

The Localization of PABPC1 in HeLa Cells

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Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant disease with an onset that usually occurs during the fifth or sixth decade of life. The disease progresses slowly and symptoms include eyelid drooping (ptosis), difficulty with swallowing (dysphagia), and proximal limb weakness. The progression of the disease varies case by case and complications can include choking, regurgitation, aspiration, pneumonia, and limitation of the visual field. Malnutrition and starvation paired with pneumonia are the leading causes of death in patients who have OPMD. While the life expectancy of patients with OPMD is not shortened, the quality of life during the last years of patients with OPMD is significantly decreased (Abu-Bake & Rouleau, 2006).

OPMD has been reported in 33 countries with two of the largest populations including patients who are French descendants of Quebec and Bukara Jews in Israel. There are also affected populations in Spain, France, Germany, the United Kingdom, and Northern New Mexico, (Becher et al., 2001). OPMD is caused by a change in a gene called *pabpn1* that then affects the production of the protein, PABPN1 that is usually found in the nucleus. OPMD is caused by the expansion of a polyalanine (GCG) triplet repeat in the encoding sequence of the poly (A) binding protein nuclear 1 (*pabpn1*) in the first exon (Abu-Baker & Rouleau, 2006). Cytoplasmic poly (A) binding protein 1 (PABPC1) is another poly (A) binding protein that is closely related to PABPN1, however it is not known if PABPC1 plays a role in OPMD. Because of the relation of PABPC1 and PABPN1 and the importance of PABPN1 in patients that have OPMD, for my project I focused on PABPC1 that is usually found in the cytoplasm rather than the nucleus. I wanted to identify the location of this protein and possibly relate it back to the dysfunction seen at the cellular level when an individual has a genetic mutation that causes OPMD.

Basics of cellular gene expression:

Mammalian cells are divided into two major compartments: the cytoplasm and the nucleus. The cytoplasm contains all of the organelles that a cell needs to survive. Within the cytoplasm is the nucleus where all of the genetic material is stored. Deoxyribonucleic acid (DNA) is found in the nucleus and this is where the hereditary information is stored. DNA is used as a template for RNA, which is made within the nucleus in a process called transcription. The RNA is then used to make proteins in a process called translation, which occurs in the cytoplasm. Proteins carry out many functions within the cell from carrying messages from one cell to another to propelling organelles through the cytoplasm. They also provide the shape and structure of the cell and constitute most of the dry mass of the cell. There are many different types of RNA. One type, messenger RNA (mRNA) carries information that is transcribed from one gene for a single protein (Alberts, 2009).

The process starts with genes within the DNA that code for the amino acid sequences of proteins. In order to make these proteins, RNA molecules are transcribed from the DNA that is then followed by the synthesis of proteins. RNA is made out of four types of nucleotides that act as building blocks

that encode proteins. The four components include the bases adenine (A), guanine (G), cytosine (C), and uracil (U) (Alberts, 2009). Messenger RNA carries information that codes for a single protein from one gene. In order for transcription for RNA to begin, the cell must first decide which of the RNAs are needed and at what rate they need to be produced. In eukaryotic cells an enzyme called RNA polymerase II randomly collides with a piece of DNA in the cell where it slides along the DNA until it reaches an area called the promoter region. This indicates where RNA synthesis should begin. Once the RNA polymerase II is bound tightly to the DNA, it opens up the double helix conformation of the DNA to expose the DNA building blocks, or nucleotides, on each of the strands of DNA. The strands of DNA act as a template for the RNA. The RNA is then transcribed based on the template of the DNA. The chain continues to grow until the enzyme reaches a signal on the DNA called the terminator. Here, polymerase II is stopped and both the DNA and the new messenger RNA are released. Once the mRNA is synthesized, it is then transported from the nucleus to the cytoplasm through small holes in the membrane surrounding the nucleus. Before the mRNA can leave the nucleus, it must be processed. In this case, polyadenylation occurs at the 3' end of the molecule. During this process, the RNA molecule is first cut by an enzyme at a specific point in the RNA chain. Another enzyme then adds a repeat of adenine nucleotides to the end of the RNA molecule. This is called the poly-A- tail and it is usually hundreds of nucleotides long. This is added to increase the stability of the mRNA molecule and the help in its export from the nucleus to the cytoplasm. The poly-A tail is also used when the mRNA is transcribed into proteins to make sure that the entire mRNA message is present and that the mRNA molecule has not been damaged (Alberts, 2009).

Once the mRNA has been processed in the nucleus, it is then allowed to pass into the cytoplasm. A complex in the nuclear pore is able to recognize which mRNA molecules are correct and allows them to pass through into the cytoplasm. Once in the cytoplasm, the building blocks of the mRNA or nucleotides are translated into the amino acid sequence of a protein. The mRNA code is read in groups of three nucleotides that code for one specific amino acid also known as codons by ribosomes. The mRNA is pulled through the ribosome and the mRNA sequence is translated into codons that are added to the growing chain of amino acids. When the synthesizing is over, the ribosome releases the mRNA and the protein is finished. The protein can then go to its specific part of the cell and perform its specified job (Alberts, 2009).

PABPN1 as well as another poly(A) binding protein, poly(A) binding protein cytoplasmic 1, (PABPC1) bind RNA poly(A) tails. PABPN1 typically localizes in the nucleus while PABPC1 localizes in the cytoplasm (Lemay et al., 2010). Recently, studies have suggested that PABPC1 is able to enter the nucleus (Afonina et al., 1998). PABPC1 and PABPN1 are shuttling proteins that enter both the nucleus and the cytoplasm and help transport mRNAs from the nucleus to the cytoplasm (Figure 1) (Abu-Baker & Rouleau, 2006). PABPN1 has been shown to be present at various stages of the break down and recycling of mRNA, while PABPC1 has been described as a regulator of mRNA translations (Bear et al., 2003; Brook & Grey, 2012). One model has proposed the remodeling of PABPN1-bound nuclear mRNAs during or after they leave the nucleus so that PABPN1 is replaced with PABPC1. Another model suggests that both PABPN1 and PABPC1 are shuttling proteins. According to this model, PABPC1 associates with pre-mRNA and contributes to the

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3' end processing with PABPN1. This model then suggests that the stoichiometry of PABPN1: PABPC1 changes in the nucleus and cytoplasm due to different concentrations of each of these molecules in cellular compartments. While the stoichiometry of PABPN1: PABPC1 may change in the cytoplasm and nucleus, it is known that PABPN1 and PABPC1 actually localize in the nucleus and cytoplasm, respectively (Lemay et al., 2010). There are suggested models of the roles of PABPC1 and PABPN1, but the exact role of these proteins has not been determined.

PABPC1 has been found in the nucleus of human cells as well as yeast cells. In an earlier study, it was shown that when transcription of the DNA was inhibited, PABPC1

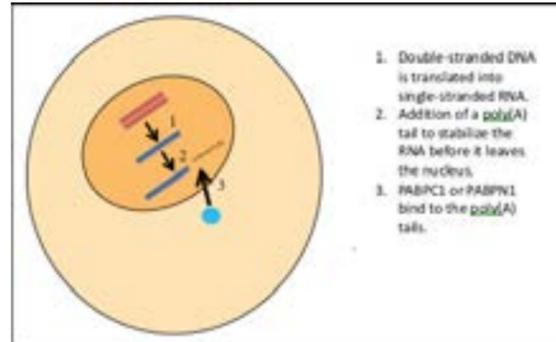


Figure 1: Proposed shuttling model of PABPC1 and PABPN1.

accumulated in the nucleus (Afonina, et al., 1998). This suggests that transcription is required for PABPC1 to be exported from the nucleus. The exact role of PABPC1 in the nucleus is not known, but it has been proposed that it is involved in one or many steps of mRNA biogenesis (Afonina et al., 1998). While this shows that PABPC1 is able to accumulate in the nucleus when DNA is inhibited, it is not known if this also occurs when DNA is not inhibited. It is possible that the DNA somehow inhibits PABPC1 from entering the nucleus, so for this reason it would be interesting to see if this experiment would have the same outcome if DNA is not inhibited.

My study looks to find if PABPC1 is found in the nucleus when the DNA is not inhibited. In this study, PABPC1 I imaged in human cells rather than yeast cells. In addition, I studied the number of cells expressing PABPC1 in the nucleus. I used HeLa cells as a model and immunofluorescence techniques to image where PABPC1 was located in HeLa cells.

In this study we used HeLa cells. These are part of a popular cell line that has recently been made famous by a book entitled *The Immortal Life of Henrietta Lacks*. These cells have been used to develop vaccines and test chemotherapy drugs among other things. HeLa cells were taken from Henrietta Lacks, a 30-year old black tobacco worker who had cervical cancer. Neither Henrietta Lacks nor her family knew that the cells from her cervix would be used for research. These cells are ideal for research because they persist indefinitely in laboratory. These cells have been used in the biological field for over 60 years because they were some of the first cells found to be "immortal" because of their ability to divide and multiply (Rao, 2010). HeLa cells were used due to the ease of the upkeep of these cells as well as the ease of obtaining HeLa. In addition, these cells are human cells and since OPMD is seen in humans and PABPC1 and PABPN1 are known to be in human cells, these were the ideal candidate for this study.

Methods (See Appendix 1 for full methods):

Immunofluorescence is used to detect the location and relative amount of a certain protein. A fluorescence microscope

is used to detect where the proteins are localized after they have been labeled with antibodies that have fluorescent tags. Antibodies are proteins that are produced by the immune system when foreign molecules such as a virus enter the body. There are many different types of antibodies and each antibody binds to a particular part of the invading molecule to mark it for destruction. For immunofluorescence staining, an antibody to the protein in study is used (in this case PABPC1 and PABPN1) to mark the protein, and then a secondary antibody is used to mark the primary antibody (Figure 2). The secondary antibody is labeled with a fluorescent dye that is covalently attached to the secondary antibody (Alberts, 2009). In order to get the antibodies into the cell, a series of steps is used to permeabilize cells. These steps are necessary because the antibodies are not able to enter the cell by themselves. Once the cells have been fixed and mounted on slides, a fluorescent microscope is used to image the cells. The dye in the fluorescent-labeled antibody is excited by a certain wavelength and emits a different wavelength that is picked up and recorded by the microscope. If more than one antibody is used, different antibodies that are excited by different wavelengths can be used. When these different dyes are imaged, they appear to be different colors because they emit different wavelengths of light.

In order to count the number of cells that expressed PABPC1 in the nucleus, we used the Slidebook software to look at the individual channels expressed by each antibody. In this case we used various antibodies for PABPC1 and PABPN1 to ensure that the antibody itself was not giving a false channel and skewing the data. A total of 220 cells were counted from

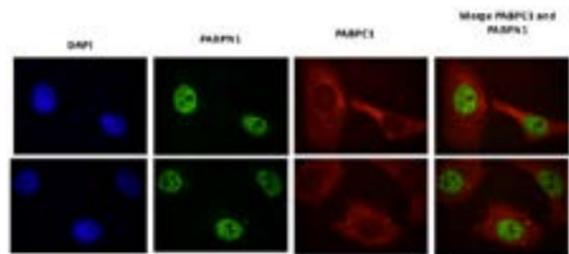


Figure 2: Confocal images of HeLa cells in which DNA (DAPI), PABPN1 (FITC), and PABPC1 (Texas Red) are visualized. Cells express PABPC1 in the nucleus.

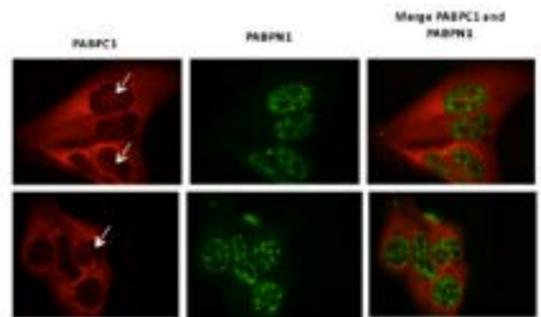


Figure 3: Confocal images of HeLa cells in which PABPC1 (Cy3) and PABPN1 (FITC) are visualized. Cells express PABPC1 in the nucleus, indicated by arrows.

3 different fixation periods also to ensure that the fixation was not causing skewed data. The data was then plotted on a bar graph to show the number of cells that expressed PABPC1 in the nucleus versus the number of cells that did not express PABPC1 in the nucleus.

Results:

To better understand the localization of PABPN1 and PABPC1, we immunofluoresced the cells with antibodies specific to each protein (Figure 2). We found that PABPC1 was localized in the cytoplasm, and we also found that there was some PABPC1 that was localized in the nucleus indicated by white arrows (Figure 3). These areas of localization seemed to be clouds of PABPC1 that formed within the nucleus.

In addition to visualizing where the proteins were located, we also quantified the number of cells that expressed PABPC1 in the nucleus (Figure 4). We counted a total of 220 cells and found that out of the 220 cells, 175 or 87.5% of the cells expressed PABPC1 within the nucleus and 45 or 20.5% of the cells did not express PABPC1 in the nucleus but was only seen in the cytoplasm. This microscope produced two-dimensional images of the cells so it is possible that with three-dimensional images of the cells, I might have been able to detect even more PABPC1 present in the nucleus of the cells.

Discussion:

Here we were able to show that not only was PABPC1 found in the nucleus, but more cells expressed PABPC1 in the nucleus than cells that not expressing PABPC1 in the nucleus. These results provide evidence that PABPC1 is prevalent in the nucleus in not just some cells, but the majority of cells. Since the PABPC1 was found in the majority of cells, this might suggest that PABPC1 plays a role in the nucleus. This has been suggested before with yeast cells with inhibited DNA, but this shows that the finding of PABPC1 in the nucleus is consistent with HeLa cells in which the DNA is not inhibited. This means that the cell environment might be more like what is seen in a normal cell because the DNA would be available to be transcribed into necessary proteins.

Other than showing that PABPC1 can be found in the nucleus and that the protein is associated with mRNA biogenesis, it is unknown what the exact role of PABPC1 is in the nucleus. While it is unknown whether PABPC1 is directly related to OPMD, mutants of PABPN1 have been shown to cause the OPMD. In patients with mutations in the PABPN1 gene, PABPN1 is over expressed and forms aggregates in the nucleus.

One hypothesis states that the sequestration into aggregates might change the function of the PABPN1 protein and change the function of the proteins that are rely on PABPN1 to work before they can begin their own work as a protein. These PABPN1 aggregates may also cause cell death in patients with OPMD and lead to symptoms such as dysphagia and ptosis (Baker & Rouleau, 2006). While there is a lot of information known about the outcomes of a mutation in the PABPN1 gene, not much is known about PABPC1 and whether it may also cause aggregates that could also contribute to the symptoms seen in patients with OPMD. With additional insight in the role of PABPC1 in the nucleus, we might be able to shed more light on

Table 1: Distances and addresses regarding the primary antibodies.

Company	Distance	Lot Number	Address	Company
PABPN1 - FITC	2 mi in 100 mi	414121	Abnova	Abnova
PABPC1 - Texas Red	1 mi in 100 mi	1138	Abnova	Abnova

Table 2: Distances and addresses regarding the secondary antibodies.

Antibody	Company	Distance	Lot Number	Address
FITC in rabbit	Abnova	30% in 100 mi	211-088-132	2 mi in 100 mi
Texas Red in mouse	Abnova	30% in 100 mi	211-088-132	2 mi in 100 mi

the causes of OPMD. Much less is known about PABPC1 than PABPN1 especially when it comes to the role of PABPC1 in the nucleus. In the future, it would be beneficial to look at the exact function of PABPC1 both in the cytoplasm and in the nucleus in live cells.

Appendix 1: HeLa Cell protocol

HeLa cells were grown on 1 cm² cover slips at 37° C for 48 hours in DMEM +10% FBS +p/S media in wells. The cover slips were then fixed by removing the media and adding 4% formaldehyde in 1 X PBS to each well. The well plate with the formaldehyde and cover slips was then agitated on a shaker for 15 minutes. The fixer was removed and 3 mL of PBS was added to each well. The well plate was agitated for 5 minutes. This was repeated 3 times. The well plate was placed on an ice pack and the HeLa cells were permeabilized by adding 0.5% normal goat serum and 5% Triton-X in 1X PBS. The well plates were then agitated for 5 minutes. The NGS and the Triton-X were removed by washing the cells for five minutes with 1X PBS. This was repeated 3 times. Dilutions were made to include both primary antibodies were made in 1X PBS (PABPC1α Mouse and PABPC1α Rabbit) (see Table 1). 45 µl of this dilution was added to a clean Corning slide and one coverslip was placed face down on top of each corning slide. The slides were left in a humid chamber at room temperature for one hour. The coverslips were removed from the slides and placed back into the wells (face up) with 3 mL of PBS. This was repeated 3 times. 45 µl

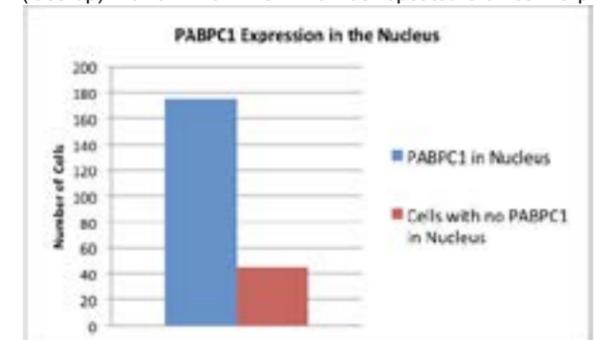


Figure 4: Cells were imaged using the techniques described in the text. The number of cells expressing PABPC1 in the nucleus was counted and plotted against the total number of cells counted. A total of 220 cells were counted.

of the secondary antibody solution containing both secondary antibodies (see Table 2) was added to the Corning slides and the coverslips were placed on the slides so that the coverslip would come in contact with the secondary antibody. The slides were kept in a humid chamber at room temperature for 30 minutes. The coverslips were then placed back into the wells face up in a solution containing 1X PBS for 5 minutes. This was repeated 3 times. A dilution of 1/20,000 DAPI in PBS was used as a DNA counter stain.

The cover slides were then mounted using 45 µl of Invitrogen Prolong Gold Antifade Reagent. The slides were imaged using a fluorescent microscope and Slidebook software.

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References

- Baumgart, D. C., & Carding, S. R. (2007). Inflammatory bowel disease: Cause and immunobiology. *Lancet*, 369(9573), 1627-1640.
- Bouma, G., & Strober, W. (2003). The immunological and genetic basis of inflammatory bowel disease. *Nature Reviews.Immunology*, 3(7), 521-533.
- Brenner, O., Levanon, D., Negreanu, V., Golubkov, O., Fainaru, O., Woolf, E., et al. (2004). Loss of Runx3 function in leukocytes is associated with spontaneously developed colitis and gastric mucosal hyperplasia. *Proceedings of the National Academy of Sciences of the United States of America*, 101(45), 16016-16021.
- Cadwell, K., Liu, J. Y., Brown, S. L., Miyoshi, H., Loh, J., Lennerz, J. K., et al. (2008). A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal paneth cells. *Nature*, 456(7219), 259-263.

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