Role of Coatamer Protein I in Virus Replication

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Abstract

Coatomer protein I (COPI) is well known as the protein coat surrounding vesicles involved in returning endoplasmic reticulum (ER)-resident proteins to the ER. COPI coats are also found in vesicles involved in other trafficking processes including endocytosis, autophagy and anterograde transport in the secretory pathway. In view of the diverse functions of COPI proteins, it is expected that they will affect virus replication, and many reports of such COPI involvement have now appeared. The experimental approaches most often employ specific siRNA to deplete COPI subunits or brefeldin A to block COPI activation. Here we briefly describe the results obtained with viruses in which COPI is found to have a role in replication. The results demonstrate that COPI affects viruses quite differently with effects observed in processes such as entry, RNA replication, and intracellular transport of viral proteins.

Keywords: Coatamer; COPI; Virus replication; Golgi; Arf1

Abbreviations: COPI: Coatomer Protein I; ER: Endoplasmic Reticulum; VLP: Virus-Like Particle; BFA: Brefeldin A; hpi: Hours Post Infection

COPI Function and Structure

Cytoplasmic vesicles containing a COPI coat are best known for their involvement in retrograde transport of cargo from the Golgi apparatus to the endoplasmic reticulum (ER). Resident ER proteins able to escape to the Golgi during anterograde transport of membrane glycoproteins are returned to the ER by way of COPI-coated vesicles (Figure 1). This sorting step contributes to the ability of the ER and Golgi to maintain their distinct identities.

Cell biological studies have demonstrated that COPI function is not limited to Golgi-to-ER transport; several other functions have been defined (Figure 1). For example, COPI has some functions required for anterograde transport at the Golgi [1,2]. COPI subunits have been identified in endosomal compartments suggesting they may have a role in endocytosis or in the maintenance of endocytic compartments [3-6]. Coatomer's functions in early endosomes are required for the maturation of autophagic vacuoles [7]. COPI also plays a role in expression of cell surface receptors, as well as in modulation of the plasma membrane lipid composition. For example, depletion of COPI alters the distribution of cholesterol, the sphingolipid GM1 and the Rho GTPases Cdc42 and Rac1 at the plasma membrane [8]. The diverse roles of coatamer provide opportunities for viruses to exploit this important protein complex.

The COPI complex is composed of seven subunits (α, β, β’, γ, δ, ε, and ζ) that form a cage-like structure expected to be similar to the clathrin coat [9-12]. Like the clathrin coat, the COPI coat consists of subcomplexes linked with three-fold symmetry and organized into rings with six-fold and five-fold symmetry (Figure 2). The αβε subcomplex forms the backbone of the COPI cage and associates at the vertices to form a triskelion structure [11]. The remaining subunits are located beneath the αβε backbone, close to the lipid membrane, where they interact with vesicle cargo [13-17]. COPI can mediate the formation of both vesicles and tubular structures, with vesicles formed primarily for retrograde transport and tubules for anterograde transport among Golgi cisternae [2].

For retrograde transport, vesicle formation begins when luminal proteins associate with the Golgi membrane. First, soluble proteins containing the ER localization signal KDEL interact with the membrane bound KDEL receptor (KDEL) [18]. Arf1-GEF then associates with the KDEL receptor activating Arf1-GDP. Arf1-GTP now associates with the Golgi membrane and recruits COPI, which in turn recruits Arf1-GAP [10,19-21]. For cargo sorting, KKXX domains on the cytoplasmic tails of membrane proteins associate with the gamma subunit of COPI [13]. Then COPI forms a cage around the membrane, inducing membrane curvature and forming a vesicle. Shortly after vesicle budding, the COPI coat is lost. Arf1-GTP is hydrolyzed by Arf1-GAP, which releases Arf1-GDP, Arf1-GAP, and COPI from the membrane [22]. For a diagram of COPI assembly see figure 2 of Nickel et al. [23]. Finally, the uncoated vesicle completes its journey to the ER, where it fuses with the membrane and unloads its cargo. The formation of COPI coated vesicles is inhibited by brefeldin A (BFA), a small molecule that prevents Arf1 activation [24].

Figure 1: Drawing of the known functional locations of coatamer. COPI, indicated in bright green, is present at many locations including the ERGIC, Golgi, early endosomes, and multi-vesicular bodies. Note that these locations correspond to the many functions of COPI in a typical cell including facilitating retrograde transport from the Golgi to the ERGIC and ER, anterograde transport among Golgi stacks, and transport among compartments in the endosomal pathway.

Figure 2: COPI subunits, indicated in red, are present at many locations including the ERGIC, Golgi, early endosomes, and multi-vesicular bodies.
COPI and Virus Replication

In view of the several functions of COPI in uninfected cells, it is reasonable to expect COPI would be involved in virus replication. Recent studies have shown this is the case. Most experiments use siRNA to deplete cells of COPI subunits or BFA to block Arf1 activation. Depleted or inhibited cells are then tested for their ability to support virus replication. For instance, siRNA depletion studies have documented the involvement of COPI in replication of vaccinia, drosophila C, polio and influenza viruses [25-27]. Further research has indicated that COPI has a variety of roles in the lifecycles of different viruses including involvement in virus entry, RNA replication, and intracellular transport.

In the remainder of this review, we describe studies that have documented involvement of COPI subunits in specific aspects of virus growth including the processes mentioned above. The studies reviewed were published prior to June 2012. The results are summarized in Table 1. For comparison, a description has been included of the role of COPI in Salmonella typhimurium infection. Overlapping material has recently been covered by Yang and Zhang [28].

COPI and Virus Entry

In several viruses, entry into the host cell has been found to involve COPI. Entry sometimes involves endosomes, where COPI is known to play a role in uninfected cells. This may account for the high proportion of viruses using COPI for entry. For example, four COPI subunits were identified in an siRNA screen for host factors important for influenza virus replication [27]. In order to identify the step(s) in the influenza lifecycle for which COPI is important, cells were treated with siRNA specific for δ-COP (si δ-COP). Cells were then infected with influenza virus-like particles (VLPs) containing reporter proteins. Results showed there was a two thirds reduction in the percentage of reporter positive cells from the si δ-COP sample, suggesting COPI is important for influenza virus entry [27].

Vesicular stomatitis (VSV) and Semliki Forest viruses showed reduced infectivity in cells deficient in e-COP [3]. In order to further define the role of COPI, cells were treated with siRNA specific for coatomer subunits, and infected with VSV encoding GFP. siRNA treatment was found to cause a 50-90% reduction in the number of cells expressing GFP, without generalized cytotoxic effects [29]. Fluorescence microscopy of VSV infected cells lacking e-COP showed a reduction in the number of parental virions attached to cells as well as a lower percentage of internalized virions. The results suggest COPI is required early in the VSV lifecycle, steps that include virus attachment and entry. Since VSV entry is clathrin-dependent, Cureton et al. [29] posit that coatomer knockdown causes a block in entry indirectly, perhaps by altering receptor expression at the cell surface.

Previous studies identified other COPI-dependent steps in the VSV lifecycle. For example, BFA treatment of VSV infected cells resulted in a two thirds reduction of viral RNA synthesis [30]. The same treatment also led to a decrease in translation of virus mRNA’s, but only if BFA was administered early in infection. The

Table 1: Viruses shown to require COPI for infection.

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<tr>
<th>Virus</th>
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<td>HPV16</td>
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<td>sh caveolin-1; co-localization IF [34]</td>
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<td>Semliki Forest Virus</td>
<td>Entry</td>
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<td>Drosophila C</td>
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<td>Polio</td>
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<td>Ebola</td>
<td>VP40 VLP formation</td>
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<td>SARS</td>
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results indicate COPI is not directly involved in viral translation, but is important for RNA synthesis. In addition, VSV-G protein glycosylation was inhibited by BFA [30]. This was suggested to result from failure of the Golgi secretory system to process glycoproteins, an effect known to follow COPI depletion [31]. The importance and versatility of COPI are highlighted by its functions at several steps in the VSV lifecycle.

A role for coatomer in simian virus 40 (SV40) entry has also been demonstrated. Microscopic analysis of infected, BFA-treated cells showed an accumulation of parental virions at the cell periphery instead of in the cytoplasm [32]. The percentage of infected cells was greatly reduced when neutralizing antibodies specific for β-COP were introduced before or after SV40 exposure. The results indicate COPI is important for entry as well as for later steps in SV40 replication [32].

Time-course experiments were carried out to further define the steps at which COPI is involved in SV40 infection. At 3-5 hours post infection (hpi) the SV40 capsid protein VP-1 was found to co-localize with β-COP and caveolin-1 [33]. By 10 hpi VP-1 co-localized with the ER marker PD-1. In contrast, when cells were treated with BFA, VP-1 did not co-localize with PD-1 at 10 hpi, but was found adjacent to the ER. The results suggest that after endocytosis, SV40 is trafficked to an intermediate compartment containing β-COP, and coatomer is required for its subsequent transport to the ER [33].

Similar time-course experiments were also performed with human papilloma virus 16 (HPV16). HPV16 was found to co-localize with the early endosome marker EEA1 shortly after clathrin-mediated endocytosis [34]. Twenty minutes to 2 hpi HPV16 also co-localized with caveolin-1. Later, at four hpi HPV16 co-localized with the ER markers Erp29 and calnexin. This progression suggests that HPV16 traffics similarly to SV40, from early endosomes to caveosomes to the ER. Treatment with BFA blocked virus transport to the ER and led to the accumulation of virions in caveosomes, indicating that COPI is important for virion transport from caveosomes to the ER [34].

Recent work by the group that first identified the caveosome has questioned its existence [35]. The results suggest the compartment that had been known as the caveosome is actually a modified late endosome/lysosome. Regardless of whether or not the caveosome exists, the results are clear that both SV40 and HPV16 are trafficked through an intermediate compartment in route to the ER, and that this trafficking requires COPI.

**COPI and Virus RNA Replication**

Many RNA viruses form membranous replication compartments in the cytoplasm. Picornaviruses, for example, carry out RNA synthesis on membrane-bound structures containing the virus-encoded RNA-dependent RNA polymerase. COPI has been shown to be involved in the formation of such structures. For instance, BFA treatment of cells infected with echo virus 11 (EV11) resulted in inhibition of virus replication and RNA synthesis. β-COP co-localized with sites of EV11 RNA synthesis early in infection, providing further evidence that COPI is important for formation of replication complexes [36].

Five of the seven COPI subunits were identified in an siRNA screen for host cell components required for replication of Drosophila C virus (DCV), a picorna-like virus [26]. In contrast, depletion of COPII did not affect DCV replication. However, depletion of β-COP inhibited formation of DCV replication compartments. Similar experiments with polio virus showed the same result, with COPI, but not COPII, required for virus replication [26]. Arf1 co-localized with polio virus RNA replication centers, further suggesting a role for COPI in the formation of these structures [37].

An siRNA screen for cellular factors required for hepatitis C virus (HCV) replication identified six coatomer subunits [38]. This intriguing observation was followed by a study in which the inhibitory effects of BFA were measured as a function of time after infection. The results showed an inhibitory effect of BFA up to 8 hpi, suggesting a role for COPI early in HCV infection [38]. In a similar study, BFA treatment of HCV-infected cells was found to cause decreased RNA production, but no decrease in viral protein synthesis [39]. BFA treatment also resulted in the mis-localization of HCV nonstructural protein 5A, a component of the replication complex [40]. Depletion of Arf1 and GBF1 by siRNA greatly reduced HCV replication and RNA synthesis [40,41]. The results suggest Arf1 and/or COPI are important for the formation or maintenance of HCV replication complexes.

**Coatomer and Intracellular Transport**

Roles for both COPI and COPII in Ebola virus infection have been suggested by the results of experiments in which cells were transfected with a gene encoding the Ebola matrix protein VP40 [42,43]. Transfected cells extend thin, cylindrical protrusions containing VP40 and release VLPs resembling the mature virus. Two observations support a link involving VP40, coatomer proteins, and VLP formation. First, VP40 expressed as a result of transfection is found to interact with Sec24C, a component of the COPII coat. Depletion of Sec24C by siRNA resulted in reduced VLP release, suggesting a role for Sec24C in VLP formation [42]. The second observation has to do with Rab1b, a small GTPase that activates GBF1. GBF1 is an Arf-GEF that regulates Arf1 activity. It was observed that a dominant negative Rab1b antagonized VP40-induced VLP release, supporting the idea that COPI is also involved in VLP formation [43].

Most viruses requiring COPI for replication appear to require it for entry, transport, RNA replication, or membrane morphogenesis. However, coronavirus and human immunodeficiency virus type 1 (HIV-1) use COPI in a unique way; dibasic motifs in viral proteins bind to coatomer. For instance, the cytoplasmic tail of the spike (S) protein of the coronavirus infectious bronchitis virus (IBV) contains a KXHXX motif that functions as a targeting signal, directing S protein to the endoplasmic reticulum-Golgi intermediate complex (ERGIC) near sites of virus replication [44]. A similar dibasic motif, KXXHXX, was identified on the cytoplasmic tail of the spike (S) protein of the coronavirus infectious bronchitis virus (IBV) contains a KXHXX motif that functions as a targeting signal, directing S protein to the endoplasmic reticulum-Golgi intermediate complex (ERGIC) near sites of virus replication [44]. A similar dibasic motif, KXXHXX, was identified on the cytoplasmic tail of the spike (S) protein of the coronavirus infectious bronchitis virus (IBV) contains a KXHXX motif that functions as a targeting signal, directing S protein to the endoplasmic reticulum-Golgi intermediate complex (ERGIC) near sites of virus replication [44]. A similar dibasic motif, KXXHXX, was identified on the cytoplasmic tail of the spike (S) protein of the coronavirus infectious bronchitis virus (IBV) contains a KXHXX motif that functions as a targeting signal, directing S protein to the endoplasmic reticulum-Golgi intermediate complex (ERGIC) near sites of virus replication [44]. A similar dibasic motif, KXXHXX, was identified on the cytoplasmic tail of the spike (S) protein of the coronavirus infectious bronchitis virus (IBV) contains a KXHXX motif that functions as a targeting signal, directing S protein to the endoplasmic reticulum-Golgi intermediate complex (ERGIC) near sites of virus replication [44]. A similar dibasic motif, KXXHXX, was identified on the cytoplasmic tail of the spike (S) protein of the coronavirus infectious bronchitis virus (IBV) contains a KXHXX motif that functions as a targeting signal, directing S protein to the endoplasmic reticulum-Golgi intermediate complex (ERGIC) near sites of virus replication [44].

**HIV-1 Nef is important for the downregulation of several cell surface proteins including CD4, CD8, CD28, and major histocompatibility complex class 1 (MHC-I) [46-48]. These proteins are targeted to endosomal compartments upon interacting with Nef. Nef binds to MHC-I via the adaptor protein AP-1, and this interaction requires Arf1 activation [49]. Nef binds to the cytoplasmic tail of CD4 to mediate its downregulation [50]. This process requires binding of the cytoplasmic tail of Nef with β-COP [41]. Nef contains two binding sites for β-COP [51,52]. Recent studies suggest that β-COP is
important for the transport of CD4, CD8, and MHC1 into lysosomes for degradation [48,52,53].

**COPI and Membrane Lipid Composition**

COPI is important for the attachment, entry, and RNA replication of VSV, as described above. In VSV-infected cells depleted of γ-COP, replenishment of both GM1 and cholesterol partially rescued the infection rate [8]. While COPI depletion may affect virus entry due to endocytic functions, the above results suggest it may also be important for entry by maintaining the plasma membrane lipid composition and the availability of receptors.

**COPI and Bacterial Invasion**

Viruses are not the only microbes to require COPI for infection. An siRNA screen for host factors important for invasion of *Salmonella typhimurium* identified five COPI subunits [8]. To initiate infection, *S. typhimurium* first binds to a host cell and then injects effectors via a type III secretion system. Effector injection stimulates actin rearrangements in host cells, resulting in the visually stunning phenomenon of membrane ruffling [54,55]. Treatment of host cells with si β- or γ-COP did not affect *S. typhimurium* attachment, but effector injection was greatly impaired. COPI subunit depletion also resulted in reduced invasion and decreased membrane ruffling [8]. Membrane lipid composition is known to be important for distribution of cell surface receptors. In COPI-depleted cells cholesterol, GM1, Cdc42 and Rac1 were mis-localized. This is noteworthy because Cdc42 and Rac1 are important for membrane ruffling [8]. The results suggest maintenance of the plasma membrane composition by COPI is important for *S. typhimurium* invasion.

**Conclusion**

Several screens for cellular factors important for virus replication identified COPI subunits. It is intriguing that these screens identify COPI so frequently as opposed to COPII, because both are important for the function and maintenance of the secretory pathway. If viruses required COPI simply to facilitate the production of viral proteins, then COPII should also be required. However, COPI is specifically required for replication by a wide variety of viruses. This suggests the functions of COPI other than retrograde transport are important for infection by several viruses and at least one bacterium. For example, coater’s roles in the endocytic pathway may be important for virus entry and its transport functions are used for the localization of coronavirus S proteins. The membrane morphogenesis capabilities of COPI are important for bacteria and virus entry, as well as required for the formation of RNA replication centers. COPI is a versatile complex with several important functions in uninfected cells, and these have been exploited for virus replication.

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**References**


