Highlight on synaptonemal complex 3D-SIM imaging on the DeltaVision™ OMX

Introduction

Early in meiosis, homologous chromosomes are held together by a zipper-like structure called the synaptonemal complex (SC). The chromosomes comprise loops of chromatin that attach to the axial elements (AE) of the SC, which form the outward facing portion of the two lateral elements (LE) (Fig 1). The LEs are analogous to the sides of the zipper. The proteins that comprise the transverse filaments (TF) and central element (CE) in the central region (CR) function as the teeth of the zipper that hold the two LE, and thus the chromosomes, together.

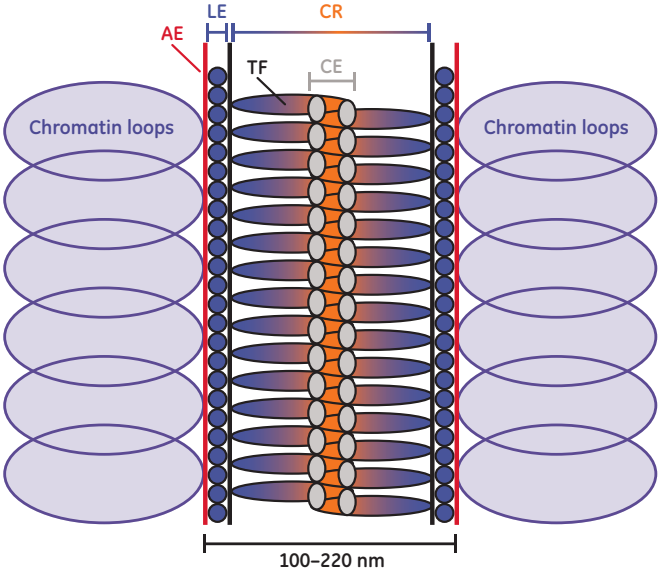

Fig 1. Schematic of the synaptonemal complex. Chromatin loops are shown in purple, the CR encompasses the CE and TF, the CE is shown in light gray, the LEs are shown in blue, TFs are shown in the purple/orange gradient, and the AEs are shown in red.

Literature shows that defects in the human SC lead to problems with chromosome segregation in meiosis that are associated with infertility, recurrent miscarriage, and genetic disorders such as Downs Syndrome (1, 2). Researchers have been studying the SC since 1956, but due to its very small size, they were not able to resolve all structural and spatial details required to understand the relationship between its structure and its function. In the past few years, there have been several important discoveries in this field involving super-resolution imaging techniques, such as 3D Structured Illumination Microscopy (3D-SIM), which have led to a better understanding of the SC and associated proteins.

The width of the SC makes it an ideal structure to study with structured illumination. 3D-SIM on the DeltaVision™ OMX can achieve resolution of 120 ± 5 nm in the x and y axes and 340 ± 10nm in the z axis (when imaged with the 488 nm laser), while the SC varies from 100 to 220 nm in most model organisms.

Here, we will tell the story of a lab in which 3D-SIM enabled the discovery of novel complexes associated with the SC and structural characterization of a mutant SC. Additionally, SIM enabled characterization of chromosome pairing and synapsis, the process of SC assembly.

A mammalian KASH domain protein coupling meiotic chromosomes to the cytoskeleton

Horn, H. *et al. J. Cell Biol.* **202(7)**, 1023–39 (2013), doi:10.1083/jcb.201304004.

Summary

- 3D-SIM revealed novel structures of both SUN1 and KASH5 in male meiosis
- The structure of the SC in *Kash5-null* spermatocytes was characterized by 3D-SIM
- Homolog pairing and synapsis were assessed by 3D-SIM
- KASH5 is required to tether meiotic chromosomes to the cytoskeleton

Hypothesis/Experiment

When Henning Horn started his postdoc in Colin Stewart's lab at the Institute of Medical Biology (IMB) in Singapore, his first project was to investigate the role of a protein named KASH5 in male meiosis of mice. His colleagues had already shown that KASH5 localized to the tips of the SC. When Horn used a widefield fluorescence or confocal microscope to image spermatocyte spreads from *Kash5-null* mice, he was unable to resolve the two axial elements (AE) of the SC. He knew that being able to do so would add an important component to their study. Horn showed his images to Graham Wright, the Head of the Microscopy Unit, an IMB core facility, who immediately recognized that the SC was a perfect biological structure to analyze using 3D-SIM on the DeltaVision OMX super-resolution imaging system. With new tools at their disposal, the authors set out to further characterize KASH5. Specifically, they wanted to determine if KASH5 functioned similarly to other previously characterized KASH proteins which tether the nucleus to the cytoskeleton.

Method

Once 3D-SIM was identified as an option, the authors were excited to see if the ability to separate AEs of the SC would allow them to identify more precisely where KASH5 localized in the context of the SC. While the authors could use their existing fluorophores and their standard sample preparation protocol, Horn recalls that some optimization was needed to acquire high-quality DeltaVision OMX images. Specifically, mounting the samples on the coverslips (rather than on the microscope slide) and changing to a soft mountant was required. Once sample preparation was optimized, this opened the door for additional experiments.

Wright noted that the DeltaVision OMX allowed them to obtain high-quality datasets in 2 to 3 days from preparing the samples. He notes that it would have taken 3 to 4 weeks to obtain results, had they decided to use electron microscopy (EM) instead of 3D-SIM. Additionally, even though EM can achieve higher resolution than 3D-SIM, it does not produce 3D datasets, thus limiting the spatial information they could gather and eliminating the ability for chromosome quantification.

Results

Horn demonstrated on a widefield DeltaVision system that KASH5 localized to foci at the ends of the SC. Together with Wright, they set out to use 3D-SIM on the DeltaVision OMX to resolve the pair of axial elements within the SC. Horn recalls being amazed when he saw the first 3D-SIM image he acquired with Wright because 3D-SIM clearly resolved the two AEs and revealed that KASH5 localizes as rings (not foci!) at the tip of each AE (Fig 2). The images they acquired went on to win an institute-wide image competition, the international GE Healthcare Image Competition in 2013, and impressively, were chosen for the cover of The Journal of Cell Biology.

Horn and Wright went on to apply 3D-SIM to examine the SC structure in *Kash5-null* (knockout) mice. The work revealed severe defects in the CR of the SC. While the AE were formed, they were abnormal in that non-homologous pairing was observed, and the TF protein, SYCP-1, had greatly reduced localization.

With this new information, the authors then shifted their focus to examine the localization of another protein in the same structural complex as KASH5, called SUN1. Again using 3D-SIM on the DeltaVision OMX, they found that SUN1, like KASH5, localizes in rings at the ends of the AE that form figure 8-like structures (Fig 2, white arrow) during homolog pairing. Further, 3D-SIM revealed that SUN1 rings are abolished in *Kash5-null* spermatocytes.

In summary, these authors used 3D-SIM on the DeltaVision OMX to identify new structures associated with the SC that function to connect chromosomes to the cytoskeleton in meiosis. Additionally, the DeltaVision OMX enabled characterization of the SC defects in *Kash5-null* mice.

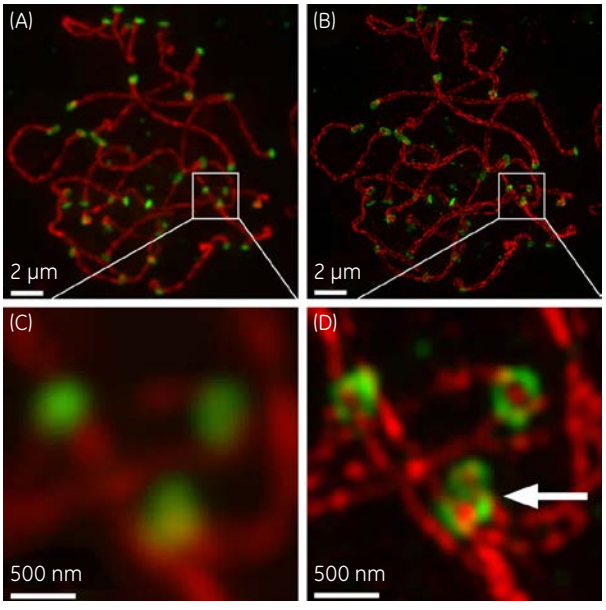

Fig 2. A. Spermatocyte spreads, labeled with anti-KASH5 in green and anti-SCP3 in red, imaged by widefield deconvolution microscopy. SCP3 is visualized as a single strand per chromosome. KASH5 localizes as a large foci to the tips of SCP3-positive SCs. B. Structured illumination microscopy of the same cells imaged in A. The two AEs of the SC are clearly resolved with SCP3 staining and KASH5 localizes as rings at the tips of the SCP3 axial strands. C. Enlargement of A. D. Enlargement of B.

Significance and future directions

3D-SIM played a key role in identifying novel structures of SUN1 and KASH5 in meiosis as well as characterizing the *Kash5-null* phenotype. In The Journal of Cell Biology publication, Horn and Wright note: *"SIM is a valuable tool in our studies of spermatogenesis. The twofold improvement in resolution over conventional widefield microscopy allowed us to directly assess homolog pairing and synapsis by following the alignment of the SC axial elements."*

Since both SUN and KASH proteins are conserved across evolution, progress in understanding meiosis in mice may have implications for understanding infertility that result from meiotic failures in humans. Ongoing work in Horn's own lab, recently established in Doha, Qatar, aims to address the requirements for KASH proteins in human meiosis.

Scientific summaries of publications imaging the SC with DeltaVision OMX

HAL-2 promotes homologous pairing during Caenorhabditis elegans *meiosis by antagonizing inhibitory effects of synaptonemal complex precursors*

Zhang, W. *et al. PLoS Genet.* **8(8)** (2012). doi:10.1371/journal.pgen.1002880.

In this publication, Zhang and colleagues demonstrate that HAL-2 promotes chromosome pairing by preventing inappropriate SC assembly. They use 3D-SIM to examine the structure of the SC in the absence of HAL-2. Specifically, the authors localize the SC components HTP-3 (LE) and SYP-1 (CR) in wildtype and *hal-2* mutants. In wildtype, SYP-1 localizes in-between the two parallel HTP-3 lateral elements. In contrast, parallel LEs are not found in *hal-2* mutants. Instead, they find that *hal-2* chromosomes are unpaired with SYP-1 localizing to both axes of unpaired homologs.

This paper advances our understanding of chromosome pairing, which has long remained an elusive part of meiosis.

Protein phosphatase 4 promotes chromosome pairing and synapsis, and contributes to maintaining crossover competence with increasing age

Sato-Carlton, A. *et al. PLos Genet.* **10(10)** (2014). doi:10.1371/journal.pgen.1004638.

Sato-Carlton, and colleagues investigate the function of PP4 in *C. elegans meiosis*. They use 3D-SIM to examine SC structure in *pph-4.1* mutants by examining SYP-1 (CR protein) and HTP-3 (LE) localization and find that SC structure is normal, with SYP-1 localizing in-between two parallel tracks of HTP-3. Intriguingly, autosomal pairing is abnormal in *pph-4.1* mutants, suggesting that non-homologous synapsis may be occurring. They went on to examine non-homologous synapsis by 3D-SIM and found defects in *pph-4.1* mutants, including multivalent synapsis, full length synapsis of non-homologous chromosomes and self-synapsis.

This work represents an advance in our understanding of chromosome pairing and synapsis, which have long been poorly understood. The authors go on to show that PPH4 is required for additional steps in meiosis in an age-dependent manner. In humans, increasing maternal age is the primary risk factor for having a Downs Syndrome child; therefore, work contributing to our understanding of meiosis in older mothers will lead to discoveries impacting human reproduction.

A mammalian KASH domain protein coupling meiotic chromosomes to the cytoskeleton

Horn, H. *et al. J. Cell Biol.* **202(7)**, 1023–39 (2013). doi:10.1083/jcb.201304004.

In this featured study, Horn and colleagues rely on 3D-SIM for their studies of KASH5 and SUN1, proteins involved in tethering meiotic chromosomes to the cytoskeleton in mouse spermatocytes. Widefield microscopy localized KASH5 to foci at the ends of the SC, but 3D-SIM resolved the pair of axial elements within the SC and revealed that KASH5 localizes as rings at the tip of each axial element. During homolog pairing, they demonstrate that KASH5 rings combine to form figure 8-like structures. In addition, they use 3D-SIM to examine the localization of SUN1 and they find that SUN1, like KASH5, localizes in rings at the ends of the AE. These rings form figure 8-like structures during homolog pairing. In *Kash5-null* spermatocytes, they find that SUN1 rings are abolished and that the SC fails to properly form. In summary, these authors used 3D-SIM to characterize the SC structure of a mutant as well as to identify novel structures that interact with paired homologs held together by SC.

As KASH proteins are conserved across evolution, this work provides a foundation for human geneticists and mouse meiosis researchers to expand upon.

High resolution analysis of meiotic chromosome structure and behavior in barley (Hordeum vulgare L.)

Phillips, D. *et al. PLoS One* **7(6)** (2012). doi:10.1371/journal.pone.0039539.

For the first time in 3D-SIM, Phillips and colleagues image barley meiocytes. They localized ASY1 (AE) with ZYP-1 (TF) and found the expected localization of ZYP-1 in-between parallel ASY1 tracks. Additionally, they discovered novel SC configurations including one in which parallel tracks of ZYP-1 run in-between the tracks of ASY-1. Finally, they identified both left-handed and right-handed twisting of ASY-1 cores.

In summary, these authors used 3D-SIM to identify novel SC configurations. While the function of these novel structures is currently unknown, if a mutant is identified that is specifically defective in this configuration, we will come to understand the significance and function of these novel SC configurations.

Corolla is a novel protein that contributes to the architecture of the synaptonemal complex of Drosophila

Collins, K. *et al. Genetics* **198(1)**, 219–28 (2014). doi:10.1534/genetics.114.165290/-/DC1.

3D-SIM is used by Collins and colleagues to localize a novel protein called Corolla to the central region of the SC in *Drosophila* oocytes. Specifically, they demonstrate that Corolla localizes in-between the C-terminal domains of the TF protein C(3)G, consistent with the finding that *corolla* mutants are severely defective in SC structure.

These results are significant because the identification of novel SC proteins in model organisms often enables identification of homologs in mice and humans. As we identify more human SC components, we will gain a better understanding of defects that impact human health and reproduction.

Vilya, a component of the recombination nodule, is required for meiotic double-strand break formation in Drosophila

Lake, C. *et al. eLIFE* **e08287**, 1-26 (2015). doi:10.7554/eLife.08287.

Lake and colleagues use 3D-SIM to localize a newly identified protein, Vilya, to the central region of the SC in foci as well as linear elements. The localization pattern of Vilya, along with additional data suggested that Vilya localizes to recombination nodules. Indeed, immuno-EM revealed that Vilya localizes to the electron-dense recombination nodules.

Recombination of chromosomes is essential for the production of normal eggs. This paper identifies and characterizes the function of Vilya to advance our understanding of this poorly understood process in meiosis.

Arabidopsis PCH2 mediates meiotic chromosome remodeling and maturation of crossovers

Lambing, C. *et al. PLOS Genet.* **11(7)**, (2015). doi:10.1371/journal.pgen.1005372.

Lambing and colleagues investigate the chromosome axis in *Arabidopsis* and show that as the SC is assembled, the chromosome axis protein, ASY1, is depleted. They also show that the AAA+ ATPase protein, PCH2, is required for chromosome axis restructuring and SC formation. In *Atpch2* mutants, ASY1 signal is not depleted on time. Ultimately in *Atpch2* mutants, maturation of crossovers is affected and the number of crossovers is reduced, leading to chromosomes which failed to recombine.

This publication reveals the role of ASY1 in chromosome axis remodeling and recombination. Recombination is an essential component of meiosis and when chromosomes fail to recombine they missegregate, leading to birth defects such as Downs syndrome. Understanding the conserved protein PCH2 in model organisms will help us understand its function in humans.

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