Botanical Studies (2011) 52: 137-144.

In vitro mitochondrial nucleoid DNA replication and transcription are affected by the associated mitochondrial membrane

Bao-Lin LI[1,2], Yih-Shan LO[1], and Hwa DAI[1,*]

[1]*Institute of Plant and Microbial Biology, Academia Sinica, Nankang, Taipei, Taiwan 11509, Republic of China*

(Received June 29, 2010; Accepted July 19, 2010)

ABSTRACT. Fractionation of lysed mitochondria of mung bean seedlings by discontinuous sucrose gradient centrifugation resulted in three major and two minor fractions of mitochondrial nucleoid-membrane complexes based on their sedimentation density. Noticeably different quantities of mitochondrial membrane were found to associate with the different mitochondrial nucleoid fractions, suggesting that mitochondrial DNA, mtDNA binding proteins and membrane may be constituted in a heterogeneous organization among those fractions and result in different sizes, shapes and densities. Moreover, mitochondrial nucleoid-membrane complexes from these five fractions displayed differential abilities of *in vitro* DNA replication/synthesis and transcription. This implies, for the first time, that some biological activities such as DNA replication/ synthesis or gene transcription of the mitochondrial nucleoids can be affected by the specific membranes associated with them.

Keywords: Mitochondrial membrane; Mitochondrial nucleoid-membrane complex; Mitochondrial nucleoids; mtDNA replication; mtDNA transcription; Mung bean.

INTRODUCTION

Mitochondrial nucleoids are relatively stable assemblies of mitochondrial nucleoproteins with mtDNA *in situ* (Nass et al., 1965, Kuroiwa, 1982, Stevens, 1981, Spelbrink et al, 2001). Purification of mitochondrial nucleoids has been accomplished by applying a moderate detergent treatment to purified mitochondria followed by gradient centrifugation (Suzuki et al., 1982; Miyakawa et al., 1987; Newman et al., 1996; Dai et al., 2005). Mitochondrial nucleoids isolated from a defined density in a 15% to 30% sucrose gradient following routine procedures (Miyakawa et al., 1987) exhibit a relatively homogeneously-sized population in higher plants (Dai et al., 2005). These mitochondrial nucleoids contain mitochondrial nucleoproteins and membrane components. Isolated mitochondrial nucleoids are self-sufficient in the processes of mtDNA replication and transcription (Kuroiwa, 1982; Miyakawa et al., 1996; Sakai et al., 2004; Dai et al., 2005). Recently, most studies on mitochondrial nucleoids have focused on the function of their binding proteins. The molecular functions of nucleoid proteins in humans and in yeast have been particularly well-studied (Newman et al., 1996; Wang and Bogenhagen, 2006; Bogenhagen et al., 2008; Spelbrink, 2010). However, the functional role played by the membrane component associated with mitochondrial nucleoids or mtDNA have been largely ignored. It was previously reported that the mitochondrial membrane plays an important role in mitochondrial DNA synthesis and mtDNA segregation (Echeverria et al., 1991; Boldogh et al., 2003; Meeusen and Nunnari, 2003). In bacteria, the ancestor of mitochondria, the initiation of chromosomal DNA replication depends on the specific association of chromosomal DNA with the membrane at a region close to the replication origin that is recognized by specific membrane proteins (Hoshino et al., 1987; Yung and Kornberg, 1988).

We have previously observed the membranes associated with mitochondrial nucleoids of mung bean seedlings under electron microscope. Moreover, the phospholipids compositions: cardiolipin (CL), phosphoglycerol (PG), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylcholine (PC) of the membranes of these mitochondrial nucleoids are similar to those of the whole mitochondria (Dai et al., 2005). Here we showed that the majority of the nucleoids derived from the detergent-treated mitochondria by the conventional processes were fractionated into five mitochondrial nucleoid-membrane complexes according to their sedimentation density. The amounts of mtDNA and membrane components of these five mitochondrial nucleoids-membrane complexes varied from one another. Moreover, the mitochondrial nucleoid-membrane complexes from each one of these five fractions displayed differential abilities of in vitro DNA replication/

[2]Current address: School of Chemistry and Material Science, Shaanxi Normal University, Xi'an, Shaanxi 710062, P.R. China.

*Corresponding author: E-mail: bodaihwa@gate.sinica.edu.tw; Tel: 886-2-27871176; Fax: 886-2-27838609.

synthesis and transcription. Taken together, we conclude that different organizations among mtDNA, mtDNA binding proteins and membrane components not only change the size, shapes or densities of mitochondrial nucleoid-membrane complexes, but also affect biological activities, such as DNA replication/synthesis and gene expression patterns, of these macromolecules. This is the first report demonstrating that the specific membrane components associated with mitochondrial nucleoids play an important role in mtDNA gene expression.

MATERIALS AND METHODS

Isolation of mitochondrial nucleoid-membrane complexes from mitochondria

Mitochondria were isolated from 3-day old etiolated mung bean seedlings (Vigna radiate*,* Tainan No. 5) as previously described (Dai et al., 1991).

Mitochondrial nucleoid-membrane complex fractions were then purified from mitochondria as described previously with some modifications (Dai et al., 2005). In brief, the purified mitochondrial pellet was suspended in NE2 buffer (7.5% sucrose, 20 mM Tris-HCl, pH 7.6, 2 mM EDTA, 0.8 mM spermidine, 7 mM 2-mercaptoethanol, 0.4 mM PMSF) to a final concentration of 5 mg protein/ml. Mitochondria were lysed by slowly adding 20% (w/v) NP-40 to a final concentration 0.5%. After remaining on ice for 5 min with gentle mixing, the lysate was centrifuged at 14,000 x *g* for 20 min to remove insoluble materials.

The clear supernatant (5 ml) was layered over five discontinuous sucrose gradients and prepared as follows: 5 ml steps containing 15% (w/v), 20% (w/v), 30% (w/v), 40% (w/v) and 50% (w/v) sucrose in buffer (20 mM Tris-HCl, pH 7.6, 1 mM EDTA, 1 mM spermidine, 7 mM 2-mercaptoethanol, 0.4 mM PMSF) and centrifuged at 46,000 x *g* for 1 h.

Mitochondrial nucleoid-membrane complexes were recovered from the supernatant lysate, bands at the 10-15%, 15-20%, 20-30%, 30-40%, 40-50% boundaries and pellet, followed by adding 2X volume of ice-cold gradient buffer and pelleting by 46,000 x *g* centrifugation for 1 h.

Thin-layer chromatographic phospholipid analysis of mitochondrial nucleoid-membrane complexes

Equivalent amounts of seven mitochondrial nucleoid-membrane fractions derived from 8 mg mitochondria were extracted with chloroform/methanol. Thin layer chromatography (Kieselgel 60 F_{254}, Merck) with the use of molybdenum blue (1.3% molybdenum oxide in 4.2 M sulfuric acid) spray reagent was performed exactly as described above (Dai et al., 2005).

Pulsed-field gel electrophoresis

Different mitochondrial nucleoid-membrane complex fractions were resuspended in equal volumes of the gradient buffer as described. An equal volume of 1% low-melting-point agarose (LGT) was added and the samples were kept at 42°C. The samples were loaded into molds and allowed to set on ice for 10 min. The plugs were treated with ESP (1 mg/ml proteinase K, 1% Sarkosyl, 0.5 M EDTA, pH 9) at 50°C overnight. Fresh ESP was replaced twice during incubation. The plugs were washed with ES buffer (1% Sarkosyl, 0. 5 M EDTA, pH 9) at 50°C for 2 h. After replacement with fresh ES buffer, pulse-field gel electrophoresis (PFGE) was performed at a 30-60 s pulse time (at the ratio A:B=1), 150 V (11.8 V/cm) on a 1.2% agarose gel for 24 h in 0.5X TBE buffer (45 mM Tris-borate, 1 mM EDTA). Gels were then stained with ethidium bromide and de-stained by washing, followed by conventional Southern blotting analysis. In mitochondrial nucleoid-membrane complex *in vitro* DNA synthesis analysis, an autoradiogram exposure was performed after the gels were dry. To count α-[^{32}p]-dCTP incorporation into newly synthesized wb- and fm-DNA, after PFGE the dried gel was sliced into well-bound and fast-moving fractions according to the autoradiography and then counted in a scintillation counter (Hewlett Packard). The amount of mtDNA in each mitochondrial nucleoid-membrane complex was measured by OD after purification of the DNA in each fraction. The ratio between wb- and fm-DNA was determined by counting the wb-DNA and fm-DNA fractions cut from the membrane after the Southern blot analysis.

Southern blot analysis

Southern blot analysis followed the conventional method (Sambrook et al., 1989). The probe used was pure mtDNA. The purity of the mtDNA was verified by PCR using primers that generate chloroplast or nuclear gene products. Neither chloroplast nor nuclear DNA was present in the probe mtDNA. A Cox3 gene fragment was generated from mitochondrial DNA by PCR using the primers: cx3-34U: gtagatccaagtccatggcct; cx3-458L: gcatgatgggcccaagttacggc; designed to generate a mitochondria-encoded *cox3* DNA product that was also used as a probe. The same results were obtained when total mtDNA was used as a probe.

In vitro DNA synthesis by mitochondrial nucleoid-membrane complexes

A method used for DNA synthesis by the membrane-associated high molecular weight complex of wheat mitochondria was used with modifications (Echeverria et al., 1991). Briefly, mitochondrial nucleoids (isolated from 2 mg of mitochondrial protein) were suspended in 800 µl of reaction buffer containing 20 mM $MgCl_2$, 25 mM KCl, 2 mM DTT, 0.1 mg/ml BSA, 50 mM Tris-HCl, pH 7.5, 2 mM ATP, 0.2 mM each of CTP, GTP and UTP, 50 µM each of dATP, dGTP and dTTP and 100 µCi of α-[^{32}p]-dCTP (3000 Ci/mmol) in Eppendorf tubes and incubated at 30°C for 30 min. The reaction was stopped by adding 100 µM of cold dCTP and 25 mM of EDTA. A 2X volume of cold gradient buffer was then added followed by

centrifugation at 46,000 x *g* for 1-2 h to pellet the mitochondrial nucleoid-membrane complexes. PFGE analysis was carried out as described above. The X-ray film was developed after drying the gel.

Mitochondrial nucleoid-membrane complex transcription *in vitro*

In vitro mitochondrial nucleoid-membrane complex transcription was adapted with some modifications from a mitochondrial RNA synthesis method (Martin et al., 1987; Dai et al., 2005). Seven mitochondrial nucleoid-membrane complex fractions prepared from 4 mg protein equivalent mitochondria as described in the above section were suspended in 300 µl reaction buffer including 10 mM Tris-base (pH 8.5), 5 mM $MgCl_2$, sucrose 0.25 M, 1 mM DTT, 0.1% BSA, 120 mM each of CTP, GTP and ATP, 90 µCi α-^{32}P-UTP and 100 units RNAsin, and then incubated at 25°C for 30 min before adding unlabeled UTP (final concentration: 20 µM) continuing to incubate at 25°C for 5 min. After stopping the reaction by adding SDS and CDTA to final concentrations of 1% and 30 mM, respectively, newly synthesized RNA was isolated and purified using phenol/chloroform/isoamyl alcohol. The RNA was analyzed with a standard MOPS gel containing formaldehyde (Dai et al., 2005). The newly-synthesized mitochondrial RNA was counted as described above.

RESULTS

The composition and amounts of phospholipids of mitochondrial nucleoid-membrane complexes derived from different sucrose gradient fractions

The phospholipid composition of mitochondrial nucleoid-membrane complexes derived from each one of the sucrose gradient fractions remained similar to that of the whole mitochondria (Figure 1). However, the amounts of phospholipids of mitochondrial nucleoid-membrane complexes varied from one sucrose gradient fraction to another (Figure 1). In general, the amounts of phospholipids of mitochondrial nucleoid-membrane complexes decreased with the increase of banding density of mitochondrial nucleoid-membrane complexes (Figure 1), suggesting that the diversity of sizes, shapes or densities among different fractions of mitochondrial nucleoid-membrane complexes may be caused by the different amounts of membrane components association with each fraction, respectively.

The mtDNA contents varied among mitochondrial nucleoid-membrane complexes with different densities from a sucrose gradient fractionation

Southern blot analysis of mitochondrial nucleoid-membrane complexes revealed that 36.6%, 25%, 17%, 8.9% and 4.55% of the original mtDNA was present, respectively, in the 15/20% sucrose boundary, lysate (7.5%)/15% sucrose boundary, the lysate layer (7.5% sucrose), 20-30% sucrose boundary and 30-40% boundary (Figure 2, Table 1). In addition, the ratios between the well-bound (wb) form and the fast-moving (fm) form of mtDNA also varied from one sucrose gradient fraction to another (Figure 2B). The highest ratio between wb form DNA to fm form DNA was found in the fraction of 15/20% sucrose boundary (Figure 2B).

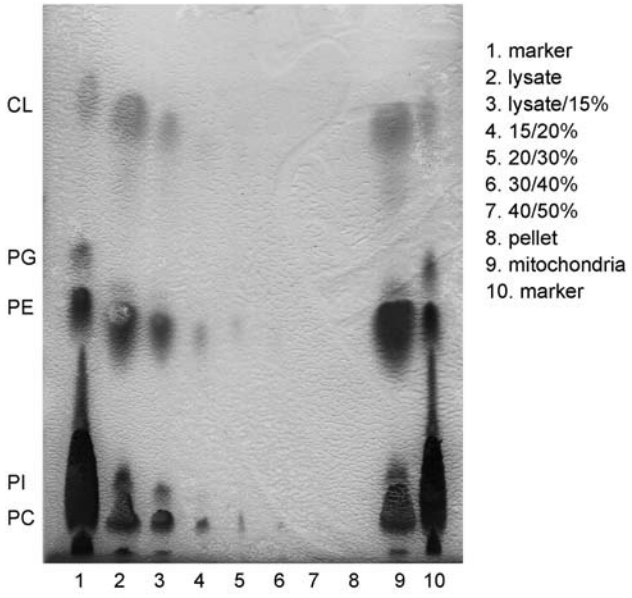

Figure 1. Phospholipid compositions of mitochondrial nucleoid-membrane complexes in different fractions after sucrose gradient centrifugation. Equivalent portions of each of the seven mitochondrial nucleoid-membrane complex fractions indicated on the right of the figure were analyzed by thin layer chromatography. A phospholipid sample extracted from the purified mitochondria containing 0.5 mg proteins was loaded in line 9 as the control. The phospholipid markers in lanes 1 and 10 are cardiolipin (CL), phosphoglycerol (PG), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylcholine (PC).

In vitro mtDNA replication by the different mitochondrial nucleoid-membrane complexes

In vitro mitochondrial nucleoid-membrane complex DNA replication was performed as described in Materials and Methods. After PFGE analysis, the complexes harvested from the lysate/15% sucrose boundary showed the most mtDNA synthesis, even though its mtDNA content was less than that in the 15/20% fraction (Figure 3 and Table 1). In addition, the wb to fm ratio of the newly synthesized mtDNA was much lower than that of the original template mtDNA (compare Figure 3, Panel B to Figure 2, Panel B). Self-sufficient mtDNA replication was found in all seven fractions, and even in the pellet, which was also able to accomplish mtDNA synthesis/replication. Newly synthesized mtDNA could barely be detected in the 40/50% boundary fraction. This result indicates that the mtDNA-protein-membrane complexes in these seven fractions carried the essential factors for DNA synthesis.

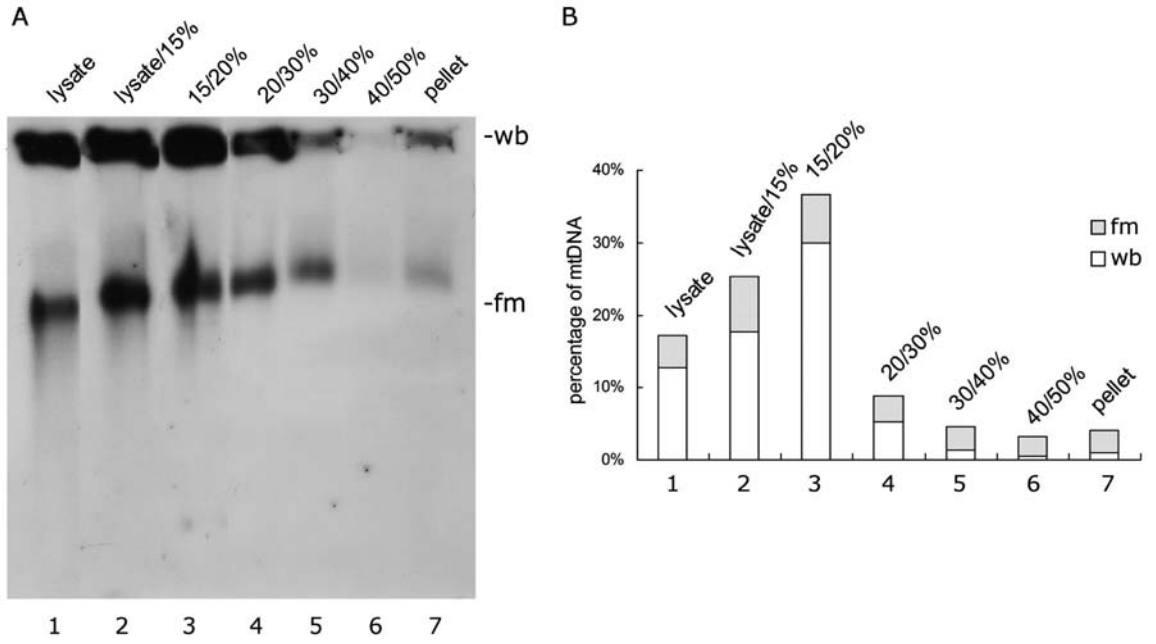

Figure 2. The amounts of mtDNA among different mitochondrial nucleoid-membrane complex fractions generated by sucrose gradient centrifugation. Panel A: Southern blot of mitochondrial nucleoid-membrane complex from various fractions generated by sucrose gradient centrifugation. Samples from the sucrose gradient fractions indicated above each lane were analyzed by pulse field gel electrophoresis followed by Southern hybridization with a probe derived from mtDNA. mtDNA migrated as the well-bound (wb) and fast-moving (fm) forms in the gel after electrophoresis. Panel B: Relative amount of mtDNA among the fractions after sucrose gradient centrifugation was determined by direct counting of the radioactivities of each fraction cut from the Southern blot membrane. Similar results were obtained from OD_{260} absorbance of the mtDNA purified from each fraction obtained from sucrose gradient. The relative amounts of mtDNA in wb and fm forms of each fraction, respectively, were measured by counting of the radioactivities cut from the Southern blot membrane.

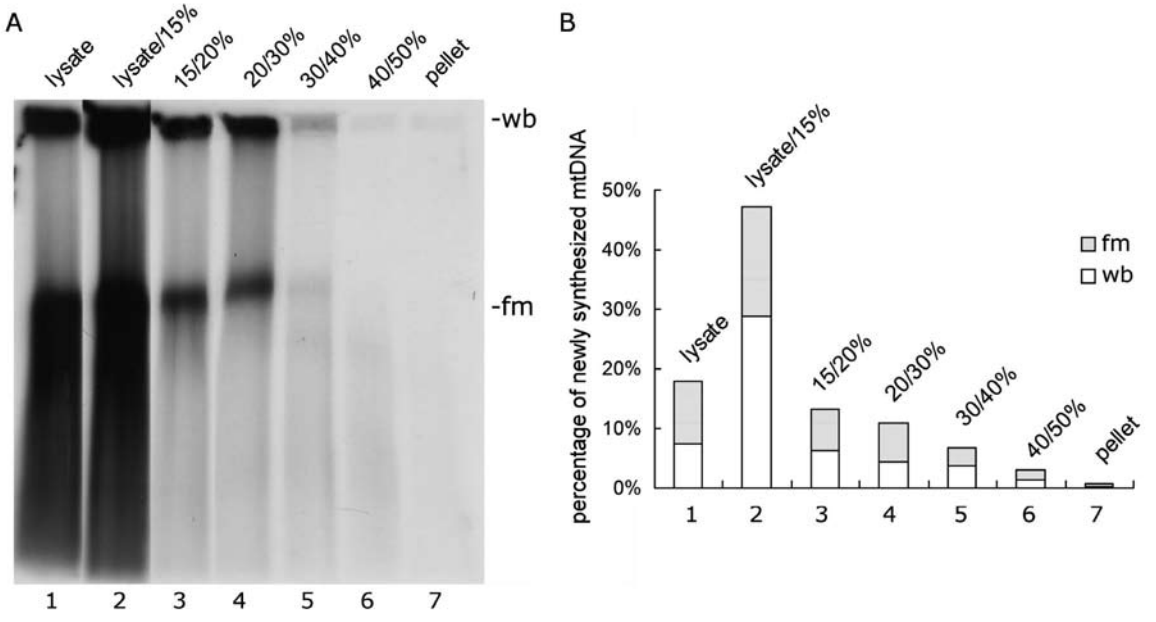

Figure 3. *In vitro* mtDNA synthesis by the different mitochondrial nucleoid-membrane complex fractions. Panel A: Autoradiograph of newly synthesized mtDNA of different mitochondrial nucleoid-membrane complexes fractionated by PFGE. Panel B: Percentage of newly synthesized mtDNA in each mitochondrial nucleoid-membrane complex. The radioactive counting was performed after cutting each lane and corresponding wb/fm from the gel.

Table 1. Comparative analysis of the relationship between mtDNA content and mitochondrial replication activity among the different mitochondrial nucleoid-membrane complex fractions.

Mitochondrial nucleoid-membrane complexes separated by sucrose gradient (SG) fractionation	Relative amount of mtDNA in each fraction (%)*	Relative amount of newly synthesized mtDNA in each fraction (%)**
Lysate	17.22	27.07
Lysate-15% SG	25.28	54.49
15-20% SG	36.60	7.31
20-30% SG	8.91	7.55
30-40% SG	4.55	2.30
40-50%SG	3.27	0.50
Pellet	4.17	0.77

*Determined by optical density of purified DNA and by radioactivity intensity of the corresponding lane cut from a Southern membrane. Similar results were obtained with the two techniques.
**Determined by counting the corresponding lane cut from the gel after PFGE.

For the first time in this research area, we found that it is not the conventional mitochondrial nucleoids (the band at the 15-30% boundary) that perform the best *in vitro* mtDNA replication. Instead, two less dense complexes containing less mtDNA but a higher amount of mitochondrial membrane show more active DNA replication capability (or completion of DNA replication).

Transcription by mitochondrial nucleoid-membrane complexes

The *in vitro* transcription activity of the mitochondrial nucleoid-membrane complexes appeared to be correlated with their associated mitochondrial membranes. The highest activity was found in the lysate fraction (26.21%, Figure 5 and Table 2), which was associated with the largest amount of mitochondrial membrane among the seven fractionated complexes. The transcriptional activity of each complex fraction decreased in parallel with the decline in the membrane content of the complex (Figure 5 and Table 2). Most of the RNA synthesized in each fraction showed a smear pattern and lacked the two distinguishing rRNA bands transcribed in mitochondria, as shown in Lane 10 of Figure 5. The smearing of the mitochondrial transcripts was most likely caused by RNA degradation during the lengthy time span required for mitochondrial nucleoid-membrane complex purification followed by *in vitro* transcription.

DISCUSSION

It is known that mtDNA binds nucleoproteins and forms mitochondrial nucleoids. Mitochondrial membranes are also associated with this macromolecular complex in higher plants (Dai et al., 2005). The conventional method for isolating mitochondrial nucleoids uses a suitable range of detergent concentrations to avoid the disruption of the organellar organization of the nucleoids (Kuroiwa, 1982; Miyakawa et al., 1996; Sakai et al., 2004; Dai et al., 2005). We demonstrated in this study that besides conventional mitochondrial nucleoids, which are density banded between 15% and 30% in sucrose gradients and have long been focused on, other fractions harvested from the same sucrose gradient at lower sedimentation densities showed even higher biological activity in terms of *in vitro* DNA

Table 2. Comparison of transcriptional activity among the different mitochondrial nucleoid-membrane complexes and its correlation with mtDNA content.

Mitochondrial nucleoid-membrane complexes separated by sucrose gradient (SG) fractionation	Relative amount of mtDNA in each fraction (%)	Percentage of newly transcribed mtRNA in each fraction (%)**
Lysate	17.22	26.21
Lysate/15% SG	25.28	21.95
15/20% SG	36.60	14.53
20/30% SG	8.91	12.89
30/40% SG	4.55	8.33
40/50% SG	3.27	7.39
Pellet	4.17	8.71

**Determined by incorporation counting and by counting the corresponding lane cut from the gel. Similar results were obtained with both techniques.

replication and RNA transcription. It is intriguing to find that these supra-molecular mtDNA complexes, containing less mtDNA but more mitochondrial membrane, can perform more active DNA replication and RNA transcription. The most prominent *in vitro* mtDNA synthesis was accomplished by the band of lysate/15% boundary (Figure 3, Panels A and B, lane 2), even though this fraction contained less mtDNA than the 15-20% fraction (Figure 4 and Table 1). It indicates that the activity of *in vitro* mtDNA replication and transcription are highly dependent on the membrane attached to mitochondrial-nucleoids. Decreasing of membrane content in mitochondrial nucleoid-complexes cause a decline in mitochondrial nucleoid replication and transcription (Figures 4-5 and Tables 1-2). It is most likely that the membranes associated with mtDNA-protein complexes lower the sedimentation density of the mitochondrial nucleoids during sucrose gradient fractionation.

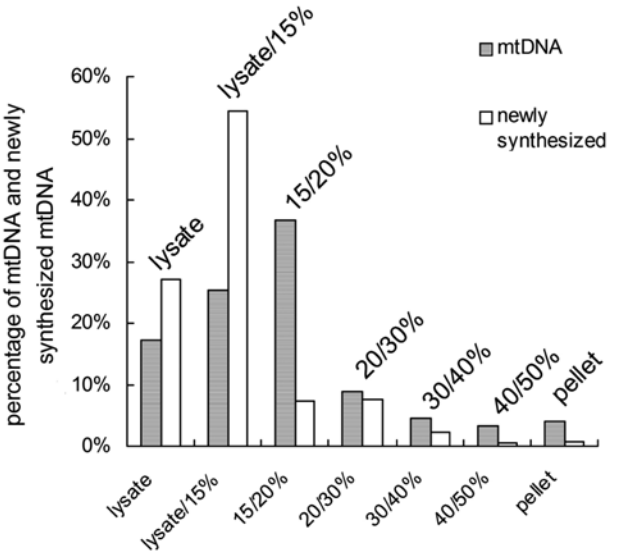

Figure 4. Correlation of mtDNA synthesis activity with mtDNA content in each mitochondrial nucleoid-membrane complex.

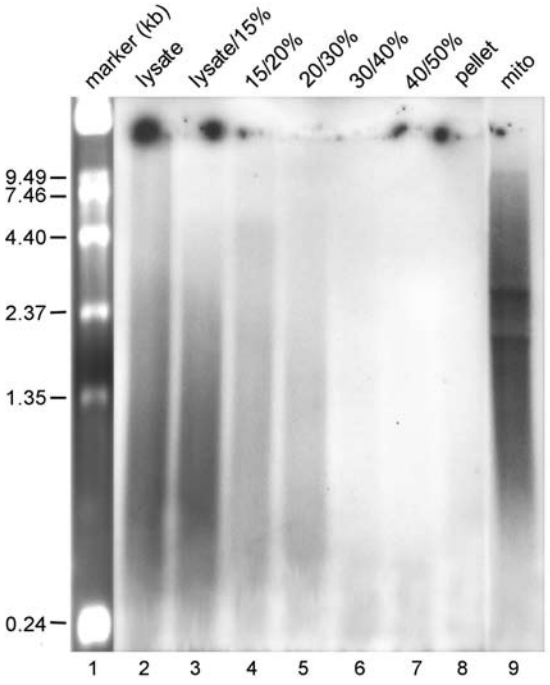

Figure 5. *In vitro* mitochondrial transcription by the different mitochondrial nucleoid-membrane complex fractions. Autoradiography of newly synthesized mitochondrial RNA is presented, and the products of *in organelle* mitochondrial transcription were run as a control in lane 9.

According to the TLC analysis shown in Figure 1, the phospholipid composition of the mitochondrial nucleoid-complexes is comprised of both outer and inner mitochondrial membrane, which mimic that of the whole mitochondria. This finding is consistent with other findings that mitochondrial DNA replication and transcription are associated with both the outer and inner membranes (Meeusen and Nunnari, 2003; Iborra et al., 2004; Dai et al., 2005).

As a moving picture of mtDNA, every mitochondrial nucleoid-membrane complex showed both well-bound (wb) and fast-moving (fm) components after pulse-field gel electrophoresis, as reported previously (Dai et al., 2005). Structural DNA that has a loose structure and greater molecular weight (>2500 kb) does not migrate into the gel and stays in the well (well-bound, wb); more compact circular or linear DNA with smaller sizes (50-200 kb) is fast-moving (fm) in the gel during pulse-field gel electrophoresis (see Figures 2A and 3A). It is clearly shown in Figure 3B that the newly-synthesized mtDNA contained less wb DNA than did the original mtDNA (compare Figure 3B with Figure 2B). We postulate that this phenomenon may be because fm DNA replication *in vitro* is much faster than that of wb DNA. Alternatively, it is also possible that conversion wb to fm DNA is more efficient *in vitro* than *in vivo*. Our previous results suggest that wb and fm mtDNA may undergo DNA replication independently and that the wb form may represent rolling circular replicating mtDNA molecules (before conversion to the fm form) that are too large to move in the pulsed electrical field. Alternatively, the immobility may be the result of an unusual mtDNA structure produced by multiple recombination events (Backert et al., 1996; Oldenburg and Bendich, 2001).

Taken together, these findings imply that there may be a defined relationship between the structural organization and the functional integrity of isolated mitochondrial nucleoids. Although the structural domains of the nucleoid membranes have yet to be characterized, more active *in vitro* DNA replication and RNA transcription are observed when larger amounts of membrane are attached to mitochondrial nucleoid-membrane complexes. We believe that the organization and conformation of a supra-molecular complex containing mtDNA, nucleoprotein and mitochondrial membranes plays an important role in mitochondrial DNA replication and RNA transcription.

Acknowledgments. This research was supported by research grants from the National Science Council and from Academia Sinica, ROC. BLL was supported by a NSC post-doctoral fellowship.

LITERATURE CITED

Backert, S., P. Dorfel, R. Lurz, and T. Borner. 1996. Rolling circle replication of mitochondrial DNA in the higher plant *Chenopodium album* (L.). Mol. Cell. Biol. **16:** 6285-6294.

Bogenhagen, D.F., D. Rousseau, and S. Burke. 2008. The layered structure of human mitochondrial DNA nucleoids. J. Biol. Chem. **283:** 3665-3675.

Boldogh, I.R., D.W. Nowakowski, H.C. Yang, H. Chung, S. Karmon, P. Royes, and L.A. Pon. 2003. A protein complex containing Mdm10p, Mdm12p, and Mmm1p links mitochondrial membranes and DNA to cytoskeleton-based segregation machinery. Mol. Biol. Cell **14:** 4618-4627.

Dai, H., Y.S. Lo, C.Y. Wu, C.L. Tsou, G.S. Hsu, C.G. Chern, M. Ruddat, and K.S. Chiang. 1991. Protein synthesis in isolated mitochondria of rice (*Oryza sativa* L.) seedlings. Plant Phys. **96:** 319-323.

Dai, H., Y.S. Lo, A. Litvinchuk, Y.T. Wang, W.N. Jane, L.J. Hsiao, and K.S. Chiang. 2005. Structural and functional characterizations of mung bean mitochondrial nucleoids. Nucleic Acids Res. **33:** 4725-4739.

Echeverria, M., D. Robert, J.P. Carde, and S. Litvak. 1991. Isolation from wheat mitochondria of a membrane-associated high molecular weight complex involved in DNA synthesis. Plant Mol. Biol. **16:** 301-315.

Hoshino, T., T. McKenzie, S. Schmidt, T. Tanaka, and N. Sueoka. 1987. Nucleotide sequence of *Bacillus subtillis* dnaB: a gene essential for DNA replication initiation and membrane attachemet. Proc. Natl. Acad. Sci. USA **84:** 635-675.

Iborra, F.J., H. Kimura, and P.R. Cook. 2004. The functional organization of mitochondrial genomes in human cells. BMC Biol. **2:** 9.

Kuroiwa, T. 1982. Mitochondrial nuclei. Inter. Rev. Cytology-a Survey Cell Biol. **75:** 1-59.

Martin, M.T., M. Echeverria, S. Litvak, and A. Araya. 1987. Studies of transcription in isolated wheat mitochondria and organelle extracts. Plant Sci. **49:** 199-207.

Meeusen, S. and J. Nunnari. 2003. Evidence for a two membrane-spanning autonomous mitochondrial DNA replisome. J. Cell Biol. **163:** 503-510.

Miyakawa, I., C. Okazaki-Higashi, T. Higashi, Y. Furutani, and N. Sando. 1996. Isolation and characterization of mitochondrial nucleoids from the yeast *Pichia jadinii*. Plant Cell Physiol. **37:** 816-824.

Miyakawa, I., N. Sando, S. Kawano, S. Nakamura, and T. Duroiwa. 1987. Isolation of morphologically intact mitochondrial nucleoids from the yeast, *Saccharomyces cerevisiae.* J. Cell Sci. **88:** 431-439.

Nass, M.M.K., S. Nass, and B.A. Afzelius. 1965. The general occurrence of mitochondrial DNA. Exp. Cell Res. **37:** 516-639.

Newman, S.M., O. Zelenaya-Troitskaya, P.S. Perlman, and R.A. Butow. 1996. Analysis of mitochondrial NDA nucleoids in wild-type and a mutant strain of *Saccharomyces cerevisiae* that lacks the mitochondrial HMG box protein Abf2p. Nucleic Acids Res. **24:** 386-393.

Oldenburg, D.J. and A.J. Bendich. 2001. Mitochondrial DNA from the liverwort marchantia polymorpha: circularly permuted linear molecules, head-to-tail concatemers, and a 5' protein. J. Mol. Biol. **310:** 549-562.

Sakai, A., H. Takano, and T. Kuroiwa. 2004. Organelle nuclei in higher plants: Structure, composition, function, and evolution. International Review of Cytology - a Survey of Cell Biology, Vol. 238. San Diego, Elsevier Academic Press Inc. **238:** 59-118.

Spelbrink, J.N. 2010. Functional organization of mammalian mitochondrial DNA in nucleoids: history, recent developments, and future challenges. IUBMB Life **62:** 19-32.

Spelbrink, J.N., F.Y. Li, V. Tiranti, K. Nikali, Q.P. Yuan, M. Tariq, S. Wanrooij, N. Garrido, G. Comi, L. Morandi, L. Santoro, A. Toscano, G.M. Fabrizi, H. Somer, R. Croxen, D. Beeson, J. Poulton, A. Suomalainen, H.T. Jacobs, M. Zeviani, and C. Larsson. 2001. Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene LF-like protein localized in mitochondria. Nature Genet. **28:** 223-231.

Stevens, B. 1981. Mitochondrial structure. *In* J.N. Strathern, E.W. Jones, and J.R. Broach (eds.), The Molecular Biology of The Yeast Saccharomyces: life cycle and inheritance. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press, pp. 471-504.

Wang, Y. and D.F. Bogenhagen. 2006. Human mitochondrial DNA nucleoids are linked to protein folding machinery and metabolic enzymes at the mitochondrial inner membrane. J. Biol. Chem. **281:** 25791-25802.

Yung, B.Y. and A. Kornberg. 1988. Membrane attachment activates dnaA protein, the initiation protein of chromosome replication in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **85:** 7202-7205.

粒線體 nucleoid DNA 複製和轉錄受其相連的粒線體膜系影響

李寶林　羅意珊　戴　華

中央研究院 植物暨微生物學研究所

這項研究顯示粒線體基因複製和轉錄除受粒線體核蛋白的影響外，與粒線體 nucleoid 關聯的特定膜元件可能也對粒線體基因的表達發揮重要作用。粒線體 nucleoids 可自給自足的行粒線體基因複製和轉錄。這研究將整個粒線體以特定方式溶解後，以常規間斷蔗糖梯度離心將其依密度的不同分為七組不同的 mitochondrial nucleoid-membrane complexes。這不同密度的 mitochondrial nucleoid-membrane complexes 含有明顯不同量的粒線體膜系。在行體外 mtDNA 複製和轉錄結果中，我們確定不同粒線體的複製和轉錄的能力深受粒線體的膜系含量影響。我們因而確認粒線體基因的表達受粒線體 DNA，粒線體核蛋白和粒線體膜系間的相關結構，組合關係密切。

關鍵詞：線粒體膜系；Mitochondrial nucleoid-membrane complex；線粒體 nucleoids；線粒體基因複製；線粒體基因轉錄；綠豆。