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Control of Adenovirus Packaging

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Abstract The results of studies of Adenovirus have contributed to our basic understanding of the molecular biology of the cell. While a great body of knowledge has been developed concerning Ad gene expression, viral replication, and effects on the infected host, the molecular details of the assembly process of Adenovirus particles are largely unknown. In this article, we would like to propose a theoretical model for the packaging and assembly of Adenovirus and present an overview of the studies that have contributed to our present understanding. In particular, we will summarize the molecular details of the process for packaging of viral DNA into virus particles and highlight the events in packaging and assembly that require further study. J. Cell. Biochem. 96: 25–35, 2005. © 2005 Wiley-Liss, Inc.

Key words: Adenovirus; assembly; DNA packaging

Since the discovery of Adenovirus (Ad) in human adenoid tissue in 1953 [Rowe et al., 1953] at least 51 human serotypes [De Jong et al., 1999; Xu et al., 2000] have been identified along with subtypes in other genera. Serotype C (Ad2 and Ad5) are among the most common infectious Ad agents and are a common cause of respiratory tract infections. The discovery in 1962 by Tenant et al. [Yabe et al., 1962] that Ad can cause tumors in rodents opened additional fields of study. More recently, the development of Ad-based vectors for gene therapeutics and vaccines (reviewed in [Roberts et al., 1986]) has again demonstrated the necessity for understanding the basic biology of this virus. The results of studies of Ad have contributed to our basic understanding of the molecular biology of the cell. Its relevance as an infectious agent is typified by the more than 95 pages of references obtained from a simple PubMed search using adenovirus and respiratory tract infection. While a great body of knowledge has been developed concerning Ad gene expression, viral replication, and effects on the infected host, the molecular details of the assembly process of Ad viral particles are largely unknown. In this article, we would like to propose a theoretical model for the packaging and assembly of Ad and present an overview of the studies that have contributed to our present understanding. In particular, we will summarize the molecular details of the process for packaging of viral DNA into Ad particles and highlight the events in packaging and assembly that require further study.

The Ad particle consists of a non-enveloped, icosahedral protein capsid that surrounds the ~36 kbp linear, double-stranded viral DNA genome. How does the Ad genome selectively go from a milieu of proteins and DNA in the nucleus of the infected cell to assemble into the icosohedral structure? We believe there are significant similarities between the packaging process of Ad and that of its distant relative, the bacteriophage. Indeed, the structural similarities between the major capsid proteins of Ad (the hexon protein), the bacterial phage PRD1 (the P3 coat protein), as well as other phage compellingly suggest that all of these viruses arose from a common ancestor in the distant past [Benson et al., 2004]. Figure 1 illustrates our concept of the packaging/assembly process of Ad.

This process simply stated involves the formation of a procapsid that is competent for packaging of the viral DNA. The procapsid contains structural proteins and nonstructural

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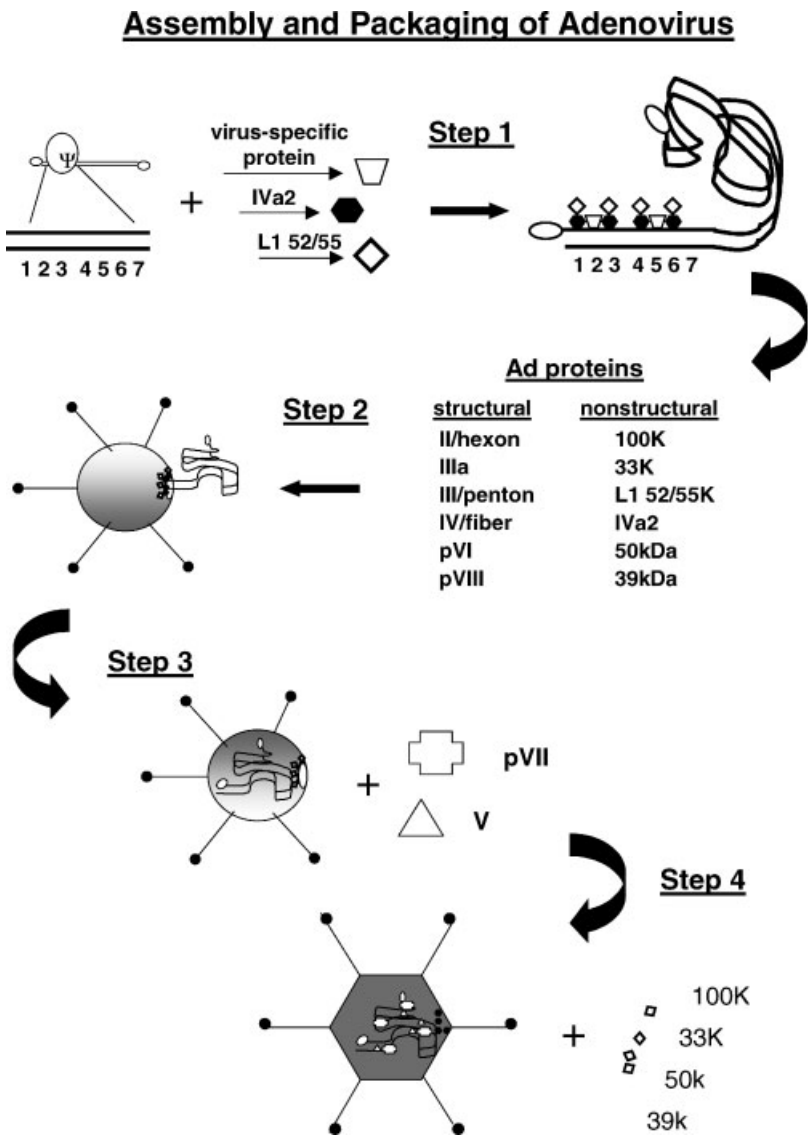

Fig. 1. Model for packaging/assembly of adenovirus. Step 1: initiation of packaging. The genome of dsDNA is represented by the double lines, ovals represent the covalently linked terminal protein, and Ψ represents the packaging domain of Ad. An enlargement of that region is shown, and the packaging A repeats 1–7 are indicated (see text). IVa2, L152/55 kDa and an unknown virus-specific protein bind to the packaging domain. Step 2: Procapsid formation. The Ad genome, along with packaging proteins, interacts with the structural and nonstructural proteins indicated resulting in the formation of a ''procapsid'' structure with the viral DNA. The circle represents the procapsid consisting of structural and nonstructural proteins; the lines and filled circles represent the fiber protein. Step 3: Encapsidation of viral DNA into the procapsid. A putative packaging motor directs the encapsidation of the complete viral genome into the immature virus particle through the portal complex. Protein V and protein pVII are found in particles containing full-length DNA. Step 4: Maturation of the virus particle. Ad particles containing full length DNA and having a density of 1.34 g/cc in cesium chloride do not contain proteins 100K, 33K, 50 kDa, and 40 kDa proteins. Substrates of the viral proteinase are cleaved in the mature Ad particle and the angular structure of the icosohedron is observed. This model is discussed in detail in the text.

proteins (referred to as scaffolding proteins) necessary for initial assembly of the procapsid. The recognition of the procapsid by the viral DNA is likely mediated by proteins binding to the packaging domain of Ad (Fig. 1, Step 1). Hypothetically, Ad DNA is incorporated into the procapsid through an opening referred to as the portal (Fig. 1, Step 2). Its location is likely at a unique vertex of the icosahedral particle. By analogy to packaging mechanisms described with bacteriophage, an ATP-driven motor is essential for moving the viral DNA into

the capsid (Fig. 1, Step 3). Once the DNA is encapsidated, the portal is sealed and final maturation of the particle occurs (Fig. 1, Step 4).

The assembly of the Ad infectious particle requires at least 12 viral proteins and the linear, double-stranded DNA molecule. Biochemical and electron microscopic studies [Macintyre et al., 1969; Boulanger et al., 1979; Adam and Nasz, 1980, 1981, 1984; Roberts et al., 1986; Furcinitti et al., 1989; Stewart et al., 1991, 1993; Stouten et al., 1992; Athappilly et al., 1994; Stewart and Burnett, 1995] (recently reviewed in [San Martin and Burnett, 2003]) have contributed to our present understanding of the structure of the mature viral particle. Still, the precise location of some of the proteins in the immature procapsid and mature capsid, as well as the organization of viral DNA within the particle, is largely unknown. Briefly, the major capsid protein forming the 20 surfaces of the icosahedron is the trimer of protein II called hexon. Located at the vertices of the icosahedron are the penton base formed by protein III and a trimer of protein IV, called fiber. The fiber and penton proteins are involved in recognition of the cellular receptor for Ad and internalization of the virus. Additional Ad structural proteins include IIIa, VI, VIII, and IX. It appears that protein IIIa is largely on the outer surface of the Ad particle, although part of the protein is also found in the interior of the virus presumably contacting four hexons at the edges of the icosahedron. Protein IX associates with hexons on the outer surface of the facets of the particle; three trimers of protein IX interact with the group of nine hexons. Proteins IIIa and IX likely play roles in stabilizing the structure of the capsid, although they may have other functions as well [Rosa-Calatrava et al., 2001, 2003; Molin et al., 2002; Parks, 2005]. Proteins VVI and VIII are located on the interior of the Ad particle, but their actual locations are not known. In addition to its structural role, protein VI also assists in the nuclear transport of hexons within an infected cell [Kauffman and Ginsberg, 1976]. The DNA in the Ad particle is associated with proteins V, VII, and mu forming the core. The linear, double-stranded Ad DNA genome is characterized by having ~100 bp inverted terminal repeats (ITRs) and terminal proteins covalently linked to the 5′ ends of the DNA; these are essential for viral DNA replication. Also located within the capsid, the viral proteinase plays an important role in maturation of the infectious virus during the last step of the assembly process. Proteins VI, VII, VIII, mu, and terminal protein are all synthesized as longer, precursor versions. They undergo processing by the Ad proteinase, a step that is essential for the production of mature, fully-infectious virus [Anderson et al., 1973; Freimuth and Anderson, 1993; Mangel et al., 2003; Gupta et al., 2004].

Several non-structural proteins are found associated with either Ad assembly intermediates or the mature viral particle. These are proteins IVa2, L1 52/55K, L4 100K, and L4 33K. The IVa2 and L1 52/55K proteins will be discussed in greater detail later. The L4 100K protein is essential for protein II to form a hexon trimer [Cepko and Sharp, 1982, 1983; Gambke and Deppert, 1983], the building block of the capsid, and it may serve as a scaffolding protein for assembly of Ad [Oosterom-Dragon and Ginsberg, 1981; Morin and Boulanger, 1986]. Results from studies of mutant viruses for the L4 33K protein have shown that this protein also plays a role in the Ad assembly process [Oosterom-Dragon and Ginsberg, 1981; Morin and Boulanger, 1986]. Similar to scaffolding proteins with other viruses, 33K is found in Ad assembly intermediates but not in the mature virus particle.

Ad is amenable to genetic study. Mutations may be introduced into the Ad genome by several methods. Traditionally, subgenomic fragments of Ad were mutagenized by standard methods and then restored into the viral genome by transfection of cells with overlapping fragments of the viral genome [Stow, 1981; Schmid and Hearing, 1999]. Recombination between these DNA fragments within the cell reconstructs the intact Ad genome. More recently, bacterial clones that contain infectious genomes of Ads have been established [Chartier et al., 1996; Crouzet et al., 1997; Lusky et al., 1998]. The Ad genomic clones may be manipulated like a standard bacterial plasmid and the final mutagenized Ad genome excised for transfection into cells. With either approach, infectious virus may be recovered by complementation if the mutations are not lethal. If the mutations are severely detrimental or lethal, then complementation using an appropriate helper cell line is required. Complementing cell lines have been established for numerous, different viral gene products [Krougliak and Graham, 1995; Amalfitano et al., 1996; Imler

et al., 1996; Yeh et al., 1996; Gustin and Imperiale, 1998; Gao et al., 2000; Oualikene et al., 2000; Zhang and Imperiale, 2003].

The initiating steps in the assembly process are the formation of the procapsid and recognition of the procapsid by viral DNA (Fig. 1, Step 1). Ad DNA is packaged in a polar fashion starting at the left end of the genome [Daniell, 1976; Tibbetts, 1977; Hammarskjold and Winberg, 1980; Hearing et al., 1987]. Extensive analyses of the Ad5 genome have shown that there is a domain between left end nucleotides 200 and 400 that is absolutely required for encapsidation of viral DNA [Hearing et al., 1987; Grable and Hearing, 1990; Grable and Hearing, 1992; Schmid and Hearing, 1997, 1998, 1999; Sandig et al., 2000; Ostapchuk and Hearing, 2003b]. This domain can function at the right end of the genome as well, and recombinant genomes carrying packaging domains at both ends of the genome are viable [Hearing and Shenk, 1983; Parks and Graham, 1997]. Although the packaging domain can be moved internally and still maintain activity, it must be situated within ~600 bp from the terminus in order for packaging to occur. It seemed possible that the limitations on location of the packaging domain reflected a functional interaction between the packaging domain (and bound proteins) and the ITR and/or the covalently-linked terminal protein. There is a precedent based on experiments with the bacteriophage ϕ29 for such an interaction to occur. ϕ29 has a genomic structure similar to Ad and the ϕ29 terminal protein appears to play a role in recognition of the capsid in the packaging process [Guo, 2002]. Recent evidence, however, shows that the ITRs, along with the covalently-linked terminal protein, are not needed for Ad packaging. Subgenomes lacking either or both ITRs and terminal proteins were found to be packaged with an efficiency similar to that observed with the WT genome [Ostapchuk and Hearing, 2003a]. What is absolutely required for Ad packaging are a series of repeated sequences located within Ad nucleotides ~200–400. Seven related sequences, termed A repeats because of their AT-rich nature, comprise the packaging domain of Ad5 (Fig. 2A) (reviewed in [Schmid and Hearing, 1999; Ostapchuk and Hearing, 2003b]). These repeats are functionally redundant, although they are not functionally equivalent. Extensive genetic analyses of the Ad5 packaging domain led to several significant findings: (1) the A repeats function in conjunction with one another and the combination of A repeats 1 and 2 or A repeats 5, 6, and 7 are the most critical for packaging activity; and, (2) a sequence homology shared by the most important A repeats, 5′-TTTG-N_8-CG-3′ (Fig. 2B), is essential for packaging domain function.

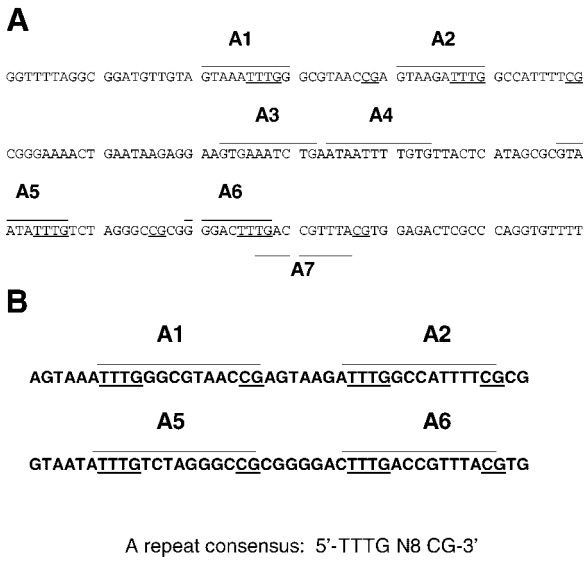

Fig. 2. Ad5 packaging domain. **A**: Nucleotide sequence of the packaging domain of Ad5 (nt 220–400 relative to the left end terminus). Lines and numbers above/below the Ad5 sequence indicate the seven AT-rich repeats that contribute to Ad5 packaging. The underlined nucleotides (TTTG, CG) depict the consensus sequence motif. **B**: Alignment of A repeats 1 and 2 with A repeats 5 and 6. Significant homology is observed between the sequences of the adjacent A repeats as well as the 21 bp spacing between A repeats 1 and 2 and A repeats 5 and 6. The consensus A repeat sequence motif is shown.

Synthetic packaging A repeats were inserted into the Ad5 genome in place of the authentic packaging domain [Schmid and Hearing, 1998]. Viruses with WT or very close to wild type levels of packaging were recovered that contained: (1) a dimerized pair of A repeats 1 and 2; (2) dimeric copies of A repeats 5, 6, and 7; or (3) six tandem copies of A repeat 1. In contrast, a genome with a synthetic packaging sequences consisting of six copies of A repeat 6 was severely defective for growth. Revertants of this defective virus were recovered that carried increased copies of A repeat 6. Based on the accumulated data, it seems likely that the packaging domain, and the critical A repeat sequences within this region, are binding sites for proteins that play a role in promoting pack-

aging of the viral genome. This idea is consistent with observations that the activity of the Ad5 packaging domain may be competed in trans by unlinked copies of packaging sequences [Grable and Hearing, 1992]. The most obvious possibility is that proteins which bind to the packaging domain also recognize the procapsid and initiate the packaging process. Therefore, identification of these proteins would be an important step to understand the process of Ad assembly.

The packaging repeats of Ad5 are situated within sequences that comprise transcriptional enhancers for the early genes [Hearing and Shenk, 1983, 1986; Hatfield and Hearing, 1991, 1993; Bolwig et al., 1992]. Therefore, the search for proteins involved in packaging is complicated by the fact that this region also binds numerous transcriptional regulatory proteins. These proteins may or may not have a dual role, one for transcription and another for packaging. Utilization of synthetic packaging elements as probes with in vitro DNA–protein binding assays narrowed the focus for relevant binding activities [Schmid and Hearing, 1998]. The cellular proteins OCT-1 (octamer-1) binding protein [Erturk et al., 2003], COUP-TF (chicken ovalbumin upstream promoter transcription factor) [Cooney et al., 1992], and CDP (CAAT displacement protein) [Erturk et al., 2003], were found to bind the packaging elements along with the viral protein, IVa2 [Perez-Romero et al., 2005], and an additional viral-specific protein of unknown origin [Ostapchuk et al., 2005]. It is not known if the virus encodes this unknown protein or the viral infection induces a cellular protein. The cumulative results from genetic analyses, in vitro DNA–protein binding studies, and chromatin immunoprecipitation assays demonstrate the best correlation between packaging activity and DNA–protein complexes formed on packaging sequences that contain the viral IVa2 protein and the unknown, viral-specific protein [Ostapchuk et al., 2005; Perez-Romero et al., 2005]. Previous studies showed that IVa2 is part of a protein complex that binds to a transcriptional element downstream of the major late promoter (MLP), termed the DE element [Tribouley et al., 1994]. The DE element consists of two parts, termed the DE1 and DE2 sites. IVa2 plays a role in transcriptional activation of the MLP [Reach et al., 1990; Pardo-Mateos and Young, 2004b]. Figure 3 shows a comparison of the binding site for the IVa2 protein in the MLP DE2 element

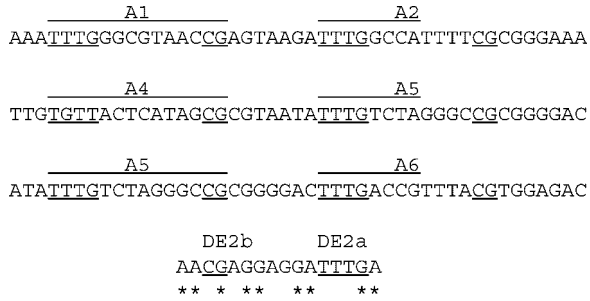

Fig. 3. Alignment of the nucleotide sequences of packaging elements with IVa2 DE2a and DE2b binding sites in the Ad MLP. Shown are the sequences of A repeats 1 and 2, A repeats 4 and 5, A repeats 5 and 6, and the IVa2 DE2a and DE2b binding sites in the MLP. The A repeat consensus motifs (5′-TTTG-N_8-CG-3′), and corresponding nucleotides in the MLP De2b/a sites, are underlined. Asterixs below the DE2b and DE2a sites denote nucleotides required for DEF binding using methylation interference assays (REF).

with IVa2 binding sites in the packaging domain. The DE2 site coincides with the CG sequence motif of the Ad5 packaging repeats. To the right of the IVa2 DE2 binding site (termed DE2b) is the binding site for another protein (termed the DE2a site, Fig. 3). This region shares sequence homology with the critical packaging sequences, TTTG (Fig. 3). It seems likely that the MLP DE2 region and Ad5 packaging repeats bind the same complex: IVa2 protein and the unknown, viral-specific protein. The MLP DE element also contains an additional protein binding site (termed DE1) located upstream of DE2. The DE1 element is critical for MLP activation along with other transcription factor binding sites in the MLP 5′ flanking region [Albrecht et al., 1988; Lutz and Kedinger, 1996]. The DE1 element does not appear to contain a counterpart in the Ad5 packaging domain [Zhang and Imperiale, 2000; Ostapchuk et al., 2005]. It seems likely that similar or identical DNA–proteins complexes that contain the IVa2 protein contribute both to activation of the MLP as well as to packaging function.

How might the complex containing the IVa2 protein and the unknown, virus-specific protein promote packaging? The IVa2 protein contains Walker A and B box consensus motifs implicating this protein may have ATPase activity [Koonin et al., 1993]. Comparison of the amino acid sequences encompassing the putative Walker boxes of the Ad5 IVa2 protein with IVa2 proteins from other Ad serotypes, including Ads that infect species other than human,

shows significant conservation supporting their importance for IVa2 protein function. Mutation of a conserved lysine residue in the Walker A box of Ad5 IVa2 resulted in the loss of virus viability [Pardo-Mateos and Young, 2004a,b]. Proteins with ATPase activity are found in cellular chromatin modifying complexes and may provide the working arm for nucleosome movement on cellular chromosomes [Tsukiyama, 2002; Langst and Becker, 2004]. ATPases also are crucial proteins in packaging motors of bacteriophages [Simpson et al., 2000; Molineux, 2001; Rao and Mitchell, 2001; Camacho et al., 2003; Serwer, 2003; Mancini et al., 2004]. Perhaps the IVa2 protein provides ATPase activity for transcriptional events at the major late promoter and this same ATPase activity is used for driving a packaging motor in the procapsid. An important step in understanding the role of the IVa2 protein during Ad infection is to determine if it indeed has ATPase activity.

A null mutation in the IVa2 coding region results in a nonviable virus [Zhang and Imperiale, 2003]. The IVa2 null virus, pm8002, may be propagated on a complementing cell line that constitutively produces IVa2 protein. The complementation of pm8002 using this cell line is not complete resulting in an ~100 fold reduction in virus yield compared to WT virus. However, this virus does replicate and express its genes albeit at a slower rate than WT virus. What is remarkable is that this IVa2 mutant does not produce any virus particles when pm8002-infected cells are examined by electron microscopy [Zhang and Imperiale, 2003]. Thus, it appears that the IVa2 protein is involved in very early steps of the virus assembly process as well as in viral DNA encapsidation. If these two events are linked is unclear. However, it was previously noted that an Ad5 mutant, with a deletion in the packaging domain that reduced virus production, also showed a reduction in empty particles that lack viral DNA [Hearing and Shenk, 1983; Hasson et al., 1992]. These complementing results suggest that IVa2 binding to the packaging domain also may be linked to initial steps in the assembly of the particle. One possibility is that the binding of the IVa2 protein and additional virus-specific proteins to the packaging domain acts as a nucleation site to initiate the formation of a procapsid, followed by the presumably ATP-dependent incorporation of the remaining viral DNA.

The IVa2 protein of Ad5 has been shown to bind the L1 52/55K protein [Gustin et al., 1996]. A temperature sensitive viral mutant in the L1 52/55K (ts369) is blocked for completion of viral DNA packaging at the nonpermissive temperature [Hasson et al., 1989]. Ts369 virus particles isolated at the nonpermissive temperature have a density lighter than that of mature virus. Examination of the DNA content of these particles demonstrated that they contain fragments of ~1,000 bp derived from the left end of the viral genome. This observation is consistent with the idea that the packaging of ts369 genome was initiated and then ceased. Similar kinds of assembly intermediates have been isolated from other Ad serotypes [Tibbetts, 1977]. That is, particles may be isolated that contain increasingly larger DNA fragments that start from the left end. It is likely that the DNA remaining outside the particle was sheared off during virus purification resulting in small DNA fragments that were protected inside the particle. The L1 52/55K protein is found associated with the ts369 particles grown at the nonpermissive temperature as well as in the empty capsids of wild type virus [Hasson et al., 1989]. In addition, immunologically related bands of 40K and 34K are seen. A putative viral proteinase site is found in the L1 52/55K protein such that cleavage may produce these smaller products. In mature WT virus particles, only the smaller products are observed. Consistent with cleavage of the L1 52/55K protein by the viral proteinase with WT virus, only full length 52/55K protein was observed in capsids isolated from Ad5 ts1-infected cells; Ad5 ts1 is temperature sensitive for viral proteinase activity [Greber et al., 1996]. In addition to the IVa2 and L1 52/55K proteins, both IVa2 and 52/55K have been shown to bind the packaging domain of Ad in vivo as analyzed using chromatin immunoprecipitation (ChIP) analyses [Ostapchuk et al., 2005; Perez-Romero et al., 2005]. The virus-specific protein that binds to the packaging domain in conjunction with IVa2 is not the L1 52/55K product [Zhang and Imperiale, 2000; Ostapchuk et al., 2005]. It would seem likely that the association of the L1 52/55K protein with the packaging domain is mediated by binding to IVa2. However, the results from ChIP experiments yielded different results since the L1 52/55K protein associated with the packaging domain in the absence of IVa2 [Perez-Romero et al., 2005]. Interestingly, electron microscopic

examination of the nuclei of cells infected with a mutant virus defective for L1 52/55K expression showed: (1) that there were many fewer particles formed than with WT virus; and, (2) the particles that did form in these cells appeared to be empty [Gustin and Imperiale, 1998]. This reduction in particles is reminiscent of, although less dramatic than, the phenotype seen with the IVa2 null virus. The roles of the IVa2 and L1 52/55K proteins in virus packaging and assembly appear to be very complex. If they play more than one role in virus assembly, e.g., as scaffolding or chaperone-type roles as well as a direct role in viral DNA packaging as part of a motor/portal complex, awaits further analysis.

The location of the L1 52/55K and IVa2 proteins in immature and mature particles is not known. If indeed IVa2 is the ATPase of a packaging motor and the L1 52/55K protein is part of the packaging structure, one might expect that these proteins would be located at a unique vertex during the assembly process, by analogy to the role of similar proteins with bacteriophage (e.g. [Stromsten et al., 2003] and herpes simplex virus [Newcomb et al., 2001; Singer et al., 2005]). Identification of the location of these proteins in immature Ad particles would assist in understanding their role in assembly and packaging.

Although many details are known about the Ad packaging domain, little is known about the procapsid of Ad. In fact, a procapsid per se has not been isolated. Importantly, however, its existence is suggested in the accumulated data from studies of Ad assembly intermediates. Various kinds of Ad particles have been identified by equilibrium and zonal centrifugation approaches, and pulse-chase experiments suggest that these putative assembly intermediates appear before the completion of the mature virus particle. Mature, infectious Ad virions band on a cesium chloride (CsCl) gradient at a density of 1.34 g/cc. Virus particles produced in cells infected with ts1 (the temperature sensitive proteinase mutant) band at a density of 1.37 g/cc in a CsCl gradient, and subsequent analyses have shown that the final event in virus maturation involves numerous internal cleavages of precursor structural proteins by the viral proteinase [Hannan et al., 1983], an event that is necessary to generate fully infectious virus that bands at 1.34 g/cc in CsCl [Greber et al., 1996].

Virus particles with densities that are lighter than mature virus (e.g., 1.29 g/cc and 1.30 g/cc) also have been identified using CsCl gradients. These appear to be capsid particles devoid of DNA and particles with a small fragment of Ad DNA, respectively [Prage et al., 1972; Ishibashi and Maizel, 1974; Edvardsson et al., 1976; D'Halluin et al., 1978b]. The major structural proteins (hexon, penton, fiber, IIIa, pVI and pVIII-p denoting the unprocessed form of the proteins) are found in these immature particles. The major proteins that contribute to the viral DNA core structure, proteins V and VII, are either missing in these immature particles or, in the case of VII, when seen is in the non-processed form. Two additional proteins have been detected in the immature virus particles of 50K and 40K, but the identity of these proteins is unknown [Hasson et al., 1989]. Speculatively, these proteins may function as scaffolding proteins for virus assembly since they appear to exit the virus particles during the maturation process.

It is not clear if the immature capsid which bands in CsCl at 1.29 g/cc is the elusive Ad procapsid, an empty dead-end particle, or a mixture of both. Certainly, assembly dead-end particles have been identified with other viruses [Homa and B, 1997]. One reason to suspect that particles banding at a density of 1.29 g/cc may be dead-end intermediates or a mixture comes from the results of experiments using approaches other than CsCl gradients as a first step in virus particle isolation. Ad assembly intermediates isolated on sucrose or Ficoll gradients have sedimentation rates (550–650S) different from that of the mature, infectious particle (750S). Cross-linking experiments have demonstrated that these intermediate particles are heterogeneous with respect to protein and DNA content when further characterized on CsCl gradients [D'Halluin et al., 1978a,b]. Interestingly, particles banding at a density of 1.29 g/cc were not observed on CsCl gradients using any of the assembly intermediates isolated first on sucrose or Ficoll gradients, suggesting that they may represent an artifact of CsCl gradient isolation. Virus particles that band at densities 1.30, 1.34, and 1.37 g/cc in CsCl were identified using intermediates isolated first on sucrose or Ficoll gradients. The lightest density particles (1.30 g/cc) contain small segments of DNA. In addition, these lightest particles have a rounded appearance by electron microscopy, a

characteristic of procapsids. The observation of particles with small fragments of DNA is consistent with the idea that the IVa2, L1 52/55K, and other packaging proteins nucleate the formation of a procapsid. More detailed characterization of these immature particles is needed to clarify the relationship between the different intermediates observed in these studies, and identification of the proteins in these structures will help to unravel the adenovirus packaging and assembly process.

Similar genomic organizations are observed among the Mastadenoviruses. DNA sequences that resemble Ad5 packaging A repeats can be found in the left ends of these viruses suggesting a common packaging process. However, serotype specificity of viral DNA packaging clearly is evident suggesting unique aspects of packaging of different Adenoviruses [Ostapchuk and Hearing, 2001]. The results suggested that this specificity is provided in *cis* by the viral genome, presumably the packaging domain. Since the terminal protein is not required for packaging, one likely possibility is that serotype specificity of packaging is mediated by virus-specific proteins that bind to packaging sequences. Results from studies using a hybrid virus composed of the replication and packaging domains of Ad5 and the coding regions of Ad7 support this idea [Zhang et al., 2001]. It appears that proteins IVa2 and L1 52/55K are likely candidates for initiating the serotype specificity of Ad DNA packaging. At present, little is known about the molecular details of this specificity.

Understanding the packaging and assembly process of Ad provides valuable information in approaching the development of antiviral agents and vaccines. Since viruses utilize the host it has been difficult to develop pharmacological agents that would block virus growth, but not affect the host. Assembly and packaging are virus-specific events and may provide useful targets for antiviral agents. Furthermore, the elucidation of the assembly and packaging process may contribute to the development of Ad-based vaccines and gene therapy vectors. The isolation and purification of a Ad packaging motor would contribute to our general knowledge of molecular motors and this information could contribute to the development of nanotechnological tools. Lastly, the study of the Ad assembly process reveals detailed information regarding protein–protein and protein–nucleic acid interactions that lead to the formation of higher order structures.

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