Analysis of SPO11 protein interaction during meiosis

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Meiosis as the specialized cell division of sexual reproduction plays a crucial role in the exchange and reorganization of genetic material between two individuals by dividing the chromosome set in half and forming gametes. Even though in the last years major findings in the field of meiosis have been achieved, especially in plants, some key questions remain concealed. For a proper meiosis the initiation of double strand breaks (DSBs) during early prophase I is essential. Without DSBs no physical connection can occur between homologous chromosomes and recombination, pairing, and crossing over are excluded. So far in all analyzed eukaryotes SPO11, a meiosis specific transesterase, is the key enzyme inducing DSBs. But other than in animals and fungi where a single SPO11 is sufficient, plants need at least two different SPO11, referred to as SPO11-1 and SPO11-2, for proper meiosis. In *Arabidopsis thaliana* both have crucial functions and are essential in a functional form for the induction of meiotic DSBs as single knock out mutants are leading to near sterility by random chromosome distribution. Despite the same function of the homologs SPO11-1 and -2, the identity between both proteins is quite low. Homology of the orthologous SPO11 from different organisms is much higher. By exchanging SPO11-1 and -2 in Arabidopsis by their orthologs from various organisms we could demonstrate a species specific function of each SPO11, as a functional complementation of sterility could only be achieved with SPO11 from closely related species from the Brassicaceae. By exchanging non conserved regions between SPO11-1 and -2 of Arabidopsis we additionally could show a sequence specific function for each SPO11, as a functional rescue could not be achieved with all chosen regions. Interestingly, we could reveal a specific pattern of aberrant spliced isoforms for each SPO11 which are also sequence as well as species specific. By producing antibodies against AthSPO11-1 and -2 we were able to analyze for the first time the binding of SPO11-2 onto the DNA and perform co-immunolocalization studies with SPO11-1 and -2.

With this novel knowledge we further want to analyze possible interactions of SPO11-1 and -2 with each other and/or with other proteins involved in meiosis. To date nothing is known about such interactions in plants. By using our self developed antibodies as well as SPO11 fused to tags, we want to perform pull down assays as well as interaction studies, aiming to identify possible interaction partners of SPO11-1 and -2. Additionally, we will mutate and exchange various regions between SPO11-1 and -2 in Arabidopsis using CRISPR/Cas9 to deepen and confirm the results gained from the previous studies.