Assembly and remodeling of viral DNA and RNA replicons regulated by cellular molecular chaperones

Takeshi Sekiya¹ · Yifan Hu² · Kohsuke Kato^{1,2} · Mitsuru Okuwaki^{1,2} · Atsushi Kawaguchi^{1,2,3} · Kyosuke Nagata⁴

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Abstract

A variety of cellular reactions mediated by interactions among proteins and nucleic acids requires a series of proteins called molecular chaperones. The viral genome encodes relatively few kinds of viral proteins and, therefore, host-derived cellular factors are required for virus proliferation. Here we discuss those cellular proteins known as molecular chaperones, which are essential for the assembly of functional viral DNA/RNA replicons. The function of these molecular chaperones in the cellular context is also discussed.

Keywords Adenovirus · Chromatin · Influenza virus · Molecular chaperone · Ribonucleoprotein

Introduction

The term “molecular chaperone” was used for the first time to describe the protein nucleoplasmin which was identified in *Xenopus laevis* egg extracts and prevents the aggregation of DNA and histones at physiological ionic strength (Laskey et al. 1978). The meaning of molecular chaperone was subsequently extended to a group of proteins that are transiently associated with target proteins to support protein folding and/or to assemble multi-protein complexes to promote many cellular processes, such as heat shock proteins (Ellis 1987). Nowadays the functions of molecular chaperones have been expanded to

include the regulation of protein unfolding and disassembly of multi-protein complexes to components. In this review, we summarize the roles of cellular proteins that function as molecular chaperones, but which were originally identified as factors involved in replication and transcription of viral genomes, such as adenovirus and influenza virus genomes (Table 1).

Molecular chaperones for adenovirus core and cellular chromatin remodeling

The Adenovirus (Ad) genome is a linear DNA that contains terminal protein (TP) covalently attached to its 5' ends. The Ad genome is assembled into a chromatin-like structure, called the Ad core, covered by viral basic proteins V, VII, and X/mu (Russell 2009). Following Ad infection, protein VII remains in the Ad genome at early phases of infection (Chatterjee et al. 1986; Xue et al. 2005), but protein V is dissociated from the Ad core during the nuclear import of the Ad core (Puntener et al. 2011). Protein X/mu is a small core protein and may be a functional partner of proteins V and VII for the packaging of the Ad core to the capsid (Ugai et al. 2007). Thus, the transcriptionally and replicationally competent Ad core is thought to be mainly composed of protein VII. A cell-free Ad DNA replication system has been developed and characterized using the TP-DNA without core proteins as a template by adding Ad DNA polymerase (Ad pol), precursor TP (pTP), Ad DNA binding protein (DBP), and nuclear extracts of uninfected cells (Enomoto et al. 1981; Ikeda et al.

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✉ Atsushi Kawaguchi
ats-kawaguchi@md.tsukuba.ac.jp

✉ Kyosuke Nagata
knagata@md.tsukuba.ac.jp

- ¹ Department of Infection Biology, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan
- ² Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Japan
- ³ Transborder Medical Research Center, University of Tsukuba, Tsukuba, Japan
- ⁴ Special Laboratory, Department of Infection Biology, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan

Table 1 Targets of chaperones

Chaperone	Targets	References
TAF-I/SET	Ad core protein VII	Matsumoto et al. 1993
	H3/H4	Okuwaki and Nagata 1998
	H1	Kato et al. 2011
TAF-II/NAP1	Unclear	Kawase et al. 1996
	H2A/H2B	Ishimi et al. 1987
	H3/H4	McQuibban et al. 1998
	H1	Shintomi et al. 2005
TAF-III/NPM1/B23	Ad core protein V	Samad et al. 2007
	Ad core protein VII	Okuwaki et al. 2001a
	H3/H4	Okuwaki et al. 2001b
	H1	Gadad et al. 2011
RAF-2p48/UAP56	Influenza nucleoprotein	Momose et al. 2001
	U2AF65	Fleckner et al. 1997
	Aly (mRNA export complex)	Luo et al. 2001
Tat-SF1	Influenza nucleoprotein	Naito et al. 2007
	U snRNPs	Fong and Zhou 2001
	p-TEFb	Zhou et al. 1998
Prp18	Influenza nucleoprotein	Minakuchi et al. 2017
	U5 snRNP	Bačíková and Horowitz 2005

1981). Biochemical fractionation of uninfected nuclear extracts has revealed three host factors, named nuclear factors (NF)-I, -II, and -III, which have been identified as essential stimulatory proteins of the cell-free system (Nagata et al. 1982, 1983; Puijn et al. 1986). However, even in the presence of NF-I, -II, and -III, the Ad core was minimally replicated in the cell-free system (Goding and Russell 1983; Leith et al. 1989), suggesting that an additional factor(s), possibly derived from the host cells, is/are required to activate the replication reaction from Ad core. Thus, we attempted to reconstitute Ad core replication in a cell-free system by the addition of uninfected cell extracts. By biochemical fractionation and complementation, we purified three host proteins, which we designated TAF-I/SET, TAF-II/NAP1, and TAF-III/NPM1/B23, as stimulatory factors of Ad core replication (Matsumoto et al. 1993; Kawase et al. 1996; Okuwaki et al. 2001a).

Cellular DNA forms nucleoprotein complexes, called chromatin, consisting of core histones (H2A, H2B, H3, and H4), linker histone H1, and a large number of non-histone proteins. Because histone proteins carry positively charged residues, they easily form non-specific aggregated structures with nucleic acids by electrostatic interaction. Furthermore, histone proteins interact with each other through the hydrophobic regions in their central core domains and tend to aggregate at physiological ionic strength in vitro. Molecular chaperones,

called histone chaperones, interact with histones to prevent non-specific aggregation between histones and DNA, transfer histones to DNA to assemble nucleosome, and remove histones from chromatin (Warren and Shechter 2017). Based on observations of the activity of template activating factor (TAF) proteins toward the Ad core (see below), these proteins were later shown to have the histone chaperone activity both in vitro and in vivo.

TAF-I/SET

TAF-I consists of two subtypes, TAF-I α and TAF-I β (Nagata et al. 1995). Although these genes are driven by each promoter sequence, *TAF-I α* and *TAF-I β* genes share the same open reading frames except for their first exon (Asaka et al. 2008). TAF-I β is identical with SET which was first identified as a part of SET-CAN fusion protein derived from a chromosomal rearrangement in patients with acute myeloid leukemia (von Lindern et al. 1992). Although TAF-I shows a diffuse localization pattern in uninfected nuclei, TAF-I accumulates in nuclear speckles with the incoming Ad genome at early phases of infection (Komatsu et al. 2015). TAF-I does not have a DNA binding activity, but it forms a ternary complex with protein VII–DNA complexes (Haruki et al. 2003; Gyurcsik et al. 2006; Komatsu et al. 2011). TAF-I interacts with protein VII and remodels the replicationally incompetent

Fig. 1 Assembly and remodeling of the Adenovirus (Ad) core mediated by template activating factor (TAF) proteins. **a** Incoming Ad core is tightly packed with protein VII and replicationally incompetent. **b** Ad core is remodeled by TAF-I at early phases of infection. **c** Histones are deposited to remodeled Ad core by histone chaperones. **d** Matured Ad core is formed. Histones are removed and replaced with core proteins by nucleophosmin 1 (NPM1)

Ad core to a replicative form (Fig. 1). TAF-I is also required for the replication of other DNA viruses, such as adeno-associated virus (Pegoraro et al. 2006), herpes simplex virus (van Leeuwen et al. 2003), and Epstein–Bar virus (Mansouri et al. 2013).

TAF-I is involved in many cellular processes, including tumorigenesis (Switzer et al. 2011; Saddoughi et al. 2013), protein acetylation (Seo et al. 2001; Kim et al. 2012; Chae et al. 2014), suppression of apoptosis (Beresford et al. 2001), mRNA transport (Brennan et al. 2000), cellular motility (ten Klooster et al. 2007), cell cycle progression (Canela et al. 2003), and DNA damage response (Kalousi et al. 2015). TAF-I also interacts with histone H3 (Okuwaki and Nagata 1998) and linker histone H1 variants (Kato et al. 2011). Compared with other core histone chaperones, such as CAF-I and ASF1, only a small part of TAF-I interacts with histone H3 (Kato et al. 2011), however TAF-I does show core histone chaperone activity via interaction with histone H3 (Fig. 2a). Biochemical analysis has also revealed that TAF-I maintains proper chromosome formation through the deposition of histone H1 onto nucleosome core particles (NCPs) and by excluding an excess amount of histone H1 from NCPs (Fig. 2b) (Kato et al. 2011; Okuwaki et al. 2016). Fluorescence recovery after photobleaching (FRAP) analyses of linker histone H1.1 suggested that TAF-I is involved in the dynamic exchange of histone H1.1 on chromatin in vivo (Kato et al. 2011).

TAF-I consists of a coiled-coil dimerization domain and an acidic carboxyl-terminal region. Not only the acidic region but also dimer formation through the coiled-coil region is essential for its chaperone activity (Miyaji-Yamaguchi et al. 1999). Although TAF-I α differs from TAF-I β only in its N-terminal region, with the former having 37 amino acids (aa) and the latter having 24 aa, the histone chaperone activity of the TAF-I α homo-dimer is significantly lower than that of the TAF-I β homo-dimer (Nagata et al. 1995). Basic amino acids within the N-terminal region of TAF-I α inhibit the histone chaperone activity via interaction with the C-terminal region (Kajitani et al. 2017). It has been reported that the expression level of TAF-I α varies among cell types compared with that of TAF-I β which is present ubiquitously at a relatively constant level (Nagata et al. 1998), suggesting that the chaperone activity of TAF-I is regulated by the expression ratio of TAF-I α and TAF-I β .

TAF-II/NAP1

TAF-I shows a low but distinct similarity to nucleosome assembly protein 1 (NAP1) (Nagata et al. 1995), which is a highly conserved histone chaperone protein. We found that NAP1 also stimulates the Ad core replication in the cell-free system (Nagata et al. 1995; Kawase et al. 1996), and thereby NAP1 could be thought of as TAF-II (Fig. 1b). NAP1 was originally identified from cell extracts as a protein that stimulates the nucleosome assembly from DNA and core histones (Fig. 2a)

Fig. 2 Histone chaperone activity of TAF proteins. **a** All TAF proteins have the histone deposition activity. TAF-I and NPM1 show a binding preference to histone H3–H4. Nucleosome assembly protein 1 (NAP1) interacts with both histone H2A–H2B and H3–H4. NAP1 disassembles nucleosomes by removing histone H2A–H2B. **b** All TAF proteins have the deposition activity of linker histone H1 to nucleosomal DNA to form chromosome structures. TAF-I and NAP1 also disassemble chromosomes by removing linker histone H1

(Ishimi et al. 1984). The C-terminal acidic domain of NAP1 is indispensable for the histone chaperone activity (Okuwaki et al. 2010). NAP1 binds to both the H2A–H2B dimer (Ishimi et al. 1987) and H3–H4 dimer (McQuibban et al. 1998). NAP1 disassembles nucleosomes by removing histone H2A–H2B (Fig. 2a) (Ito et al. 2000; Okuwaki et al. 2005). Biochemical analysis shows that the acidic domain of NAP1 is not essential for binding to the H3–H4 dimer but it is critical for binding to the H2A–H2B dimer (Ohtomo et al. 2016). NAP1 has also been identified as a linker histone chaperone from *Xenopus laevis* egg extracts. *Xenopus* NAP1 is associated with egg-specific linker histone B4 and deposits linker histones onto NCPs (Shintomi et al. 2005). Biochemical analyses showed that human NAP1 possesses the linker histone chaperone activity in the process of both depositing histone H1 onto NCPs and excluding an excess amount of histone H1 from NCPs (Fig. 2b) (Kato et al. 2011). However, FRAP assays showed that the exchange kinetics of histone H1.1 is minimal in human NAP1 knockdown HeLa cells (Kato et al. 2011), suggesting that NAP1 could have a species- or cell type-specific linker histone chaperone activity.

TAF-III/NPM1/B23

TAF-III activity was purified from uninfected cell extracts as a factor that stimulates the cell-free replication of the Ad core (Okuwaki et al. 2001a). TAF-III contains two polypeptides that are splicing variants of nucleophosmin 1 (NPM1), and the longer splicing variant of NPM1 (called B23.1) is

responsible for TAF-III activity. NPM1 interacts directly with Ad core proteins V, VII, and the precursor of protein VII (pre-VII) (Samad et al. 2007). Although viral DNA replication decreases in TAF-I knockdown cells (Haruki et al. 2006), knock down of NPM1 has no significant effect on viral DNA replication (Samad et al. 2012). However, the production of infectious viral particles decreases in infected NPM1 knockdown cells (Samad et al. 2012). It has been reported that newly synthesized Ad genomes are packed into a chromatin-like structure with cellular histones and pre-VII, and then histones are replaced with core proteins to form matured Ad core (Giberson et al. 2012). Chromatin immunoprecipitation (ChIP) assays revealed that excess amounts of proteins V, VII, and histone H3 interact with progeny Ad genome in NPM1 knockdown cells even at late phases of infection (Samad et al. 2012). These results suggest that B23 is required for the maturation of progeny Ad core (Fig. 1d).

NPM1 is involved in a variety of cellular processes, such as ribosome biogenesis (Borer et al. 1989), apoptosis (Ye 2005), centrosome duplication (Okuda et al. 2000), and tumorigenesis (Grisendi et al. 2006). NPM1 is a member of the nucleophosmin (NPM) family of proteins that also includes NPM2 and NPM3. The members of this protein family share a conserved N-terminal core domain that is essential for their homo-oligomerization (Okuwaki et al. 2012). NPM1 functions as a core histone chaperone through interaction with histone H3-H4 (Fig. 2a) (Okuwaki et al. 2001b; Murano et al. 2008). NPM1 also mediates the deposition of linker histones onto NCPs, as does TAF-I (Gadad et al. 2011; Okuwaki et al. 2016). However, an excess amount of TAF-I, but not of NPM1, removes histone H1 from H1-bound NCPs, even though both TAF-I and NPM1 recognize the C-terminal domain of histone H1 (Okuwaki et al. 2016). These results suggest that TAF-I and NPM1 may play different roles in the assembly and disassembly of chromatosomes (Fig. 2b).

Molecular chaperones for the assembly of influenza viral RNP complexes

The genome of influenza type A viruses consists of single-stranded RNAs of negative polarity. The viral genome (vRNA) is covered by multiple copies of viral protein NP and interacts with heterotrimeric viral RNA-dependent RNA polymerases to form ribonucleoprotein (designated vRNP) complexes. NP is a highly basic viral protein that binds single-stranded RNA without sequence specificity. This protein is essential to maintain the RNA template in an ordered conformation suitable for viral RNA syntheses (Honda et al. 1988; Yamanaka et al. 1990; Portela and Digard 2002; Kawaguchi et al. 2011; Minakuchi et al. 2017). Cryo-electron microscopy analysis has revealed that the oligomerization of NP is important to form a double helical structure with the

anti-parallel strand of vRNP (Arranz et al. 2012; Moeller et al. 2012). Oligomeric NP shows higher RNA binding activity than monomeric NP (Tarus et al. 2012). Although crystal structures of RNA-free NP have shown that NP forms oligomers in a crystallized state, a broad size distribution was observed by gel filtration chromatography in solution at physiological salt concentrations (Ye et al. 2006; Ng et al. 2008; Tarus et al. 2012; Ye et al. 2012), suggesting that NP exists in an equilibrium between monomers and oligomers, including trimers and tetramers (Ruigrok and Baudin 1995). Thus, an exact structure of NP that assembles with vRNP remains poorly understood.

For efficient viral transcription and replication, not only viral factors but also host factors are required (Shimizu et al. 1994). To identify host factors, systematic screening systems in which the functional activity can be monitored are needed. To date, we have reconstituted the influenza viral polymerase activity in a cell-free system (Momose et al. 1996) and in yeast cells (Naito et al. 2007), respectively. In the cell-free system, the efficient viral transcription and replication of influenza virus could be reconstituted by a combination of vRNP complexes and nuclear extracts prepared from uninfected cells. This has allowed us to systematically identify host factors that stimulate the viral RNA synthesis in biochemical complementation assays through fractionation of nuclear extracts (Shimizu et al. 1994; Momose et al. 1996). By reconstitution and dissection of the cell-free system, RAF-2p48/UAP56/BAT1, RAF-1/Hsp90, IREF-1/MCM, and IREF-2/pp32/APRIL were identified as host factors that stimulate viral RNA syntheses (Momose et al. 2001; Momose et al. 2002; Kawaguchi and Nagata 2007; Sugiyama et al. 2015). We also developed an influenza virus replicon system in yeast by transfecting vRNP complexes. Using a genome-wide set of yeast single gene deletion libraries, we identified Tat stimulatory factor 1 (Tat-SF1) and Prp18 as stimulatory host factors in the viral RNA synthesis (Naito et al. 2007).

Hsp90 is involved in the assembly and nuclear transport of viral RNA polymerase subunits, possibly as a molecular chaperone for the polymerase subunits prior to the formation of a mature ternary polymerase complex (Naito et al. 2007; Minakuchi et al. 2017). The minichromosome maintenance (MCM) complex stabilizes the replicating polymerase complexes by promoting the interaction between the viral polymerase and nascent cRNA (Kawaguchi and Nagata 2007). pp32 (also known as ANP32A) is required for vRNA synthesis from complementary RNA replication intermediates and is thought to be involved in host range restriction of avian influenza viruses (Sugiyama et al. 2015; Long et al. 2016). UAP56, Tat-SF1, and Prp18 function as molecular chaperones for NP to assemble viral RNA into vRNP complexes as described in the following sections and shown in Fig. 3 (Momose et al. 2001; Naito et al. 2007; Minakuchi et al. 2017).

Fig. 3 NP chaperone activity of UAP56, Tat-SF1, and Prp18, host factors that stimulate viral RNA syntheses. UAP56 stimulates the assembly of trimeric NP from monomer. The UAP56–NP complex is composed of two trimeric NP connected by the UAP56 dimer. The newly synthesized viral genome is co-replicationally assembled into RNP complexes by UAP56. The function of Tat-SF1 may be similar to that of UAP56. Prp18 dissociates the nascent RNA chain from the template and stimulates elongation after early elongation step

RAF-2p48/UAP56

RAF-2 activity was purified from uninfected nuclear extracts as a factor that stimulates cell-free viral RNA synthesis (Momose et al. 2001). RAF-2 was identified as a heterodimer composed of 48-kDa (RAF-2p48) and 36-kDa (RAF-2p36) polypeptides. RAF-2p48 is identical to UAP56, which is a cellular splicing factor required for spliceosome assembly (Fleckner et al. 1997) and nuclear export of cellular mRNA to the cytoplasm (Luo et al. 2001). UAP56 was separately identified as an NP-interacting protein, designated NPI-5, in a yeast two-hybrid screening assay. UAP56 binds to NP free of RNA but not to NP–RNA complexes (Momose et al. 2001). UAP56 interacts with 20-aa residues from the N-terminus of NP through the C-terminal region of UAP56 (Momose et al. 2001). Although UAP56 belongs to the DExD-box family of ATP-dependent RNA helicases (Linder and Stutz 2001), the putative RNA helicase activity and ATPase activity are not needed for the NP chaperone activity (Momose et al. 2001). The newly synthesized viral genome is co-replicationally assembled into RNP complexes by UAP56 (Kawaguchi et al. 2011). Recently, we found that UAP56 is complexed with trimeric NP, but not with monomeric NP, and stimulates the assembly of trimers from monomeric NP even at physiological salt concentrations (Hu et al. 2017). The molecular size of the UAP56–NP complex on the gel filtration column is around 440 kDa, and the complex was observed as a dumbbell-shaped complex of approximately 40 nm by atomic force microscopy

analysis. Since the size of the trimeric NP and UAP56 dimer is approximately 15 and 6 nm, respectively, the complex seems to be composed of two trimeric NP connected by the UAP56 dimer (Fig. 3). In the absence of UAP56, monomeric NP first binds to RNA, and the additional monomers randomly and slowly bind to the monomeric NP on the NP–RNA complex (Tarus et al. 2012). In contrast, UAP56 represses the binding of an excess amount of NP to viral RNA, possibly by transferring trimeric NP.

Tat-SF1

Using a genome-wide set of yeast single-gene deletion strains, the yeast *CUS2* gene was identified as a stimulatory host factor of the influenza viral replicon system in yeast (Naito et al. 2007). *CUS2* shares 37% identity with human Tat stimulatory factor 1 (Tat-SF1) protein. Tat-SF1 interacts with the spliceosomal U small nuclear ribonucleoproteins (snRNPs) and stimulates cellular RNA polymerase II elongation activity (Fong and Zhou 2001). This is likely to be mediated through the binding of Tat-SF1 to positive transcription elongation factor b (p-TEFb) and nascent splicing substrate, respectively (Zhou et al. 1998). Tat-SF1 specifically interacts with NP free of RNA but not with RNA-bound NP. NP or the NP–RNA complex is released from Tat-SF1 by the addition of RNA, thereby the RNA–NP–Tat-SF1 trimeric complex cannot be formed. NP is produced as a form free of RNA in infected cells, and the interaction of NP with Tat-SF1 may repress the non-specific aggregation of NP. The function of Tat-SF1 is quite similar to that of UAP56 as described above. NP may

use redundant host factors for efficient viral RNA synthesis. The exact functional difference between Tat-SF1 and UAP56 in NP chaperone activity should be addressed.

Prp18

The screening of a single-gene deletion yeast library also demonstrated that the viral RNA synthesis level was decreased in Prp18 deletion yeast cells (Naito et al. 2007). Yeast Prp18 shares 39% identity with human Prp18 protein. It interacts with U5 snRNP and functions in catalytic step II in pre-mRNA splicing as a splicing factor (Bačíková and Horowitz 2005) and also interacts with NP and viral polymerases (Minakuchi et al. 2017). A deletion mutant of Prp18, which binds to NP but not to viral polymerases, stimulated viral RNA synthesis in the cell-free system, suggesting that Prp18 activates viral RNA synthesis through the interaction between Prp18 and NP. Prp18 has NP chaperone activity (Minakuchi et al. 2017). Although UAP56 and Tat-SF1 stimulate viral RNA synthesis from the naked RNA template by recruiting NP, Prp18 enhances viral RNA synthesis from vRNP complexes, suggesting that Prp18 functions as a chaperone of NP onto the virus genome (Fig. 3). Biochemical studies showed that Prp18 dissociates the nascent RNA chain from the template and stimulates the elongation after early elongation step. The binding of NP to the virus genome is required to form the vRNP template for the efficient elongation reaction by the influenza virus RNA polymerase (Kawaguchi et al. 2011). It is therefore quite possible that Prp18 maintains the structural integrity of vRNP for processive template reading.

Perspectives

Template activating factor proteins commonly have acidic stretches as functional domains for the chaperone activity. This property is not exclusive to TAF proteins, as other histone chaperones also have acidic stretches (Warren and Shechter 2017). It has been proposed that the acidic regions of histone chaperones provide non-specific electrostatic interactions with histones (Warren and Shechter 2017). It is supposed that the acidic stretches are intrinsically disordered (Warren and Shechter 2017). This unstructured domain has a potential to bind a diverse range of partner proteins and to fold them into ordered structures (Sugase et al. 2007). Despite recent extensive biochemical analyses, the mechanism of molecular chaperones remains unclear. Indeed, further structural analyses, including X-ray crystallography and cryo-electron microscopy analyses, are required to provide detailed mechanistic insights. Determination of the dynamic structures of the DNA/RNA replicon during the reaction with molecular chaperones is needed to understand the functional redundancy of each molecular chaperone.

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Compliance with ethical standards

Conflict of interest Takeshi Sekiya declares that he has no conflicts of interest. Yifan Hu declares that he has no conflicts of interest. Kohsuke Kato declares that he has no conflicts of interest. Mitsuru Okuwaki declares that he has no conflicts of interest. Atsushi Kawaguchi declares that he has no conflicts of interest. Kyosuke Nagata declares that he has no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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