Induction of chromatin condensation by nuclear expression of a novel arginine-rich cationic protein genetically engineered from the enhanced green fluorescent protein

（アルギニンに富む人工タンパク質の核局在発現とクロマチン構造変換誘導に関する研究）

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【背景・目的】

細胞増殖・分化・生存・死・発癌などの高次生命現象は‘細胞核’により制御される。核内 DNA は、ヒストンに代表される核内タンパク質と結合し機能的に折り畳まれることで、クロマチンと呼ばれる高次構造を形成している。クロマチン構造はダイナミックに組織化されており、分子密度が低く転写活性を示す‘ユークロマチン領域’と分子密度が高く遺伝子サイレンス領域である‘ヘテロクロマチン領域’とに動的に構造変換することで、その遺伝子機能の on-off が厳密に制御されている。

クロマチン構造は核内タンパク質の正電荷と DNA のもつ負電荷によって成立しており、構造制御には核内の電荷の均衡が重要である。本研究では、オワンクラゲ由来緑色蛍光タンパク質変異体 EGFP (Enhanced Green Fluorescent Protein) をコードする遺伝子をフレームシフトさせると核内タンパク質と同様に正電荷に富んだタンパク質をコードする遺伝子になることが予想されたことから、人工的に塩基性に富んだタンパク質を作製することとした。作製した塩基性タンパク質を細胞核内に発現させ、細胞核及びクロマチン構造への影響を解析することで、クロマチン構造変換誘導機構の解明を目指した。

【方法・結果】

1）アルギニンに富んだ人工タンパク質の作製

EGFP 遺伝子の開始コドンより 30 番目及び 31 番目のヌクレオチドを削除し、フレームシフト変異体を作製した。本変異体を構成するアミノ酸の 31%が塩基性アミノ酸であり、特にアルギニンに富んでいることから、Arginine-rich cationic protein (以下、Arg-CAP) と名付けた。Arg-CAP はアルギニンのクラスター配列を多数有しており、これらは核移行シグナルとして機能していることがデータベース解析により予想された。

2）Arg-CAP の核局在とクロマチン凝縮の誘導

培養細胞株 COS-1 細胞において、FLAG エピトープタグした Arg-CAP (Arg-CAPF) が主に核内に局在することが免疫蛍光染色法を用いた共焦点レーザー顕微鏡の結果から明らかとなった (Fig.1)。

また、高倍率での顕微鏡観察により、Arg-CAPF 発現細胞の核ではクロマチン凝縮が誘導されている

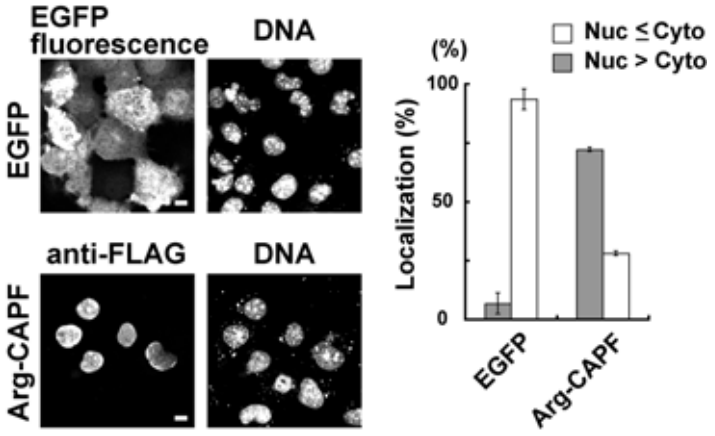

Fig.1. EGFP 及び Arg-CAPF の局在
DNA 染色された場所が核を表す．EGFP は主に細胞質に局在する.

ことが分かった（Fig.2）。フローサイトメトリーを用いた細胞周期解析により、本凝縮は通常の培養細胞で見られる細胞分裂期、及びアポトーシスに伴うクロマチン凝縮ではないことが分かった。核内における Arg-CAPF の局在部位を詳しく見ると、DNA 染色像と一致しないことから、クロマチン間領域（インタークロマチン）に局在していることが示唆された（Fig.2）。

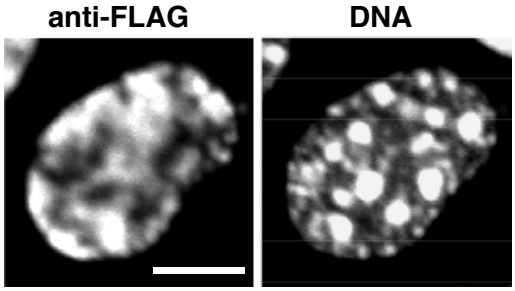

Fig.2. Arg-CAPF の核内局在部位とクロマチン凝縮
Arg-CAPF によって誘導されたクロマチン凝縮. anti-FLAG 抗体染色で表される Arg-CAPF の局在部位は DNA 染色部位とは異なる.

3）アルギニンクラスター配列によるクロマチン凝縮

Arg-CAPF によるクロマチン凝縮に、アルギニンのクラスター配列が寄与していると予想し、クラスター配列の一つ（RRRKR 配列、R：アルギニン、K：リジン）に GFP を融合させた RRRKR-GFP 変異体を作製し、発現細胞の核内構造を観察した。

RRRKR-GFP 発現細胞では、Arg-CAP と同様にクロマチン凝縮が誘導されており、また、驚く事に R を塩基性アミノ酸である K に入れ替えた KKKRK-GFP 変異体ではクロマチン凝縮が誘導されなかった（Fig.3）。このことから、クロマチン凝縮にはアルギニンのクラスター配列が寄与していることが示された。単に正電荷アミノ酸のクラスターがあればクロマチン凝縮が誘導される訳ではなく、アルギニンというアミノ酸がクラスターをつくる事がクロマチン凝縮に重要であることが判明した。

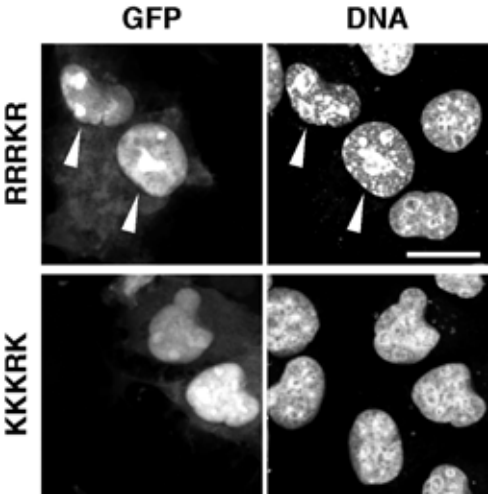

Fig.3. アルギニンクラスター配列による クロマチン凝縮
Arrow-head:クロマチン凝縮した細胞.

4）核骨格における RNA と Arg-CAPF の結合

アルギニンは RNA との結合能を有することが知られている。近年、RNA は遺伝子情報を司るだけでなく、クロマチン間領域に存在する様々な核内小器官や構造体の構成成分の一つとなり、核内構造の制御や維持にも寄与していることが報告されている。そこで、細胞核を高塩濃度溶液による抽出、及び DNase 処理して DNA を消化して核骨格分画を調整すると、RNA と Arg-CAPF との共局在が観察された。また、RNase 処理により RNA を消化すると Arg-CAPF タンパク質の存在も消失した（Fig.4）。従って、RNA 消化により、RNA と結合していた Arg-CAPF も同時に細胞核から洗い流されたものと考えられ、核骨格分画における RNA と Arg-CAPF との結合が示唆され、核内での RNA-Arg-CAPF 複合体の形成がクロマチン構造に影響を与えるものと推測された。

【総括】

本研究の結果より、EGFP 遺伝子をフレームシフトさせることで作製した、アルギニンに富んだ人工タンパク質は核内のクロマチン間領域に局在し、クロマチン凝縮を誘導することが明らかとなった。クロマチン凝縮にはアルギニンクラスター配列が関与しており、クロマチン間スペース領域に存在する核内 RNA に結合し作用することによって、クロマチン構造変換を誘導していることが示唆された。

クロマチンのダイナミックな構造変換は遺伝子発現やエピジェネティックな遺伝子制御に関与している。そして、クロマチン凝縮は細胞周期進行の過程、細胞分化、細胞老化、アポトーシスなどで観察される。現在、クロマチンの凝縮と脱凝縮は、ヒストンタンパク質のアセチル化、メチル化、ユビキチン化、リン酸化など、いわゆるヒストン修飾反応により制御されていることが分かってきた。さらに、核内には機能未知の RNA が豊富に存在していることが知られてきた。

従って、本研究で作製した人工タンパク質を新たなツールとして用いることで、RNA による核内構造変換及びクロマチン構造変換制御メカニズムの解明に繋がることが期待される。

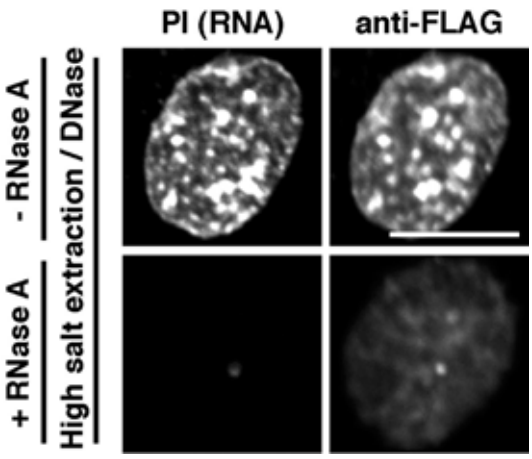

Fig.4. 核骨格分画における Arg-CAPF と RNA の結合

高塩濃度溶液による抽出と DNase 消化後に anti-FLAG 抗体を用いて Arg-CAPF を免疫染色. RNase 未処理では、PI (propidium iodide) に染まる核酸と Arg-CAPF が共局在していた. しかしながら、DNA と特異的に反応する DAPI(4',6-diamidino-2-phenyl-indole)では染色されなかったことから DNase 消化は十分であったこと、及び、RNase 処理により PI 染色が消失したことから、PI で染色されているのは RNA であると考えられる.

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Abstract

In the interphase nuclei of cultured cells, chromatin is compacted and organized higher-order structures through the condensation and decondensation processes. Chromosomes in the interphase nucleus are known to occupy distinct territories. The chromosome territory-interchromatin compartment model premises that the interchromatin compartment is separated from compact higher-order chromatin domains and expands in between these chromatin-organized territories. Chromatin in cultured cells is compacted under some conditions, such as the stress of heat shock and high osmolarity, and Src-mediated nuclear tyrosine phosphorylation. We report here that a novel arginine-rich cationic protein is created by frameshift mutation of enhanced green fluorescent protein (EGFP). The arginine-rich cationic protein is highly hydrophilic and contains potential arginine-based nuclear localization signals. Expression of the arginine-rich cationic protein shows its predominant localization to the nucleus and induces striking chromatin condensation in the interphase, which might be involved in interchromatin spacing or euchromatinization. Thus, the arginine-rich cationic protein as a new tool would be useful for dissecting chromatin architecture dynamics.

Introduction

A striking feature of nuclear architecture is the existence of distinct structural and functional compartments. In the interphase nuclei of cultured cells, chromatin is compacted and organized higher-order structures through the condensation and decondensation processes (Horn and Peterson 2002). Chromosomes are dynamically organized as distinct territories in the interphase nucleus and gene activation or silencing is often associated with repositioning of the genomic regions in nuclear space (Spector 2003; Lanctôt et al. 2007). Although nuclear compartmentalization, chromatin accessibility, and spatial sequestration of genes and their regulatory factors serve to modulate the output and functional status of genomes, the principles of the cellular organization of genomes and reorganization of nuclear architecture are still elusive (Misteli 2007).

Proteins destined for transport into the nucleus contain amino acid targeting sequences called nuclear localization signals (NLSs). The classical NLS consist of either one (monopartite) or two (bipartite) stretches of basic amino acids. Monopartite and bipartite NLSs

are exemplified by the SV40 large T antigen NLS (PKKKRKV) and the nucleoplasmin NLS (KRPAATKKAGQAKKKK), respectively (Dingwall and Laskey 1991). Even though lysine-rich sequences generally serve as effective NLSs, the HIV Tat-NLS (RKKRRQRRR) is a semiconsensus arginine-rich motif and is found in several proteins, including the HIV-1 Rev protein (Truant and Cullen 1999; Cardarelli et al 2008).

The green fluorescent protein (GFP) was originally identified from the jellyfish *Aequorea victoria* and cloned GFP has subsequently been modified to an enhanced, humanized version of GFP (enhanced green fluorescent protein EGFP, Clontech Laboratories) (Tsien 1998), which is often used to tag a target protein of interest in living cells owing to its high brightness and stability (Cubitt et al 1995; Lippincott-Schwartz et al 2001). We noticed that frameshift mutation of EGFP with deletion of two nucleotides (positions 30 and 31 downstream from the ATG start codon) is expected to generate a novel arginine-rich cationic protein. It would therefore be worthwhile examining the characteristics of this novel protein.

In this study, we examined the expression and localization of this novel arginine-rich cationic protein and showed the induction of chromatin condensation by this novel protein.

Materials and methods

Plasmid construction

To construct an arginine-rich cationic protein (Arg-CAP), the pBluescript II SK (+) vector (Stratagene) encoding EGFP (pBluescript/EGFP) was prepared from the pcDNA4/TO vector (Invitrogen) encoding ChkΔSH3ΔSH2-EGFP (pcDNA4/TO/ChkΔSH3ΔSH2-GFP) (Nakayama and Yamaguchi 2005) and the pBluescript II SK (+) vector. Then, to alter the reading frame, pBluescript/EGFP was digested with *Bse*RI, blunted and ligated, thereby resulting in generation of the pBluescript II SK (+) vector encoding Arg-CAP (pBluescript/Arg-CAP). pBluescript/Arg-CAP was subsequently digested with *Age*I and *Sma*I to obtain the Arg-CAP fragment. After removing the NLS-Chk(PTK) fragment from the pcDNA4/TO vector encoding NLS-Chk(PTK)-FLAG (pcDNA4/TO/NLS-Chk(PTK)-FLAG) (Nakayama and Yamaguchi 2005) by digestion with *Eco*RI and *Sma*I and blunting, the Arg-CAP fragment was ligated into the resulting pcDNA4/TO vector containing the FLAG epitope to create Arg-CAP tagged with the FLAG epitope at the C-terminus (Arg-CAPF).

Antibodies

The following antibodies were used: the FLAG epitope (M2; Sigma), lamin B1 (L-5; Zymed), GFP (Medical and Biological Laboratories, Co., Nagoya) and α-tubulin (MCA78G; Serotec). Horseradish peroxidase (HRP)-F(ab')$_2$ secondary antibodies were purchased from Amersham Bioscience. FITC-F(ab')$_2$ of IgG or TRITC-IgG secondary antibodies were from BioSource International and Sigma-Aldrich.

Cell culture and transfection

COS-1 cells were cultured in Iscove's modified Dulbecco's medium supplemented with 5% fetal bovine serum. Transient transfection was performed using *Trans*IT transfection reagent (Mirus), according to the manufacturer's instructions, as recently described (Sato et al 2009). Cells were analyzed at 24 h or 36 h after transfection.

Western blotting

Cells were seeded into 35-mm culture dishes (1×10^5 cells per dish) and cultured for 1 day, and ~1 μg of plasmid DNA with *Trans*IT was added to each culture dish. Cells were cultured for 36 h, and then directly lysed in 100 μL of SDS-PAGE sample buffer and cell lysates were analyzed by SDS-PAGE (~1 x 10^4 cells per lane) and Western blotting using the enhanced chemiluminescence (ECL) detection system (GE Healthcare), as described (Kasahara et al 2007; Kuga et al 2008). Images of chemiluminescence were obtained using an Image Analyzer LAS-1000plus (Fujifilm, Tokyo). Composite figures were prepared using Photoshop 5.0 and Illustrator 9.0 software (Adobe).

Immunofluorescence

Immunofluorescence staining was detected using a Fluoview FV500 confocal laser scanning microscope with a 40x 1.00 NA oil or a 60x 1.00 NA water-immersion objective (Olympus, Tokyo) as described (Kasahara et al 2004; Sato et al 2009). COS-1 cells were fixed in PBS

containing 4% paraformaldehyde for 20 min, and permeabilized in phosphate-buffered saline (PBS) containing 0.1% saponin and 3% bovine serum albumin at room temperature. FLAG-tagged Arg-CAP (Arg-CAPF) was reacted with anti-FLAG antibody for 1 h and subsequently stained with FITC-conjugated $F(ab')_2$ secondary antibody for 1 h. DNA was stained with 20 μg/mL propidium iodide (PI) for 30 min after treatment with 200 μg/mL RNase A for 1 h, and cells were mounted with Prolong Antifade™ reagent (Molecular Probes). For detection of lamin B1, cells were fixed with 100% methanol at -30ºC for 1 min and stained with anti-lamin B1 antibody, as described previously (Nakayama and Yamaguchi 2005). Emission signals were detected at between 505 and 530 nm for fluorescein, and at more than 650 nm for PI. Care was taken to ensure that there was no bleed-through from the fluorescein signal into the red channel (Tada et al 1999). One planar (xy) section slice (0.6-μm thickness) is shown in all experiments. Composite figures were prepared using Photoshop 5.0 and Illustrator 9.0 software (Adobe).

Cell cycle analysis

Cells transfected with nothing, Arg-CAPF or EGFP were detached by trypsinization, fixed in 1.5% paraformaldehyde at 4^{o}C for 1 h, and then fixed/permeabilized with 70% ethanol at -30^{o}C for more than 1 h. Fixed cells were washed twice with PBS containing 3% FBS, and stained with anti-FLAG antibody for 1 h, washed with PBS and stained with FITC-conjugated secondary antibody for 1 h. After treatment with 200 μg/mL RNase A and 50 μg/mL PI at 37^{o}C for 30 min to stain DNA, cell cycle phase was analyzed in cells expressing Arg-CAPF or EGFP by flow cytometry using a MoFlo cell sorter equipped with a 488-nm argon laser (Beckman Coulter), as described (Nakayama and Yamaguchi 2005; Takahashi et al 2009).

Results

Frameshift mutation of EGFP yielding an arginine-rich cationic protein

There are different types of DNA mutations, such as insertion and deletion mutations, and point mutations. Regardless of deletion or insertion, a frameshift mutation usually translates into a protein that does not function properly. However, we noticed that frameshift mutation of EGFP with deletion of two nucleotides (positions 30 and 31 downstream from the ATG start codon) is expected to generate a novel arginine-rich cationic protein. We therefore generated a novel polypeptide containing a large number of basic amino acid residues (Arg, His, and Lys), as described under Materials and methods, and we named it an arginine-rich catioinic protein (Arg-CAP). To detect protein expression of Arg-CAP, we tagged Arg-CAP with the FLAG epitope at the C-terminus (Arg-CAPF) (Fig. 1a).

Arg-CAPF (1-280; with 1 designating the initiator methionine) is composed of an N-terminal EGFP region (1-10), a newly created region by frameshift (11-241), a spacer (242-272), and the FLAG epitope (273-280) (Fig. 1a). Arg-CAPF (280 amino acid residues) is

a highly cationic protein with a calculated isoelectric point of 12.37 and contains 31% basic amino acid residues. Arg-CAPF has amino acid composition of 59 Arg, 24 His, 4 Lys, 14 Asp, 16 Glu, 26 Gly, 31 Ala, 9 Ser, 2 Thr, 0 Asn, 27 Gln, 29 Pro, 13 Val, 20 Leu, 1 Ile, 1 Phe, 2 Tyr, 0 Trp, 1 Cys, and 1 Met, whereas EGFP (239 amino acid residues) has that of 6 Arg, 9 His, 20 Lys, 18 Asp, 16 Glu, 22 Gly, 8 Ala, 10 Ser, 16 Thr, 13 Asn, 8 Gln, 10 Pro, 18 Val, 21 Leu, 12 Ile, 12 Phe, 11 Tyr, 1 Trp, 2 Cys, and 6 Met. A search of the amino acid sequence of Arg-CAPF using the PSORT II program (http://psort.ims.u-tokyo.ac.jp/) indicates that Arg-CAPF has two clusters of eight Arg-rich stretches (Fig. 1a, underlines). To exhibit the regions of Arg-CAPF that are charged and hydrophilic, a hexapeptide hydrophilicity analysis (Hopp and Woods 1981) was performed. More than 25 stretches of amino acid sequence are highly hydrophilic (Fig. 1b), suggesting that most of the regions of Arg-CAPF may be exposed to the molecular surface.

Nuclear expression of Arg-CAPF

COS-1 cells transiently transfected with Arg-CAPF or EGFP were lysed in SDS-PAGE sample buffer, and cell lysates were analyzed by Western blotting. Fig. 2a shows that Arg-CAPF was detected as a single band at approximately 35 kDa using anti-FLAG antibody but EGFP was at approximately 32 kDa with anti-GFP antibody. Although anti-GFP antibody is able to react with wild-type GFP and its variants, such as EGFP, EBFP, ECFP and EYFP, it is underscored that anti-GFP antibody did not recognize Arg-CAPF. Therefore, these results indicate that the EGFP cDNA can be read in an alternative reading frame for Arg-CAPF, which is unrelated to EGFP.

Clusters of basic amino acid residues are considered to play an important role for protein localization to the nucleus. Since Arg-CAPF contains two clusters of eight Arg-rich stretches of predicted NLSs, we examined whether Arg-CAPF was capable of localizing to the nucleus. COS-1 cells were transiently transfected with EGFP or Arg-CAPF and visualized with EGFP fluorescence or anti-FLAG antibody and PI for DNA. Arg-CAPF per se was nonfluorescent,

but it was clearly visualized using anti-FLAG antibody. Unlike EGFP, Arg-CAPF was found to localize predominantly in the nucleus (Fig. 2b). We recently created NLS-EGFP, which includes the classical lysine-rich NLS of the SV40 large T antigen, and showed that more than 90% of NLS-EGFP restrictedly localized to the nucleus (Takahashi et al 2009). These results suggest that the Arg-rich stretches in Arg-CAPF function as NLSs despite being less efficient compared with the classical SV40 NLS.

Induction of chromatin condensation by Arg-CAPF

High magnification images show that expression of Arg-CAPF but not EGFP induced striking chromatin condensation in COS-1 cells and a large fraction of Arg-CAPF present in the nucleus was accumulated in the areas of interchromatin compartments or hypocondensed chromatin/euchromatin (Fig. 3a). However, we recently showed that nuclear expression of NLS-EGFP only induces subtle changes in chromatin condensation (Takahashi et al 2009), suggesting the importance of arginine residues for chromatin condensation. Arg-CAPF-induced

chromatin condensation was also seen in other types of cultured cells, such as HeLa, HEK293, and fibroblastic cells (data not shown). Since chromatin condensation normally occurs in mitosis, apoptosis, gene regulation, and cell cycle progression, we examined whether Arg-CAPF expression was linked to mitosis or apoptosis. However, Arg-CAPF did not induce breakdown of the nuclear envelope, which associates with the nuclear intermediate filament protein lamin B1, indicating that the chromatin condensation is not due to mitotic progression (Fig. 3b). FACS analysis showed that Arg-CAPF did not affect the cell cycle nor did it induce subG_1-cell population (Fig. 3c), suggesting no apoptotic induction. Upon fixation with methanol but not paraformaldehyde, most Arg-CAPF was not retained in the nucleoplasm except the nuclear envelope (Fig. 3b, right panels), suggesting a weak or indirect interaction of Arg-CAPF with chromatin. Taken together, these results suggest that induction of chromatin condensation by Arg-CAPF takes place in interphase without leading to mitosis or apoptosis, suggesting that Arg-rich stretches may be involved in chromatin structural dynamics.

Discussion

In the present study, we demonstrate that frameshift mutation of EGFP generates nuclear localized Arg-CAPF, which contains clusters of arginine-rich stretches and induces striking chromatin condensation. Foci of condensed chromatin are poorly colocalized with Arg-CAPF (Fig. 3a), suggesting that Arg-CAPF is largely present in the areas of interchromatin compartments or hypocondensed chromatin/euchromatin. These results lead to an intriguing hypothesis that nuclear expression of arginine-rich stretches perturbs proper regulation of chromatin dynamics.

Chromatin dynamics in the interphase nucleus is often reflected by gene expression and epigenetic gene regulation (Misteli 2007). Moreover, the increase in the osmolarity of the culture medium and the stress of heat shock can induce the change of chromatin condensation in living cells from normally condensed chromatin to hypercondensed chromatin, leading to inhibition of RNA synthesis and DNA replication (Flannery and Hill 1988; Albiez et al 2006). Growth factor stimulation increases levels of euchromatic hypocondensation and concomitant

heterochromatic hypercondensation through nuclear tyrosine phosphorylation mediated by Src (Takahashi et al 2009). Taken together, endogenous and exogenous factors may influence chromatin structural dynamics through the repositioning of the genetic material in the nucleus.

Along with functioning as NLSs, the arginine-rich clusters in Arg-CAPF affect the higher-order chromatin structures. It is of interest to note that Arg-CAPF contains a large number of arginine residues, because the cationically charged guanidinium group in arginine has the potential to form pentadentate hydrogen bonds (Patel 1999) and to associate with the RNA (Burd and Dreyfuss 1994). Chromatin condensation by Arg-CAPF is induced by a weak or indirect interaction of Arg-CAPF with chromatin. Assumingly, Arg-CAPF might directly interact with nucleoplasmic RNAs present in interchromatin compartments for chromatin architecture changes. Given a potential role of noncoding RNAs in chromatin organization (Prasanth and Spector 2007), the creation of Arg-CAPF will provide us with a new tool to seek RNA species that can interact with chromatin and to dissect chromatin structure dynamics in terms of RNA-chromatin interactions.

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Figure Legends

Fig. 1. Frameshift mutation of EGFP cDNA.

(a) Nucleotide and deduced amino acid sequences of the EGFP-frameshifted mutant Arg-CAPF. Deletion of two nucleotides (positions 30 and 31 downstream from the ATG start codon) is shown. Cationic amino acid residues are marked: arginine (circle), histidine (square) and lysine (oval). Thin and a thick underlines indicate predicted monopartite and bipartite NLSs, respectively. **(b)** A hydrophilicity plot of Arg-CAPF using Hopp-Woods hexapeptide hydrophilicity values.

Fig. 2. Expression of Arg-CAPF.

(a) Whole cell lysates from COS-1 cells transfected with nothing, EGFP, or Arg-CAPF after 36 h were subjected to Western blotting, probed with anti-FLAG antibody, and reprobed with anti-GFP and anti-α-tubulin antibodies. **(b)** COS-1 cells transfected with EGFP or Arg-CAPF were cultured for 24 h, fixed with paraformaldehyde and doubly visualized with propidium iodide (PI) for DNA and GFP fluorescence or anti-FLAG antibody. Scale bars, 10 μm.

Localizations of EGFP and Arg-CAPF were quantitated from three independent experiments. Nuc, nuclear expression; Cyto, cytoplasmic expression.

Fig. 3. Induction of chromatin condensation by Arg-CAPF.

(a) COS-1 cells transfected with EGFP or Arg-CAPF were cultured for 24 h, fixed with paraformaldehyde and doubly visualized with PI and GFP fluorescence or anti-FLAG antibody. Two types of Arg-CAPF expression were shown as (i) Nuc > Cyto (Nuc) and (ii) Nuc = Cyto (Nuc + Cyto). Scale bars, 10 μm. **(b)** COS-1 cells transfected with nothing or Arg-CAPF were cultured for 24 h, fixed with methanol and doubly stained with anti-lamin B1 antibody and PI or with anti-lamin B1 and anti-FLAG antibodies. Scale bars, 10 μm. **(c)** COS-1 cells transfected with nothing, EGFP, or Arg-CAPF were cultured for 24 h. DNA contents were analyzed by flow cytometry.

(a)

```
                                cg
atggtgagcaagggcgaggagctgttcacgggtggtgcccatcctggtcgagctggacggcgacgtaaacggcca
M  V  S  K  G  E  E  L  F  T  G  G  A  H  P  G  R  A  G  R  R  R  K  R  P    25

caagttcagcgtgtccggcgagggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgcaccac
Q  V  Q  R  V  R  R  G  R  G  R  C  H  L  R  Q  A  D  P  E  V  H  L  H  H    50

cggcaagctgcccgtgccctggcccaccctcgtgaccaccctgacctacggcgtgcagtgcttcagccgctaccc
R  Q  A  A  R  A  L  A  H  P  R  D  H  P  D  L  R  R  A  V  L  Q  P  L  P    75

cgaccacatgaagcagcacgacttcttcaagtccgccatgcccgaaggctacgtccaggagcgcaccatcttctt
R  P  H  E  A  A  R  L  L  Q  V  R  H  A  R  R  L  R  P  G  A  H  H  L  L   100

caaggacgacggcaactacaagacccgcgccgaggtgaagttcgagggcgacaccctggtgaaccgcatcgagct
Q  G  R  R  Q  L  Q  D  P  R  R  G  E  V  R  G  R  H  P  G  E  P  H  R  A   125

gaagggcatcgacttcaaggaggacggcaacatcctggggcacaagctggagtacaactacaacagccacaacgt
E  G  H  R  L  Q  G  G  R  Q  H  P  G  A  Q  A  G  V  Q  L  Q  Q  P  Q  R   150

ctatatcatggccgacaagcagaagaacggcatcaaggtgaacttcaagatccgccacaacatcgaggacggcag
L  Y  H  G  R  Q  A  E  E  R  H  Q  G  E  L  Q  D  P  P  Q  H  R  G  R  Q   175

cgtgcagctcgccgaccactaccagcagaacaccccccatcggcgacggccccgtgctgctgcccgacaaccacta
R  A  A  R  R  P  L  P  A  E  H  P  H  R  R  R  R  P  R  A  A  A  R  Q  P  L   200

cctgagcacccagtccgccctgagcaaagaccccaacgagaagcgcgatcacatggtcctgctggagttcgtgac
P  E  H  P  V  R  P  E  Q  R  P  Q  R  E  A  R  S  H  G  P  A  G  V  R  D   225

cgccgccgggatcactctcggcatggacgagctgtacaagtccggatagctcgagcccgggagctacgcagtgca
R  R  R  D  H  S  R  H  G  R  A  V  Q  V  R  I  A  R  A  R  E  L  R  S  A   250

ggtgccccagcctccgtctcagggcaggacgccgacggctccacctcgccccgaagccaggagcccgattataaa
G  A  P  A  S  V  S  G  Q  D  A  D  G  S  T  S  P  R  S  Q  E  P  D  Y  K   275

gatgatgatgataaatga
D  D  D  D  K  STOP
```

(b)

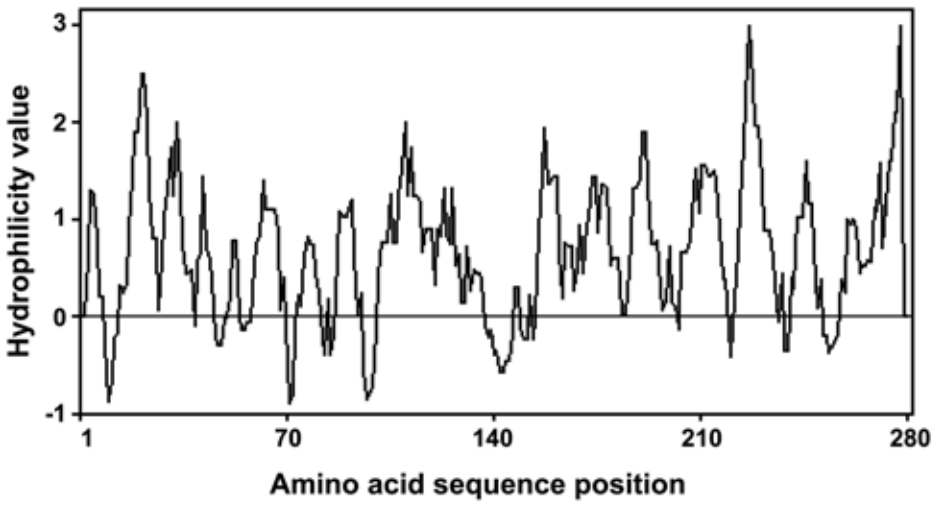

Figure 1

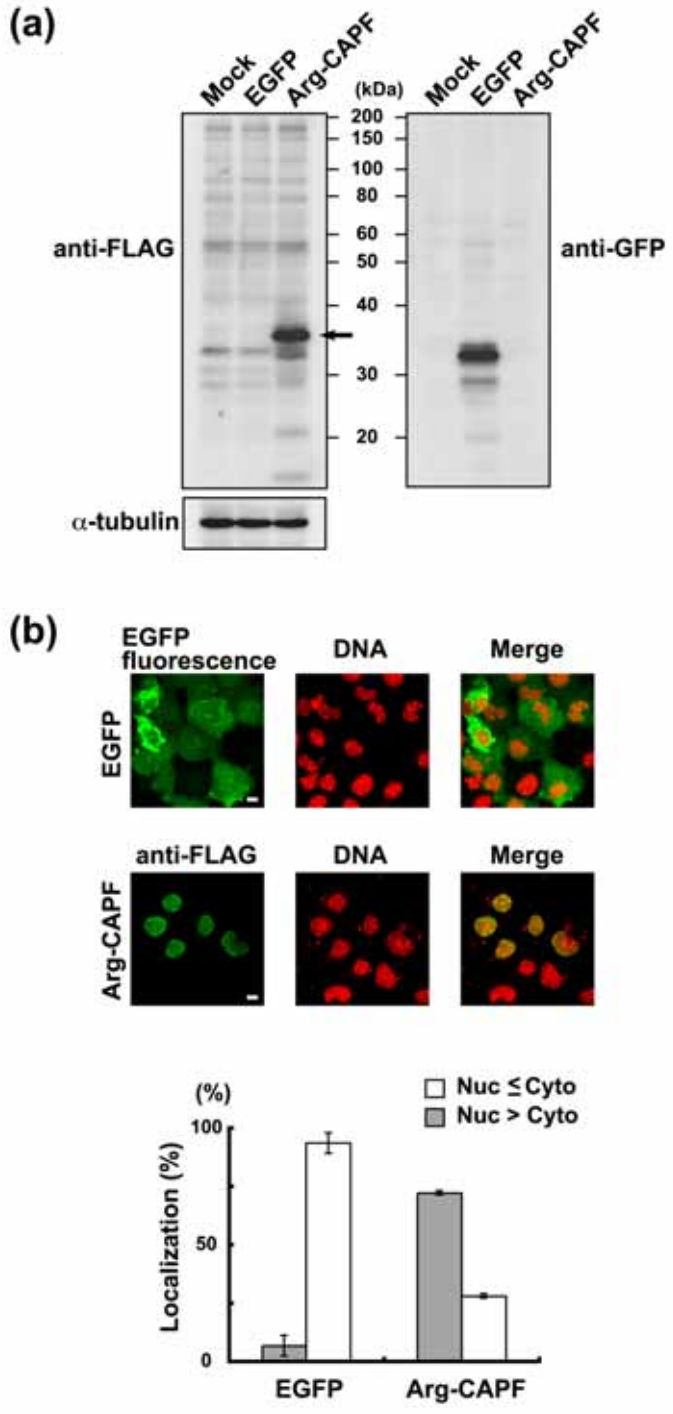
(a)
Mock
EGFP
Arg-CAPF
(kDa)
Mock
EGFP
Arg-CAPF
200
150
100
80
60
50
40
30
20
anti-FLAG
anti-GFP
α-tubulin
(b)
EGFP fluorescence
DNA
Merge
EGFP
anti-FLAG
DNA
Merge
Arg-CAPF
(%)
Nuc ≤ Cyto
Nuc > Cyto
Localization (%)
100
50
0
EGFP
Arg-CAPF

Figure 2

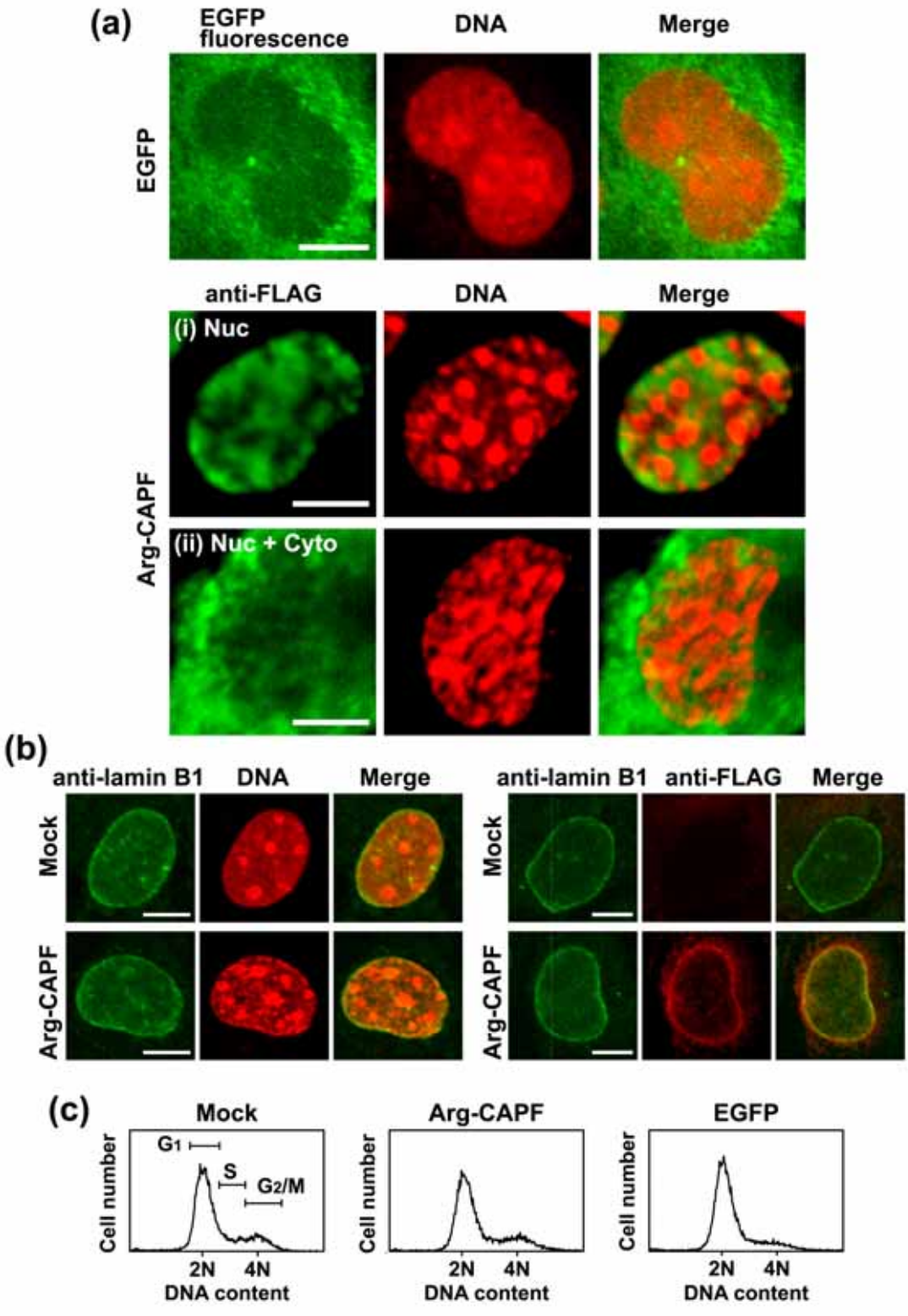
(a)
EGFP fluorescence
DNA
Merge
EGFP
anti-FLAG
DNA
Merge
(i) Nuc
Arg-CAPF
(ii) Nuc + Cyto
(b)
anti-lamin B1
DNA
Merge
anti-lamin B1
anti-FLAG
Merge
Mock
Arg-CAPF
(c)
Mock
Arg-CAPF
EGFP
Cell number
G1
S
G2/M
2N
4N
DNA content

Figure 3

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