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Disruption of Cellular P-bodies During an Adenovirus Infection

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Abstract

Adenovirus has made major contributions in medicine by serving as a model DNA virus to study other viruses, such as human papillomavirus (HPV). Adenoviruses are a diverse family of nonenveloped, double-stranded DNA viruses that are ubiquitous to animals and humans. There are over 67 serotypes of human adenoviruses that can cause a variety of illnesses including, gastroenteritis, conjunctivitis and respiratory infections. Adenovirus can cause these infections by invading host cells and producing an environment that is favorable for viral replication. During the early phases of infection, adenovirus expresses various viral proteins such as E4 11k, which has multiple functions. One of these functions involves controlling host cell gene expression by preventing the expression of host proteins and enabling viral protein production. Recently, it was discovered that E4 11k also disrupts processing bodies (p-bodies) by relocalizing cellular proteins. It is hypothesized that disruption of p-bodies allows E4 11k to regulate gene expression. One protein found in p-bodies, Pat1b, is a scaffolding protein that participates in p-body assembly and gene expression. Its localization during an adenovirus infection is not well understood. In this study, we will observe the localization of Pat1b and other p-body proteins with E4 11k using immunofluorescence microscopy. We hypothesize that E4 11k will induce a different localization pattern for Pat1b during an adenovirus infection. Three variations of adenovirus will be utilized. If E4 11k is responsible for relocalization of Pat1b, it will only be observed with wild type virus and a virus that only expresses E4 11k. Different localization patterns are not expected with a virus that is deleted for E4 11k. Since cellular p-bodies are not well-understood, and other viruses have been shown to disrupt p-bodies, the effect of E4 11k on p-bodies could assist us in understanding the impact of p-bodies on healthy and virally-infected cells.

Introduction

- Adenovirus causes multiple diseases including:
  - Respiratory tract infections, such as the common cold
  - Conjunctivitis
  - Gastrointestinal tract infections
- The adenovirus genome consists of early and late genes.
  - Early genes target a variety of host cell machinery while late genes are involved in building progeny viruses for future infections.
- One early protein, E4 11k, has multiple important functions, one of which is involved host cell shutoff and synthesis of late viral proteins.
- Cytoplasmic processing bodies (p-bodies) are involved in mRNA degradation and translational repression.
  - E4 11k has been shown to disrupt p-bodies.
- P-body protein, Ddx6, has previously been shown to be relocalized by E4 11k
- Pat1b is another major p-body protein.
  - It has been demonstrated that Pat1b binds to Ddx6.
  - Pat1b plays a role in p-body assembly and translational regulation.
  - Pat1b has not been observed in adenovirus-infected cells.
- Our goal is to determine the localization pattern of Pat1b during an adenovirus infection.

Materials and Methods

Cell culture: A549 cells were grown in Dulbecco`s Modified Modified Eagle`s Medium (DMEM) containing 10% calf serum, penicillin, streptomycin and glutamine and incubated in 5% CO_2 at 37°C. Cells were passaged at approximately 80-90% confluency. Prior to infection, cells were split 1:7 in 24 well-plates on collagen-covered coverslips.

Viral Infections: Wild-type adenovirus serotype 5 (Ad5), an Ad5 E1-replacement HA-E4 11k virus and the E4 11k-deleted mutant were used for viral infections. Viral stocks were diluted in DMEM for viral infections. After 29 hours post-infection, the cells were fixed and stained.

Cell Fixation and Immunofluorescence: Cells were fixed in ice cold methanol for 5 minutes and washed in phosphate buffer saline (PBS). Cells were blocked in 10% goat serum for 1 hour. Primary antibodies were added and incubated at room temperature for an hour then washed in PBS. Secondary antibodies were added and incubated in the dark for 45 minutes at room temperature. Cells were washed in PBS three times before staining cell nuclei with DAPI (150µg/mg). Coverslips were mounted and visualized on the Olympus FV3000 confocal microscope.

Results

In order to determine the role of E4 11k in the localization pattern of Pat1b, cells were infected with wild type virus, a virus expressing only E4 11k, and a virus deleted in the E4 11k gene. Cells were stained with antibodies against Pat1b and a viral marker of infection (DBP in wild type and the deletion mutant and HA in the E4 11k virus) and with DAPI to stain the nuclei (Figure 1). The number of Pat1b foci in the cytoplasm was counted and compared to each other (Table 1).

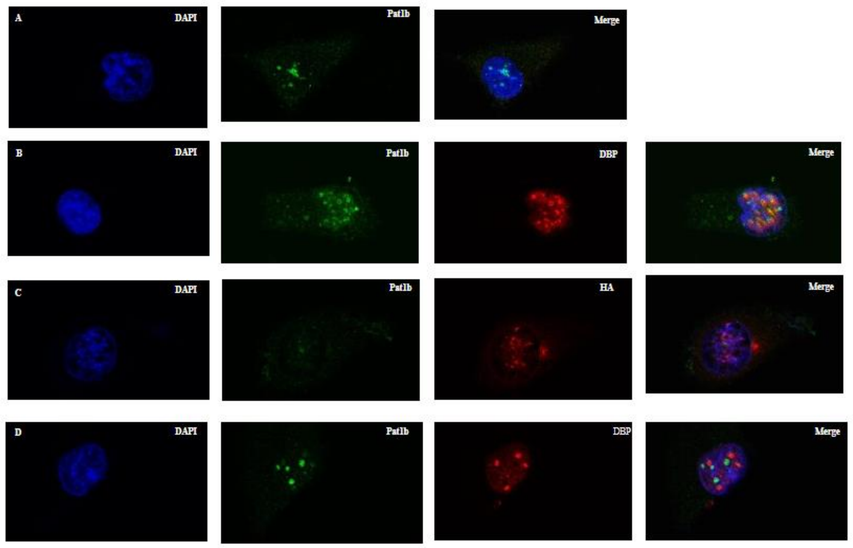

Figure 1. Localization of Pat1b during an Ad5 infection. A549 cells were left uninfected (A), infected with wild type virus (B), virus expressing only E4 11k (C), or an E4 11k-deleted mutant (D) for 29 hours. Cells were immunostained with antibodies against the indicated proteins and stained with DAPI to visualize cell nuclei.

Results (continued)

Table 1. Pat1b cytoplasmic foci. Pat1b cytoplasmic foci from the experiment from Figure 1 were counted in 30 cells for each experiment. For infected cells, only cells expressing DBP or HA (viral markers) were counted. The mean and standard deviation for each infection was calculated. A t-test was used to determine the statistical difference from one sample to the other. T-test (uninfected) shows the p values for each sample compared to uninfected cells. T-test (deletion mutant) shows the p values for each sample compared to the E4 11k-deleted mutant. An ANOVA and Tukey's test confirmed a statistically significant difference between the different groups and between uninfected and the different infected cells, respectively.

	Mean	Standard Deviation	T-test (uninfected)	T-test (deletion mutant)
Uninfected	6.03	3.11	-	-
Wild-type Ad5	11.6	7.52	2.84e-4	5.4e-1
HA-E4 11k	14.0	6.22	3.96e-8	7.29e-2
E4 11k deleted	10.5	6.13	7.5e-4	-

Conclusions

- According to our data, there is a significant increase between the number of Pat1b cytoplasmic foci in uninfected cells compared to the cells infected with the different viruses. This indicates that infection alone is enough to increase the number of Pat1b cytoplasmic foci.
- There is not a statistically significant difference between the different viruses. This suggests that another protein may be involved in the relocalization of cytoplasmic Pat1b. In the future we do more replicates of this experiment and increase our sample size.

Future Directions

- We will repeat the experiment to increase our sample size and number of replicates.
- We will used RFP-tagged Ddx6 and stain for Pat1b to observe the colocalization of the two p-bodies during an infection.
- Additionally, we plan to use siRNAs to knockdown Ddx6 and Pat1b to determine their role in the late phases of the adenovirus life cycle.

References

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