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Visualizing the Tuberin-CyclinB1 Interaction at the G2/ M Transition

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Importance of the Project

      Tuberous Sclerosis (TS) is an autosomal dominant, multisystem disorder that affects 1 in 6000 live births. It is characterized by the development of hamartomas (benign tumours) in various organs as well as various neurological disorders. TS arises due to mutations in the tumour suppressor genes TSC2 and TSC1, which encode Tuberin and Hamartin, respectively. Tuberin has been recently shown to bind Cyclin B1, a mitotic cyclin, to control progression from G2 to mitosis of the cell cycle. My project aims to characterize the cellular timing and localization of the Tuberin and Cyclin B1 complex, using the bimolecular fluorescence complementation (BiFC) system. Understanding this interaction would allow for a greater understanding of the pathology of Tuberous Sclerosis, therefore contributing to the development of effective treatments.

Existing State of Knowledge

      Tuberous Sclerosis (TS) is an autosomal dominant, multisystem disorder that affects 1 in 6000 live births. It is characterized by the development of hamartomas (benign tumours) in various organs including skin, heart, lung, and brain, as well as neurologic manifestations such as epilepsy, mental retardation, and autism. TS arises due to mutations in the tumour suppressor genes TSC2 and TSC1. Mutations in TSC2 are five times more common than mutations in TSC1, and patients with mutations in TSC2 appear to be more severely affected. The protein products of TSC2 and TSC1, Tuberin and Hamartin respectively, interact to form a heterodimer complex, which regulates the cell cycle as well as other cellular processes such as growth, and differentiation through the inhibition of mTOR. It is well known that the Tuberin-Hamartin complex functions during G1/S to control these cellular processes. However, it has recently been shown that Tuberin also functions during the G2/M transition. Tuberin binds to Cyclin B1, a mitotic cyclin that partners with Cdk1 (Cyclin-dependent kinase 1), to regulate mitotic onset.

Research Question

      What is the timing and localization of the Tuberin-Cyclin B1 interaction, and how does this interaction influence progression through the cell cycle?

Methodology

      My research focuses on the construction of a set of bimolecular fluorescence complementation (BiFC) vectors using molecular cloning techniques. The BiFC vectors contain the gene of the protein to be studied (Tuberin or Cyclin B1) conjugated with the amino (N) or carboxi (C) terminus of the yellow fluorescence protein (YFP). When Tuberin and Cyclin B1 interact, a yellow fluorescent complex is formed. Furthermore, I transfected mammalian HEK293 cells with these vectors and

analyzed the expression and interaction of Tuberin and Cyclin B1 in real time using live cell fluorescence microscopy.

Your Findings

I have successfully created a set of mammalian BiFC vectors where the N- or C-terminus of the YFP gene is conjugated to either the N- or C-terminus of the Tuberin or Cyclin B1 genes. Furthermore, I have tested the expression of these vectors in HEK293 cells and visualized the fluorescent complex formation using live cell microscopy.

My results up to now have demonstrated that two of the four Tuberin-Cyclin B1 vector combinations produce yellow fluorescence, while the other two combinations exhibit no fluorescence. Knowing which combinations fluorescence provides information about the 3D conformation of Tuberin, as its crystal structure is currently unknown. Knowing which combinations fluoresce also allows us to determine which vector combinations are most useful in terms of studying the Tuberin-Cyclin B1 complex.

Moreover, when HEK293 cells are transfected with these two constructs, the fluorescent cells arrest in G2 and do not appear to progress to mitosis. This is significant because it confirms that the Tuberin-Cyclin B1 complex inhibits mitotic onset. Additionally, literature suggests that bimolecular fluorescent complexes are irreversible. However, my results suggest that the BiFC Tuberin-Cyclin B1 complex is reversible, perhaps dependent upon concentration of transfected DNA.

Furthermore, immunofluorescence procedures together with the BiFC system are being used to better visualize the cellular localization of the interaction between Tuberin and Cyclin B1. Preliminary results suggest that Tuberin and Cyclin B1 interact in a peri-nuclear fashion during the G2 phase of the cell cycle, before the onset of mitosis.

Finally, the success of using the BiFC system to analyze the Tuberin-Cyclin B1 interaction suggests that this model would be advantageous in examining other factors and pathways involved in the control of cell size and proliferation. The BiFC system could be used to deduce the various mechanisms of not only Tuberous Sclerosis, but many other cell reproduction diseases, such as cancer.

Abstract:

Tuberous Sclerosis (TS) is an autosomal dominant, multisystem disorder that affects 1 in 6000 live births. It is characterized by the development of hamartomas (benign tumours) in various organs as well as numerous neurologic manifestations such as epilepsy, mental retardation, and autism. TS arises due to mutations in the tumour suppressor genes TSC2 and TSC1. Mutations in TSC2 are five times more common than mutations in TSC1, and patients with mutations in TSC2 appear to be more severely affected. The protein products of TSC2 and TSC1, Tuberin and Hamartin respectively, interact to form a heterodimer complex. This complex regulates the cell cycle as well as other cellular processes such as growth and differentiation through the inhibition of mTOR. It is well known that the Tuberin-

Hamartin complex functions during G1/S to control these cellular processes. However, it has recently been shown that Tuberin also functions during the G2/M transition. Tuberin binds to Cyclin B1, a mitotic cyclin that partners with Cdk1 (Cyclin-dependent kinase 1) to regulate mitotic onset. My project aims to characterize the cellular timing and localization of the Tuberin-Cyclin B1 interaction, through the construction of a variety of mammalian expression vectors using the bimolecular fluorescence complementation (BiFC) system. This unique system employs the use of yellow fluorescence protein (YFP), in which the N- and C-terminus of this gene are conjugated to either the N- or C-terminus of the Tuberin or Cyclin B1 genes. Through fluorescence microscopy, I have observed in real time two of the four vector combinations interacting via the YFP fragments binding one another to produce yellow fluorescence. Moreover, these fluorescent cells appear to arrest in G2 and do not progress to mitosis, confirming that the Tuberin-Cyclin B1 complex does in fact inhibit mitotic onset. Furthermore, immunofluorescence procedures suggest that Tuberin and Cyclin B1 interact in a peri-nuclear fashion prior to the onset of mitosis.

Finally, the success of using the BiFC system to analyze the Tuberin-Cyclin B1 interaction will allow for a greater understanding of the pathology of Tuberous Sclerosis and other growth related diseases, like cancer, contributing to the development of effective treatments for these diseases.

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