Isolation of a 25-kDa Protein Binding to a Curved DNA Upstream the Origin of the L Strand Replication in the Rat Mitochondrial Genome*

Gemma Gadaleta‡§, Domenica D'Elia§, Lara Capaccio, Cecilia Saccone, and Gabriella Pepe

From the Centro di Studio sui Mitocondri e Metabolismo Energetico-CNR and Department of Biochemistry and Molecular Biology, University of Bari, via Orabona 4A, 70126 Bari, Italy

The presence of a curved DNA sequence in the gene for the NADH-dehydrogenase subunit 2 of rat mitochondrial genome, upstream from the origin of the light strand replication have been demonstrated through theoretical analysis and experimental approaches. Gel retardation assays showed that this structure makes a complex with a protein component extracted from the mitochondrial matrix. The isolation and purification of this protein is reported. With a Sepharose CL-6B and magnetic DNA affinity chromatography a polypeptide was purified to homogeneity having 25-kDa mass as shown by gel electrophoresis. To functionally characterize this protein, its capability to bind to other sequences of the homologous or heterologous DNA and to specific riboprobes was also investigated. A role for this protein as a trans-acting agent required for the expression of the mammalian mitochondrial genome is suggested.

Mammalian mtDNAs[1] have two separate and distinct replication origins. The origin of the heavy strand replication (Ori-H), inside the so-called D-loop containing region which also contains the promoters for the transcription of both strands (H and L strand promoters); and the origin of the L strand synthesis (Ori-L), nested within a cluster of five tRNA genes at two-thirds of the molecule, clockwise with respect to the Ori-H (for review, see Ref. 1). Initiation of the L strand synthesis only occurs after Ori-L is exposed as a single-stranded template. The outline of mtDNA replication in mammals has been elucidated (2) and the γ-DNA polymerase has been identified and partially purified in humans (3). Several other proteins take part in this process with different roles. An RNA polymerase and an RNase-MRP have a role in the initiation of RNA priming, the very first step of the new H strand synthesis; a DNA primase is involved in priming the L strand synthesis; unwinding factors have helicase or topoisomerase functions. Other protein candidates for process regulation by specific targeting to mtDNA sequences associated with replication have been identified from different eukaryotes, including mammals (for review, see Ref. 4). For many years we have deeply studied the genetic and structural organization of the mt genome in mammals, especially in rat (5, 6).

Moreover, we analyzed the rat mtDNA sequence searching for primary or higher order structures that could be recognized by functional proteins. Namely, we checked for sequence-dependent DNA curvatures, frequently present in the regulatory regions of a genome, involved in protein binding (7–11). Then we concentrated our efforts on the isolation, purification, and characterization of the proteins able to interact with these structures. We have already reported the presence of a curved DNA in the D-loop containing region, located close to Ori-H. This structure acts as a binding site for a nuclearly coded 67-kDa polypeptide isolated from the matrix and we suggested that it is a regulatory factor promoting the first step of the H strand replication through the RNA → DNA transition (12, 13). Thereafter we focused our attention on the region of the genome containing the other replication origin, the Ori-L.

In this paper we report the localization of a curved DNA sequence inside the gene for the NADH dehydrogenase subunit 2 (ND2) upstream Ori-L. We also present the purification to homogeneity and characterization of a 25-kDa protein unknown so far, able to bind this DNA structure specifically. This is one of the few proteins, nuclearly coded, purified until now from higher eukaryotes which should participate in the biogenesis of mitochondria and for which we suggest a possible physiological role.

MATERIALS AND METHODS

Source of DNA—Several recombinants were constructed by cloning different portions of the *Rattus norvegicus* mtDNA around the Ori-L. The organization of this mtDNA region, its restriction map, and the position of the cloned fragments are shown in Fig. 1.

The larger recombinant, called BS-C, was a Blue Scribe plasmid containing the entire 2982-bp long *Eco*RI-C restriction fragment as the insert. From BS-C, another recombinant was derived inserting the *Eco*RI-*Hin*cII subfragment, called C1, in the pUC8 vector. The third recombinant, called BS-A, was a *Sma*I digested Blue Scribe containing the 520-bp-long *Eco*RI-*Taq*I subfragment, inserted in the direction T3 → T7 of the promoters contained in the vector.

Curvature Analysis—The theoretical analysis was performed on the entire *Eco*RI-C sequence according to Plaskon and Wartell (14). For the experimental approach the above mentioned recombinants were digested with suitable enzymes and the restriction products underwent gel electrophoresis in retarding conditions, as described previously (12).

Electrophoretic Mobility Shift Assay (EMSA)—The DNA probe was the 520-bp insert excised by suitable digestion from the BS-A recombinant. Alternatively a smaller probe was obtained by PCR amplification of BS-A using a mitochondrial upstream primer (about 200 bp from the *Eco*RI site) and the T7 downstream primer. This probe was 350 bp long, out of which 320 bp were mtDNA. EMSA analysis was carried out as in Gadaleta *et al.* (13), by incubating the ^{32}P-end-labeled probe with proteins prepared according to Greenawalt (15) and purified as described

[1] The abbreviations used are: mt, mitochondria(l); L strand, light strand; H strand, heavy strand; Ori-L, origin of the L strand replication; Ori-H, origin of the H strand replication; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; EMSA, electrophoretic mobility shift assay; bp, base pair(s); ND2, NADH dehydrogenase subunit 2.
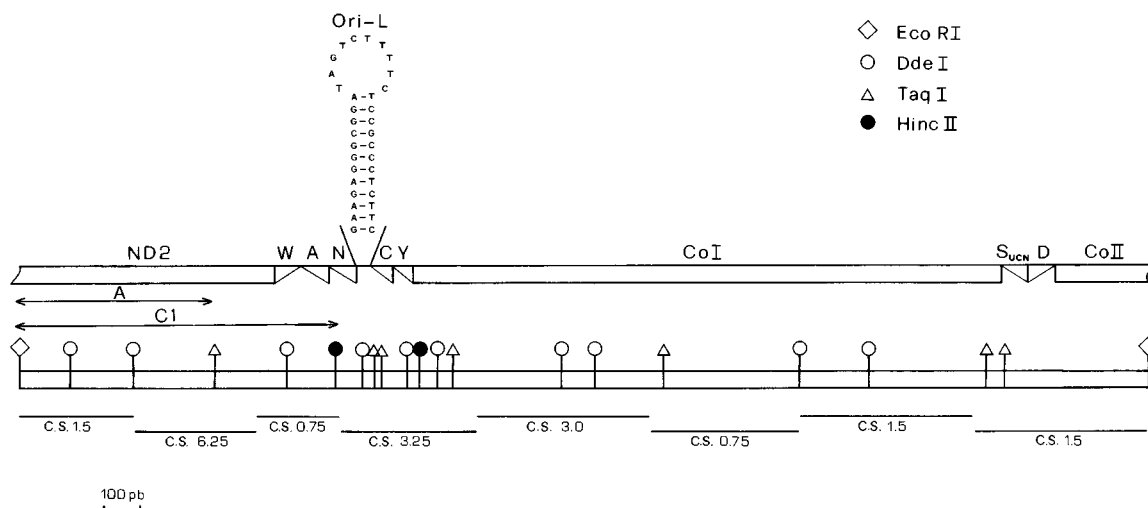
FIG. 1. **Structure and genic organization of the *Eco*RI-C fragment from rat mtDNA.** *Top*, genic content (part of NADH dehydrogenase subunit 2, cluster of five tRNAs, cytochrome oxidase subunit 1, two more tRNAs, and part of cytochrome oxidase subunit 2) and the stem-loop structure of Ori-L. The sequence goes from nucleotides 4253 to 7235 of rat mt genome (for this and other numerations in the paper see Ref. 6). *Middle*, physical map of the restriction enzymes used in the experiments, with the limit of the cloned subfragments A and C1. *Bottom*, curvature scores (*c.s.*) calculated according to Plaskon and Wartell (14) on different regions along the sequence.

below. The stability of the binding was measured in terms of dissociation rate of the protein-DNA complex in the presence of an excess of unlabeled homologous DNA as a competitor (16).

Protein Purification and Characterization—First mitoplastic proteins were partially purified by heparin-Sepharose CL-6B chromatography, as we have already reported (13). The fractions eluted at 0.5 M KCl were able to bind the DNA probe when analyzed by EMSA. The active fractions were pooled and further purified by a highly selective procedure, the magnetic DNA affinity purification, developed by Gabrielsen *et al.* (17, 18). Streptavidin-coated Dynabeads M-280 (Unipath) were used. For this approach no DNA fragment purification was performed to avoid loss of material: about 2 mg of the recombinant BS-A were digested in the polylinker *Sac*I-*Bam*HI sites and both the plasmid and the insert were biotinylated at the *Bam*HI end by biotin-dUTP and Klenow enzyme. The presence of competing nonspecific binding sites on the beads was highly reduced cleaving off the biotinylated *Bam*HI end of the vector by a further digestion at the close *Pst*I site. Then the DNA was desalted by Sephadex G50 and Centricon 30 (Amicon). The suspension of 10 mg of beads in 1 ml of TEN buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA, 100 mM NaCl) was mixed with the DNA solution for the coupling to the labeled fragment. A reusable magnetic solid-phase was formed with a specific affinity for the target protein (10 μg of specific DNA fragment per mg of beads). The beads were then washed with TGED buffer, 50 mM NaCl (20 mM Tris-HCl, pH 8, 10% glycerol, 1 mM dithiothreitol, 0.01% Triton X-100, 50 mM NaCl) and mixed with the pooled fractions from the heparin-Sepharose active in the EMSA. The beads were overloaded so that the limiting number of available DNA sites were certainly saturated only by the specific proteins. The contaminating unbound proteins remained in the solution after the magnetic separation and were discarded. Finally, the bound protein component was eluted by TGED buffer, 1 M NaCl and characterized by 11% SDS-PAGE as in Laemmli (19).

RESULTS

Detection of the Curved DNA—The entire *Eco*RI-C sequence was carefully checked for the characteristic An-Tn stretches consistent with a curved DNA (7–12). This property was indeed present in several regions along the fragment. Plaskon and Wartell algorithm was applied which assigns a curvature numerical score based on the phasing of the An-Tn tracts and allows prediction of the presence of a curved DNA in fragments with higher values. Plaskon and Wartell also suggested that the curved DNA is a characteristic feature of regulatory regions in a genome (14). Fig. 1 shows a schematic representation of the *Eco*RI-C fragment. The analysis showed a curvature score of 6.25, significantly high according to Plaskon evaluation, only in the second half of the ND2 gene upstream Ori-L. Thus, the region seemed to be a good candidate for containing a DNA

curvature in rat mt genome.

The region was experimentally tested by gel electrophoresis. It is known that an anomalously low migration of a fragment can reveal the presence of a curved DNA and the retardation due to the bending phenomenon is increased at lower temperatures and reduced at higher temperatures (20–22). Thus, a number of experiments was performed in which the recombinant DNAs described under "Materials and Methods" were digested with different enzymes as shown in Fig. 1 and analyzed on a monodimensional gel electrophoresis (at 4 and at 60 °C) or on a two-dimensional gel (12). BS-C was double digested by *Eco*RI-*Hinc*II giving three subfragments of 1913, 849, and 220 bp, according to the sequence data (6). However, on polyacrylamide at 4 °C the 849-bp fragment migrated as a 913-bp long one with $R = 1.1$, where R is the ratio of the apparent size, obtained at 4 °C, *versus* the actual size (results not shown). This region (C1) was cloned in pUC8 and the derived recombinant was carefully analyzed to localize the curvature. It was double digested by *Taq*I-*Hinc*II giving a 976-bp subfragment with the apparent size of 1291 bp ($R = 1.33$). Fig. 2*a* shows the two-dimensional gel that highlights this retarded fragment consisting of 520 bp from rat mtDNA and a vector portion. To verify that the anomalous migration was due to a curvature present in the mtDNA sequence, the pUC8-C1 recombinant underwent *Eco*RI-*Taq*I digestion and the new products were analyzed by two-dimensional gel electrophoresis as shown in Fig. 2*b*. This experiment definitely localized the curvature in the 520-bp sequence of rat mt DNA inside ND2 gene from nucleotides 4252 to 4772. The fact that this curved DNA did not correspond to that found in human mt genome by Welter *et al.* (23) is discussed below. Finally, the sequence containing the curvature was cloned in the Blue Scribe vector giving the BS-A recombinant that was thereafter used in most of our experiments. Fig. 2*c* shows gel retardation performed with the shorter 350-bp sequence obtained by PCR as described under "Materials and Methods." This sequence had almost normal migration with respect to the marker fragments. We demonstrated by EMSA that both the 520-bp and the 350-bp fragments bound a nuclearly coded protein component extracted from the organelle matrix. These results are consistent with the presence of the curvature roughly in the middle of the 520-bp and near the 5′ terminus of the 350-bp fragment. Thus, both these probes were used in EMSA and
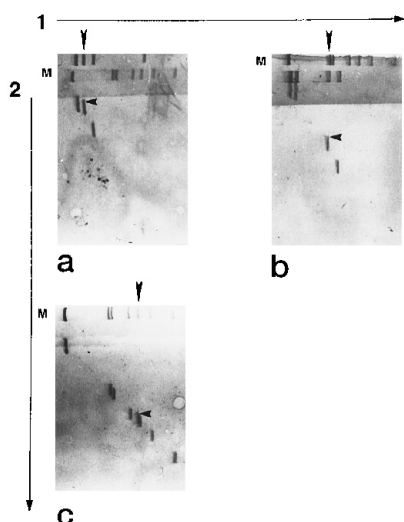
FIG. 2. **Gel retardation experiments.** Two-dimensional gel electrophoresis in 6% polyacrylamide of different restriction products: *a*, pUC8-C1 digested by *Taq*I-*Hin*cII; *b*, pUC8-C1 digested by *Eco*RI-*Taq*I. *c*, mixture of the pBR322 digested by *Hin*fI with the 350-bp fragment obtained by PCR. The *arrows 1* and *2* indicate, respectively, the first dimension at 60 °C and the second dimension at 4 °C. In the first dimension, *M* indicates the pBR322 marker. *Small arrows* label the fragments with abnormal mobility, due to the presence of a curvature.

FIG. 3. **Protein-DNA interaction analysis.** *a*, EMSA: *lane* −, free labeled probe (2 ng); *lanes 2, 4,* and *10*, probe incubated with increasing amounts of the pooled fractions eluted at 0.5 M KCl from the heparin-Sepharose column. The proteins were quantified according to Waddel (24). *b*, proteinase K sensitivity. The experiment was carried out by incubating 10 μg of pooled eluted fractions with 100 μg/ml proteinase K for 15 min at 37 °C before the binding reaction.

affinity purification experiments.

Protein-DNA Interaction Analysis—The functional role of the identified curved structure was studied. EMSA experiments were performed by incubating the selected DNA probe with the mt proteins extracted from the matrix and partially purified by heparin-Sepharose CL-6B. Fig. 3*a* shows the experiment with different amounts of proteins: with 10 μg the probe was completely saturated in the complex. When the same proteins were pretreated with proteinase K the complex disappeared (Fig. 3*b*) indicating the real protein nature of the component bound to the DNA. To evaluate the stability of the protein-DNA interaction, the dissociation rate of the complex was monitored by EMSA as described under "Materials and Methods" (Fig. 4). The *in vitro* estimated lifetime was about 3.5 h, when the complex appeared destabilized. Binding assays were also carried out in the presence of a competing ribonucleotide synthesized by using the 520-bp sequence of BS-A as the template and corresponding to the ND2-RNA or the ND2-anti-RNA. The competition causes the decreasing or disappearing of the complex and the increasing of the free probe. Fig. 5 shows the inhibition of the protein-DNA binding activity by both riboprobes, although the anti-RNA is less efficient. In Figs. 4 (*lane 9*) and 5 (*lanes 3–5*) intermediate complexes appeared, suggesting the interaction of more protein subunits at the DNA binding site.

Protein Purification—The result of the affinity purification performed as described under "Materials and Methods" is presented in the SDS-PAGE of Fig. 6. The polypeptide eluted by the activated beads is essentially pure, with a 25-kDa mass as measured by the marker. A purification of 26.3-fold with respect to the heparin chromatography was calculated by gel densitometry of the lanes loaded with the proteins after and before magnetic separation.

Binding Properties of the 25-kDa Polypeptide—As shown in Fig. 7*a*, the purified factor still retained a strong activity in EMSA, whereas the unbound contaminant proteins lost this property. In order to investigate if the 25-kDa factor recognizes any DNA curvature, the binding experiment was performed with the other curved region present in the rat mt genome upstream Ori-H (12). Indeed, this probe formed a complex,
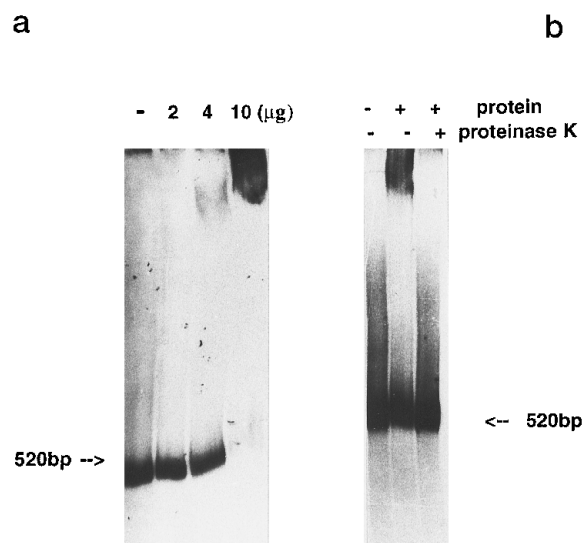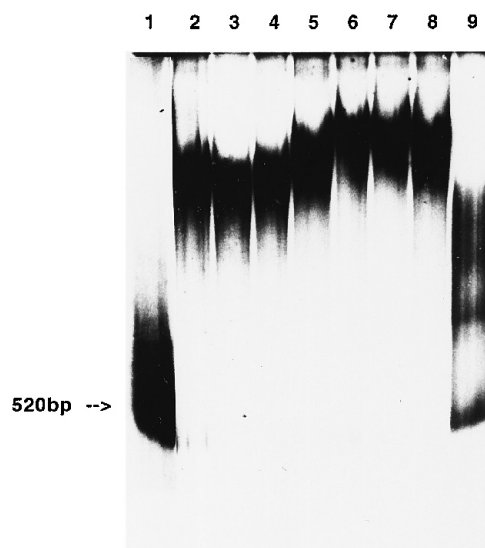

FIG. 4. **Stability of the protein-DNA complex.** The complex between 2 μg of the labeled 520-bp probe and 10 μg of the heparin-eluted proteins was performed and allowed to reach equilibrium in 30 min incubation. The binding reaction was allowed to continue in the presence of 100-fold molar excess of the unlabeled probe for periods of multiples of 30 min. Then the samples were simultaneously analyzed by a nondenaturing PAGE. *Lane 1*, free labeled probe; *lane 2*, complex at 30 min; *lanes 3–9*, complex in the presence of unlabeled homologous competitor, at different incubation times, up to a maximum of 3.5 h.

although it was a much less efficient target for this protein (Fig. 7*b*).

The binding capability of the 25-kDa factor was also tested on the two corresponding regions from the human mtDNA. In both cases a much lower intensity complex was observed (data not shown).

DISCUSSION

As far as the mtDNA replication and transcription is concerned the knowledge of regulatory factors is still poor and only a few protein factors have been purified (see Refs. 1 and 2, for review). Thus it is of great interest to determine the require-
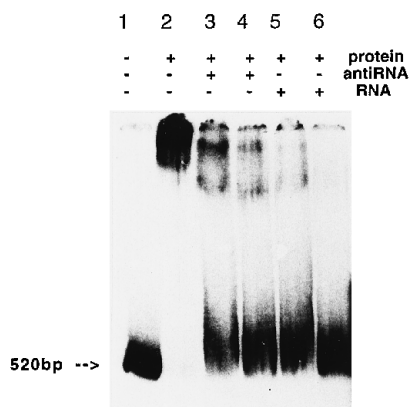
FIG. 5. **EMSA with competing riboprobes.** *Lane 1*, free labeled probe. Binding assay between 10 µg of the labeled 520-bp probe and 10 µg of the heparin-eluted proteins was carried out in the absence (*lane 2*) and in the presence of 100- and 200-fold molar excess of the cold anti-RNA (*lanes 3* and *4*) and RNA (*lanes 5* and *6*).
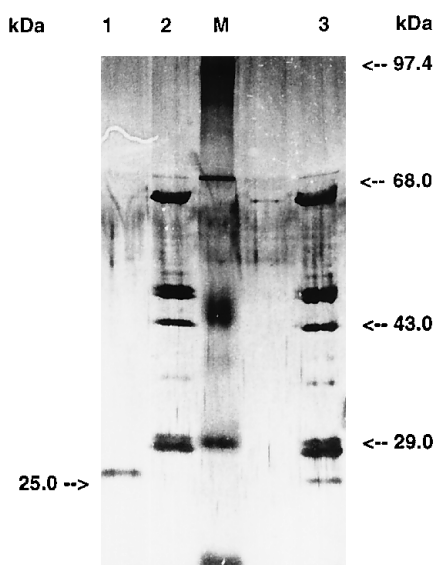

FIG. 6. **SDS-PAGE characterization of the proteins purified by magnetic affinity.** The gel (11% polyacrylamide, 0.1% SDS) was silver stained. *Lane 1*, polypeptide eluted with 1 M NaCl by the activated beads (one-tenth of the eluted volume); *lane 2*, contaminant unbound proteins (1/100 of the total volume); *lane 3*, pooled protein fractions eluted from heparin-Sepharose and used for magnetic purification (1/100 of the total). *M*, prestained marker proteins whose mass is reported on the *right*. On the *left*, the mass of the purified protein.

FIG. 7. **EMSA of the purified protein on 4% PAGE.** *a*: *lane 1*, free labeled 350-bp probe obtained by PCR; *lane 2*, binding with the contaminant protein discarded from magnetic affinity; *lane 3*, binding with the protein eluted by the magnetic beads. *b*: *lane 1*, free labeled 520-bp sequence containing the curvature upstream Ori-L (−) and the complex formed with this probe (+). *Lane 2*, free end labeled 270-bp sequence containing the curvature upstream Ori-H (−) and the faint complex formed with this probe (+). 1 ng of probe and 200 ng of the protein purified by magnetic affinity were used.

located in proximity of the origins of replication, could play a role in this process, via the recognition and binding of proteins that facilitate the positioning of the multienzyme replicative complex.

In a previous study we demonstrated the presence in rat mt genome of a curved DNA inside the main regulatory region, the D-loop containing region. This structure, localized close to Ori-H, was a conformational signal able to bind a specific 67-kDa protein with a presumptive regulatory role (13).

In human mtDNA a curvature was localized inside the ND2 gene, 310 bp upstream of Ori-L, which bound a protein factor of about 100 kDa isolated from mitochondria and the authors proposed that this complex could promote the initiation of replication at the correct site (23).

In the present paper we demonstrate in rat mtDNA the existence of a curvature in a different sequence of ND2 gene, over 600 bp upstream Ori-L. It corresponds to the 520-bp fragment of rat showing gel retardation (Fig. 2*b*) and excludes the human curved DNA. On the contrary, the region corresponding to the human curved DNA has normal migration (not shown). Indeed this sequence has a poor similarity in the two mammals (about 55%) and the An-Tn blocks, which are probably responsible for the curvature in human DNA, are not completely conserved in rat. As discussed under "Results," although our experiments did not localize exactly the locus of the curvature, we have good reason to believe it is roughly in the middle of the 520-bp fragment cloned in BS-A and near the 5′ end of its 350-bp subfragment, obtained by PCR.

The binding protein factor was isolated to an excellent degree of purification by magnetic affinity and appeared in the SDS-PAGE as a single polypeptide of 25 kDa. However, Figs. 4 and 5, that showed the intermediate complexes, suggest that more protein subunits could be involved in the curved DNA interaction. The existence of different co-migrating polypeptides is excluded by preliminary sequencing data which reveal only one NH$_2$ terminus.[2]

ment and role of structural elements and accessory protein factors involved in the molecular apparatus and mechanism of mitochondrial biogenesis and expression.

Here we provide evidence for another structural element at the level of Ori-L beside the stem-loop, namely a curved DNA which recognizes a nuclearly coded protein extracted from the rat mt matrix. We purified this protein to homogeneity and studied its possible functional role in the machinery of the L strand synthesis.

It has been reported by *in vitro* studies on human cells that the synthesis of the L strand is accomplished by the enzymes DNA primase and polymerase co-assisted by protein factors that interact with the stem-loop DNA structure of Ori-L. The evolutionary conservation of this secondary structure suggests it may play a functional role in vertebrate mt genomes (4). Furthermore, over the last years the importance of DNA higher-order structures and of specifically binding proteins in the regulation of molecular processes has emerged clearly. DNA regions with peculiar conformations, such as the curved DNA,
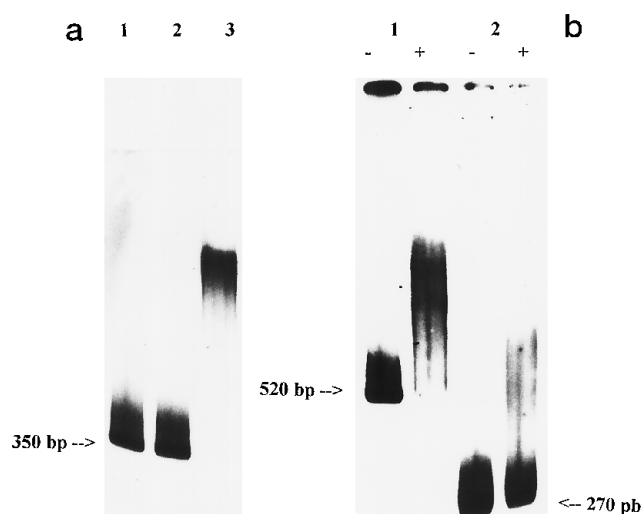
---

[2] G. Gadaleta, D. D'Elia, L. Capaccio, C. Saccone, and G. Pepe, unpublished data.

The data discussed above strongly support the need of a curvature near Ori-L to bind a protein factor required in the L strand replication mechanism. The position of this structure seems not to be constrained strictly and it is likely to have different locations, in different species, not too far upstream of Ori-L.

In order to functionally characterize the 25-kDa protein, we performed a series of binding assays. The dissociation rate experiment indicated a strong stability of the complex *in vitro*, compatible with its functional role. Other experiments using different probes showed that the protein recognizes the rat curved DNA close to Ori-H, and the human curved DNA within ND2 gene. The low efficiency of these bindings can be explained by a general capability of the protein to recognize a curved structure.

Furthermore, the two riboprobes synthesized by the curvature-containing DNA as the template, corresponding to the ND2-RNA and its antisense, were able to compete with the DNA probe in forming the complex with the protein component. The competition was complete when the higher amount of the ND2-RNA was used; the antisense only decreased the complex. The incomplete competition gave faster complexes (Fig. 5), again suggesting that a multimeric protein factor interacts with the curved DNA. The anti-RNA seems to interfere more generally with the complex through its secondary structure; whereas the binding of the sense-RNA to the protein seems to reflect a more specific recognition between the two molecules. Further studies will be necessary to clarify the functional role of these interactions.

Therefore, we can hypothesize that the replication of the L strand and the correct positioning of the DNA primase at the initiation site within Ori-L need an accessory protein, probably a multimeric one. A possible function for the 25-kDa protein in DNA replication is suggested by the proximity of its binding site to the L strand origin. By analogy with other genetic systems (25–27), this protein, when bound, could stall an advancing replication complex involving the newly synthesized H strand, by antagonizing the action of an associated DNA helicase (whose activity in mammalian mitochondria has been demonstrated so far in bovine (28)). This could facilitate the formation, on the displaced strand, of the priming structure for L strand initiation. Consequently, the local presence of DNA polymerase and other components of the replication machinery initiates efficiently the L strand synthesis.

In conclusion, the reported data are relevant in several respects. The importance of the structural elements in regulatory regions of mt genomes is stressed. The 25-kDa protein we isolated from rat mt matrix is the first purified factor that interacts at the level of Ori-L, with a presumptive regulatory function in the initiation of L strand replication. Finally, the different position of the curved structure in rat and human mtDNA and the isolation from the two systems of two proteins with different molecular weight offer new interesting topics to investigate mitochondrial and nuclear genome co-evolution in the biogenesis of mammalian organelles.

REFERENCES

1. Saccone, C., and Sbisà, E. (1993) in *Principles of Medical Biology* (Bittar, E. E., ed) Vol. 1B, pp. 37–70, Jai Press Inc., Greenwich, CT
2. Wong, T. W., and Clayton, D. A. (1985) *Cell* **42,** 951–958
3. Gray, H., and Wong, T. W. (1992) *J. Biol. Chem.* **267,** 5835–5841
4. Clayton, D. A. (1992) *Int. Rev. Cytol.* **141,** 217–232
5. Brown, G. G., Gadaleta, G., Pepe, G., Saccone, C., and Sbisà, E. (1986) *J. Mol. Biol.* **192,** 503–511
6. Gadaleta, G., Pepe, G., De Candia, G., Quagliariello, C., Sbisà, E., and Saccone, C. (1989) *J. Mol. Evol.* **28,** 497–516
7. Trifonov, E. N. (1985) *CRC Crit. Rev. Biochem.* **19,** 89–106
8. Lilley, D. (1986) *Nature* **320,** 487–488
9. Eisemberg, H. (1986) *Trends Biochem. Sci.* **11,** 350–351
10. Diekmann, S. (1987) *EMBO J.* **6,** 4213–4218
11. Mizuno, T. (1987) *Nucleic Acids Res.* **15,** 327–341
12. Pepe, G., Gadaleta, G., Palazzo, G., and Saccone, C. (1989) *Nucleic Acids Res.* **17,** 8803–8819
13. Gadaleta, G., D'Elia, D., Saccone, D., and Pepe, G. (1995) *Gene* (*Amst.*) **160,** 229–234
14. Plaskon, R. R., and Wartell, R. M. (1987) *Nucleic Acids Res.* **15,** 785–796
15. Greenawalt, J. W. (1974) *Methods Enzymol.* **31,** 310–323
16. Carey, J. (1991) *Methods Enzymol.* **208,** 103–117
17. Gabrielsen, O. S., Hornes, E., Korsnes, L., Ruet, A., and Øyen, T. B. (1989) *Nucleic Acids Res.* **17,** 6253–6267
18. Gabrielsen, O. S., and Huet, J. (1993) *Methods Enzymol.* **218,** 508–525
19. Laemmli, U. K. (1970) *Nature* **227,** 680–685
20. Diekmann, S., and Wang, J. C. (1985) *J. Mol. Biol.* **186,** 1–11
21. Koo, H. S., Wu, H. M., and Crothers, D. M. (1986) *Nature* **320,** 501–506
22. Hagermann, P. J. (1985) *Biochemistry* **24,** 7033–7037
23. Welter, C., Dooley, S., Zang, K. D., and Blin, N. (1989) *Nucleic Acids Res.* **17,** 6077–6086
24. Waddel, W. J., and Hill, C. (1956) *J. Lab. Clin. Med.* **48,** 311–314
25. Lee, E. H., Kornberg, A., Hidaka, M., Kobayashi, T., and Hoziuchi, T. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 9104–9108
26. Mayhook, A. G., Rinaldi, A. M., and Jacobs, H. T. (1992) *Proc. R. Soc. Lond. B.* **248,** 85–94
27. Qureshi, S., and Jacobs, M. T. (1993) *Nucleic Acids Res.* **21,** 2801–2808
28. Hehman, G. L., and Hauswirth, W. W. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 8562–8566

Isolation of a 25-kDa Protein Binding to a Curved DNA Upstream the Origin of the L Strand Replication in the Rat Mitochondrial Genome

Gemma Gadaleta, Domenica D'Elia, Lara Capaccio, Cecilia Saccone and Gabriella Pepe

Access the most updated version of this article at http://www.jbc.org/content/271/23/13537

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 28 references, 3 of which can be accessed free at
http://www.jbc.org/content/271/23/13537.full.html#ref-list-1