Supplementary Material

The general scheme for mitochondrial nucleoid purification is shown in Figure 1. In some cases, nucleoids were prepared by isopycnic centrifugation as reported (Bogenhagen et al., 2003) as an alternative to immunoaffinity purification. Slowly- and rapidly-sedimenting nucleoid fractions were applied separately to preformed 25%-45% Nycodenz gradients which separate molecules and complexes based on their buoyant densities, as shown for the gradients containing the related compound, metrizamide. Interestingly, both subsets of nucleoids shifted to a similar density range during equilibrium centrifugation (Fig S1A,B). The presence of mtDNA in both fractions is confirmed by *Hin*dIII digestion (Fig S1C,D) and corresponding protein analysis shows that the nucleoid marker, TFAM, copurifies with the mtDNA. The rapidly-sedimenting nucleoid fraction appears to be highly purified, since virtually all of the input protein in the glycerol gradient fraction co-bands with mtDNA (data not shown). However, the cytoskeletal proteins vimentin and actin are both abundant in this fraction, as shown in Fig S1E. Protein sequencing revealed a number of other cytoskeletal-associated proteins in this rapidly-sedimenting fraction (data not shown). This fraction consistently banded as a very tight peak, as expected for a complex having a very high molecular weight. Buoyant density gradient analysis of the slowly-sedimenting nucleoids shows that a significant fraction of the protein in the slowly-sedimenting region of the velocity gradient profile is not tightly-associated with mtDNA nucleoids. However, it is striking that this fraction is essentially free of detectable contamination by cytoskeletal proteins. We suggest that association with cytoskeletal proteins determines the sedimentation behavior of mtDNA nucleoids.

To confirm that the presence of proteins in nucleoids was dependent on the presence of mtDNA, glycerol gradient purified nucleoids were treated with DNase I in parallel with a mock-

incubated control. Both samples were banded in Nycodenz gradients as in Fig. S2. The results in Fig. S2 document that digestion of the mtDNA in nucleoids resulted in dispersal of nucleoid proteins through a broad region of the gradient.

To test the possibility that the composition of nucleoids might vary significantly depending on the conditions used during the preparation, we prepared HeLa mitochondria and performed detergent lysis, gradient sedimentation and immunopurification with the anti-TFAM affinity column under a variety of conditions. In one experiment, the mitochondrial preparation was divided into three equal fractions: one fraction was processed as usual with 1.2% Triton X-100 at 70 mM NaCl, another was lysed and processed with 25 mM octyl glucoside in place of Triton X-100 and the third aliquot was lysed with 1.2% Triton X-100 in the presence of 160 mM NaCl. In a second experiment, mitochondria were lysed with 1.2% Triton X-100 at 70 mM NaCl and one-half of the lysate was treated with 10 μg/ml RNaseA for 10 min at 37 °C and throughout the incubation with the anti-TFAM coated beads while the second half was mock-incubated as a parallel control. Fig. S3 shows the analysis of the proteins bound to the beads after elution with SDS. The silver stained protein gel shows few changes in the protein patterns. Immunoblots with antibodies against TFAM, mtSSB, HSP60, Complex I (CI, NDUFA9) and VDAC are shown below the stained gel. These show a consistent recovery of TFAM and mtSSB, but with some losses during RNase treatment. Under most conditions, HSP60 and NDUFA9 were retained while VDAC was purified away. Lane 3 shows that more VDAC was retained at higher salt, as may be expected if its presence is due to a hydrophobic interaction with ANT. We conclude that the protein composition is not drastically altered by changes in the nucleoid purification procedure.

Supplemental Figures

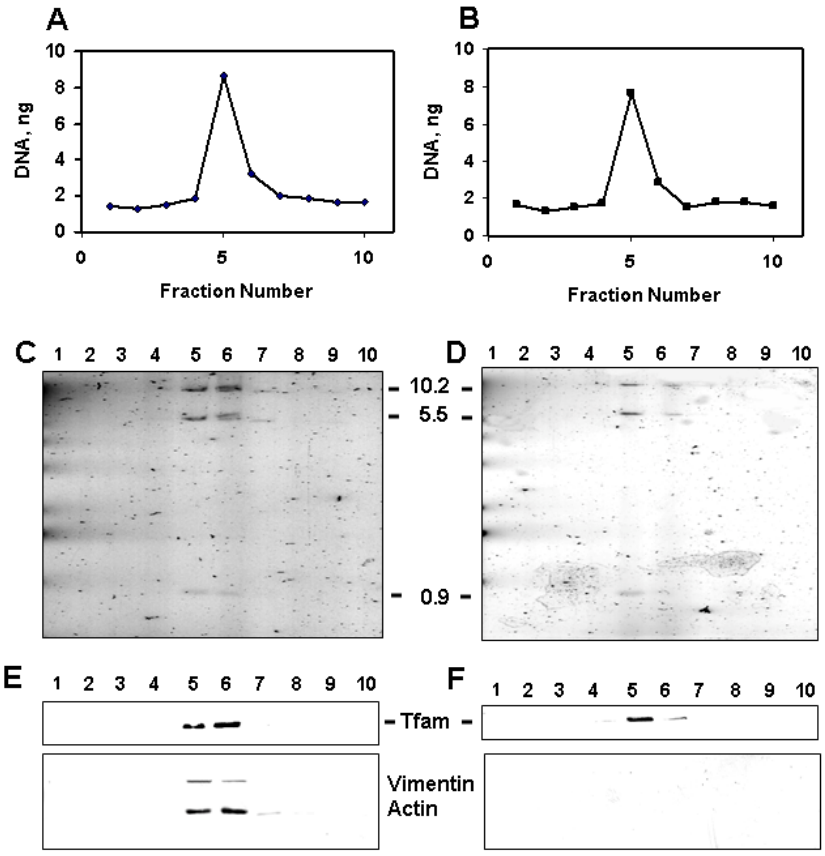


Fig S1. HeLa cell mitochondrial nucleoid proteins in 25%-45% Nycodenz isopycnic gradients.

A, B. DNA analysis. Nucleoids in rapidly- and slowly-sedimenting fractions, respectively, were further purified by isopycnic centrifugation on 25%-45% Nycodenz gradients. DNA was detected as described in the legend to Fig. 2.

C, D. MtDNA in rapidly- and slowly-sedimenting fractions, respectively, was digested with *Hin*dIII and detected as described in Fig. 2.

E, F. Western blot of proteins in rapidly- and slowly-sedimenting fractions probed for TFAM, vimentin and actin.

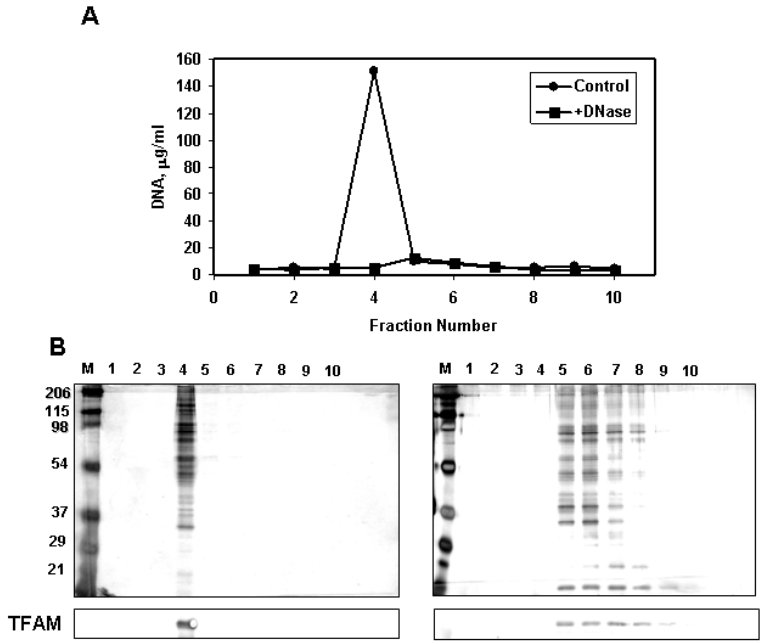
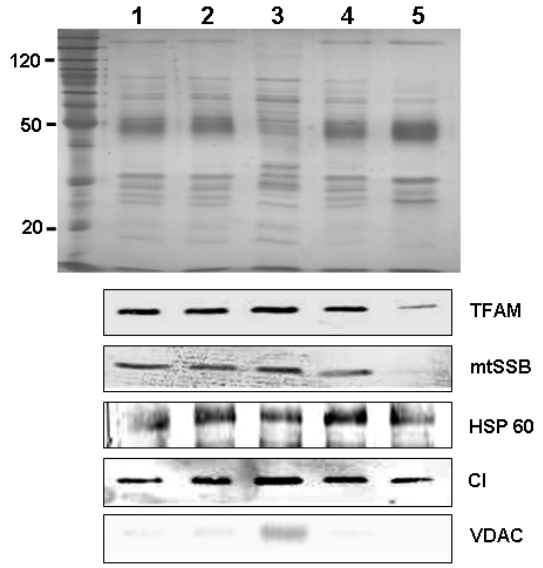


Fig. S2. DNase treatment shows the size and density of rapidly-sedimenting nucleoids depends on mtDNA. A preparation of mtDNA nucleoids purified through the glycerol gradient step was divided into two portions. One sample was treated with 30 µg/ml DNase I at 20 °C for 5 min while the other was an untreated control. Both samples were then banded to equilibrium in separate Nycodenz gradients. Panel A shows a superimposition of the DNA distribution in the gradients assayed by Picogreen fluorescence. Panel B shows the polypeptides in the two gradients after SDS-PAGE. The *left* panel shows a tightly-focused distribution of protein cobanding with the DNA in the control sample; the *right* panel shows that the protein in the nuclease-treated sample is distributed in a broad zone at lower density than the intact nucleoids. Western blots to detect TFAM confirmed that this nucleoid marker behaved like other proteins in the nucleoid.



1. Standard TFAM antibody IP (TX-100, 70 mM NaCl)
2. TFAM antibody IP (DG, 70 mM NaCl)
3. TFAM antibody IP (TX-100, 160 mM NaCl)
4. Standard TFAM antibody IP (TX-100, 70 mM NaCl, duplicate of lane 1)
5. TFAM antibody IP (TX-100, 70 mM NaCl, 10 µg/ml RNAse)

Fig. S3. Effect of varying conditions used for nucleoid purification. Nucleoids were prepared in two experiments described in the supplementary text. In the first experiment, represented by lanes 1-3, nucleoids were prepared under standard conditions (lane 1; 1.2% Triton X-100 and 70 mM NaCl) or using 25 mM octyl glucoside in place of Triton X-100 (lane 2, at 70 mM NaCl), or with 160 mM NaCl (lane 3, with 1.2% Triton X-100). In the second experiment, represented by lanes 4 and 5, nucleoids were prepared under standard conditions (lane 4) or with RNase treatment prior to and during the affinity chromatography step. The image of a silver stained 10% SDS-PAGE gel is shown at the top along with the results of immunoblots to detect the indicated proteins. Note that lanes 1 and 4 represent two equivalent independent controls.