FEBS Open Bio 2 (2012) 145–150

journal homepage: www.elsevier.com/locate/febsopenbio

The manner in which DNA on transcription activation and inhibition

Ryo Furukawa [a,1], Yuma Yamada [a,1], Yuichi Matsushima [b], Yu-ichi Goto [b], Hideyoshi Harashima [a,*]

[a] Laboratory for Molecular Design of Pharmaceutics, Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan
[b] Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi, Kodaira, Tokyo 187-8502, Japan

ARTICLE INFO

Article history:
Received 9 May 2012
Revised 7 June 2012
Accepted 7 June 2012

Keywords:
Mitochondria
Mitochondrial transcription factor A (TFAM)
DNA packaging
Mitochondrial DNA (mtDNA)
Mitochondrial transcription

ABSTRACT

For successful mitochondrial transgene expression, an optimal packaging exogenous DNA is an important issue. We report herein on the effects of DNA packaged with mitochondrial transcription factor A (TFAM), which packages mitochondrial DNA (mtDNA), on the transcription process. Our initial findings indicated that the transcription of the TFAM/DNA complex was activated, when the complex was formed at an optimal ratio. We also found that TFAM has a significant advantage over protamine, a nuclear DNA packaging protein, from the viewpoint of transcription efficiency. This result indicates that TFAM can be useful packaging protein for exogenous DNA to achieve mitochondrial transgene expression.

1. Introduction

It has been reported that mutations and defects in the mitochondrial genome (mtDNA) can form the basis for a variety of human diseases [1,2]. Therefore, mitochondrial gene therapy and diagnosis would be expected to have substantial medical benefits. Mitochondrial transgene expression represents an attractive methodology for achieving such an innovative medical goal. However, successful examples of mitochondrial transgene expression have not been reported to date. On the other hand, many reports have appeared describing the use of a variety of applications for nuclear transgene expression, including cationic liposomes, cell-penetrating peptides, polycations, etc. [3]. The formation of nanoparticles between plasmid DNA (pDNA) and polycations have been extensively investigated by many researchers, because it is known that the manner in which pDNA is packaged with polycations has a substantial effect on gene expression [4,5]. Protamine is well known as a nuclear DNA condenser with a high transfection activity [6,7].

mtDNA is packaged with specific core proteins in a complex referred to as a mitochondrial nucleoid, which has been implicated, not only in replication and transcription but also in the maintenance of mtDNA [8–12]. Mitochondrial transcription factor A (TFAM), one of the major components of nucleoids [8,13], was initially identified as a factor that activates transcription on heavy and light strand mtDNA promoters (HSP and LSP) via sequence-specific binding [14–17]. TFAM also binds DNA non-specifically, a property that is essential for mtDNA packaging to play a crucial role in mtDNA maintenance [18–21]. The expression levels of TFAM and mtDNA are sufficiently interactive that the knockdown of TFAM results in mtDNA depletion, and a reduction in mtDNA copy number results in the degradation of TFAM [22,23]. Thus, TFAM largely contributes to mtDNA maintenance and would be expected to affect the replication and transcription.

Based on previous reports, we predicted that TFAM represents a potentially optimal DNA condenser for mitochondrial transgene expression. To validate this prediction, we compared DNA complexes produced using TFAM and protamine in terms of their physicochemical properties and transcription efficiency. We first prepared DNA complexes with TFAM and protamine at various molar ratios, and evaluated their physicochemical properties. We then evaluated the extent to which transcription was affected by the conformation of DNA packaged with DNA condensers using T7 RNA polymerase, an enzyme that is homologous to mitochondrial RNA polymerase [24,25]. We also compared the transcription efficiency of long circular DNA (pDNA, 6445 bp) and short linear DNA (1324 bp), to examine how the conformation in different-structured DNA packaged with TFAM affected transcription.

* Corresponding author. Fax: +81 11 706 4879.
E-mail address: harasima@pharm.hokudai.ac.jp (H. Harashima).
[1] These authors contributed equally to this work.

2211-5463
http://dx.doi.org/10.1016/j.fob.2012.06.001

2. Material and methods

2.1. Chemicals and materials

Protamine was purchased from Calbiochem (Darmstadt, Germany). T7 RNA polymerase, ribonuclease inhibitor and SYBR Green 2 were purchased from Takara (Shiga, Japan). The pTriEx-3 Neo vector (6445 bp) was obtained from Novagen (Madison, WI, USA). pDNA was purified using a Qiagen EndoFree Plasmid Mega Kit (Qiagen GmbH, Hilden, Germany). Ribonucleoside 5′-triphosphates were from GE Healthcare Bio-Sciences (Piscataway, NJ, USA). For the preparation of short linear DNA (1324 bp), pDNA was digested by *Tth111*I and *Xba*I, and subjected to agarose gel electrophoresis. Short linear DNA (1324 bp) was then extracted and purified using a QIAquick Gel extraction kit (Qiagen GmbH).

2.2. Purification of Recombinant TFAM

A fragment of human TFAM, corresponding to residues 43–246 (NCBI NP_003192) was amplified by PCR using the following pair of primers; 5′-ggggcatatgtcatctgtcttggcaagttg-3′ (forward) and 5′-ggggctcgagtctacttttaacactcctca-3′ (reverse). N-terminal protein region was reported to be mitochondrial targeting signal [26], and was, therefore, removed from our construct. To express human TFAM in *Escherichia coli*, the PCR fragment was cloned into pET-28a (Novagen) that had been cleaved with *Nde*I and *Xho*I. Bacterial cells harboring the plasmid were grown in LB medium containing 100 mg/L kanamycin, and expression was induced by treatment with 1 mM isopropylthiogalactoside. The N-terminal His tag was utilized for nickel-nitrilotriacetic acid affinity purification of the recombinant human TFAM according to the manufacturer's instructions (Qiagen GmbH). The purified protein was finally dialyzed against a buffer containing 10 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM dithiothreitol, and 20% glycerol. Protein concentrations were determined using a BCA protein assay kit (Pierce, Rockford, IL, USA).The purified protein was analyzed by SDS–PAGE to confirm that the recombinant human TFAM was purely prepared (Fig. S1).

2.3. Preparation of TFAM/DNA complex and protamine/DNA complex

For preparation of the DNA complex, DNA and TFAM (or protamine) solutions at various molar ratios were mixed in 10 mM HEPES buffer (pH 7.4) and the preparations then incubated at 25 °C for 30 min. Dynamic light scattering (DLS) was employed to determine the hydrodynamic diameters of the complexes in the suspension (Zetasizer Nano ZS; Malvern Instruments, Herrenberg, Germany). The ζ-average diameter (mean diameter) was calculated from a cumulants analysis based on the intensity of the scattered light. The ζ-potential, an indicator of surface potential, was determined electrophoretically using laser doppler velocimetry (Zetasizer Nano ZS).

2.4. Electrophoretic mobility shift assay of TFAM/DNA complex and protamine/DNA complex

To evaluate the packaging of DNA, TFAM/pDNA complex and protamine/pDNA complex were subjected to agarose gel electrophoresis before and after treatment with the polyanion preparation. The samples were incubated for 30 min at 25 °C with a polyanion, *i.e.*, a 1 mg/ml solution of poly (L-aspartic acid) (pAsp), to release the pDNA. A 0.1 μg sample of pDNA was subjected to electrophoresis. Electrophoresis was performed on a 1% agarose gel in TAE (40 mM Tris–HCl, 40 mM acetic acid, 1 mM EDTA, pH 8.0) at 100 V for 30 min. The gel was stained with EtBr and analyzed by ImageQuant LAS 4000 (GE Healthcare Bio-Sciences).

2.5. In vitro transcription assay

Transcription efficiencies of TFAM/DNA complex and protamine/DNA complex were evaluated by in vitro transcription assay using T7 RNA Polymerase as described below. DNA complexes containing pDNA (30 fmol) or short linear DNA (60 fmol) were incubated with T7 RNA Polymerase (17 U) at 37 °C for 2 h, in a reaction mixture (5.5 μL) containing incubation buffer (40 mM Tris–HCl, pH 8.0, 8 mM $MgCl_2$, 2.0 mM spermidine, 5.0 mM dithiothreitol), 1.0 mM ribonuclease inhibitor and the four ribonucleoside 5′-triphosphates (2 mM each). The reaction was terminated by the addition of 14.5 μL of blocking buffer (29.6 mM Mops, pH 7.0, 7.4 mM sodium acetate, 1.5 mM EDTA, 18.5% formaldehyde, 74.1% formamide) and then incubated at 65 °C for 15 min. The reaction mixtures were analyzed by 18% formaldehyde–1% agarose gel electrophoresis and stained with SYBR Green 2. Fluorograms were obtained with an ImageQuant LAS 4000 to evaluate transcription efficiency. Transcription efficiency was calculated as follows;

Transcription efficiency = intensity of the transcript band from samples/intensity of the transcript band from naked DNA.

2.6. Investigation of transcription inhibition by ultrafiltration and in vitro transcription assay

pDNA were packaged with TFAM at various molar ratios, and the resulting complexes were then subjected to ultrafiltration to remove unbound TFAM using Amicon Ultra Centrifugal Filters (14,000*g*, 25 °C,15 min). An in vitro transcription assay before and after ultrafiltration were performed, and as result, electrophoresis gel data of transcripts after in vitro transcription reactions were obtained (Fig. 4A). Relative transcription values were calculated as follows, the transcription efficiency of samples was normalized by maximum transfection efficiency among the samples (Fig. 4B).

3. Results

3.1. Physicochemical characteristics of TFAM/DNA complex and protamine/DNA complex

We first prepared pDNA complexes using several different molar ratios of pDNA (circular DNA, 6445 bp) and TFAM, or protamine that is a nuclear DNA packaging protein, which is a polycationic peptide rich in arginine residues [27]. Their particle sizes and ζ potentials are summarized in Fig. 1. The particle sizes of the pDNA complexes became smaller with increasing molar ratio in the case of both TFAM and protamine (Fig. 1A), however, major differences were observed in the case of ζ potentials (Fig. 1B). In the case of protamine/pDNA complex, as the molar ratio increased, negatively charged particles were converted into positively charged particles, with diameters of ~100 nm (Fig. 1, open diamonds). On the other hand, negatively charged particles were formed when TFAM packaged pDNA, and no positively charged ones were observed (Fig. 1, closed diamonds). These results suggest that DNA packaging between TFAM and protamine occur in a different manner.

3.2. Investigation of the stabilities of TFAM/DNA complex and protamine/DNA complex

We evaluated the stability of TFAM/pDNA complex and the protamine/pDNA complex by means of an electrophoretic mobility shift assay (Fig. 2). In this experiment, pDNA packaged with TFAM or protamine at various molar ratios, and the pDNA complexes were subjected to gel electrophoresis before and after treatment

with a counter polyanion (pAsp). A fluorescent band is observed by emitted as the result of EtBr intercalating with pDNA, when pDNA is released from the complex or is loosely packaged with TFAM (or protamine).

As shown in Fig. 2A, TFAM/pDNA complexes were detected by EtBr at any molar ratio in the absence of pAsp, although their mobilities were shifted to shorter values with increasing molar ratio (Fig. 2A, lanes 1–6). We also observed a similar tendency in the electrophoretic mobility shift of pDNA after pAsp treatment (Fig. 2A, lanes 7–12). On the other hand, the stability of the pDNA complex formed with protamine was quite different from that formed with TFAM (Fig. 2B). Protamine/pDNA complexes formed at molar ratios of 500 or more were undetectable by EtBr in the absence of pAsp (Fig. 2B, lanes 5 and 6), suggesting that pDNA is tightly packaged at high molar ratios. We also observed the release of pDNA from the protamine/pDNA complex after pAsp treatment (Fig. 2B, lanes 7–12), suggesting that electrostatic interactions between pDNA and protamine are major contributors to the packaging pDNA.

3.3. Comparison of the transcription efficiencies between TFAM/DNA complex and protamine/DNA complex

We examined the issue of how transcription efficiency was affected by the conformation of the pDNA packaged with TFAM (Fig. 3A). Transcription efficiency was estimated by using T7 RNA polymerase, an enzyme that is homologous to mitochondrial RNA polymerase [24,25]. The transcription from pDNA packaged with TFAM was activated in the optimal molar ratio, and an approximate twofold increase in transcription efficiency was found compared to the naked pDNA (Fig. 3A, closed diamonds). We also observed a decrease in the transcription efficiencies, when pDNA was packaged with TFAM at molar ratios of 500 and more. On the other hand, in the case of protamine, no activation of transcription was observed when pDNA was packaged at any molar ratio, and the transcription was progressively inhibited with increasing molar ratio (Fig. 3A, open diamonds).

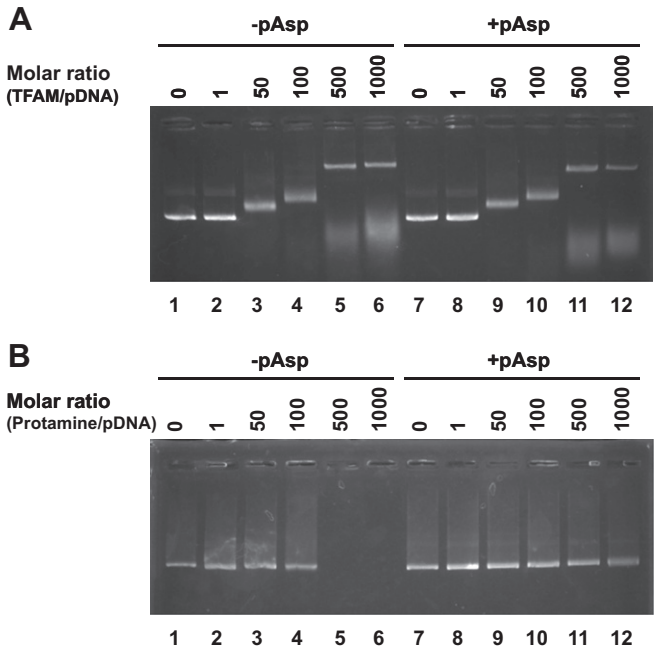

Fig. 2. Electrophoretic mobility shift assay of pDNA packaged with TFAM and protamine. Gel shift analysis of pDNA packaged with TFAM (A) and protamine (B). pDNA was packaged with TFAM or protamine at various molar ratios (molar ratio = 0; lane 1 and 7, 1; lane 2 and 8, 50; lane 3 and 9, 100; lane 4 and 10, 500; lane 5 and 11, 1000; lane 6 and 12), and then the resulting products were subjected to agarose gel electrophoresis with pAsp (lanes 1–6) or without pAsp (lanes 7–12).

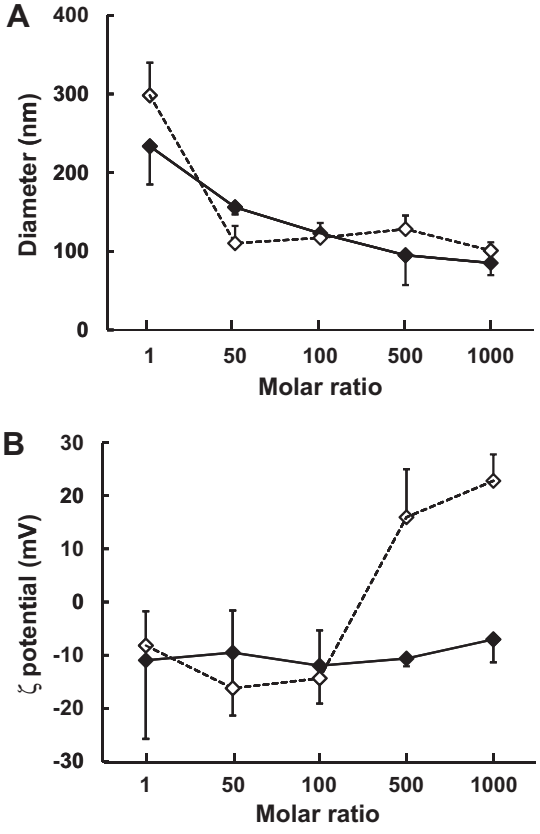

Fig. 1. Characteristics of pDNA packaged with TFAM or protamine. pDNA were packaged with TFAM (closed symbols) and protamine (open symbols) at various molar ratios, and their diameters (A) and ζ potentials (B) were then measured. Data are presented as the mean ± SD (n = 3).

3.4. Evaluation of transcription efficiency of different structured DNA packaged with TFAM

To determine whether the transcriptional activation of DNA packaged with TFAM is influenced by the structure of DNA, we compared the transcription efficiency of a TFAM/DNA complex in the case of pDNA (circular structure, 6445 bp) and short linear DNA (linear structure, 1324 bp) preparations. Activation and inhibition of transcription were observed in the case of both pDNA and short linear DNA, but the optimal molar ratio for activating transcription was different (Figs. 3A, B, closed symbols). The transcription efficiency of pDNA was the highest when pDNA was packaged at a molar ratio of 375 (Fig. 3A, closed diamonds), while, for short linear DNA, the highest value was observed at a molar ratio of 75 (Fig. 3B, closed triangles). The highest transcription efficiency of the TFAM/pDNA complex was 1.8-fold greater than that for naked pDNA, while the value for short linear DNA was 1.3-fold. A previous report showed that super coiled DNA was activated to a greater extent by TFAM than relaxed circular DNA [28]. It seemed that the conformational change for transcription activation might be limited to linear DNA. In the case of protamine, the transcription of the DNA complex was greatly inhibited and no activation was found in the case of both pDNA and short linear DNA (Fig. 3A, B, open symbols).

Based on the data shown in Fig. 3A, B (closed symbols), we converted the molar ratios to the base-pair intervals required to TFAM to bind to DNA (bp intervals) on the X-axis (Fig. 3C, closed

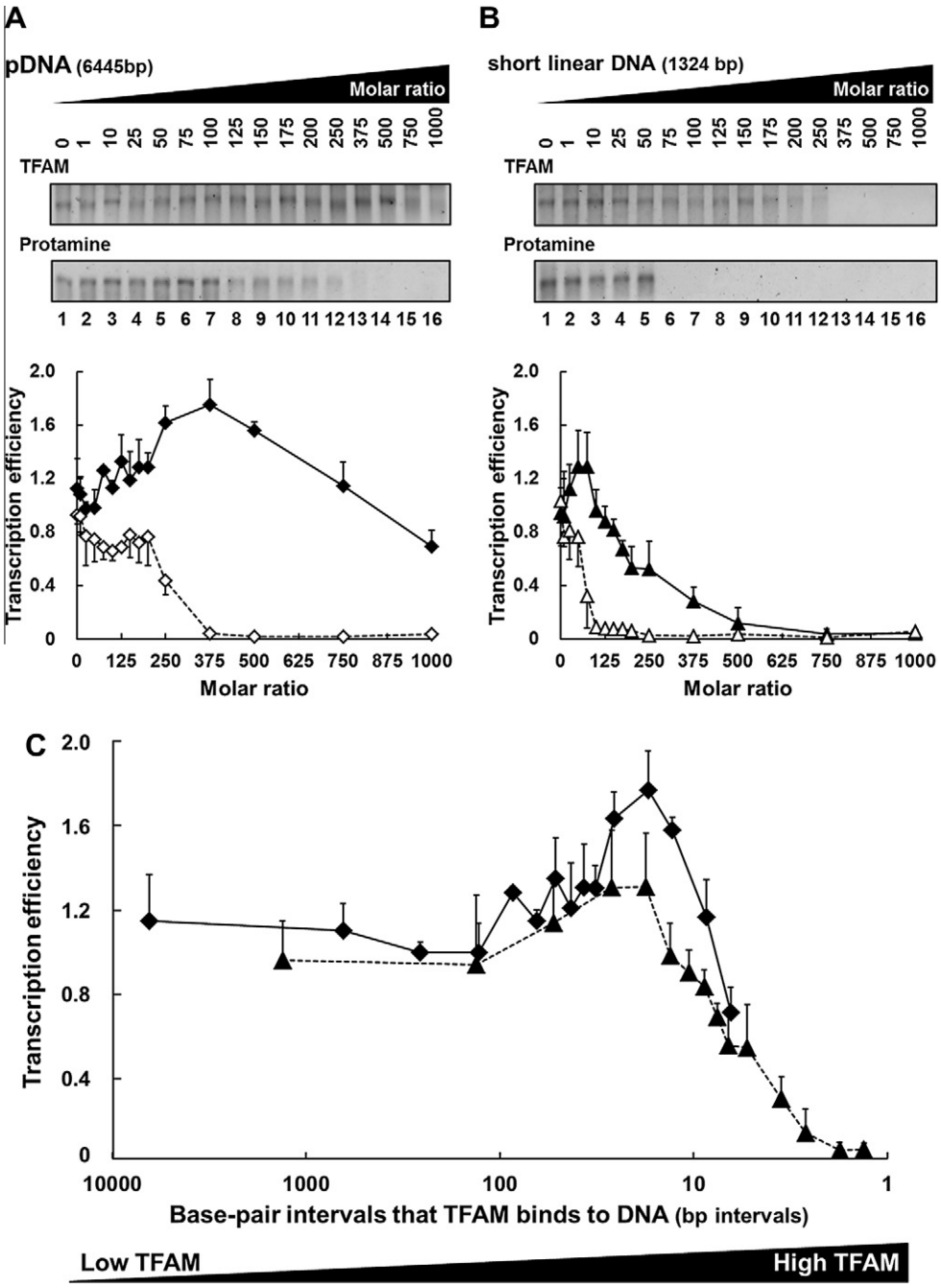

Fig. 3. In vitro transcription assay of DNA packaged with TFAM and protamine. pDNA (A, circular structure, 6445 bp) and short linear DNA (B, linear structure, 1324 bp) were packaged with TFAM and protamine at various molar ratios, and then subjected to an in vitro transcription assay. Electrophoresis gel data for transcripts after in vitro transcription reactions (upper part). Transcription efficiencies of DNA packaged with TFAM (closed symbols) and protamine (open symbols) were calculated based on the relative intensity of transcript band (lower part). Data are presented as the mean ± SD (n = 3). Based on the data shown in Fig. 3A, B (closed symbols), we converted the molar ratios to base-pair intervals that TFAM binds to DNA (bp intervals) in the X-axis (C). Closed diamonds and closed triangles indicate the values using pDNA and short linear DNA, respectively. Data are presented as the mean ± SD (n = 3).

diamonds, pDNA; closed triangles, short linear DNA).The base-pair intervals were calculated, when a DNA complex was formed at the optimal molar ratio for transcriptional activation (see Supplementary material for details).The values were calculated to be 17.1 and 17.6 in the case of pDNA and short linear DNA, respectively. These values are in general agreement with values previously reported by Kang and co-workers. They showed that one molecule of TFAM for every 20 bp activated transcription by in vitro transcription using mitochondrial RNA polymerase and mtDNA [21,28]. Based on this stoichiometric consideration, we speculated that transcription might be activated at the optimal DNA-conformation, when TFAM binds to DNA at the appropriate interval, as shown in Fig. 5A.

3.5. Investigation of transcription inhibition by excess TFAM using in vitro transcription assay

At high molar ratios, the transcription of DNA packaged with TFAM was inhibited (Fig. 3A, B). In such situation, a number of unbound TFAM molecules might interfere with the transcription reaction by T7 RNA polymerase. To validate this consideration, a

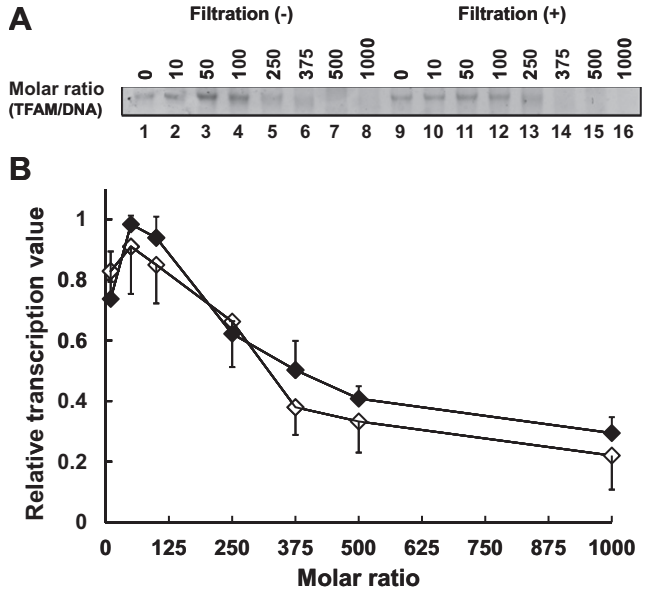

Fig. 4. In vitro transcription assay of a TFAM/pDNA complex before and after ultrafiltration. Electrophoresis gel data of transcripts after in vitro transcription reactions (A). pDNA were packaged with TFAM at various molar ratios (molar ratio = 0; lane 1 and 9, 10; lane 2 and 10, 50; lane 3 and 11, 100; lane 4 and 12, 250; lane 5 and 13, 375; lane 6 and 14, 500; lane 7 and 15, 1000; lane 8 and 16), and then subjected to in vitro transcription assay before ultrafiltration (lanes 1–8) and after ultrafiltration (lanes 9–16). Relative transcription value was calculated as follows (B), transcription efficiency of samples was normalized by maximum transfection efficiency among samples. Relative transcription values are indicated before (closed symbols) ultrafiltration and after (open symbols) ultrafiltration. Data are presented as the mean ± SD ($n = 3$).

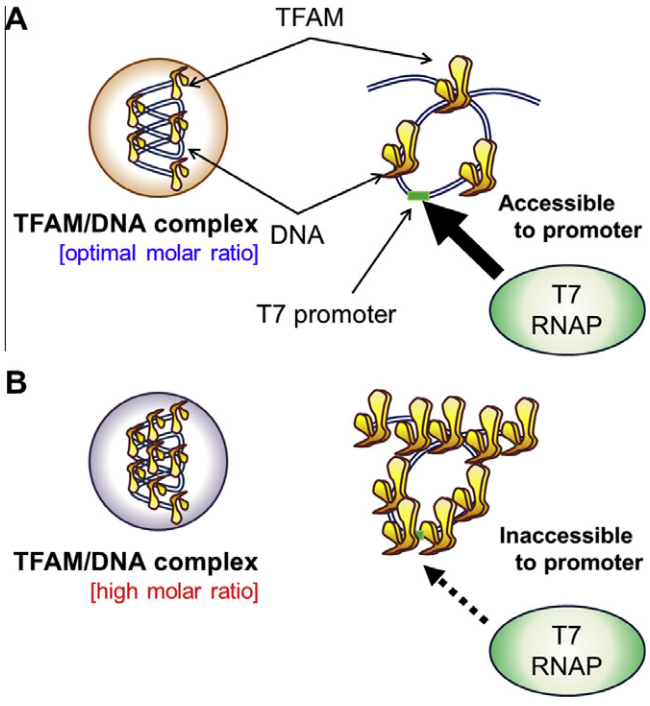

Fig. 5. Schematic illustration of DNA packaged with TFAM and the transcriptional manner. TFAM might form active DNA conformation for the transcription process, where DNA is bent or supercoiled for recruiting T7 RNA polymerase (T7 RNAP) (A). On the other hand, the overpackaging of DNA by excess TFAM would inhibit transcription, because T7 RNA polymerase might be inaccessible to the T7 promoter (B).

transcription assay using TFAM/pDNA complex was carried out after removing unbound TFAM by ultrafiltration. pDNA were packaged with TFAM at various molar ratios, and then subjected to in vitro transcription assay before and after ultrafiltration to remove unbound TFAM. Fig. 4A shows electrophoresis gel data of transcripts before ultrafiltration (Fig. 4A, lanes 1–8) and after ultrafiltration (Fig. 4A, lanes 9–16). Relative transcription value was normalized by maximum transfection efficiency among samples (Fig. 4B, closed diamonds, before filtration; open diamonds, after filtration).

If unbound TFAM interferes with the transcription reaction by T7 RNA polymerase, the transcription would be expected to be accelerated after the removal of unbound TFAM. However, the quantitative data showed that the transcription was inhibited to the same extent both before and after ultrafiltration (Fig. 4), indicating that transcriptional inhibition was not largely involved with the interference of transcription reaction by T7 RNA polymerase. This supports the view that the inhibition of transcription by TFAM is dependent on another factor, excess TFAM might bind to DNA and, as a result, decrease the recognition affinity of the T7 promoter by T7 RNA polymerase via the overpackaging of DNA (Fig. 5B).

4. Discussion

We first investigated the physicochemical properties of pDNA complexes when packaged with TFAM and protamine. In the case of protamine, negatively charged particles were converted into positively charged particles at a molar ratio of 500 (Fig. 1B, open diamonds). In this electrostatic inversion point, which is calculated to be a ± charge ratio of 2.4, excess protamine would contribute to the formation of positively charged particles. On the other hand, in the case of TFAM, the ζ potentials of the pDNA complexes were negatively charge at all molar ratios (Fig. 1B, closed diamonds). TFAM possesses high mobility group domains that contain both cationic and anionic amino residues [13,29]. Based on our results and this report, we conclude that the binding of cationic amino residues in TFAM to DNA is possible and, when this occurs, the anionic amino residues would be displayed on the surface of the TFAM/pDNA complex.

Electrophoretic mobility shift assay data showed that pDNA was efficiently released from a protamine/pDNA complex in the presence of pAsp (Fig. 2B, lanes 7–12), whereas pDNA release was not observed in the case of the TFAM/pDNA complex with pAsp treatment (Fig. 2A, lanes 7–12). It is presumed that pAsp might not be accessible to the TFAM/pDNA complex, because the TFAM/pDNA complex is negatively charged. In addition, EtBr was able to intercalate with the TFAM/pDNA complex, even at high molar ratios (Fig. 2A, lanes 5 and 6), although the protamine/pDNA complex was not detected under the same conditions (Fig. 2B, lanes 5 and 6). These results suggest that a TFAM/pDNA complex might be more loosely formed compared to a protamine/pDNA complex.

From the viewpoint of the unique structure of TFAM, we considered the reasons for why transcription is activated at specific conditions as shown in Fig. 3. One possibility is that TFAM might form a DNA conformation that is beneficial for activating transcription via non-specific binding, in which DNA would be easily recognized by T7 RNA polymerase, as shown in Fig. 5A. A recent crystallographic analysis of TFAM provides support for this possibility [29]. In the study, the crystal structure of full-length TFAM in complex with an oligonucleotide containing the LSP sequence was analyzed and the results suggest that TFAM causes the LSP to bend, thus creating an optimal DNA arrangement for transcriptional initiation. It was hypothesized that TFAM might play a role in the

structural change to strengthen the affinity of mitochondrial RNA polymerase for mtDNA, resulting in transcription activation.

Our findings indicated that DNA packaged with TFAM would have a great advantage over one packaged with protamine from the viewpoint of transcription efficiency. We also found that the TFAM could activate the transcription process with packaged DNA at the optimal molar ratio of DNA/TFAM. Moreover, a noteworthy point is that this study is the first trial to evaluate the effect of DNA packaged with TFAM on the transcription process using T7 RNA polymerase and DNA containing the T7 promoter. Collectively, our findings would largely contribute to the development of mitochondrial transgene methodology. In the future, we plan to investigate the function of DNA packaged with TFAM in mitochondria of living cells, using our mitochondrial matrix delivery system, a MITO-Porter that delivers cargoes into the mitochondrial matrix via membrane fusion [30–33]. Studies in this area are currently underway.

Acknowledgements

This work was supported, in part by, a Grant-in-Aid for Young Scientists (A) and a Grant-in-Aid for Scientific Research (S) from the Ministry of Education, Culture, Sports, Science and Technology of Japanese Government (MEXT), the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation, Japan (NIBIO). We thank Dr. Milton Feather for his helpful advice in writing the manuscript.

Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fob.2012.06.001.

References

[1] Wallace, D.C. (2005) A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. Annu. Rev. Genet. 39, 359–407.
[2] Suzuki, T. and Nagao, A. (2011) Human mitochondrial tRNAs: biogenesis, function, structural aspects, and diseases. Annu. Rev. Genet. 45, 299–329.
[3] Pichon, C., Billiet, L. and Midoux, P. (2010) Chemical vectors for gene delivery: uptake and intracellular trafficking. Curr. Opin. Biotechnol. 21, 640–645.
[4] Matsumoto, Y., Itaka, K., Yamasoba, T. and Kataoka, K. (2009) Intranuclear fluorescence resonance energy transfer analysis of plasmid DNA decondensation from nonviral gene carriers. J. Gene Med. 11, 615–623.
[5] Yamada, Y., Nomura, T., Harashima, H., Yamashita, A. and Yui, N. (2012) Post-nuclear gene delivery events for transgene expression by biocleavable polyrotaxanes. Biomaterials 33, 3952–3958.
[6] Li, S., Rizzo, M.A., Bhattacharya, S. and Huang, L. (1998) Characterization of cationic lipid–protamine–DNA (LPD) complexes for intravenous gene delivery. Gene Ther. 5, 930–937.
[7] Warrant, R.W. and Kim, S.H. (1978) Alpha-helix-double helix interaction shown in the structure of a protamine-transfer RNA complex and a nucleoprotamine model. Nature 271, 130–135.
[8] Kanki, T., Nakayama, H., Sasaki, N., Takio, K., Alam, T.I., Hamasaki, N. and Kang, D. (2004) Mitochondrial nucleoid and transcription factor A. Ann. N. Y. Acad. Sci. 1011, 61–68.
[9] Iborra, F.J., Kimura, H. and Cook, P.R. (2004) The functional organization of mitochondrial genomes in human cells. BMC Biol. 2, 9.
[10] Bogenhagen, D.F., Rousseau, D. and Burke, S. (2008) The layered structure of human mitochondrial DNA nucleoids. J. Biol. Chem. 283, 3665–3675.
[11] Gilkerson, R.W. (2009) Mitochondrial DNA nucleoids determine mitochondrial genetics and dysfunction. Int. J. Biochem. Cell Biol. 41, 1899–1906.
[12] Holt, I.J., He, J., Mao, C.C., Boyd-Kirkup, J.D., Martinsson, P., Sembongi, H., Reyes, A. and Spelbrink, J.N. (2007) Mammalian mitochondrial nucleoids: organizing an independently minded genome. Mitochondrion 7, 311–321.
[13] Gangelhoff, T.A., Mungalachetty, P.S., Nix, J.C. and Churchill, M.E. (2009) Structural analysis and DNA binding of the HMG domains of the human mitochondrial transcription factor A. Nucleic Acids Res. 37, 3153–3164.
[14] Fisher, R.P. and Clayton, D.A. (1988) Purification and characterization of human mitochondrial transcription factor 1. Mol. Cell. Biol. 8, 3496–3509.
[15] Dairaghi, D.J., Shadel, G.S. and Clayton, D.A. (1995) Addition of a 29 residue carboxyl-terminal tail converts a simple HMG box-containing protein into a transcriptional activator. J. Mol. Biol. 249, 11–28.
[16] Fisher, R.P., Lisowsky, T., Parisi, M.A. and Clayton, D.A. (1992) DNA wrapping and bending by a mitochondrial high mobility group-like transcriptional activator protein. J. Biol. Chem. 267, 3358–3367.
[17] Scarpulla, R.C. (2008) Transcriptional paradigms in mammalian mitochondrial biogenesis and function. Physiol. Rev. 88, 611–638.
[18] Kang, D., Kim, S.H. and Hamasaki, N. (2007) Mitochondrial transcription factor A (TFAM): roles in maintenance of mtDNA and cellular functions. Mitochondrion 7, 39–44.
[19] Pohjoismaki, J.L., Wanrooij, S., Hyvarinen, A.K., Goffart, S., Holt, I.J., Spelbrink, J.N. and Jacobs, H.T. (2006) Alterations to the expression level of mitochondrial transcription factor A, TFAM, modify the mode of mitochondrial DNA replication in cultured human cells. Nucleic Acids Res. 34, 5815–5828.
[20] Kaufman, B.A., Durisic, N., Mativetsky, J.M., Costantino, S., Hancock, M.A., Grutter, P. and Shoubridge, E.A. (2007) The mitochondrial transcription factor TFAM coordinates the assembly of multiple DNA molecules into nucleoid-like structures. Mol. Biol. Cell 18, 3225–3236.
[21] Kukat, C., Wurm, C.A., Spahr, H., Falkenberg, M., Larsson, N.G. and Jakobs, S. (2011) Super-resolution microscopy reveals that mammalian mitochondrial nucleoids have a uniform size and frequently contain a single copy of mtDNA. Proc. Natl. Acad. Sci. U S A 108, 13534–13539.
[22] Matsushima, Y., Goto, Y. and Kaguni, L.S. (2010) Mitochondrial Lon protease regulates mitochondrial DNA copy number and transcription by selective degradation of mitochondrial transcription factor A (TFAM). Proc. Natl. Acad. Sci. U S A 107, 18410–18415.
[23] Seidel-Rogol, B.L. and Shadel, G.S. (2002) Modulation of mitochondrial transcription in response to mtDNA depletion and repletion in HeLa cells. Nucleic Acids Res. 30, 1929–1934.
[24] Nayak, D., Guo, Q. and Sousa, R. (2009) A promoter recognition mechanism common to yeast mitochondrial and phage t7 RNA polymerases. J. Biol. Chem. 284, 13641–13647.
[25] Shutt, T.E. and Gray, M.W. (2006) Bacteriophage origins of mitochondrial replication and transcription proteins. Trends Genet. 22, 90–95.
[26] Claros, M.G. and Vincens, P. (1996) Computational method to predict mitochondrially imported proteins and their targeting sequences. Eur. J. Biochem. 241, 779–786.
[27] Sorgi, F.L., Bhattacharya, S. and Huang, L. (1997) Protamine sulfate enhances lipid-mediated gene transfer. Gene Ther. 4, 961–968.
[28] Fukuoh, A., Ohgaki, K., Hatae, H., Kuraoka, I., Aoki, Y., Uchiumi, T., Jacobs, H.T. and Kang, D. (2009) DNA conformation-dependent activities of human mitochondrial RNA polymerase. Genes Cells 14, 1029–1042.
[29] Rubio-Cosials, A. et al. (2011) Human mitochondrial transcription factor A induces a U-turn structure in the light strand promoter. Nat. Struct. Mol. Biol. 18, 1281–1289.
[30] Yasuzaki, Y., Yamada, Y. and Harashima, H. (2010) Mitochondrial matrix delivery using MITO-Porter, a liposome-based carrier that specifies fusion with mitochondrial membranes. Biochem. Biophys. Res. Commun. 397, 181–186.
[31] Yamada, Y. et al. (2008) MITO-Porter: a liposome-based carrier system for delivery of macromolecules into mitochondria via membrane fusion. Biochim. Biophys. Acta 1778, 423–432.
[32] Yamada, Y., Furukawa, R., Yasuzaki, Y. and Harashima, H. (2011) Dual function MITO-porter, a nano carrier integrating both efficient cytoplasmic delivery and mitochondrial macromolecule delivery. Mol. Ther. 19, 1449–1456.
[33] Yamada, Y. and Harashima, H. (2012) Delivery of bioactive molecules to the mitochondrial genome using a membrane-fusing, liposome-based carrier, DF-MITO-Porter. Biomaterials 33, 1589–1595.