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Physical Biology of Meiotic Chromosome Pairing in the Nematode Lineage

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Meiosis is a specialized cell division in which haploid gametes are generated from diploid precursors. Fundamental aspects of meiosis are widely conserved. However, many features of the process have diversified in various species. For example, in the nematode, Caenorhabditis elegans early steps in meiotic recombination, including the formation of programmed double-strand breaks by the enzyme Spo11, end resection, and strand invasion, are not required for homologous chromosomes to pair and synapse, as they are in budding yeast, mammals, and plants. The emergence of recombination-independent homolog-pairing mechanisms in this organism has been accompanied by loss of the gene encoding DMC-1, the meiosis-specific paralog of the RAD-51 recombinase protein. Whole-genome sequence information has recently revealed that loss of DMC-1, as well as its cofactors HOP-2 and MND-1, has occurred independently in several roundworm lineages. In Pristionchus pacificus, a nematode species that diverged from C. elegans around 200 MYA, genes encoding all three proteins are present. We have developed tools to investigate how meiotic mechanisms have changed during nematode evolution. Using immunofluorescence we have examined the dynamics of the recombinase RAD-51, the meiotic chromosome axis protein HOP-1 and the synaptonemal complex component SYP-4 during meiotic progression. We find that HOP-1 staining initially localizes along the full lengths of the chromosomes. Shortly after synapsis, both SYP-4 and HOP-1 become restricted to a limited segment along each pair of chromosomes. Additionally, analysis of targeted deletion alleles of Ppa-spo-11 and Ppa-dmc-1 has revealed that SPO-11, as well as DMC-1 are required for pairing and synapsis. In addition, we apply simple polymer models with confinement and tethering to quantitatively address the physical principles of chromosome pairing in P. pacificus. Our results indicate P. pacificus shares some core meiotic mechanisms with other eukaryotes.

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A Genome Edited Pig with the Hypertrophic Cardiomyopathy-Mutation R723G in the *MYH7*-gene

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Familial Hypertrophic Cardiomyopathy (HCM) is the most common inherited cardiac disease. About 30% of the patients are heterozygous for mutations in the *MYH7* gene. This gene encodes the ß-myosin heavy chain (MyHC), the motor protein of the sarcomeres in human heart. Hallmarks of HCM are cardiomyocyte disarray and hypertrophy of the left ventricle, the symptoms range from slight arrhythmias to sudden cardiac death or heart failure. To gain insight into the underlying mechanisms of the diseases' etiology we aimed to generate genome edited pigs with an HCM-mutation.Designer nucleases have been successfully employed for editing of the porcine genome. Here, we used TALEN-mediated genome editing and successfully introduced the orthologous HCM-point mutation R723G into the *MYH7* gene of porcine fibroblasts. These cells were successfully used for somatic cell nuclear transfer based cloning of domestic pigs that were heterozygous for the HCM-mutation R723G. No off-target effects were determined in the R723G-pigs. Surprisingly, the animals died within 24 h *post partem,* probably due to heart failure as indicated by a shift in the alpha/beta-MyHC ratio in the left ventricle and lung edema. Most interestingly, the neonatal pigs displayed features of HCM, including mild myocyte disarray, malformed nuclei, and *MYH7*-overexpression. Mutated mRNA and protein were expressed, albeit at very low levels. Force generation of cardiomyocytes was essentially unaffected. The finding of HCM-specific pathology in neonatal R723G-piglets suggests a very early onset of the disease and highlights the importance of novel large animal models for studying causative mechanisms and long-term progression of human cardiac diseases.

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Vertebrate Endonuclease G Preferentially Cleaves Holliday Junctions and Specifically Recognizes 5-Hydroxymethylcytosine

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This work provides evidence for a DNA recombination pathway where 5-hydroxymethylcytosine (^{5hm}C) is a recombination marker that is recognized and cleaved by Endonuclease G (Endo G). We hypothesize that EndoG acts as a resolvase to terminate Holliday junction migration by specifically cleaving ^{5hm}C sites. EndoG is historically characterized as a non-specific DNA cutting protein that cleaves G/C rich sequences of DNA. EndoG has been implicated in a variety of cellular recombination functions, and was recently found to preferentially cleave ^{5hm}C DNA in a sequence-specific context to promote recombination. ^{5hm}C is an epigenetic marker that represents up to 1% percent of cytosines in the mammalian genome, and it is found in all vertebrate organisms. In this study, we demonstrate that the Holliday junction, the 4-stranded DNA intermediate in homologous recombination, is a preferred substrate for EndoG. EndoG cuts ^{5hm}C-modified Holliday junctions to produce unique cleavage products, suggesting ^{5hm}C is a marker in EndoG mediated recombination. Furthermore, we present the single-crystal structure of mouse EndoG and propose a mechanism for vertebrate EndoG recognition of ^{5hm}C. An α-helix seen at the DNA binding site of the homologous enzymes from Drosophila and *C. elegans* has unraveled into a long structured loop, allowing the side chains of Ala109 and Cys110 to enter the binding site and potentially recognize the ^{5hm}C modification. The unraveling of this helix is attributed to a two amino acid deletion near the binding site, which is conserved for all vertebrate EndoG sequences. Although EndoG is found in all eukaryotic species, we suggest EndoG has evolved to recognize ^{5hm}C in vertebrate species.

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Computational Analysis of DNA Homologous Recombination Pathway in a Foldback Intercoil Structure

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Four-stranded DNA structures including foldback intercoil (FBI) have been studied not only to understand various life phenomena in vivo, but also to design new DNA nanostructures. A stem-loop FBI structure is constructed by intertwining two DNA duplexes in the major groove. Each strand shares the same helical axis and its diameter almost coincides with a conventional B-DNA. Although DNA homologous recombination as well as deletion can be successfully explained by the FBI structure, only few studies have been reported unlike other four-stranded DNA structures such as DNA G-quadruplex structure and DNA supercoil structure.

In this work, we first constructed a 3D computer model of FBI structure and then carried out several computational analyses including MD and NMA in order to investigate the DNA homologous recombination process. First of all, a 3D computer model of FBI structure was generated with homologous and palindromic DNA base sequences. Its diameter and helicity are 22 Å and 10.5 bp/turn, respectively. Validity of this 3D FBI model was confirmed by comparison of a variety of distances such as adjacent P-P distance, hydrogen bond length, and adjacent base-base distance with those of B-DNA. When homologous recombination occurs in the FBI structure, it is also required to secure enough space for base flipping of the intercoil structure. For this, the unwinding FBI structure was also constructed with 26 Å diameter and 13 bp/turn helicity. Consequently, this computational approach enables us to better understand that homologous recombination occurs through base flipping of the FBI structure.

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Molecular Dynamics Simulation Study of DNA Mismatch Recognition by Complementary Strand Interactions in Thymine DNA Glycosylase

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Base excision repair (BER) is a conserved repair mechanism of the cell that restores the genetic material back to its original state. Defects in BER components lead to elevated mutation rates, eventually pushing the cell to a cancerous state. Thymine DNA glycosylase is an essential component of BER that corrects G·T mutagenic mismatches, while A·T pairs are not subject to base excision by TDG. How TDG achieves this specificity is not well understood. One hypothesis is that rejection of A·T pairs is due to unfavorable contacts with the complementary strand. In this study, we use molecular dynamics (MD) simulations to elucidate the substrate recognition of TDG in the presence of several different complementary bases including guanine, adenine and inosine. MD simulations are performed on DNA alone and the enzyme-DNA complex using the CHARMM36 additive and Drude polarizable force field, with