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USING TARGETED MUTAGENESIS BY CRISPR/CAS9 IN C. ELEGANS TO STUDY THE STRUCTURE AND FUNCTION OF SYP-3 IN MEIOSIS

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The process of meiosis is crucial for organisms to produce offspring via sexual reproduction and to generate genetic variation by the recombination process. The synaptonemal complex (SC) is a protein complex that assembles between chromosomes during meiosis. However, our understanding of the SC is still limited. Some research indicates that certain functions of the SC are related to SYP-3. SYP-3 is a coiled-coil protein that plays an important role in assembling the SC. During SC assembly, this protein then forms protein complexes that connect meiotic chromosomes and allow them to pair. In my proposed research, I used the Clustered Regularly Interspaced Short Palindromic Repeat (CRIPSR) technology to mutagenize the coding sequence of SYP-3 in the nematode C.*elegans* in order to study the structure and function of SYP-3 in meiosis. Since the SC is conserved in human cell division during meiosis prophase I, my research work on the SC in C. *elegans* could contribute and apply to human research and health in the future.

My research project used a nematode called C. *elegans* as an experimental system because of its unique meiotic cytology. In meiotic nuclei, chromosomes can be directly observed at each stage, and many nuclei in many stages can be observed at the same time. C.*elegans* is a small nematode about 1 mm long. C. *elegans* exists primarily as a self-fertilizing hermaphrodite; this means that they produce sperm and eggs by themselves. It takes the C. *elegans* approximately 4 days to grow to their adult stage. Each C. *elegans* that is a hermaphrodite adult can produce about 200 embryos for the first generation (F1). This rapid life cycle allows to obtain a large amount of data in a very short time period

I injected CRISPR/Cas9 with a designed “repair template” into the worm gonad in order to mutagenize the progeny. The repair template includes many random mutations that are caused by increasing 10 times the concentration of guanine (dGTP) in the PCR reaction. This helps to increase the mutation rate on the targeted region of syp-3, which allows us to study the function and structure of SYP-3 protein. The Cas9 nuclease subunit is targeted to the original gene sequence; this cut the original sequence, which repairs using the repair template to achieve mutagenesis