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

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Abstract In the interphase nuclei of cultured cells, chromatin is compacted and organized in 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 generated 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.

Keywords Arginine-rich · Chromatin condensation · Enhanced green fluorescent protein · Frameshift · Nuclear architecture · Nuclear localization signal

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 in 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

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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 *TransIT* transfection reagent (Mirus), according to the manufacturer's instructions, as recently described (Sato et al. 2009). Cells were analyzed at 24 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 $\sim 1 \mu\text{g}$ of plasmid DNA with *TransIT* 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 ($\sim 1 \times 10^4$ 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 40×1.00 NA oil or a 60×1.00 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 AntifadeTM reagent (Molecular Probes). For detection of lamin B1, cells were fixed with 100% methanol at -30°C 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°C for 1 h, and then fixed/permeabilized with 70% ethanol at -30°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°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 cationic 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.

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

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. Figure 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 subG₁-cell population (Fig. 3c),

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

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

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 μ m. **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 μ m. **c** COS-1 cells transfected with nothing, EGFP, or Arg-CAPF were cultured for 24 h. DNA contents were analyzed by flow cytometry

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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References

- Albiez H, Cremer M, Tiberi C, Vecchio L, Schermelleh L, Ditttrich S, Küpper K, Joffe B, Thormeyer T, Hase JV, Yang S, Rohr K, Leonhardt H, Solovei I, Cremer C, Fakan S, Cremer T (2006) Chromatin domains and the interchromatin compartment form structurally defined and functionally interacting nuclear networks. *Chromosome Res* 14:707–733. doi:[10.1007/s10577-006-1086-x](https://doi.org/10.1007/s10577-006-1086-x)
- Burd CG, Dreyfuss G (1994) Conserved structures and diversity of functions of RNA-binding proteins. *Science* 265:615–621. doi:[10.1126/science.8036511](https://doi.org/10.1126/science.8036511)
- Cardarelli F, Serresi M, Bizzarri R, Beltram F (2008) Tuning the transport properties of HIV-1 Tat arginine-rich motif in living cells. *Traffic* 9:528–539. doi:[10.1111/j.1600-0854.2007.00696.x](https://doi.org/10.1111/j.1600-0854.2007.00696.x)
- Cubitt AB, Heim R, Adams SR, Boyd AE, Gross LA, Tsien RY (1995) Understanding, improving and using green fluorescent proteins. *Trends Biochem Sci* 20:448–455. doi:[10.1016/S0968-0004\(00\)89099-4](https://doi.org/10.1016/S0968-0004(00)89099-4)
- Dingwall C, Laskey RA (1991) Nuclear targeting sequences—a consensus? *Trends Biochem Sci* 16:478–481. doi:[10.1016/0968-0004\(91\)90184-W](https://doi.org/10.1016/0968-0004(91)90184-W)
- Flannery AV, Hill RS (1988) The effect of heat shock on the morphology of amphibian lampbrush chromosomes. *Exp Cell Res* 177:9–18. doi:[10.1016/0014-4827\(88\)90020-1](https://doi.org/10.1016/0014-4827(88)90020-1)
- Hopp TP, Woods KR (1981) Prediction of protein antigenic determinants from amino acid sequences. *Proc Natl Acad Sci USA* 78:3824–3828
- Horn PJ, Peterson CL (2002) Chromatin higher order folding—wrapping up transcription. *Science* 293:1824–1827. doi:[10.1126/science.1074200](https://doi.org/10.1126/science.1074200)
- Kasahara K, Nakayama Y, Ikeda K, Fukushima Y, Matsuda D, Horimoto S, Yamaguchi N (2004) Trafficking of Lyn through the golgi caveolin involves the charged residues on α E and α I helices in the kinase domain. *J Cell Biol* 165:641–652. doi:[10.1083/jcb.200403011](https://doi.org/10.1083/jcb.200403011)
- Kasahara K, Nakayama Y, Nakazato Y, Ikeda K, Kuga T, Yamaguchi N (2007) Src signaling regulates completion of abscission in cytokinesis through ERK/MAPK activation at the midbody. *J Biol Chem* 282:5327–5339. doi:[10.1074/jbc.M608396200](https://doi.org/10.1074/jbc.M608396200)
- Kuga T, Hoshino M, Nakayama Y, Kasahara K, Ikeda K, Obata Y, Takahashi A, Higashiyama Y, Fukumoto Y, Yamaguchi N (2008) Role of Src-family kinases in formation of the cortical actin cap at the dorsal cell surface. *Exp Cell Res* 314:2040–2054. doi:[10.1016/j.yexcr.2008.03.018](https://doi.org/10.1016/j.yexcr.2008.03.018)
- Lancôt C, Cheutin T, Cremer M, Cavalli G, Cremer T (2007) Dynamic genome architecture in the nuclear space: regulation of gene expression in three dimensions. *Nat Rev Genet* 8:104–115. doi:[10.1038/nrg2041](https://doi.org/10.1038/nrg2041)
- Lippincott-Schwartz J, Snapp E, Kenworthy A (2001) Studying protein dynamics in living cells. *Nat Rev Mol Cell Biol* 2:444–456. doi:[10.1038/35073068](https://doi.org/10.1038/35073068)
- Misteli T (2007) Beyond the sequence: cellular organization of genome function. *Cell* 128:787–800. doi:[10.1016/j.cell.2007.01.028](https://doi.org/10.1016/j.cell.2007.01.028)
- Nakayama Y, Yamaguchi N (2005) Multi-lobulation of the nucleus in prolonged S phase by nuclear expression of Chk tyrosine kinase. *Exp Cell Res* 304:570–581. doi:[10.1016/j.yexcr.2004.11.027](https://doi.org/10.1016/j.yexcr.2004.11.027)
- Patel DJ (1999) Adaptive recognition in RNA complexes with peptides and protein modules. *Curr Opin Struct Biol* 9:74–87. doi:[10.1016/S0959-440X\(99\)80010-4](https://doi.org/10.1016/S0959-440X(99)80010-4)
- Prasanth KV, Spector DL (2007) Eukaryotic regulatory RNAs: an answer to the ‘genome complexity’ conundrum. *Genes Dev* 21:11–42. doi:[10.1101/gad.1484207](https://doi.org/10.1101/gad.1484207)
- Sato I, Obata Y, Kasahara K, Nakayama Y, Fukumoto Y, Yamasaki T, Yokoyama KK, Saito T, Yamaguchi N (2009) Differential trafficking of Src, Lyn, Yes and Fyn is specified by the state of palmitoylation in the SH4 domain. *J Cell Sci* 122:965–975. doi:[10.1242/jcs.034843](https://doi.org/10.1242/jcs.034843)
- Spector DL (2003) The dynamics of chromosome organization and gene regulation. *Annu Rev Biochem* 72:573–608. doi:[10.1146/annurev.biochem.72.121801.161724](https://doi.org/10.1146/annurev.biochem.72.121801.161724)
- Tada J, Omine M, Suda T, Yamaguchi N (1999) A common signaling pathway via Syk and Lyn tyrosine kinases generated from capping of the sialomucins CD34 and CD43 in immature hematopoietic cells. *Blood* 93:3723–3735
- Takahashi A, Obata Y, Fukumoto Y, Nakayama Y, Kasahara K, Kuga T, Higashiyama Y, Saito T, Yokoyama KK, Yamaguchi N (2009) Nuclear localization of Src-family kinases is required for growth factor-induced euchromatinization. *Exp Cell Res* 315:1117–1141. doi:[10.1016/j.yexcr.2009.02.010](https://doi.org/10.1016/j.yexcr.2009.02.010)
- Truett R, Cullen BR (1999) The arginine-rich domains present in human immunodeficiency virus type 1 Tat and Rev function as direct importin β -dependent nuclear localization signals. *Mol Cell Biol* 19:1210–1217
- Tsien RY (1998) The green fluorescent protein. *Ann Rev Biochem* 67:509–544. doi:[10.1146/annurev.biochem.67.1.509](https://doi.org/10.1146/annurev.biochem.67.1.509)