript levels to be maintained following partial outlet obstruction is increased mt transcriptional initiation.

Transcription of mt DNA is accomplished by a mt RNA polymerase which, like DNA polymerase γ, is encoded by nuclear genes and which functions exclusively within the organelle [17–19]; in addition to the mt RNA polymerase, a *trans*-acting factor designated mtTFA is required for transcriptional initiation in mammalian mt systems (e.g., 35). The mt RNA polymerase-mtTFA system initiates transcription of both heavy and light strands of the mt genome at specific sites within the D-loop region, and transcripts of each strand are full genome length [18, 19]. Thus, regardless of the rate of transcriptional initiation on either strand, mt mRNAs, tRNAs, and rRNAs are produced at stoichiometric levels from each strand from each transcriptional initiation event. Our observations suggest that for the first 3 d or so following outlet obstruction, the rate of mt transcriptional initiation, and probably the rates of elongation and turnover for mt RNAs, remain essentially unadjusted by cellular control mechanisms. However, between d 3 and d 5, the rate of initiation of mt transcripts is increased to produce ever more transcripts from the declining number of mt DNA templates. The system appears to reach a sort of equilibrium between d 5 and d 7 post-obstruction, when mt genome copy number reaches is lowest, steady level. The

detailed mechanism by which the rate of mt transcriptional initiation is increased under these circumstances remains to be elucidated.

The recovery of cytochrome oxidase activity, and the return toward normal of NADH cytochrome *c* reductase activity, by 7 d post-obstruction supports the idea that increased mt transcription is an attempt to maintain mt energy production under severely adverse conditions. It is therefore surprising that transcript levels for a mt-related nuclear gene, *COIV*, are attenuated during the same period. Evidence from other laboratories has indicated that under normal conditions, expression of some functionally-related nuclear and mt genes is coordinately controlled [32]. Indeed, recent evidence has suggested that such coordinate control is mediated by transcriptional *trans*-activators which bind to commonly-held *cis*-regulatory elements in the D-loop of the mt genome and 5′ to relevant nuclear genes [36, 37]. Our results showing increased mt transcript levels but decreased *COIV* transcript levels suggest that the coordinate transcriptional control system is at least partly disengaged in bladder tissue after partial outlet obstruction, and that this disengagement begins between d 1 and d 3 post-obstruction. How activity for the mosaic complexes of the respiratory chain is maintained under such circumstances is not clear, but we suspect that maintenance of such activity, and maintenance of an extremely high mt transcription rate, can only be accomplished for a circumscribed period. We suggest here that in large part, the previously-described period of compensated function in bladder tissue, which has been observed to follow the initiation of partial outlet obstruction [1, 2], corresponds to the period when mt transcriptional activity, and mosaic respiratory complex activity, can be supported; decompensated function would therefore begin when the huge mt transcriptional increase, and mosaic complex activity, can no longer be maintained. Reversal of obstruction, with its attendant return to normal function in bladder tissue, would represent a return of mt genome copy number and mt transcription parameters to normal levels. We are now assessing mt genetic parameters following reversal of outlet obstruction, and we are studying the long term reaction of the mt genetic system to extended periods of partial outlet obstruction.

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