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The Impact of Processing Body Proteins on an Adenovirus Infection

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Table of Contents

Table of Contents

List of Figures

Abstract

Adenovirus is a nonenveloped, double-stranded DNA virus that contains a 36 Kbp genome. The E4 ORF3 region of its genome consists of an E4 11k protein that can reorganize host cell components by relocalizing cellular proteins. One such cellular component is the cytoplasmic processing body, which consists of proteins involved in translational repression of mRNA and mRNA degradation. During an adenovirus infection, processing body proteins Ddx6 and Pat1b have been shown to colocalize and form aggresomes. Aggresomes induced by E4 11k are specific to the adenovirus serotype 5 (Ad 5). In this study, cytoplasmic Pat1b foci were observed and quantified to determine E4 11k's effect on Pat1b during an infection. Our data reveals that Ad 5 E4 11k is necessary and sufficient for increasing Pat1b foci in Ad 5-infected cells. To determine whether this increase in Pat1b foci is serotype-specific, we also studied the localization pattern of Pat1b in Ad 9 and Ad 12 E1-replacement viruses. There was a significant increase in Pat1b foci in Ad 5-infected cells compared to mock and the other two serotypes suggesting that rearrangement of Pat1b is serotype-specific. Additional studies were done to determine the effect of p-body rearrangement by E4 11k on late gene expression, but the results are currently inconclusive. Altogether, our data contributes to the understanding of how viruses can disrupt p-body proteins during an infection.

Introduction

Human adenoviruses (HAdVs) are ubiquitous in humans. Depending on their surrounding environmental conditions, these viruses can survive between seven and three months outside of their host (Kramer et al. 2006). HAdVs belong to the genus *Mastadenovirus* in the Adenoviridae family. These viruses are further classified into seven distinct species (HAdV-A to HAdV-G) and contain over 67 serotypes. Among the 67 serotypes are 70 different viral genotypes. Human adenoviruses serotypes 52-68 are a result of interspecies recombination between the preexisting 51 serotypes. Recombination of this virus can potentially lead to increased viral fitness, enhanced virulence, and altered host cell tropism. Adenoviruses can cause an array of medical conditions associated with respiratory and gastrointestinal symptoms or a mixture of both (Cook and Radke 2017).

Human adenoviruses are usually transmitted through aerosolized droplets (respiratory infections) or through the fecal-oral route (gastrointestinal infections). Different subgroups tend to be associated with certain clinical conditions. Subgroups (A, B type 1 and 2, C, D, F and G) can cause gastrointestinal infections. Respiratory infections are caused by subgroups (A, B type 1 and 2, C and E). Subgroups (B type 1, D and E) can result in keratoconjunctivitis, and subgroups (A, B type 1 and 2, and C) are responsible for urinary tract infections (Ghebremedhin 2014). Not many people with HAdVs will develop severe illnesses. Most severe HAdV infections occur in immunosuppressed adults and young children; however, it is possible for individuals with no preexisting risk factors to become severely infected (Munoz et al. 1998).

Currently, there are no drugs approved to specifically treat Adenovirus (Ad) infections; however, Cidofovir, an antiviral drug, has clinically been used in stem cell transplant patients when Ad DNA has been detected inside of a patient. One of Cidofovir`s functions is that it terminates synthesis of Ad DNA, but a major concern regarding Cidofovir`s use is that it can

lead to kidney toxicity (Wold and Toth 2013). Another important issue is that antibodies that are produced to target adenoviruses are serotype-specific. Since not all of the serotypes for adenoviral infections have been studied, this presents a challenge for creating vaccines for the general public. There is a live, oral enteric-coated vaccine that is available to military recruits to prevent acute respiratory diseases. HAdV species B consists of multiple serotypes that are associated with acute respiratory diseases. HAdV species B serotypes 3 and 7 are the most common adenovirus linked to respiratory outbreaks in the US military resulting in approximately 80% of respiratory diseases among military recruits. These vaccines are used to treat Ad 4 and Ad 7 serotypes (Chen and Tian 2018).

Advances in molecular techniques have re-established interest in adenoviral biology. As a result, adenovirus serotype 5 (Ad 5) is well characterized. Modified versions of Ad 5 displays promising use for future vaccination purposes and gene therapy. Additionally, adenoviral biology has expanded our knowledge of cellular and molecular biology. For example, mRNA splicing was first discovered in adenovirus serotype 2 (Ad 2) and contributed to our understanding of mRNA splicing. Research on protein-protein interactions during an Ad 5 infection has also led to the discovery of multiple transcription factors (Berget et al. 1977). Because of the vital role Ad 5 has in molecular and cellular biology, understanding the life cycle of Ad 5 during an infection can be beneficial.

The life cycle of the adenovirus is generally categorized into two stages. This classification is based on whether proteins are being expressed before or after viral DNA replication. Early proteins (E1B, E2, E3, and E4) are under the control of the E1A protein. Early proteins can target and form complexes with key cellular components. As a result, cellular activities are manipulated to produce an environment favorable for viral DNA replication, to

inhibit immune responses, apoptotic pathways and promote viral gene expression. The Ad genome also consists of a major late promoter (MLP) (Figure 1) that directs the synthesis of a late pre-mRNA. The pre-mRNA undergoes alternative splicing to produce five late mRNAs (L1-L5) (Figure 1). Late transcriptional units encode structural proteins that are responsible for virion assembly and encapsidation (Parks et al. 2005).

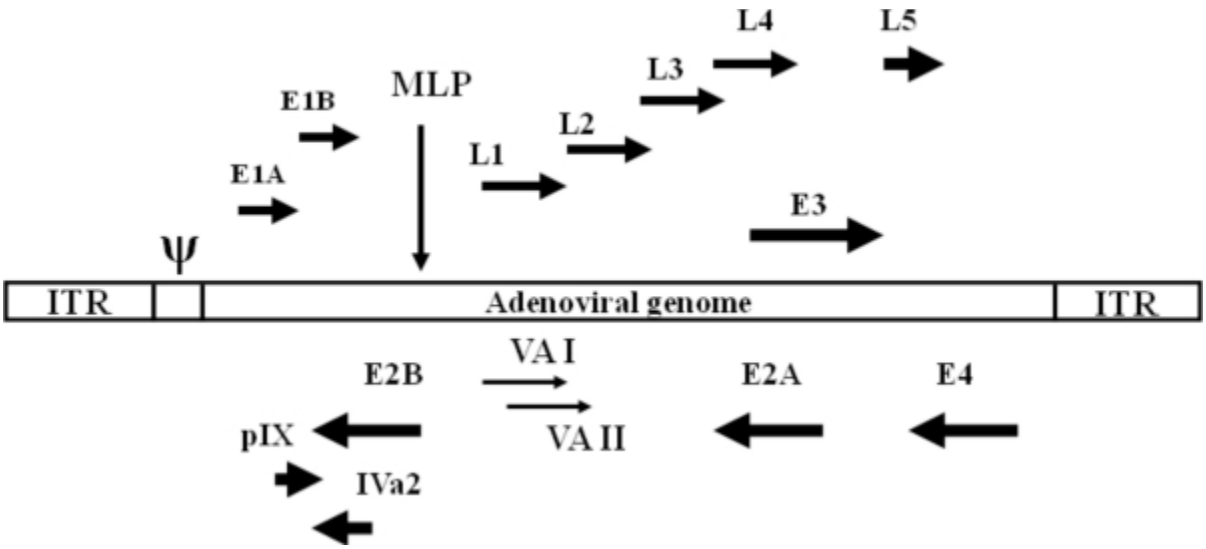

Figure 1: Adenovirus transcriptional map (Vetrini and Ng 2010).

In this study, we focused on the early region 4, which encodes an E4 11k protein and E4 34k protein (products of E4 ORF3 and E4 ORF6, respectively). The E4 region of the early genes is involved in DNA replication, shutoff of cellular protein synthesis, and the production of late viral proteins (Bridge and Ketner 1990). Mutations in the E4 region, specifically the E4 11k and E4 34k proteins, affect viral replication and gene expression. The E4 11k protein can influence viral replication of the Ad virus by disrupting cellular antiviral response systems and has demonstrated that it can relocalize processing body (P-body) proteins; Lsm1, and Ddx6 to aggresomes (Greer et al. 2011). Processing bodies are suggested to be sites for mRNA

degradation. During an adenovirus infection, multiple p-body proteins, including Lsm1 and Ddx6, are relocalized to juxtanuclear aggregates, called aggresomes. Aggresomes are aggregates of misfolded proteins and are believed to be degradation sites for proteins that are not functioning properly (Greer et al. 2011).

Adenovirus Structure and Gene Expression

Human adenoviruses (HAdVs) are double-stranded DNA viruses that range from 65nm to 90 nm in diameter. They consist of an inner core, which contains six proteins: 23k maturation protease, terminal protein, V, VII, Mu, and IVa2 (Figure 2). These proteins are bundled with the viral DNA. The core is enclosed by an icosahedral capsid that consists of major proteins and minor proteins: IIIa, VI, VIII and IX (Figure 2). Protein VI is located below the penton base, which allows it to interact with protein V (Vellinga et al. 2005).

Hexon (protein II), fiber (protein IV) and penton base (protein III) are the major capsid proteins (Figure 2). The hexon capsid consists of 720 hexon monomers that form 240 hexon homotrimers, which gives the capsid its icosahedral shape. Protein II has a hexagonal shape that allows for optimal packaging of viral DNA. The penton protein constructs 12 penton base complexes on the 12 vertices located on the capsid. The elongated fiber protein protrudes from each penton base complex and initiates adenovirus infections by attaching to cellular receptors, such as the coxsackie and adenovirus receptor (CAR) or membrane cofactor protein (MCP) (Vellinga et al. 2005).

Once the fiber and penton base proteins bind to the host cell receptors, integrin-mediated endocytosis occurs. Protein VI then engages in the nuclear transportation of the Ad genome by prompting pH-independent disruptions of endosomes. Protein VI then aids the virion particles in the departure from endosomal compartments. As a result, endosome lysis occurs and protein VI

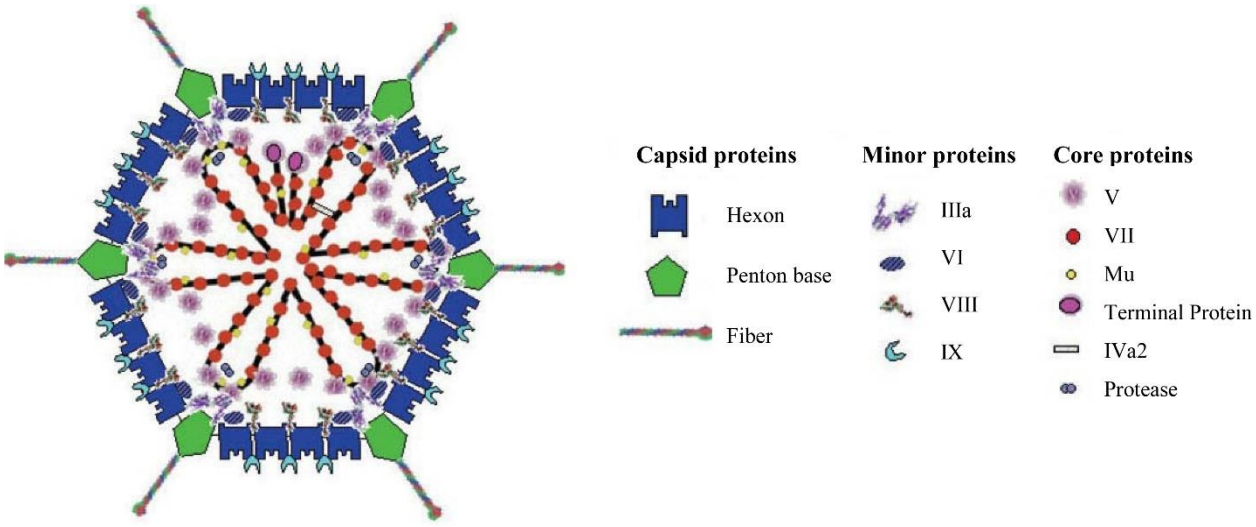

Figure 2: Structure of the adenovirus core and capsid (Russell 2000).

is able to enter into the cytoplasm (Vellinga et al. 2005). After protein VI has allowed for the virus content to be released from endosomes, virus particles are then transported to the nucleus on microtubules by dynein motor proteins. It is taken to the microtubule organization center and then interacts with the nucleoporin NUP214/CAN cytoplasmic filament. During its import to the nucleus, viral DNA is coated with VII protein. Nuclear import of the Ad genome is usually a result of nuclear localization sequence (NLS)-associated proteins binding to nucleic acids (Johnson et al. 2004). Both protein VI and protein VII have NLSs that transport the virus from the cytoplasm to the nucleus. Protein VII's NLS binds to importin beta via the adapter importin alpha or independently. This then allows for the protein VII-DNA complex to enter the nuclear pore complex (NPC). Once protein VII and the viral DNA have entered the nucleus, they continue to bind and remain in the nucleus until the onset of viral DNA replication. E1A becomes the first protein expressed and initiates transcription of other viral genes (E1B, E2, E3 and E4). E1A's ability to associate with DNA is dependent on its interaction with VII, which is

suggested to be the initial step in stimulating transcription of other early genes. (Johnson et al. 2004). Early proteins have functions in manipulating host cell machinery to produce environments favorable for viral replication. After approximately 12 hours post infection (hpi), viral DNA replication occurs and late viral genes are expressed.

The late proteins (L1-L5) (Figure 1) are responsible for forming the capsid and viral assembly and maturation (Russell 2000). After Ad DNA replication, the IX and IVa2 proteins become highly expressed and the major late promoter (MLP) becomes fully functional for transcription. Late viral messenger RNAs are exported from the nucleus to the cytoplasm via the E1B 55k and E4 ORF6 complex. The IVa2 and L4 33k proteins bind to the MLP and stimulate transcription (Ali et al. 2007). The L4-100k protein promotes viral mRNA translation by eliminating the cap-dependent translational pathways and suppressing cellular mRNA translational activity (Koyuncu and Dobner 2009). Efficient translation of viral RNAs requires the L4 100k protein. In addition, L4 100k and L4 33k are involved in capsid assembly (Koyuncu and Dobner 2009). When the L4 33k protein is mutated, it impairs the genome packaging process by producing empty capsids (Wu et al. 2013). This means that the L4 33k protein is important for encapsidation of the Ad viral genome. Once a sufficient number of virions are produced, lysis of the cell occurs.

Early Adenoviral Proteins

The human adenovirus genome encodes approximately 40 proteins, which are categorized into two major stages. Classification of Ad transcriptional units is dependent on whether a protein is expressed before DNA replication (early) or after DNA replication (late). Both early and late gene products have functions in transcriptional activation and translation.

E1A is sufficient in disrupting the S-phase of the cell cycle to ensure that the virus can regulate the host's cellular activities and has access to cellular DNA precursors for viral replication (Liu et al. 2021). E1A regulates the expression of other early genes: E1B, E2, E3 and E4 (Figure 2), and numerous cellular genes (Berk 1986). Early transcriptional units (E1-E4) consist of proteins whose primary functions are manipulating the host cells to produce environments favorable for viral replication, initiating expression of Ad genes and directly participating in Ad DNA replication.

The two most abundant proteins transcribed from E1A are the 12S and 13S protein. Both proteins have roles in transcription of early viral products and modulating host cell gene expression and proliferation. The 13S protein also interacts with the tumor suppressor protein, p53, and can induce apoptosis independently of p53 (Putzer et al. 2000). The E1A 12S protein is an oncoprotein involved in tumorigenesis by rescuing cells from apoptosis and senescence (Gopalakrishnan et al. 1997) by stabilizing the p53 protein.

In Ad 12-infected cells, expression of E1A has shown there is an increase in tumorigenicity (Eager et al. 1986) and a decrease in the major histocompatibility complex (MHC) class I expression. The major histocompatibility complex is a large locus that contains genes that code for multiple cell surface proteins crucial for recognizing foreign substances. This complex binds to foreign peptides and arranges them on the cell surface to be recognized by T cells.

After E1A is expressed, it activates transcription of E1B, E2, E3 and E4. The E3 region consists of six known proteins. The gp 19k protein binds to the Class I antigens of the major histocompatibility complex (MHC) and down-regulates Class I antigens by blocking glycosylation. This prevents Class I antigens from transporting to the surface of the cell. It has

been demonstrated that deletion of the gp 19k protein increases lymphocytes and macrophage inflammatory response (Ginsberg et al. 1989)

Two other E3 proteins, E3 4.7k and E3 10.4k, prevent apoptosis mediated by the tumor necrosis factors (TNF). The E3 10.4k and E3 14.5k can also control cellular signaling pathways by down-regulating the EGF receptor (Wold and Gooding 1991). In addition, the E3 14.5k can re-establish cell survival by inhibiting apoptotic pathways (Russell 2000). E3 11.6k is more abundant during the late stages of Ad infection and mediates the release of Ad progeny through apoptosis. Ad viruses that lack the E3 11.6k protein can still replicate, but viral progeny is released at slower rates (Tollefson et al. 1996).

The E1B region codes for the E1B 19k and E1B 55k proteins. Both are known to inhibit p53-dependent induction of apoptosis. The E1B 19k protein inhibits apoptosis mediated by the E1A 13S and p53 proteins. E1B 19k inhibits p53 functions by preventing mitochondrial pore formation (Cuconati and White 2002). On the other hand, E1B 55k appears to cooperate with E1A 12S and p53 by directly binding to p53 to alleviate repression of transcriptional activation by p53 (Kannabiran et al. 1999). The E1B 55k protein can influence the activity of p53 and affects late viral mRNA transcription (Russell 2000).

The E4 gene produces 18 different messenger RNAs. Each mRNA encodes one of seven distinct polypeptides ORF1-ORF3, ORF3/4, ORF4, ORF6 and ORF6/7 (Tauber and Dobner 2001). E4 gene products are involved in the shutoff of cellular protein synthesis, regulating apoptosis, the production of progeny virions, prevention of concatenation of viral genomes, viral mRNA splicing and transportation (Greer et al. 2011). In the late phase of Ad infection, the ORF1 mRNA causes cytoplasmic accumulation suggesting that ORF1 contributes to cell lysis

(Dix and Leppard 1993). The E4 ORF1 region has been observed interacting with cellular factors and acting as a scaffolding protein in cell signaling (Fanning and Anderson 1999).

Currently, there are no known functions of the E4 ORF2 protein. The E4 ORF3 and E4 OR6 proteins work independently of each other, but they have some redundant functions. They are able to amplify late viral gene expression by stabilizing late mRNAs (reviewed in Imperiale et al. 1995), and stimulating viral DNA synthesis (Medghalchi et al. 1997). In addition, E4 ORF3 and E4 ORF6 proteins inhibit the double-stranded DNA (dsDNA) break repair (DBR) system by binding to the DNA-dependent protein kinase (DNA PK) (Boyer et al. 1999). It has also been revealed that the E4 ORF3 region binds to E1B 55k and induces small ubiquitin modifiers (SUMO) proteins. These proteins regulate the localization of proteins and the DNA response system. The SUMO2/3 proteins are relocalized to viral replication centers during Ad infections (Higginbotham and O'Shea 2015).

The E1B 55k and E4 34k complex leads to the degradation of the Mre11-Rad50-Nbs1 protein complex (MRN) (Stracker et al. 2002), which is crucial for detecting double-stranded DNA breaks. E4 11k also inhibits the double-stranded repair system by binding to the DNA-dependent protein kinase (DNA PK) (Boyer et al. 1999). In the absence of E1B 55k or E4 34k, E4 11k has demonstrated that it is essential for viral replication (Evans and Hearing 2005). The E1B 55k-E4 34k complex and E4 11k protein work independently of one another simultaneously to prevent viral concatenation during an Ad infection (Stracker et al. 2002). E1B 55k and E4 11k also function in shutting off cellular protein synthesis at late stages (Shepard and Ornelles 2004).

One mechanism E4 11k may use to shut off cellular protein synthesis is by disrupting processing bodies (p-bodies). E4 11k, specifically adenovirus serotype 5 (Ad 5), is known to target multiple p-body proteins, such as Lsm1 and Ddx6, into aggresomes. Other serotypes at

least relocalize Lsm1 and Ddx6 to various distinct cytoplasmic structures but have not been found to relocalize them to aggresomes (Greer et al. 2011). Relocalization of these proteins may allow Ad viruses to effectively replicate and produce virions.

Processing Bodies

Messenger ribonucleoprotein (mRNP) granules are cytoplasmic structures that regulate gene expression within cells. There are two types of RNA granules conserved in eukaryotic cells: stress granules (SGs) and processing bodies (p-bodies). Stress granules form under cellular stress and contain multiple translational initiating factors. While p-bodies form in response to stimuli that affect mRNA translation and contain protein involved in mRNA suppression and degradation. Both of these RNA granules are known to be associated with mRNA storage and decay (Reineke and Lloyd 2013) and have demonstrated their role in antiviral responses by remodeling of cellular mRNP structures. P-bodies and stress granules interact with each other during and after cellular stress. During the recovery period, mRNA stored in SGs and p-bodies can be recycled and returned to polysomes for translation. In contrast, other mRNA may be directed to p-bodies for degradation. Disruption of polysomes have shown to promote the formation of p-bodies while stabilization of polysomes lead to p-body degradation (Kedersha et al. 2000).

P-bodies are a network of multiple protein-protein and protein-RNA interactions that are involved in decapping and deadenylation (Sheth and Parker 2003). Among those proteins are translational factors, mRNA decapping enzymes (Dcp1 and Dcp2), decapping activators (Pat1b and Ddx6), deadenylation factor and exonucleases (Xrn1), which degrades mRNA after decapping. Inhibition of decapping activities result in increase in p-body size and abundance while blocking deadenylation resulted in a decrease in p-body abundance. In addition to mRNA

processing proteins, microRNA(miRNAs) have also been shown to localize to p-bodies. miRNAs are small, noncoding RNAs involved in gene silencing. miRNAs have been demonstrated to bind with the RNA-induced silencing complexes (RISCs). It is suggested that transportation of miRNAs and RISC complexes to p-bodies is crucial for miRNA-mediated gene silencing (Jakymiw et al. 2005).

Ddx6 and P-bodies

Two p-body proteins Ddx6 and Pat1b have shown that they are essential for the formation of p-bodies.The protein Ddx6 (also known as RCK and p54) is a member of the DEAD-box helicase family, and it is conserved among eukaryotes (Yu et al. 2011). Homologs of Ddx6 have multiple functions involved in mRNA metabolism processes, which gives them an important role in regulating post-transcriptional gene expression. The Ddx6 protein is crucial for gene silencing of several pathways. Ddx6 has functions in assembly of the decapping complex (Nissan et al. 2010) and activation of Dcp2 (Fischer and Weis 2002). When decapping enzymes are not available, Ddx6 is sufficient in silencing protein expression via translational suppression (Ostarek et al. 2014).

In addition, Ddx6 is able to control various post-transcriptional activities by interacting with the miRNA-induced silencing complex (miRISC). Two of the RISC components (Ago1 and Ago2) localize to p-bodies and directly interact with Ddx6. The absence of Ddx6 leads to the dispersal of Ago2, p-body dissociation, and the decrease of miRISC- mediated repression (Ostarek et al. 2014). These interactions are suggested to be mediated by binding of Ddx6 and Pat1b (Ozgur and Stoeklin 2013).

Pat1b and P-bodies

Pat1b and Ddx6 are both important for the formation of p-bodies, and the absence of one or both of these proteins result in reduced p-body formation (Ozgur and Stoeklin 2013). Pat1 proteins are conserved in eukaryotes and have evolved into two proteins (Pat1a and Pat1b). It is suggested that Pat1a is expressed in maternally expressed germline cells and functions as a translational repressor, whereas Pat1b is expressed in somatic cells and have functions involved in mRNA decay (Marnef et al. 2012). Pat1b is a scaffolding protein that interacts with both mRNA and proteins involved in translational repression. Pat1b has multiple binding sites that are protein specific for protein decapping and deadenylation factors, which indicates that Pat1b proteins have functions throughout the different stages of translational repression and mRNA decapping (Pilkington and Parker 2008). The Pat1b N-terminal region has been reported to bind Caf1 (CCR4-Not deadenylation complex subunit) while its C-terminal domain binds to decapping proteins (Marnef et al. 2012). Pat1 interacts with Lsm1-7 through the N-terminal edge of its C-terminal end. Pat1b's interaction with Lsm1 is vital for the Pat1-Lsm1-7 complex to mediate translational repression (Garre et al.2018).

The Lsm1-7-Pat1 complex interacts with the 3' end of messenger RNAs to promote the decay of mRNAs. The Lsm1-7-Pat1 complex has revealed that it has some involvement in the life cycle of positive-stranded RNA viruses by promoting viral translation and recruitment to viral replication complexes (Jungfleisch et al. 2015). It has also been demonstrated that Pat1 interacts with at least eight helical leucine rich motifs on the C-terminal end of yeast decapping enzyme Dcp2. This in return could allow Dcp2 the ability to recruit multiple Lsm1-7-Pat1 complexes that contain mRNA ready for degradation (Charenton et al. 2017).

In eukaryotic cells, Pat1b has demonstrated that it is crucial for the formation of p-bodies and control of post-transcriptional gene expression. The number of p-bodies in multiple cell types have shown to increase when exogenous Pat1b is overexpressed (Ozgur et al. 2010), and knockdown of Pat1b led to a decrease in p-bodies (Ozgur and Stoeklin 2013).

E4 11k and Ddx6 Aggresomes

E4 11k is able to rearrange nuclear and cytoplasmic structures, such as PML nuclear bodies and p-bodies, by relocalizing specific host cell proteins. E4 11k shows that it interacts with Ddx6 and relocalizes it to aggresomes. In Ad 5-infected cells, p-bodies containing Ddx6 decreases, which may be a consequence of E4 11k relocalizing Ddx6 to aggresomes. During a wild type Ad 5 infection, E4 11k-expressing viruses have been shown to co-precipitate with Ddx6 and colocalize in aggresomes with Ddx6 (Greer et al. 2011). Moderate reduction of Ddx6 in p-bodies, and its relocalization to aggresomes may be important for E4 11k to carry out its functions. Aggresomes are cytoplasmic bodies that contain aggregates of misfolded proteins, and they are usually observed in cells that are stressed by chemical and physical factors such as heat shock, chemical treatments and viral infections (Garcia-Mata et al. 2002). In some cases, proteins are able to form aggresomes when chemical stressors are present. This is the case for Lsm-1 and Ge-1 when cells undergo cadmium treatment, however, Ddx6 remains unaffected. This suggests that Ddx6 relocalizing to aggresomes is specifically due to its interaction with E4 11k (Greer et al. 2011).

Greer et al. observed whether E4 11k's ability to form aggresomes was serotype-specific (2011). The distribution of E4 11k and Ddx6 was observed in Ad 3, Ad 4, Ad 5, Ad 9 and Ad 12, which represents five of the seven human adenovirus subgroups. It was determined

that E4 11k's ability to form aggresomes was specific to E4 11k`s ability to interact with both RecA-like domains on Ddx6. Further studies need to be conducted to find the physical binding sites of Ddx6 on E4 11k; however, single amino acid substitutions ($N_{82}A$, $L_{103}A$) and a double amino acid substitution ($D_{105}A/L_{106}A$) on E4 11k prevents it from rearranging key cellular proteins, such as PML proteins, which results in knockdown of many of its functions. These amino acid substitutions also prevent E4 11k and Ddx6 binding (Greer et al. 2011). As a result, E4 11k is not able to form aggresomes with Ddx6, which is proposed to play an important role in viral growth. When E4 11k-induced aggresomes are present, it appears that relocalization of Ddx6 to aggresomes reduces the number of p-bodies containing Ddx6 (Greer et al. 2011). Some p-bodies containing Ddx6 are still observed, indicating that absolute removal of Ddx6 is not essential for E4 11k's functions (Greer et al. 2011).

E411k and Pat1b

More recently, Pat1b has been observed during an Ad 5 infection, and it appears to be relocalized as well. Ddx6 and Pat1b have shown that they can colocalize in small cytoplasmic foci as well as large juxtanuclear aggregates that have been proposed to be aggresomes (Friedman and Karen 2017). Initial studies showed that E4 11k increased Pat1b cytoplasmic foci during Ad infections. It was also discovered that the E4 11k protein alone was efficient in altering the localization of Pat1b (Friedman and Karen 2017).

Interestingly, cytoplasmic foci containing other P-body proteins, such as Ddx6 and Lsm1, tend to decrease when aggresomes are present (Greer et al. 2011) while Pat1b foci tend to increase during an Ad 5 infection. It has yet to be determined if E4 11k is required for this relocalization of Pat1b, and if this relocalization is serotype-specific. Pat1b's role during Ad

infections are still unclear, but it is thought that Pat1b may act as a defense mechanism and promote the decapping of viral mRNAs. Alternatively, the Ad virus may use Pat1b for its own benefit by causing decapping of cellular mRNAs instead. Pat1b may also have functions related to viral translation and replication. Pat1b has previously been discovered to affect viral RNA translation in the brome mosaic virus (BMV) in yeast (Jungfleisch et al. 2015) as well as affect translation and replication of the hepatitis C virus (Scheller et al. 2009).

Effect of E4 11k on the Synthesis of Late Viral Proteins

During the late stages of an adenovirus infection, cytoplasmic shuttling of most cellular mRNA is inhibited. In return, viral mRNA is able to accumulate in the cytoplasm. Early proteins E1B 55k, E4 34k, and E4 11k regulate nucleo-cytoplasmic shuttling of late viral mRNA to promote viral replication (Leppard 1998). One late viral protein, L4 100k, promotes viral mRNA translation by eliminating the cap-dependent translational pathways and suppressing cellular mRNA activities. Multiple studies have demonstrated that mutant viruses with mutations in E1B 55k, E4 34k or E4 11k leads to reduced viral mRNA accumulation in the cytoplasm, which decreases viral production (Leppard 1998). In a study conducted by Shepard and Ornelles (2004), the E1B 55k-E4 ORF3 double mutant significantly reduced the accumulation of late viral mRNA in the cytoplasm by 30-fold compared to the wild type infection. To determine whether this decrease in viral mRNA reflected on the production of late viral protein synthesis, late viral gene expression was measured in the double mutant, wild type, the E1B 55k-deleted mutant and E4 ORF3-deleted mutant. The double mutant revealed that it was extremely defective in its ability to shutdown host protein synthesis in order to produce late viral proteins. The E4 ORF3-deleted mutant showed late viral protein expressions similar to that of the wild type while the

E1B 55k-deleted mutant showed a significant decrease in late viral protein expression. However, this decrease in late viral protein production was not as severe as the double mutant. These results indicated that E4 ORF3 has functions involved in promoting late viral translation while inhibiting transcription of early cellular proteins. We hypothesized that if the knockdown of Ddx6 and Pat1b looked similar to the E1B 55k-deleted mutant then Ddx6 and Pat1b may have roles in late viral production. Furthermore, if our hypothesis is true, then relocalization of Ddx6 to aggresomes and the increase in Pat1b foci may be mechanisms E4 11k uses to promote late viral protein expression.

Research Goals

E4 11k functions to regulate host cell machinery to allow it to successfully replicate as well as inhibit pathogenic cellular response pathways that may interfere with the virus' life cycle. It also stimulates late mRNA splicing. E4 11k is known to bind and relocalize p-body proteins, which are involved in mRNA degradation and translational repression. Ddx6 is one p-body protein that is relocalized by E4 11k to aggresomes (Greer et al. 2011). The reason for this rearrangement is unknown. However, since E4 11k has a role in late gene expression, it is possible that relocalizing Ddx6 prevents degradation of viral mRNA and enables the virus to express late viral genes. Pat1b is another p-body protein that is possibly rearranged by E4 11k. It has been demonstrated that the number of Pat1b foci increase during an Ad infection.

In order to determine if E4 11k is necessary for relocalization of Pat1b, I investigated Pat1b and its localization prior to and post-Ad infection. The wild type virus, E1-replacement E4 ORF3-expressing virus, and an Ad virus with the E4 ORF1-3 genes deleted were used to determine the effect E4 11k has on Pat1b. Once E4 11k was determined to be necessary and

sufficient for Pat1b's localization, different serotypes for the E4 ORF1-3 mutant were used to determine if this relocalization was serotype-specific.

In order to determine the role of various p-body proteins being relocalized by E4 11k, we knocked down Ddx6 to determine its role in the production of late viral proteins. The experiment done by Shepard and Ornelles (2004) was modified to include the Ddx6 knockdowns. Similar to Shepard and Ornelles, a mock, wild type, E1B 55k-E4 ORF3-deleted and 55k-deleted infection was conducted. In addition to determine the role Ddx6 has on late viral protein production, Ddx6 was knocked down during a mock, wild-type, and 55k-deleted infection. We intended to knock down Pat1b as well, but we had issues with finding an appropriate antibody to test the efficiency of the knock down. An AHA metabolic labeling reagent and Click-iT reaction kit was used to look at newly synthesized proteins. This will allow us to have a better understanding of the role Ddx6 has late during an Ad infection.

Materials and Methods

The materials and methods used in this study are as previously described by Friedman and Karen (2017).

Cell Culture and Cell Line: Adenocarcinomic human alveolar basal epithelial cells (A549 cells) were propagated in Dulbecco`s modified Eagle`s medium (DMEM) supplemented with 10% calf bovine serum, 2mM glutamine, penicillin and streptomycin and seeded on collagen-coated coverslips, and 60 mm plates and incubated at 37°C in 5% CO_2.

Viruses and Infections: Wild type Ad 5, Ad 5 E1-replacement HA tagged E4-ORF3 and the E4-ORFs 1-3-deleted (*dl*1013) viruses were used to determine the effect E4 11k had on relocalization of Pat1b. The E1-replacement viruses were deleted of the E1A gene to prevent expression of other early viral genes. E1 was then replaced with E4 ORF3 under the control of the CMV promoter to only express the E4 ORF3 gene. To determine serotype-specificity, in addition to Ad 5 HA-ORF3, Ad 9 HA-ORF3 and Ad 12 HA-ORF3 were also used as E1 replacement viruses. For analysis of newly synthesized proteins, E1B 55k-E4 ORF3-deleted (*dl*1016), and 55k-deleted (*dl*110) viruses were also utilized. Cells were infected with 200 virus particles per cell. For immunofluorescence assays, A549 cells were seeded on collagen-coated coverslips in a 24-well plate and infected with each virus for 30 hours. For the Click-iT protein analysis, A549 cells were seeded on 100mm plates, transfected for 36 hours, and then infected for 36 hours.

Antibodies: Antibodies were prepared in 10% goat serum in PBS for immunofluorescence. The antibodies that were used were anti-Ddx6 polyclonal antibody from Novus Biologicals, anti-HA monoclonal antibody clone number (16B12), anti-Pat1b polyclonal antibody from Abnova, and anti-DBP monoclonal antibody (B6-8). The secondary antibodies used were Alexa Fluor 594

goat anti-mouse IgG, Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen). For western blotting anti-Ddx6 polyclonal antibody (Novus Biologicals) was used and monoclonal gamma-tubulin clone 4D11 (Invitrogen). The secondary antibodies used were Goat anti-rabbit 680 RD and Goat anti-mouse 800 CW (IRDye).

Immunofluorescence: At 30 hours post-infection, cells were fixed and permeabilized using 100% methanol at -20°C for five minutes and then washed with 1X phosphate-buffered saline (PBS). The cells were blocked using 10% goat serum and allowed to sit for an hour. The Pat1b polyclonal antibody was diluted at 1:300, DBP was added at a 1:100 dilution, and anti-HA was diluted 1:200 in 10% goat serum. The diluted antibodies were then incubated at room temperature for 1 hour. Cells were washed with 1X PBS three times and placed on a rocker after each wash. Secondary antibodies Alexa Fluor 594 and 488 were diluted 1:300 in 10% goat serum and were added and incubated at room temperature for 45 minutes in the dark. The cells were once again washed in 1X PBS three times. Afterwards, the nuclei were stained using 1 µl DAPI for five minutes and washed with 1X PBS three times. After the final wash, fluoromount-G was placed on slides and coverslips were mounted. The slides were viewed and images were obtained using the Olympus FV3000 confocal microscope.

Transfection: A549 cells were transfected with Lipofectamine RNAiMax or Lipofectamine 3000 at 60% confluency on 60 mm plates. A549 cells were either mock transfected, or transfected with siRNA against Ddx6 (ID: 121483) or siRNA against Pat1b (ID: 124106) for 36 hours. Some samples were then harvested and prepared for western blotting. For the Click-iT analysis, cells were transfected for 36 hours then infected for 36 hours. Cells were then labeled with the AHA metabolic labeling reagent for 4 hours. Cells were then harvested and lysed according to the manufacturers protocol to be prepared for the Click-iT reaction.

SDS-PAGE: A 40% Acrylamide gel was used. Acryl:Bisocryl, 1M pH 8.8 Tris, 10% SDS, 10% APS, dH_2O, and TEMED was used to make the resolving gel. The resolving gel set at room temperature for about 15 minutes. Acryl:Bisocryl, 1M pH 6.8 Tris, 10% SDS, 10% APS, dH_2O, and TEMED were used for the stacking gel. 40µL of each sample with 4X loading buffer was added to the wells. Gels were run at 120 volts for approximately 1 hour.

Transfer: Gels were transferred to a nitrocellulose membrane. The protein was electrophoretically transferred (Bio-Rad) in Towbin buffer with SDS, which consisted of 25 mM Tris, 192 mM glycine, 20% methanol, 0.01% SDS. The transfer was run at 100 mA for about an hour.

Western Blot: Following transfer, the membrane was blocked in Odyssey blocking buffer in TBS. The membrane was stained with primary antibodies gamma tubulin and Ddx6 in odyssey buffer at 1:1000 Ddx6 and 1:5000 dilutions, respectively. The membrane was then washed with TBS-Tween20 three times. Secondary antibodies, Alexafluor anti rabbit 680 and Alexafluor anti-mouse 800, were diluted at 1:5000 in 5% milk then incubated in the dark for 45 minutes. The membrane was washed with TBS-Tween20 and then washed with TBS for 5 minutes each. Images of the samples were then taken using the LICOR Odyssey machine. Images were imaged at 600 nm and 700 nm.

Click-iT AHA labeling: After transfection and infection, the Click-iT AHA labeling was performed according to the manufacturer's protocol. Cells were washed in 1X PBS warmed to 37°C then incubated in methionine-free media at 37°C for 1 hour. Five µL of 1000X AHA (Invitrogen C10102) metabolic labeling reagent was added to the plates and incubated for 4 hours. AHA is a modified amino acid that contains an azido moiety that is delivered to cells and incorporated in newly synthesized proteins. The Click-iT reaction is able to recognized azide

labeled proteins and emits fluorescence at 532 nm allowing for visualization of the proteins. Cells were then harvested and lysed using 1% SDS in 50 mM Tris-HCl at a pH of 8.0. Protein concentration was determined using the BCA Protein Determination Kit. Up to 200µg of labeled protein samples were used to perform the Click-iT reaction. After the Click-iT reaction, the pellets were left to dry for 10 minutes and resolubilized in 2X sample buffer. Electrophoresis was then performed. The gel was visualized using the LICOR Odyssey machine at 600 nm.

Results

Pat1b is found in a nuclear pattern in mock-infected and Ad 5-infected cells.

Immunofluorescence studies were conducted to determine the localization pattern of Pat1b during an Ad 5 infection. A549 cells were either mock infected, infected with wild type Ad 5, the E1 replacement HA-ORF3 virus only expressing HA-ORF3, or the d*l*1013 virus (E4 ORF3 deleted virus) for 30 hours. We stained for Pat1b as well as the viral markers DBP and HA to determine, which cells were infected. We did not observe any colocalization between Pat1b and E4 11k. We expected to find Pat1b in cytoplasmic foci. However, we noticed large nuclear aggregates in both mock and infected cells (Figure 3). The function of these aggregates is not known and are most likely unrelated to the infections.

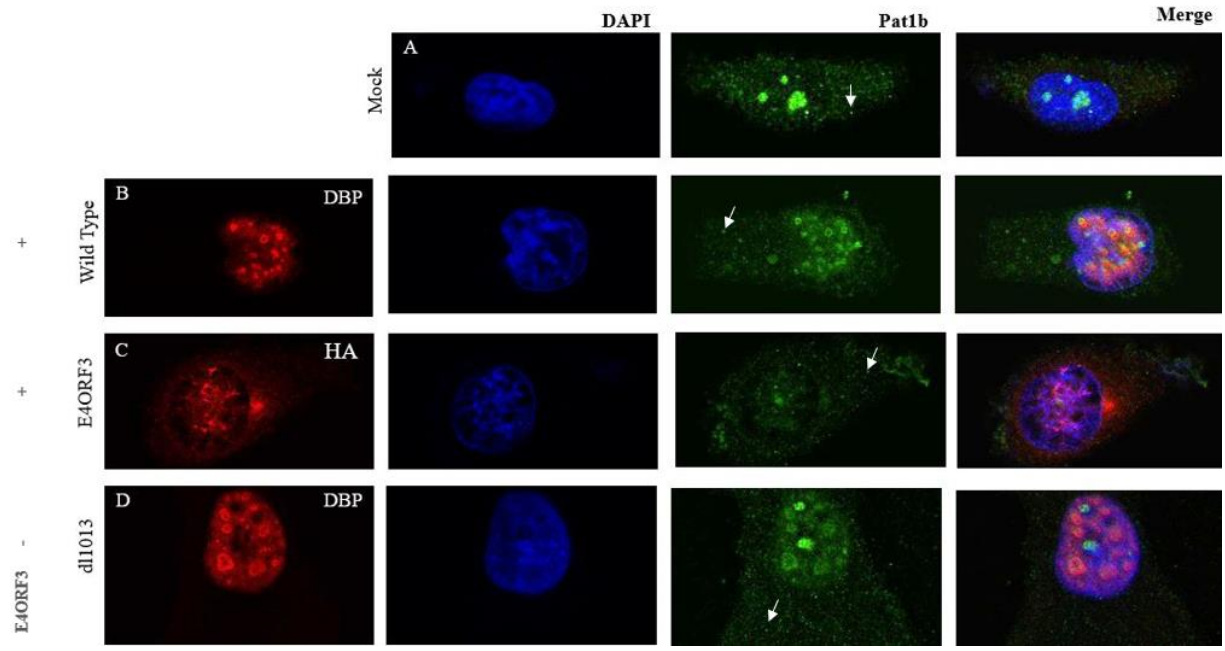

Figure 3. Localization of Pat1b during an Adenovirus Infection. A549 cells were mock-infected (A), infected with wild-type virus (B), infected with the E1-replacement virus expressing only the E4 ORF3 gene (C), or E4 ORF3-deleted virus (dl1013) (D). Cells were fixed at 30 hours post-infection (hpi) and immunostained for DBP (B and D) or HA (C), and Pat1b. Nuclei were stained with DAPI.

E4 11k is necessary and sufficient for relocalization of Pat1b during an Ad 5 infection

When Pat1b foci were observed, we noticed that infection with an Ad 5 virus led to an increase in Pat1b foci (Figure 4). Our data also revealed that there was a significant increase in the number of Pat1b foci in the wild-type and the E4 ORF3 only virus when compared to the mock (Figure 4). However, no significant difference was found between the mock and the E4 ORF3-deleted virus (Figure 4). These results show that E4 11k is necessary and sufficient in the relocalization of Pat1b.

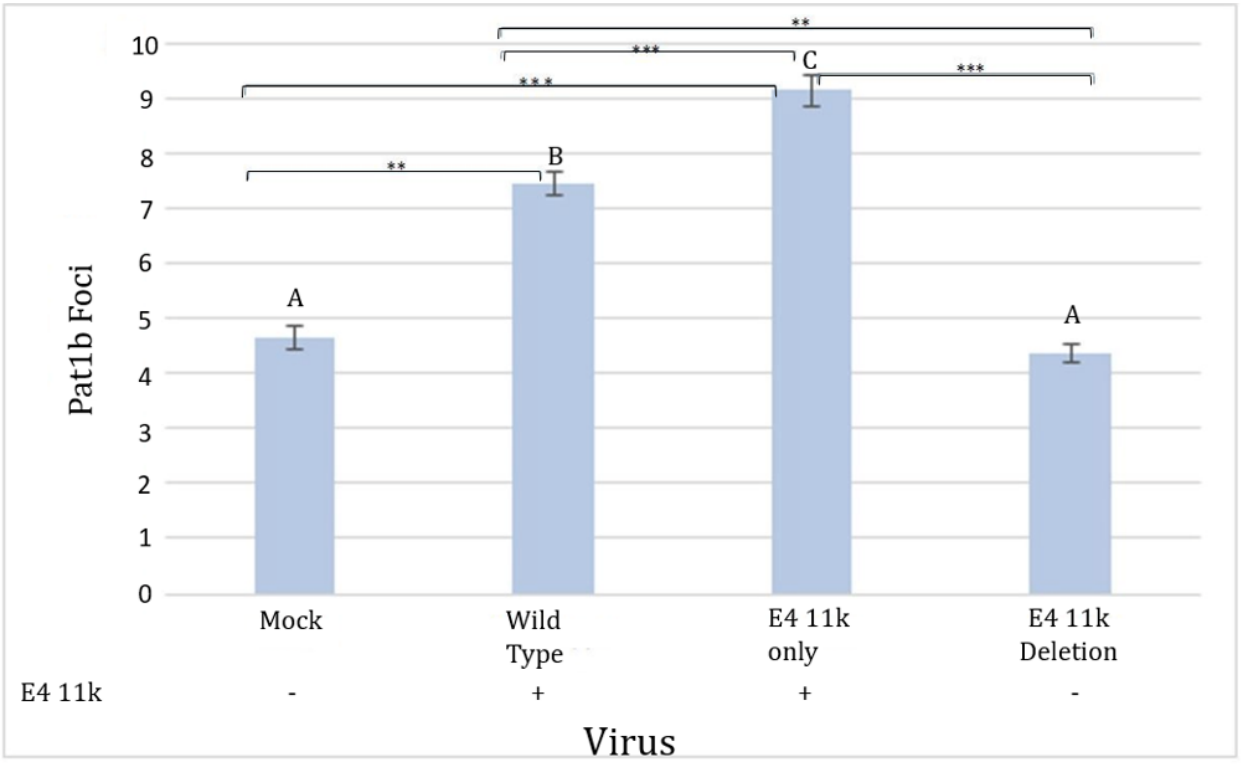

Figure 4. Quantification of Pat1b foci during different viral infections. A549 cells were infected and stained as in Figure 3. The number of Pat1b foci were counted in 30 infected cells under each condition. This was performed in duplicate and combined averages were plotted with error bars representing standard error. Differences were determined by one-way ANOVA and Tukey's test. * represents $p<0.01$, ** represents $p<0.001$, and *** represents $p<0.0001$.

Rearrangement of Pat1b is serotype-specific

To determine if relocalization of Pat1b by E4 11k was serotype-specific, Pat1b foci were counted in cells infected with E1-replacement viruses expressing HA-tagged Ad 5 E4 ORF3 (subgroup C), Ad 9 E4 ORF3 (Subgroup D) and Ad 12 E4 ORF3 (subgroup A) as seen in Figures 5 and 6. Similar to Figure 4, the Ad 5 E4 ORF3-infected cells showed a significant increase in Pat1b foci when compared to mock-infected cells. Pat1b foci in Ad 5 and Ad 12 infected cells showed a significant increase of 123% and 27%, respectively. An 18% increase was observed between mock and Ad 9, which was not a significant difference according to the Tukey's post-hoc test. Since there was a significant difference between Ad 5, when compared to mock, Ad 9, and Ad 12, and Ad 12 was only slightly significantly different from mock, this suggests that relocalization of Pat1b is serotype-specific.

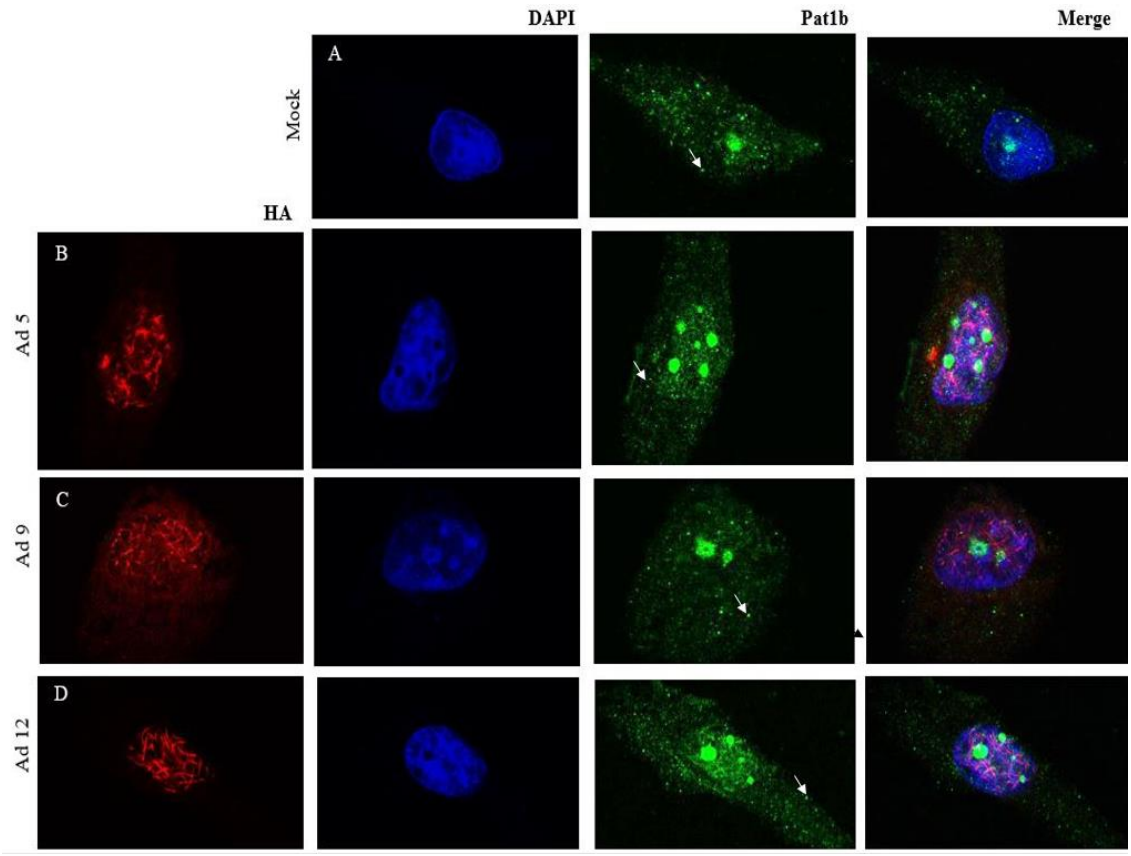

Figure 5. Relocalization of Pat1b by E4 11k. A549 cells were mock-infected (A), infected with Ad5 E4 ORF3 (B), infected with Ad9 E4 ORF3 (C) or infected with Ad12 E4 ORF3 (D). Cells were fixed at 30 hours post-infection (hpi) and immunostained for HA and Pat1b. Nuclei were stained with DAPI.

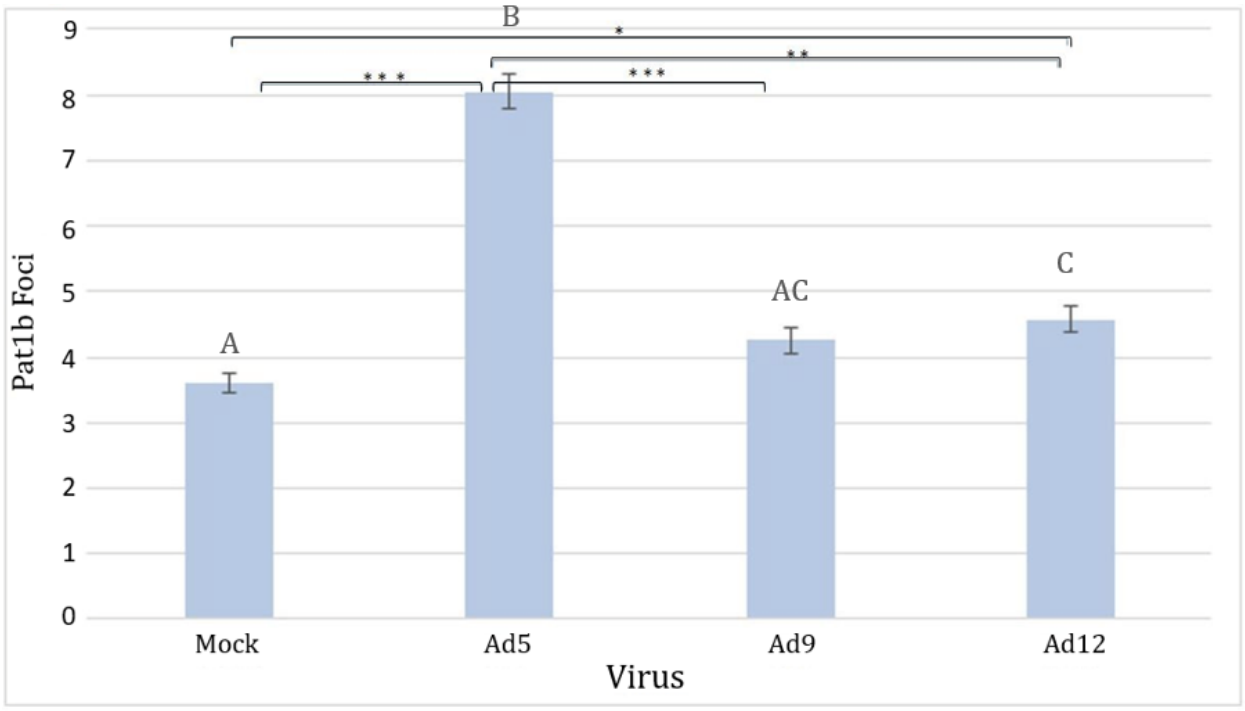

Figure 6. Serotype-specificity and E4 11k's effect on Pat1b foci. A549 cells were infected and stained as shown if Figure 3. Pat1b foci were counted in 30 mock-infected and Ad-infected cells. The experiment was duplicated and the combined averages were graphed with error bars representing standard error. A one-way ANOVA and Tukey's test was used to indicated any significant differences between any two groups. P-values <0.01 is indicated by *, ** represents p-values <0.001 and p-value <0.0001 is indicated by ***.

To determine that knockdown of Ddx6 was successful, A549 cells were mock transfected or transfected with siRNA against Ddx6. Gamma tubulin was used as a control and was seen in both the mock and the Ddx6 knockdown. Expression of Ddx6 was reduced by 50%, which confirmed that Ddx6 was successfully knockdown.

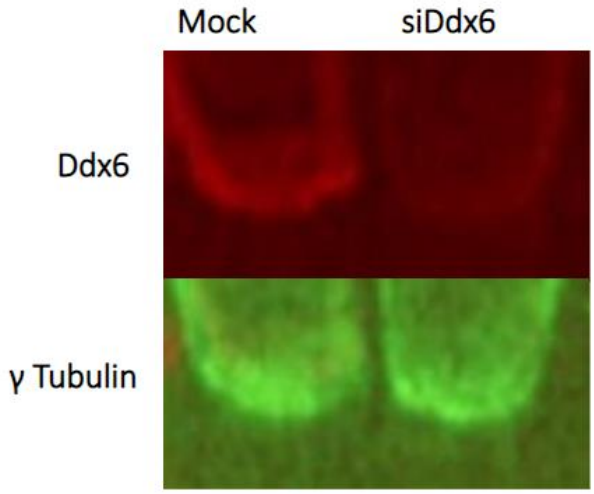

Figure 7. Knockdown of Ddx6. A549 cells were transfected with siRNA against Ddx6 for 36 hours. Lipofectamine RNAi Max was used for the delivery of siRNA against Ddx6. Cells were harvested and lysed. Lysed samples were then used for SDS-PAGE. A western blot was conducted using antibodies against γ tubulin and Ddx6. The membrane was then imaged at 600 nm and 700 nm using the LICOR Odyssey Machine.

Newly synthesized proteins were observed on a gel to determine the function E4 11k and Ddx6 have during late viral protein production. Although we are still optimizing to get bands that are more distinguishable, the viral proteins, hexon, penton or L4 100k, does not appear to be present in both the mock and mock siDdx6 lanes as expected. Similarly, E1B 55K-E4 ORF3-deleted virus revealed that deletion of these two proteins inhibited synthesis of late viral proteins. In the wild-type and 55k-deleted virus, hexon, penton and L4 100k are expressed. However, it appears that knockdown of Ddx6 in wild-type infected cells prevented the production of late viral protein, while late proteins can still be observed when Ddx6 is knocked down during a 55k-deleted infection. These results were unexpected and suggests that E4 11k is not relocalizing Ddx6 to aggresomes as a mechanism for E4 11k to produce late viral proteins.

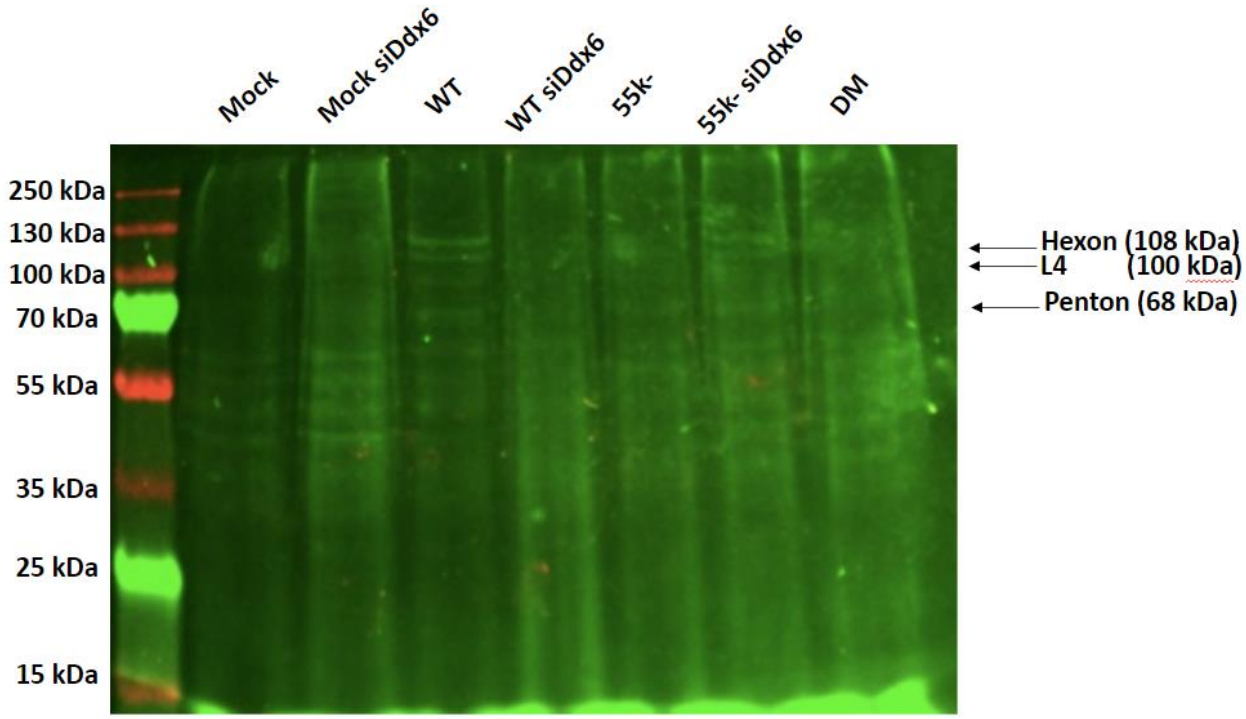

Figure 8. E4 11k's and Ddx6's effect on synthesis of new proteins. A549 cells were transfected for 36 hours followed by a 36-hour infection. Cells were labeled with AHA for 4 hours, then harvested and lysed. The newly synthesized proteins were then labeled with the Click-iT metabolic reagent. The samples then underwent SDS-PAGE gel electrophoresis. The gel was then imaged at 600 nm using the LICOR Odyssey Machine.

Since we ran out of Lipofectamine RNAiMAX, we switched to Lipofectamine 3000. Lipofectamine 3000 has also shown that it can be used for delivery of siRNA. A549 cells were mock transfected of infected with siRNA against Ddx6. Gamma tubulin was used as a control. As expected, gamma tubulin was observed in both mock and Ddx6 knockdown cells. Transfection with siRNA against Ddx6 reduced expression of the protein by 97%.

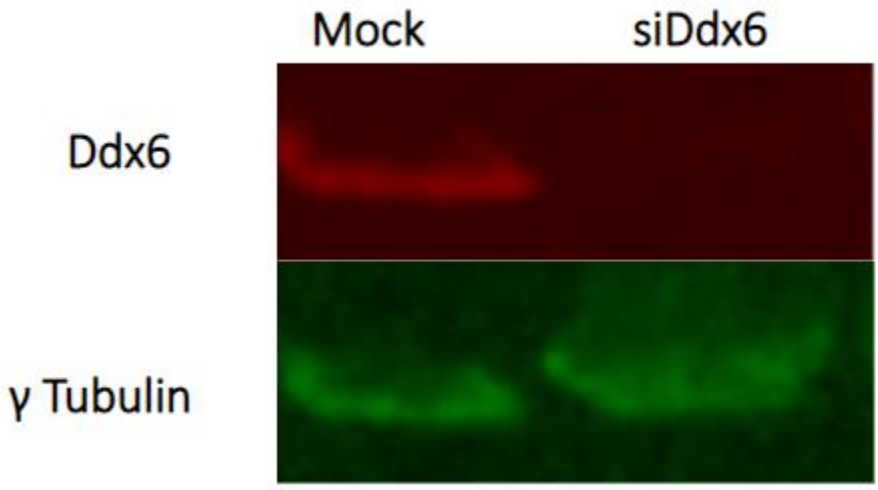

Figure 9. Knockdown of Ddx6. A549 cells were transfected with siRNA against Ddx6 for 36 hours. Lipofectamine 3000 was used for the delivery of siRNA against Ddx6. Cells were harvested and lysed. Lysed samples were then used for gel electrophoresis. A western blot was conducted using antibodies against γ tubulin and Ddx6. The membrane was then imaged at 600 nm and 700 nm using the LICOR Odyssey machine.

Discussion

Eukaryotic cells respond to viral infections and other external and internal stress conditions by placing the host cell under cellular arrest and preventing the translation of both cellular and viral protein translation. These translationally repressed mRNAs have been shown to localize in cytoplasmic RNP granules, such as p-bodies. The majority of p-body proteins are involved in mRNA repression or decay, and their size and number vary depending on the physiological conditions of the cell (Teixeira et al. 2005). Numerous studies have demonstrated multiple patterns of p-body disruption in a variety of viral infections. Specific viruses have been shown to rearrange specific p-body proteins. In our study, we observed that Pat1b foci increased during an Ad 5 infection, but this increase was only observed when E4 11k was present. This increase in Pat1b foci was found to be specific to Ad 5 (Figure 6).

It is possible that the increase in Pat1b foci may be due to dispersal of the protein by E4 11k. Alternatively, this could be due to the structural integrity of the p-bodies becoming compromised from Ddx6 rearrangement. As Ddx6 and Pat1b both have key scaffolding roles in the formation of p-bodies, removal of one may result in the dispersal of the other. The significant increase could also be a result of the protein becoming overexpressed; however, we do not have evidence that overexpression of Pat1b is occurring at this time. Ad infections also resulted in finer Pat1b foci among all observed serotypes. Finer foci were also observed in an E4 ORF3-deleted mutant suggesting that E4 11k is not responsible for the reduction in p-body size. The observed finer foci in Ad infected cells could be caused by cellular stress.

Other viruses have been shown to disrupt p-bodies during their infections. West Nile virus (WNV) has been shown to diminish p-bodies during infection. Interestingly, the protein expression level of these p-body proteins (Ddx3, Ddx6 Xrn1, Lsm1, Gw182 and Dcp1) remained

the same while their mRNA level increased (Chahar et al. 2012) Gw182, Lsm1, Ddx3, Ddx6 and Xrn1 are recruited to viral replication sites and colocalize with the viral protein NS-3. Knockdown of these proteins decrease WNV replication. Dcp1a and Edc3 on the contrary are not recruited viral replication centers. An opposite pattern is seen in HIV infections. Depletion of p-body proteins such as Ddx6, Lsm1 and Xrn1 enhances HIV replication. Depletion of Ddx6 also releases translational repression and enhances production of viral proteins (Nathans et al. 2009).

During adenoviral infection, E4 11k disrupts p-bodies and has shown that it colocalizes with Ddx6 in juxtanuclear aggresomes. When aggresomes are present, p-bodies containing Ddx6 are diminished (Greer et al 2011). To determine whether Ddx6 has a function in synthesis of viral proteins, Ddx6 was knocked down. Although we cannot conclude the role Ddx6 has during late times of infection, our data appears to indicate that Ddx6 does not affect the synthesis of viral proteins in Ad infections (Figure 8). This suggests that there could be another function E4 11k uses that involves rearranging Ddx6 from p-bodies to aggresomes. In order to determine the impact that other p-body proteins in addition to Ddx6 have during an adenoviral infection, it would be interesting to examine the effect that knockdown of p-body proteins such as Lsm1, Xrn1, Dcp1a, Edc3 and Pat1b have on adenoviral infections.

In a continuation of previous research regarding the localization of Pat1b during an adenoviral infection (Friedman and Karen 2018), our data confirm that E4 11k is sufficient in increasing the number of cytoplasmic foci containing Pat1b during an Ad 5 infection. In contrast, cells containing E4 11k-induced aggresomes, revealed a decrease in cytoplasmic p-bodies containing Ddx6 (Greer et al. 2011). The reason for this contrast is unknown. However; the increase in Pat1b foci may be a consequence of the decrease in Ddx6 found in p-bodies.

Relocalization of Ddx6 to E4-11k-induced aggresomes, disrupts, and as a result of this disruption, Pat1b may become dispersed into smaller foci.

Previously it has been shown that the presence of E4 11k is sufficient in increasing Pat1b foci. To determine if it required for relocalization of Pat1b, we added an E4 ORF3-deleted mutant. We observed that there was no significant difference between mock cells and E4 ORF3-deleted infected cells. This suggests that Pat1b is necessary for the relocalization of Pat1b. We also aimed to determine whether this rearrangement of Pat1b was serotype-specific. We initially planned to count and compare Pat1b foci in cells infected with Ad 3 E4 ORF3 (subgroup B), Ad 4 E4 ORF3 (subgroup E), Ad 5 E4 ORF3 (subgroup C), Ad 9 E4 ORF3 (subgroup D), and Ad 12 E4 ORF3 (subgroup A). However, we were not able to infect with Ad 3 and Ad 4. According to our data, we cannot officially conclude that this relocalization is serotype-specific, but it was interesting to see that there was a significant increase in the observed Pat1b foci in Ad 5-infected cells compared to mock, Ad 9 and Ad 12, which indicates that relocalization of Pat1b is specific to Ad 5. The reason for such an increase has not been determined. There was also an observed increase in Pat1b foci in Ad 5 and Ad 12 infected cells compared to mock This may suggest that E4-11k disrupts Pat1b in p-bodies in other Ad serotypes to some extent. A similar pattern was observed with the localization of Ddx6 during adenoviral infections with Ad serotypes 3, 4, 5, 9 and 12. Ad 5 was the only serotype that could relocalize Ddx6 to aggressomes, but all serotypes disrupted p-bodies due to the presence of E4 11k and relocalized Ddx6 to different distinct cytoplasmic structures (Greer et al. 2011). The reason for the observed increase in Pat1b foci is unknown; however, this increase could be a response to cellular stress caused by over expression of E4-11k. Since p-bodies are sites for mRNA degradation, E4-11k's ability to relocalize Ddx6 and Pat1b may promote the stimulation of late viral protein accumulation in the cytoplasm. In

order to determine the role Ddx6, Pat1b and E4 11k have on late viral protein production, Ddx6 was knocked down in an E1B 55k-mutant background. The Click-iT reaction was used to determine the presence of newly synthesized proteins. These preliminary results suggest that Ddx6 does not play a role in the production of late viral proteins, however, there are some controls of the experiment that do not appear to make sense so it will need to be repeated to make any solid conclusions. If confirmed, this would indicate that relocalization of Ddx6 to aggresomes is not a mechanism E4 11k uses to stimulate late viral protein production,

In addition to confirming the role E4 11k and Ddx6 have on late viral protein production, Pat1b knockdowns will be added. It is also important to get an accurate representation of Pat1b foci across all known subgroups to shed light on whether the rearrangement of Pat1b is truly serotype-specific. The sample size will be increased and Pat1b foci from serotypes Ad 3, Ad 4, Ad 5, Ad 9 and Ad 12 will be counted.

While our data suggest that E4 11k increase the number of Pat1b foci in Ad 5 and Ad 12 infected cells, it's important to note that we encountered some difficulties with counting Pat1b foci. The size of Pat1b foci varied in both the mock and Ad-infected cells. The intensity of the fluorescence also differed from foci within the same cell. In an attempt to avoid any discrepancies while counting Pat1b foci, we experimented with the CellSens counting software, but were unable to successfully find a way to use the software for our purposes. In the future, software that can measure the intensity of fluorescence and foci size will be sought, to prevent any bias in the results.

Currently, there are not many studies that demonstrate the effect DNA viruses have on the structural integrity of p-bodies or p-body proteins stability. P-bodies have the ability to degrade viral mRNAs; however, p-bodies may also give rise to an opportunity for viruses to

regulate cellular gene expression. Altogether, our results demonstrated that E4 11k plays a crucial role in relocalizing Pat1b to more numerous foci. E4 11k is known to modulate host gene expression. Since Pat1b is a scaffolding protein and is essential for p-body assembly, relocalization of Pat1b by E4 11k may also lead to the dissociation of other proteins from p-bodies. This may explain the reason for the observed smaller Pat1b foci during an Ad infection. It is possible that disruption of p-bodies by E4 11k is a mechanism the virus uses to allow for accumulation of viral mRNAs in the cytoplasm. Although the roles p-bodies have during a viral infection are still not well understood, our results provide insight on how p-bodies are disrupted during a viral infection.

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