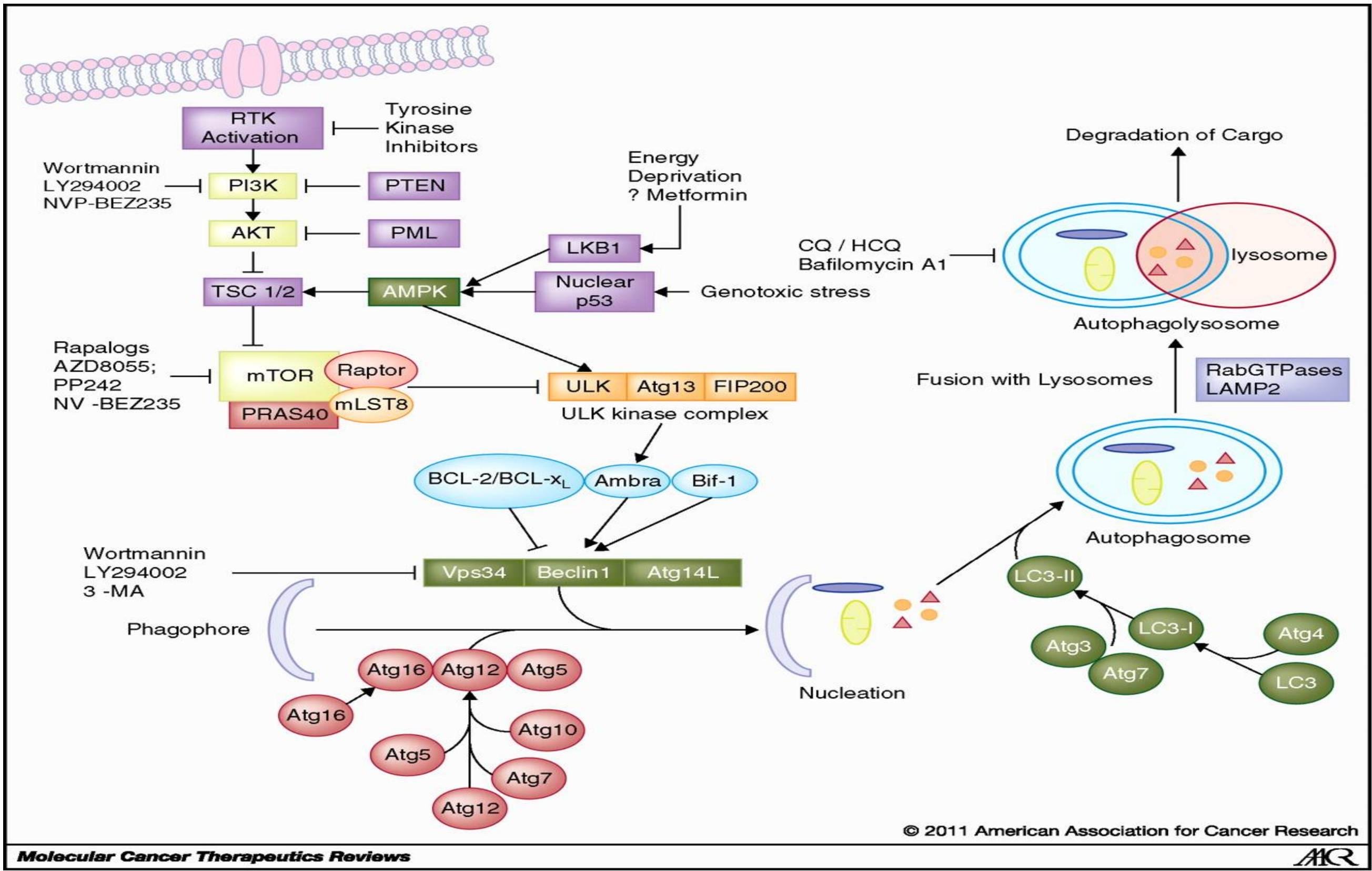


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Abstract



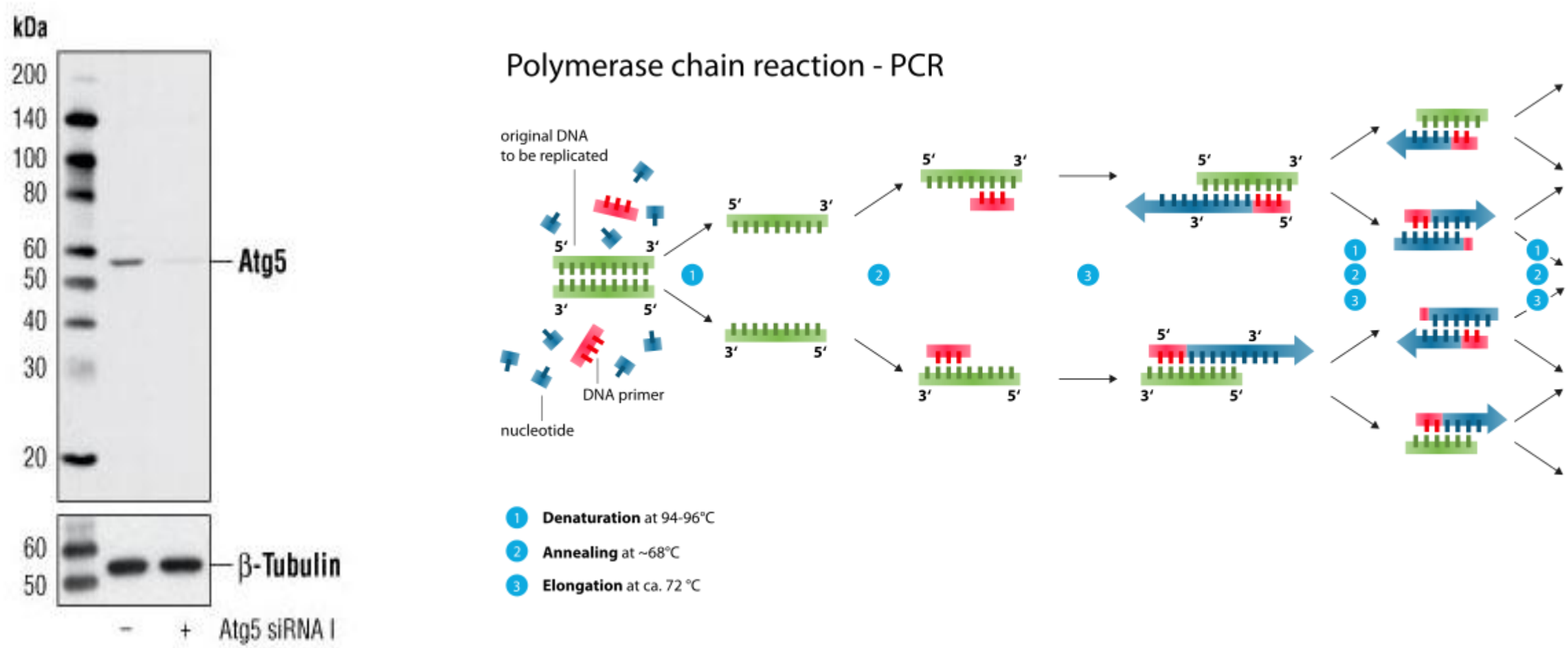
Cancer is the name given to a collection of related diseases. In all types of cancer, some of the body’s cells begin to divide without stopping and spread into surrounding tissues. Cancer is a genetic disease that is caused by changes to genes that control the way our cells function, especially how they grow and divide. Autophagy is a self-degradative process that is important for balancing sources of energy at critical times in development and in response to nutrient stress. Autophagy is generally thought of as a survival mechanism.

ATG5 is an autophagy related protein which has an important role in autophagy. ATG5 modulates the immune system and cross talks with apoptosis.

Objectives

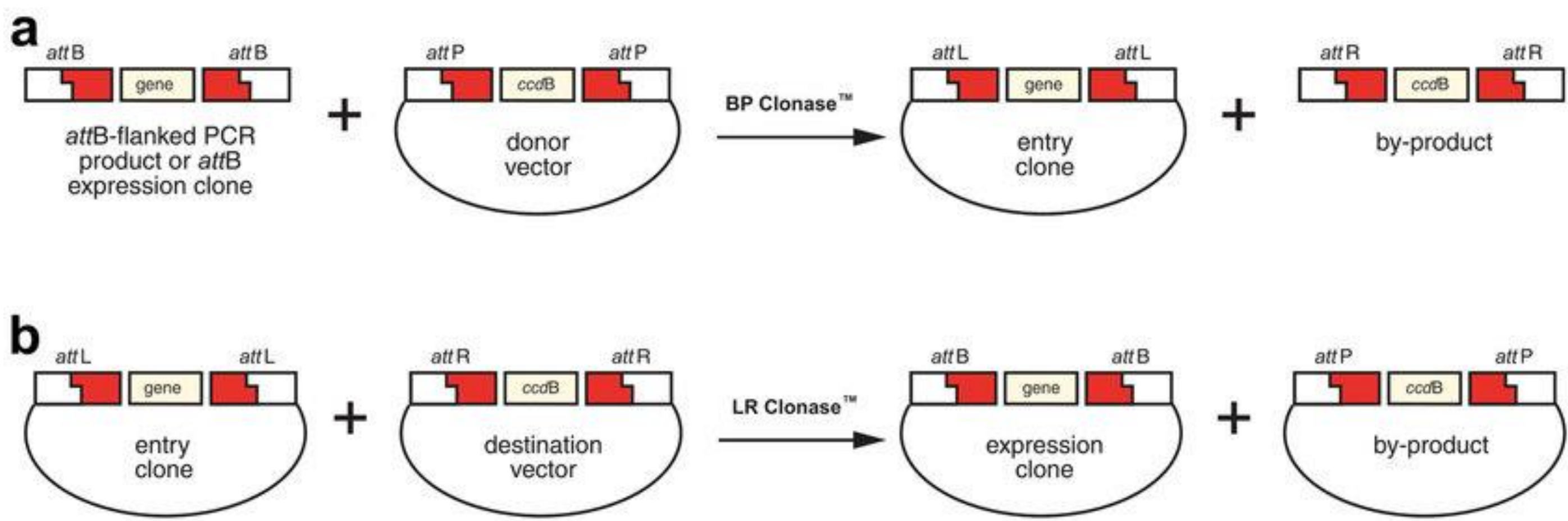
Understanding the role of ATG5 in HEK cells.

Project Details



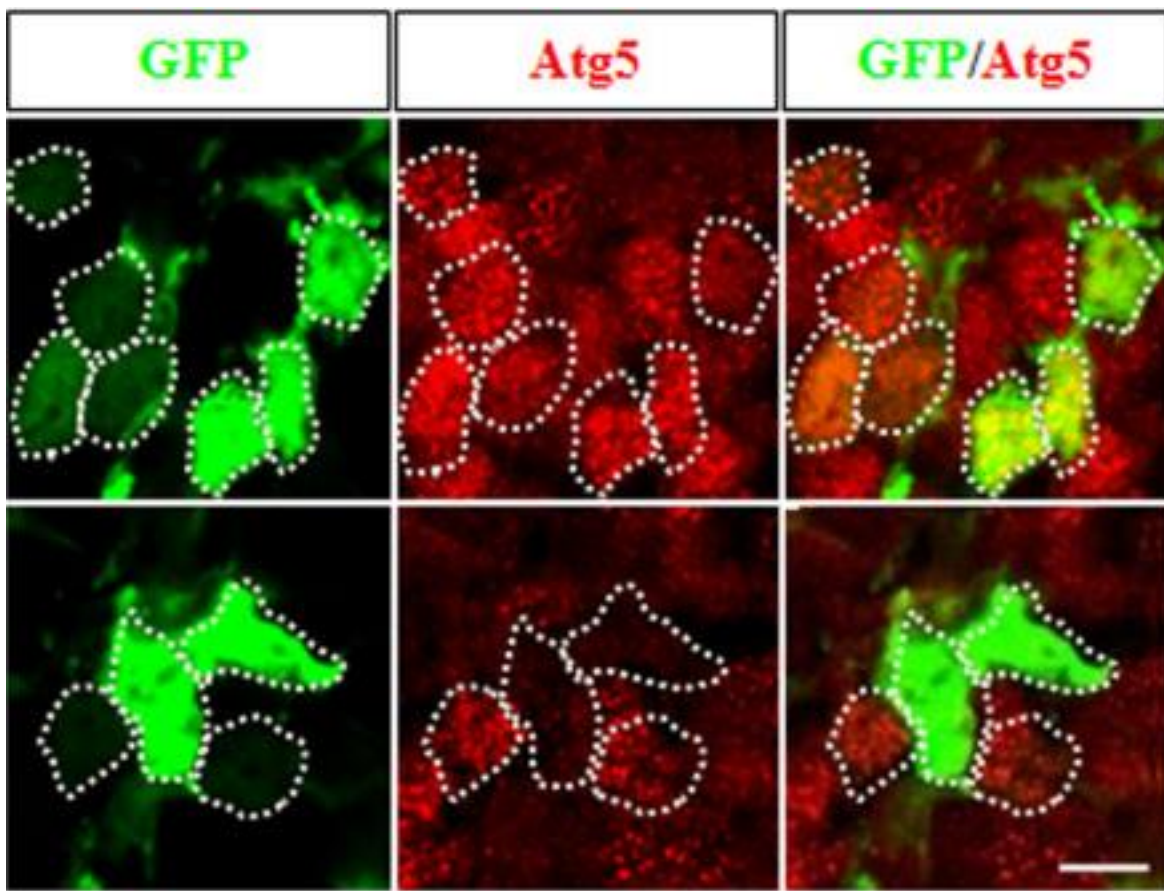
PCR is based on using the ability of DNA polymerase to synthesize new strand of DNA complementary to the offered template strand. Because DNA polymerase can add a nucleotide only onto a preexisting 3'-OH group, it needs a primer to which it can add the first nucleotide. This requirement makes it possible to delineate a specific region of template sequence that the researcher wants to amplify. At the end of the PCR reaction, the specific sequence will be accumulated in billions of copies. After PCR, DNA copies were inserting into plasmids and these plasmids transformed into bacteria to make bacteria colony. In order to control the results colony PCR had done. Colony PCR is a rapid, high throughput PCR method to determine the presence or absence of the inserted DNA into plasmid directly from the bacterial colonies.

Project Details



The BP Reaction is a recombination reaction which the gene of interest is amplified with the help of an attB tagged primer pair. The donor vector includes attP sites. The PCR product that includes the attB sites combines with the donor vector that includes the attP sites resulting in the formation of an entry clone. This integration reaction between the attB and the attP sites forms the basis of this reaction. The resulting entry clone contains the gene of interest flanked by attL sites. The LR Reaction, again is a recombination reaction between attL and attR sites. The reaction generates an expression clone. The entry clone generated from the BP reaction includes the attL sites. The Destination vector is designed to include the attR sites. The LR reaction is carried out to transfer the sequence of interest to one or more destination vectors in simultaneous reactions. The entry clone is mixed with the appropriate vector and Clonase enzyme. Recombination between these sites generates two molecules. One molecule contains the DNA segment of interest, the other molecule is a by-product. Then expression vector will inserted into bacteria to make bacteria colony. After overnight incubation colony PCR will be done to check expression vector have the insert. If the results is correct expression vector is given into HEK cells to find out role of ATG5.

Conclusion



There are many roles of ATG5 protein and I wanted find out another roles of ATG5 however due to the time limitations I could not finish my project.

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