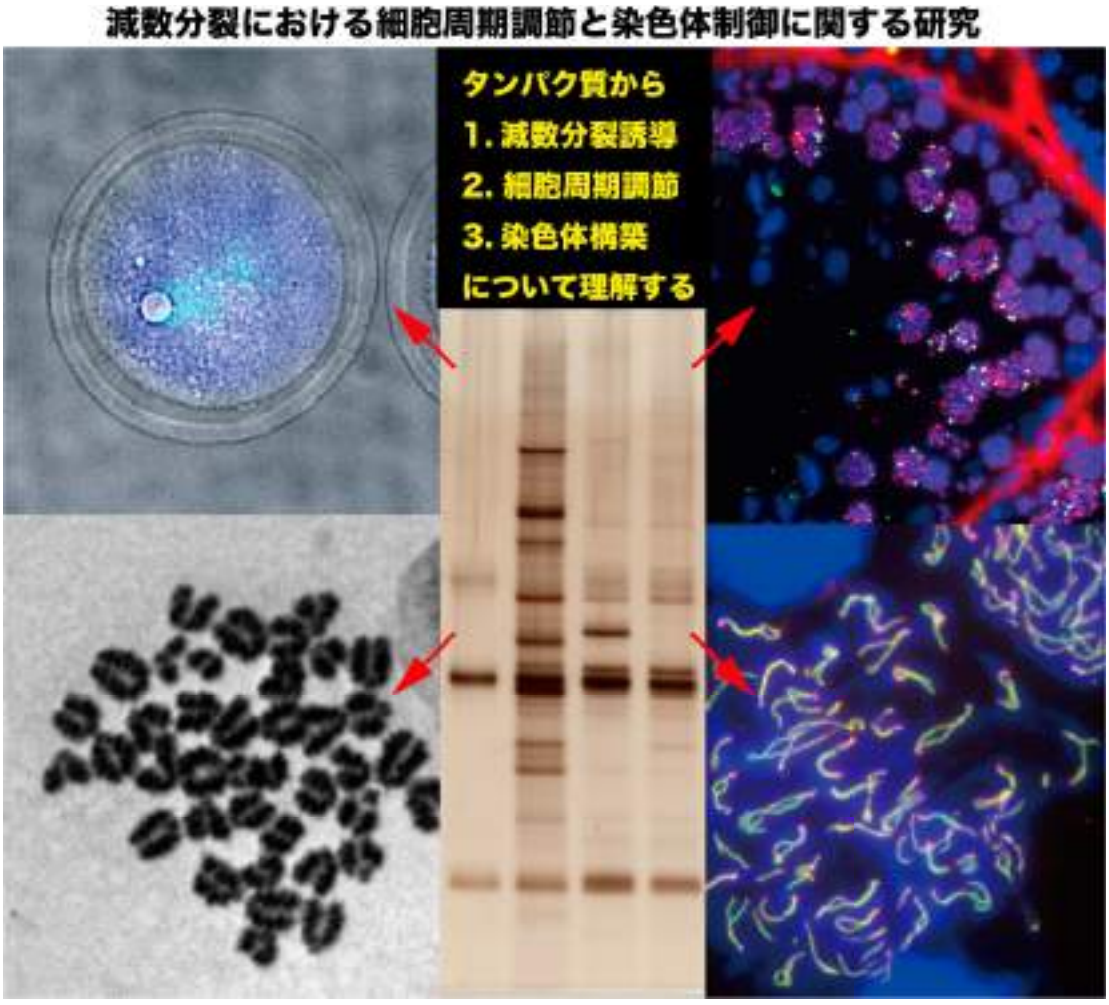
染色体制御分野
Department of Chromosome Biology

減数分裂は、染色体の次世代への正確な継承と初期胚の正常発生の観点から極めて重要なイベントである。当分野では高等動物の減数分裂における染色体構築とその制御のメカニズムについて研究を推進する。とりわけ、当分野では(1)体細胞分裂から減数分裂への切り替え制御、(2)減数分裂型細胞周期の制御、(3) 減数分裂における染色体構築の制御、これら 3 つの角度から基礎研究を行う。内容的には高齢出産、少子化の観点で、医学分野のみならず社会的にも強くアピールできる研究課題に取り組む。

Meiosis is supposed to be a “special cell cycle process” that modifies the canonical mitotic cell cycle. Understanding the mechanisms how meiotic cell cycle is regulated for correct assembly of specialized chromosome structure and timely order of chromosomal events is important because dysregulation of meiosis often leads to infertility and pregnancy losses. Our laboratory is investigating the molecular mechanisms of meiosis from three aspects as following: (1) molecular mechanisms of induction of meiosis; (2) cell cycle regulation that provide crucial difference between meiosis and mitosis; (3) molecular basis of meiosis-specific chromosomal structure.



構成員　Staff (2018.3)

名前	職名	Name and Position
石黒　啓一郎	独立准教授	Kei-ichiro Ishiguro, Associate Professor
福田　恵	文部科研技術支援員	Megumi Fukuda, Secretary Assistant
本岡　千聡	大学院生	Chisato Motooka, Graduate Student
丹野　修宏	大学院生	Nobuhiro Tanno, Graduate Student

元在籍者　Staff in the past (2016.9～2018.3)

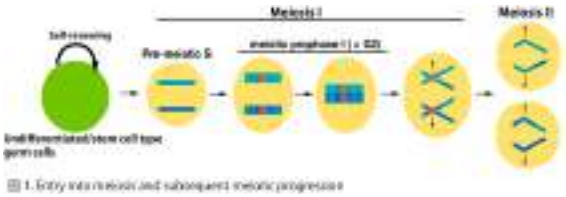
名前	Name	在籍期間	在籍時職名	転出先
喜多村　悦至	Etsushi Kitamura	2017.1.1-2017.12.6	助教	

研究概略　Projects

減数分裂は発生過程の中でも染色体構成の次世代への正確な継承と着床前胚の全能性再獲得に向けたゲノム半数化を達成するための極めて重要なステップとして位置づけられる。生殖細胞には、あらゆる生物種に共通する原理的な保存性と、生物種に固有のメカニズムが存在する。事実、高等動物の減数分裂に特有で他の種には見られない現象があることや、アミノ酸配列上はまったく相同性のないものが高等動物とそれ以外の種で似た役割を果たす事例が見出されている。このように生殖細胞には種にユニークな機構が備わっている例が多く知られることからも当分野では主にマウスを用いた研究を行う。とりわけヒトの不妊・不育などのメカニズムを考えた場合には、ヒトの疾患の原因解明に道筋をつけるためにも敢えてマウス個体や胚性幹細胞を用いることに本研究を行う意義がある。当分野では以下の３つの確度から、減数分裂の分子機構の解明を目指す。

1.体細胞分裂から減数分裂への切り替え制御

マウスの場合、減数分裂に進行する前段階では、未分化型・幹細胞型の生殖細胞(♀胎児期始原生殖細胞、成体♂精原細胞)が存在する。この未分化型・幹細胞型の生殖細胞は体細胞型の増殖を経た後に、その一部の集団が spontaneous に減数第一分裂の pre-meiotic S 期へと進行する(図１)。この時、体細胞型増殖から減数分裂型 cell cycle への切り替えが起きていると推測される



が、その分子機構は多くの点が不明のままである。特にこの減数第一分裂への移行期においては染色体構造が減数分裂仕様に再構成されると推測され、染色体制御を理解する上でキーとなるステージであるにもかかわらず材料の量的・数的な制限のため研究は膠着している。最近我々は、体細胞増殖→減数分裂への遷移期に該当する生殖細胞集団を効率良く分離する手法を確立し、特異的発現を示すタンパク質のスクリーニングを行った。その結果、減数分裂の開始や進行に重要な役割を果たす新規の因子が複数見出された。これらの未解析因子の解析を通して、減数分裂の減数分裂誘導に決定的な分子機構の解明を目指す。

2.減数分裂型細胞周期の制御

本研究では体細胞型と減数分裂型の細胞周期の制御機構の違いを見出すことを課題とする。生殖細胞は通常の体細胞と同様の細胞周期の機構を巧みに転用しながらも、染色体構造に減数分裂特異性が与えられるようにプログラムされている。減数分裂を細胞周期調節という観点から体細胞と比較すると、両者は様々な点で異なっている。例えば減数第一分裂前期(meiotic prophase I)と呼ばれる時期は、通常の体細胞の細胞周期の G2 期に相当する(図１)。この meiotic prophase I は、そのタイムスパンが通常の細胞周期 G2 期と比べて際だって長いこと(精母細胞でおよそ 7-8 日にも及ぶ)が特徴で、染色体上の様々なイベントを達成させる猶予を与える期間

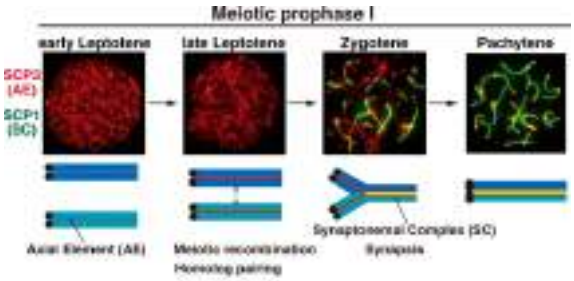


図2. 減数第一分裂前期に見られる染色構造の変化

であると考えられる(図 2)。さらに、第一分裂 M 期が完了しても次の S 期が開始されずに直ちに第二分裂 M 期へ進行する点も、通常の細胞周期と極めて異なる特徴の一つである。このように細胞周期の調節は減数分裂仕様に大幅に特殊化されているが、この分子機構はほとんど理解されていない。

体細胞で見られる細胞周期調節が染色体の動態に決定的な役割を果たしていることからも明白であるように、減数分裂における細胞周期調節の観点から染色体制御を理解することが重要

となろう。本研究では、細胞周期の進行に重要とされるユビキチンリガーゼ複合体の減数分裂の素過程における働きを酵素・基質・調節の観点から研究する。

3. 減数分裂における染色体構築の制御

これまで我々は減数分裂に特異的な新規因子の同定と染色体構造変換の解明に取り組んできた。とりわけマウスなどの高等動物では、酵母、ショウジョウバエ、線虫のような順遺伝学的スクリーニング手法が容易に適用できないために、その減数分裂における染色体制御機構の解明は国際的にも攻め倦んでいる背景があった。我々はマウス生殖細胞クロマチン画分からの蛋白質複合体の精製・質量分析法および抗体ライブラリーを駆使したスクリーニングにより、減数分裂型の新規コヒーシン複合体(Ishiguro et al. *EMBO Rep* 2016,Ishiguro et al.*Genes Dev* 2014, Ishiguro et al.*EMBO Rep* 2011)、染色体末端と核膜とを繋ぐテロメア結合因子(Shibuya et al. *Nat Cell Biol* 2014)、減数第一分裂に特異的なセントロメア局在因子(Kim et al.*Nature* 2015)など、複数の新規染色体結合因子を同定した。奇しくもこれらはすべてデータベースに眠る未

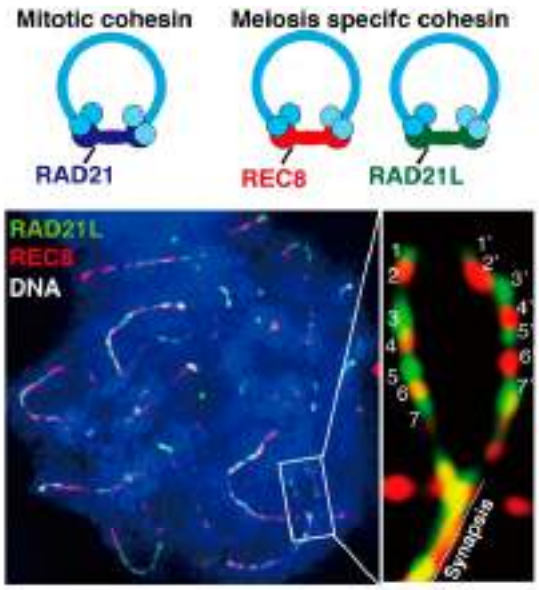


図 3. 染色体構造の土台となる減数分裂型コヒーシン

解析のhypothetical因子であったが、欠損マウスの解析から減数分裂における染色体動態に極めて重要な役割を果たしていることが明らかとなった。

コヒーシンは S 期で複製された姉妹染色分体がばらばらとならないように、それらの接着に働くタンパク質複合体である。さらに体細胞においては、コヒーシン複合体はインスレーターターンパク質 CTCF と協調して遠方のエンハンサーとプロモーターとを手繰り寄せるようにして、Topologically Associated Domain(TAD)と呼ばれる高次クロマチンドメインを規定することにより遺伝子発現の調節に働くことも知られている。重要なことに、このコヒーシン複合体には体細胞型と減数分裂型の使い分けがある(Ishiguro et al., *J. Cell Sci.*2007)。すなわち、体細胞では RAD21 をサブユニットとして含むコヒーシン複合体があるのに対して、減数分裂では、RAD21L または REC8 をサブユニットとして含むものが存在する(Ishiguro et al.*EMBO Rep* 2011)。この減数分裂型コヒーシン複合体は、姉妹染色分体接着のみならず、体細胞では見られない減数分裂に特有の染色体構造の骨組みとしても極めて重要な役割を果たしている(図 3) (Ishiguro et al. *EMBO Rep* 2016,Ishiguro et al.*Genes Dev* 2014)。

また新規の減数分裂特異的動原体因子MEIKINはコヒーシン複合体のセントロメア領域への局在を制御する(図4) (Kim et al. *Nature* 2015)。

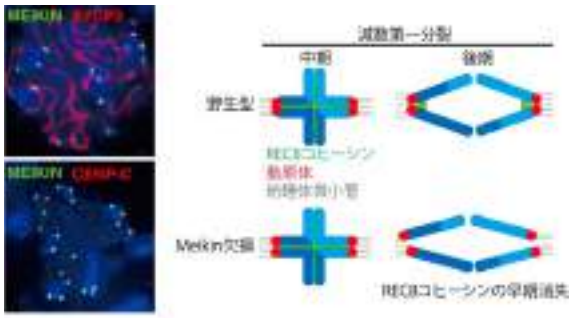


図 4. 減数第一分裂に特異的な動原体因子

MEIKINはPLK1と呼ばれるキナーゼを動原体に呼び込むことが判明しいているが、依然この基質は不明とされる。このように減数分裂を特徴付ける未知の染色体構成因子は未だデータベースに埋もれているものと推測され、今後この全貌を明らかにすることが大きな課題である。

The meiotic cell cycle consists of a single DNA replication followed by two round of chromosome segregation (meiosis I and meiosis II), halving

chromosome number in haploid gametes. Remarkably, structure and dynamics of chromosomes during meiosis are remarkably different to that in mitosis.

Meiosis is supposed to be a "special cell cycle process" that may modify the canonical mitotic cell cycle. Understanding the mechanisms how meiotic cell cycle is regulated for correct assembly of specialized chromosome structure and timely order of chromosomal events is important because dysregulation of meiosis often leads to infertility and pregnancy losses. Our laboratory is investigating the molecular mechanisms of meiosis from three aspects as following. Our current work is mainly focused on (1) investigating the molecular mechanisms of induction of meiosis from mitotic state in precursor germ cells; (2) studying the molecular mechanisms of the cell cycle regulation that provide crucial difference between meiosis and mitosis; (3) studying the molecular basis of meiosis-specific chromosomal structure that ensures unique chromosomal events during meiosis.

1. Molecular mechanisms of induction of meiosis

In mammals, undifferentiated/stem cell type germ cells have a potential to enter meiosis. After mitotic growth, a subpopulation of such germ cells spontaneously progress to meiotic S-phase, which is triggered by external signal of retinoic acid. Importantly, it is assumed that induction of meiosis accompanies switching the cell cycle regulation from "mitotic type" to "meiotic type". Probably, the cell cycle switching is also negatively or positively regulated by expression of germ cell specific RNA-binding proteins, accompanying downregulation of somatic cell cycle program. However, the molecular mechanism of meiotic induction has been largely elusive, because of limited materials available for study. Our current aim is to identify putative germ cell-specific factors, which are crucial for induction of meiosis.

2. Cell cycle regulation in meiosis

Our specific aim in this project is to elucidate molecular differences in cell cycle regulation between mitosis and meiosis. In germ cell, meiosis-specific events are programed in the context of cell cycle regulation. Notably, meiotic prophase I, which is equivalent to G2 phase in somatic cell cycle, is prolonged so that meiosis-specific chromosomal events are sequentially progressed. Moreover, it should be mentioned that one round of S phase is missing between the first (M I) and second M phases (M II), which is sharp contrast to somatic cell cycle. Thus, it is assumed that canonical cell cycle regulation is diversified and modified in meiosis. However, the underlying mechanism of meiotic cell cycle is largely elusive.

As it is well known that chromosomal events, such as DNA replication, recombination, repair and chromosome segregation, are driven by cell cycle regulation in somatic cell, it would be important to consider the mechanisms of meiotic chromosome dynamics in the aspect of cell cycle. Thus, our current approach for this aim is to identify meiosis-specific substrates and regulatory subunits for E3 ubiquitin ligases in germ cells, that are crucial for cell cycle progression.

3. Meiosis-specific chromosomal structure

When chromosomes are replicated in S-phase, sister chromatids are held together by a mechanism, sister chromatid cohesion, which enables accurate chromosome segregation in both mitosis and meiosis. Sister chromatid cohesion is mediated by an evolutionary conserved multi-protein complex, cohesin. Moreover, cohesin acts for transcriptional regulation collaborating with insulator binding factor in somatic cells. Importantly, there exist two types of cohesins : mitotic-type and meiotic-type cohesins.

During meiotic prophase I, sister chromatids are organized into proteinaceous structures called chromosome axis, on which the synaptonemal complex is assembled. Thus, meiotic cohesin acts as a structural basis for chromosomes. Then, homologous chromosomes undergo pairing, synapsis and meiotic recombination, yielding a physical linkage between homologs called chiasmata. Meiosis-specific cohesin plays crucial roles in all of these sequential chromosomal events during meiosis. In mammalian meiotic cells, two different types of cohesin complexes exist, which contains either subunit REC8 or RAD21L. These REC8 and RAD21L subunits determine spatiotemporal distribution patterns of distinct cohesin complexes on chromosomes during meiosis. Thus, REC8- and RAD21L-cohesins play different roles in chromosomal dynamics during meiosis. Meiotic cohesins also play a crucial role in regulating kinetochore structure, collaborating with the newly identified centromeric protein MEIKIN during meiosis I.

Thus, it still remains elusive how chromosomes are reorganized during meiosis. Our specific aim in this project is to identify meiosis-specific chromatin proteins and elucidate the molecular mechanisms of reorganization of chromosomes during meiosis.

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Expression analysis of the endogenous *Zscan4* locus and its coding proteins in mouse ES cells and preimplantation embryos

Kei-ichiro Ishiguro[1,2] · Yuhki Nakatake[1] · Nana Chikazawa-Nohtomi[1] · Hiromi Kimura[1] · Tomohiko Akiyama[1] · Mayumi Oda[1] · Shigeru B.H. Ko[1] · Minoru S.H. Ko[1]

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Abstract Mouse Zinc finger and SCAN domain containing 4 (Zscan4) is encoded in multiple copies of *Zscan4* genes, which are expressed in late two-cell stage preimplantation embryos and in 1–5% of the embryonic stem (ES) cell population at a given time. Due to the highly identical nucleotide sequences of multiple copies of *Zscan4* paralogs and pseudogenes in the mouse *Zscan4* genomic cluster, previous analyses have been done using exogenous transgenes under the regulation of *Zscan4c* promoter. In this manuscript, we generated knock-in mouse ES cell lines and mouse lines, in which the expression of endogenous *Zscan4c*, one of the *Zscan4* genes, can be specifically monitored with a green fluorescent protein variant, Emerald. Interestingly, we found that only ~30% of Zscan4-immunopositive ES cells were Emerald positive, suggesting that even when the Zscan4 locus is active, not all *Zscan4* genes are expressed synchronously. We also carried out mass spectrometry of protein complexes associated with endogenous Zscan4 proteins. Taken together, our genetic engineering at an endogenous *Zscan4c* gene provides the first clue for the expression and function of each gene copy of *Zscan4* locus in a physiological context.

Keywords Zscan4 · ES cell · Preimplantation embryo · Two-cell stage · Knock-in

✉ Minoru S.H. Ko
ko.minoru@keio.jp

[1] Department of Systems Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan

[2] Present address: Institute of Molecular Embryology and Genetics, Kumamoto University, 2-2-1 Honjo, Chuo-ku, Kumamoto 860-0811, Japan

Introduction

Zinc finger and SCAN domain containing 4 (*Zscan4*) genes were originally identified as genes that are specifically expressed during zygotic genome activation (ZGA) in the late two-cell stage of mouse preimplantation embryos (Falco et al. 2007). The mouse genome encodes nine members of the *Zscan4* paralogs (*Zscan4a*, *Zscan4b*, *Zscan4c*, *Zscan4d*, *Zscan4e*, and *Zscan4f* and three pseudogenes *Zscan4-ps1*, *Zscan4-ps2*, and *Zscan4-ps3*). In the human genome, there is only one copy of *Zscan4* gene (Falco et al. 2007). Among the mouse *Zscan4* genes, *Zscan4c*, *Zscan4d*, and *Zscan4f* encode a full-length 506-aa protein, whereas *Zscan4a*, *Zscan4b*, and *Zscan4e* encode truncated proteins (360 amino acids (aa), 195 aa, and 195 aa, respectively) (Falco et al. 2007). In two-cell stage embryos, the knockdown of *Zscan4* by small interfering RNA (siRNA) leads to a delay of progression from the two-cell to four-cell stage and, consequently, implantation failure (Falco et al. 2007). In mouse embryonic stem (ES) cells, the expression of *Zscan4* is transient and reversible with infrequent transcriptional activation in only 1–5% of the cell population at a given time point (Falco et al. 2007) (Zalzman et al. 2010). A burst of *Zscan4* transcription (Z4 events) is accompanied by biological events including transient expression of other ZGA-specific genes (Amano et al. 2013; Akiyama et al. 2015) rapid derepression and rerepression of heterochromatin regions (Akiyama et al. 2015), rapid telomere extension (Zalzman et al. 2010), and blockage of global translation (Hung et al. 2013). Additionally, Zscan4 has also been shown to enhance the efficiency of generating mouse-induced pluripotent stem (iPS) cells and their quality (Hirata et al. 2012; Jiang et al. 2013). These data suggest that Zscan4 plays diverse biological roles during Z4 events of ES cells and in two-cell stage preimplantation embryos.

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Zscan4 is expressed specifically during late meiotic prophase in both spermatogenesis and oogenesis

Kei-ichiro Ishiguro[1,4] · Manuela Monti[2] · Tomohiko Akiyama[1] · Hiromi Kimura[1] · Nana Chikazawa-Nohtomi[1] · Miki Sakota[1] · Saeko Sato[1] · Carlo Alberto Redi[2,3] · Shigeru B. H. Ko[1] · Minoru S. H. Ko[1]

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Abstract Mouse zinc finger and SCAN domain containing 4 (Zscan4) proteins, which are encoded by multiple copies of *Zscan4* genes, are expressed specifically in preimplantation embryos in vivo and embryonic stem (ES) cells in vitro. However, the expression patterns of mouse Zscan4 in vivo have been largely elusive. Here, we show that Zscan4 proteins are expressed in adult ovaries and testes. In ovaries, Zscan4 proteins were detected in germinal vesicle (GV) stage oocytes in antral follicles, indicating that *Zscan4* genes are activated during the diplotene/dictyate stage in meiotic prophase I. Remarkably, Zscan4 showed different spatial localization patterns between two distinct GV oocytes, which can be distinguished by global chromatin organization—surrounded nucleolus (SN) and non-surrounded nucleolus (NSN). These spatiotemporal differences in Zscan4 localizations correlated with the transition of RNA polymerase II-mediated transcriptional status during GV oocyte maturation. In testes, Zscan4 proteins were detected in spermatocytes at late pachytene/diplotene stages and in Sertoli cells. These results suggest that Zscan4 may play critical roles during late meiotic prophase in both males and females.

Keywords GVoocyte · SN and NSN oocyte · Ovary · Testis · ES cell · Preimplantation embryo

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✉ Minoru S. H. Ko
ko.minoru@keio.jp

[1] Department of Systems Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan

[2] Research Center for Regenerative Medicine, Fondazione IRCCS Policlinico San Matteo, viale Golgi 19, 27100 Pavia, Italy

[3] Dipartimento di Biologia e Biotecnologie L. Spallanzani, University of Pavia, via A. Ferrata 9, 27100 Pavia, Italy

[4] Present address: Institute of Molecular Embryology and Genetics, Kumamoto University, 2-2-1 Honjo, Chuo-ku, Kumamoto 860-0811, Japan

Introduction

In the mouse genome, nine copies of zinc finger and SCAN domain containing 4 (*Zscan4*) genes are encoded in the *Zscan4* locus: six members of *Zscan4* genes (*Zscan4a*, *Zscan4b*, *Zscan4c*, *Zscan4d*, *Zscan4e*, and *Zscan4f*) and three pseudogenes (*Zscan4-ps1*, *Zscan4-ps2*, and *Zscan4-ps3*) (Falco et al. 2007). Because it is difficult to distinguish between the copies of the *Zscan4* genes and since there is only a single copy of *ZSCAN4* in the human genome (Falco et al. 2007), the mouse Zscan4 genes are collectively called *Zscan4* (Falco et al. 2007; Zalzman et al. 2010; Amano et al. 2013). The mouse *Zscan4* genes were originally identified for their unique expression during zygotic genome activation (ZGA) in late two-cell stage embryos, but they are also expressed in mouse embryonic stem (ES) cells (Falco et al. 2007; Akiyama et al. 2015).

In mouse ES cells, the transcription of *Zscan4* is transient and reversible, resulting in a small population (1–5%) of Zscan4+ cells in culture at a given time point (Zalzman et al. 2010). A burst of *Zscan4* transcription is accompanied by unique biological events, including transient expression of other ZGA-specific genes (Akiyama et al. 2015), rapid derepression and rerepression of heterochromatin regions (Akiyama et al. 2015), rapid telomere extension (Zalzman et al. 2010), and blockage of global translation (Hung et al. 2013). Moreover, Zscan4 has been shown to enhance the efficiency of generating mouse induced pluripotent stem (iPS)

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EMBO reports

The cohesin REC8 prevents illegitimate inter-sister synaptonemal complex assembly

Kei-ichiro Ishiguro† & Yoshinori Watanabe

During meiosis, a specialized chromosome structure is assembled to promote pairing/synapsis of homologous chromosomes and meiotic recombination, a process yielding chiasmata between homologs to ensure accurate segregation. Meiosis-specific cohesin complexes mediating sister chromatid cohesion play pivotal roles in almost all these events, including synaptonemal complex (SC) formation. In this issue of *EMBO Reports*, Agostinho and colleagues have examined chromosome axes and SC structures by taking advantage of a hypomorphic *Stag3* mutant in which the levels of the cohesin subunit REC8 are partly reduced [6]. Using super-resolution microscopy, the authors illuminate previously unforeseen chromosome axis structures, showing locally separated axes in regions where REC8 is absent, regardless of RAD21L or RAD21 cohesin localization. Furthermore, they assessed the relationship between sister chromatid cohesion and inter-sister SC formation, demonstrating that "axial opening" in the REC8-free region is accompanied by illegitimate SC formation between sister chromatids. This study highlights the physiological importance of REC8 in sister chromatid cohesion and proper SC formation during meiosis, suggesting a new model in which a high density of REC8 deposition along the chromosome prevents illegitimate inter-sister SC formation.

See also: **A Agostinho *et al*** (June 2016)

When chromosomes replicate in S-phase, sister chromatids are held together by a mechanism termed sister chromatid cohesion that enables accurate chromosome segregation in both mitosis and meiosis. In somatic cells, sister chromatid cohesion is mediated by the cohesin multi-protein complex, which is comprised of four core subunits: SMC1α and SMC3, the kleisin family protein RAD21, and either one of the accessory subunits SA1 and SA2. In mammalian germ cells, the cohesin complex contains in addition to RAD21 the meiosis-specific kleisin subunits REC8 and RAD21L [1–3] (Fig 1A). Furthermore, SMC1α and SA1/SA2 are largely replaced by other meiosis-specific cohesin subunits, SMC1β and STAG3, respectively [4,5]. Given that STAG3, SMC1β, and SMC3 are commonly shared in these meiotic cohesin complexes, it is assumed that the kleisin subunits REC8, RAD21L, and RAD21 have specific roles in the cohesin complexes during meiosis [1,2].

In the meiotic prophase, REC8 is localized along chromosomes before meiotic DNA replication and persists throughout the first meiotic division and, at least at centromeres, until metaphase II. In contrast, RAD21L transiently appears on the chromosomes after DNA replication and culminates at the leptotene/zygotene stage. While RAD21 is detectable in testicular mitotic cells, it is not detected in nuclei from early leptotene until zygotene, in contrast to the patterns of RAD21L and REC8. Thus, the kleisin subunits endow distinct cohesin complexes with different spatiotemporal localization patterns on chromosomes during meiotic prophase. Accumulating evidence suggests that meiosis-specific cohesin complexes play essential roles not only in sister chromatid cohesion, but also in SC formation. However, it is largely elusive how distinct cohesin complexes exert their specific functions in sister chromatid cohesion and SC formation.

Using super-resolution microscopic analyses (SIM and STED), Agostinho and colleagues have now examined chromosome axis and SC structures in mouse mutants defective in various cohesin subunits (*Rec8, Stag3,* or *Smc1β*) [6]. In wild-type spermatocytes, the chromosome axis, which consists of two sister chromatids, is labeled by a single line of the axial element (AE) component SYCP3. Strikingly, however, the entire axis of univalent chromosomes can be resolved as two separate SYCP3-labeled structures in *Rec8* knockout (KO) cells, which is consistent with previous electron microscopic observations [7,8]. Moreover, similar to these observations in *Rec8* KO spermatocytes, the chromosome axis is also regionally separated in *Stag3* mutant and *Smc1β* KO cells, which has previously not been observed, due to the low resolution of ordinary optical microscopic tools (Fig 1B).

Notably, the inter-axis distance at these "axial opening" regions in *Stag3* mutant and *Smc1β* KO cells is comparable to that of synapsed homologous chromosomes in the wild type (150–175 nm). Previous reports have indicated that illegitimate SCs might be assembled between sister chromatids in *Rec8* KO cells [7,9]. Similarly, the authors detected SC components (SYCP1, SYCE1, SYCE2, and TEX12) at these local "axial opening" regions in the *Stag3* mutant and *Smc1β* KO cells. This suggests that tripartite SC assembly, which is discernible from that between synapsed homologs in the wild type, occurs in local "axial opening" regions in the absence or at reduced levels of particular cohesin subunits (Fig 1B).

Laboratory of Chromosome Dynamics, Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan. E-mail: ywatanab@iam.u-tokyo.ac.jp
†Present address: Institute of Molecular Embryology and Genetics, Kumamoto University, Chuo-ku, Kumamoto, Japan
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ARTICLE

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Meikin is a conserved regulator of meiosis-I-specific kinetochore function

Jihye Kim[1]*, Kei-ichiro Ishiguro[1]*, Aya Nambu[1], Bungo Akiyoshi[1], Shihori Yokobayashi[1], Ayano Kagami[1], Tadashi Ishiguro[1], Alberto M. Pendas[2], Naoki Takeda[3], Yogo Sakakibara[4], Tomoya S. Kitajima[4], Yuji Tanno[1], Takeshi Sakuno[1] & Yoshinori Watanabe[1]

The kinetochore is the crucial apparatus regulating chromosome segregation in mitosis and meiosis. Particularly in meiosis I, unlike in mitosis, sister kinetochores are captured by microtubules emanating from the same spindle pole (mono-orientation) and centromeric cohesion mediated by cohesin is protected in the following anaphase. Although meiotic kinetochore factors have been identified only in budding and fission yeasts, these molecules and their functions are thought to have diverged earlier. Therefore, a conserved mechanism for meiotic kinetochore regulation remains elusive. Here we have identified in mouse a meiosis-specific kinetochore factor that we termed MEIKIN, which functions in meiosis I but not in meiosis II or mitosis. MEIKIN plays a crucial role in both mono-orientation and centromeric cohesion protection, partly by stabilizing the localization of the cohesin protector shugoshin. These functions are mediated mainly by the activity of Polo-like kinase PLK1, which is enriched to kinetochores in a MEIKIN-dependent manner. Our integrative analysis indicates that the long-awaited key regulator of meiotic kinetochore function is Meikin, which is conserved from yeasts to humans.

In mitosis, the establishment of sister chromatid cohesion is dependent on cohesin in S phase and maintained until metaphase when the sister chromatids are captured by spindle microtubules from opposite poles and aligned on the spindle equator. For the onset of anaphase, the anaphase-promoting complex (APC) triggers the degradation of securin, an inhibitory chaperone for separase that cleaves the cohesin subunit RAD21 and removes cohesin complex along the entire chromosome. This removal of cohesin triggers the separation of sister chromatids and their movement to opposite poles, a process called equational division[1–3]. However, during meiosis, the meiotic cohesin REC8 mainly replaces RAD21 along the entire chromosomes; one round of DNA replication is followed by two rounds of nuclear division, which results in four haploid nuclei or gametes (Fig. 1a).

In the first division of meiosis (meiosis I), homologous chromosomes connected by chiasmata are captured from the opposite poles, whereas sisters are captured from the same pole (mono-orientation). At the onset of anaphase I, REC8 cohesin is cleaved by separase along the arm regions, but protected at centromeres until metaphase II (refs 4–6). Thus, mono-orientation and centromeric cohesion protection are two hallmarks of meiotic kinetochore function, which are widely conserved among eukaryotic organisms[7–9] (Fig. 1a). There is increasing evidence that cohesion protection is mediated by the centromeric protein shugoshin (SGO) and its partner protein phosphatase 2A (PP2A)[10–15], which antagonizes REC8 phosphorylation, a prerequisite of cleavage[16,17]. So far, meiosis-specific kinetochore proteins have been identified only in two yeasts (*Saccharomyces cerevisiae* Spo13 and Mam1 (monopolin subunit), and *Schizosaccharomyces pombe* Moa1)[18–23]. Puzzlingly, however, because their structural and functional similarities remain to be identified, conservation of meiotic kinetochore regulation is questionable even between yeasts[8,9]. Therefore, in this study, we address the long-standing question of whether meiotic kinetochore regulation is conserved from yeasts to mammals, and, if so, how.

Mammalian meiotic kinetochore protein MEIKIN

Fission yeast protein Moa1 interacts directly with the conserved kinetochore protein Cnp3 (CENP-C homologue), and localizes to the kinetochore in meiosis I (ref. 24). To identify an equivalent meiosis-specific kinetochore protein in mammals, we searched for proteins that interact with mouse CENP-C in a yeast two-hybrid assay using a cDNA library prepared from mouse testis (Extended Data Fig. 1a). The most frequently obtained clones (*4930404A10Rik* gene) encoded a novel protein, which we named MEIKIN (for meiosis-specific kinetochore protein). *Meikin* shows specific expression in germ cells (both testis and ovary) but not in other organs (Extended Data Fig. 1b). Immunoprecipitation assays using testis chromatin extracts indicate that MEIKIN indeed forms a complex with CENP-C (Extended Data Fig. 1c). Blast search analysis revealed that MEIKIN is a novel uncharacterized protein conserved among vertebrates (Extended Data Fig. 2).

To determine the localization of MEIKIN, we immunostained for MEIKIN in spermatocytes along with SYCP3, a component of the axial elements, and ACA (anti-centromeric antibodies), which stains constitutive centromeric proteins including CENP-C (Fig. 1b and Extended Data Fig. 3a). MEIKIN appears at centromeres during the pachytene stage when homologous chromosomes (homologues) are synapsed. Centromeric MEIKIN signals reach a peak during diplotene stage, persist with gradual reduction until metaphase I, and finally disappear in anaphase I. In meiosis II, MEIKIN does not reappear on chromatin. This localization contrasts with that of ACA (or CENP-C), which increase during zygotene and persist throughout meiosis I and meiosis II (Fig. 1b and Extended Data Fig. 3a). A similar localization pattern of MEIKIN was observed in oocytes (Extended Data Fig. 3b, c). To determine the mechanism of MEIKIN localization, we narrowed down the kinetochore localization sequences of MEIKIN by expressing GFP-fusion versions in testis, and identified carboxy terminus conserved sequences that have an essential role in localization to kinetochores (Fig. 1c and

[1]Laboratory of Chromosome Dynamics, Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1Yayoi, Tokyo 113-0032, Japan. [2]Instituto de Biologia Molecular y Celular del Cáncer (CSIC-USAL), 37007 Salamanca, Spain. [3]Center for Animal Resources and Development, Kumamoto University, 2-2-1 Honjo, Kumamoto 860-0811 Japan. [4]Laboratory for Chromosome Segregation, RIKEN Center for Developmental Biology, 2-2-3 Minatojima-Minamimachi, Chuo-ku, Kobe 650-0047, Japan.
*These authors contributed equally to this work.

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RESEARCH ARTICLE

MEI4 – a central player in the regulation of meiotic DNA double-strand break formation in the mouse

Rajeev Kumar[1,*], Norbert Ghyselinck[2], Kei-ichiro Ishiguro[3], Yoshinori Watanabe[4], Anna Kouznetsova[4], Christer Höög[4], Edward Strong[5], John Schimenti[5], Katrin Daniel[6], Attila Toth[6] and Bernard de Massy[1,‡]

ABSTRACT

The formation of programmed DNA double-strand breaks (DSBs) at the beginning of meiotic prophase marks the initiation of meiotic recombination. Meiotic DSB formation is catalyzed by SPO11 and their repair takes place on meiotic chromosome axes. The evolutionarily conserved MEI4 protein is required for meiotic DSB formation and is localized on chromosome axes. Here, we show that HORMAD1, one of the meiotic chromosome axis components, is required for MEI4 localization. Importantly, the quantitative correlation between the level of axis-associated MEI4 and DSB formation suggests that axis-associated MEI4 could be a limiting factor for DSB formation. We also show that MEI1, REC8 and RAD21L are important for proper MEI4 localization. These findings on MEI4 dynamics during meiotic prophase suggest that the association of MEI4 to chromosome axes is required for DSB formation, and that the loss of this association upon DSB repair could contribute to turning off meiotic DSB formation.

KEY WORDS: Meiosis, Recombination, DNA double strand break, Synapsis

INTRODUCTION

Sexual reproduction relies on faithful chromosome transmission during meiosis to generate viable gametes. At the prophase of meiosis I, the programmed formation of DNA double-strand breaks (DSBs) is a key step that initiates homologous recombination events on each chromosome. Physical connections resulting from reciprocal exchanges (crossovers) generated by DSB repair between homologous chromosomes (homologs) play a mechanical role to ensure the accurate segregation of homologs at the first meiotic division (Hunter, 2007). As DSBs pose a potential threat to genome integrity, the timing and frequency of DSB formation, as well as their localization, are highly regulated. Meiotic DSBs are formed in a narrow time window at the leptotene stage of meiotic prophase after the completion of meiotic DNA replication (S phase). Moreover, DSBs occur preferentially in specific regions of the genome called hotspots. The number of DSBs per meiosis varies from tens in worms and flies to one hundred or more in yeast, plants and mammals (de Massy, 2013) and recent studies have identified several regulatory pathways for the control of timing and level of meiotic DSBs (Keeney et al., 2014). The evolutionarily conserved SPO11 protein, which carries the catalytic activity for DSB formation, is not sufficient on its own and other proteins that are essential for meiotic DSB formation have been identified in several organisms. Some of these DSB factors are proposed to act in sub-complexes and show limited sequence conservation during evolution (de Massy, 2013).

A more precise view of the factors involved in DSB formation has emerged from studies in *S. cerevisiae*, where at least nine proteins are required for Spo11-dependent DSB formation. Among these DSB factors Rec114, Mei4 and Mer2 form a complex (RMM complex), localized on chromosome axes before DSB formation and this complex is also conserved in *S. pombe* (Miyoshi et al., 2012). Recruitment of both Rec114 and Mei4 on meiotic chromosomes is dependent on Mer2, which associates with chromatin during meiotic S phase (Henderson et al., 2006; Li et al., 2006; Panizza et al., 2011). The phosphorylation of Mer2 by replication-associated kinases has been shown to allow the coordination between DNA replication and DSB formation (Murakami and Keeney, 2014). The DNA damage checkpoint turns off Rec114 activity, by a phosphorylation mechanism that is dependent on Tel1 and Mec1 (the budding yeast ATM and ATR orthologs), to limit DSB frequency (Carballo et al., 2013). The axis localization of the RMM suggests that DSB formation takes place on the axis. The chromosome axis is a proteinaceous structure that has been proposed to serve as a platform for different meiotic recombination steps. On this structure, called the axial element, chromatin is organized as an array of loops of both sister chromatids that are anchored to the axis at sites that correspond to cohesin-binding sites (Kleckner, 2006; Zickler and Kleckner, 1999). Axis formation is as yet poorly characterized but is known to involve chromosomal architectural proteins, such as condensins and type II DNA topoisomerases (Wood et al., 2010; Zickler and Kleckner, 1999), and other meiosis-specific factors. Among these, the *S. cerevisiae* Hop1 and Red1 proteins are spatially enriched in similar domains along chromosomes (Börner et al., 2008) and their localization is partly dependent on the meiosis-specific cohesin subunit Rec8 (Klein et al., 1999; Panizza et al., 2011). The *S. cerevisiae rec8A* strain shows region-specific variations in meiotic DSB levels (Kim et al., 2010; Kugou et al., 2009) that correlate with Hop1 localization in these regions (Panizza et al., 2011). In addition, Hop1 and Red1

[1]Institute of Human Genetics, UPR 1142, CNRS. 141, Rue de la Cardonille, 34396 Montpellier, France. [2]Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), CNRS UMR7104 - INSERM U964, Department of Functional Genomics and Cancer, 1 rue Laurent Fries, BP10142, 67404 ILLKIRCH CEDEX, France. [3]Laboratory of Chromosome Dynamics, Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan. [4]Department of Cell and Molecular Biology (CMB), Berzelius Väg 35, Box 285, Karolinska Institutet, S-171 77 Stockholm, Sweden. [5]Cornell University, College of Veterinary Medicine T9014A, Ithaca, NY 14853 USA. [6]Institute of Physiological Chemistry, Medical Faculty of TU Dresden, Fiedlerstrasse 42, 01307 Dresden, Germany.
*Present address: Institut Jean-Pierre Bourgin, UMR1318 INRA AgroParisTech, INRA Centre de Versailles-Grignon, Batiment 7, Route de St-Cyr (RD10), 78026 Versailles Cedex, France.

‡Author for correspondence (bernard.de-massy@igh.cnrs.fr)

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Meiosis-specific cohesin mediates homolog recognition in mouse spermatocytes

Kei-ichiro Ishiguro,[1] Jihye Kim,[1,2] Hiroki Shibuya,[1,2] Abrahan Hernández-Hernández,[3] Aussie Suzuki,[4] Tatsuo Fukagawa,[4] Go Shioi,[5] Hiroshi Kiyonari,[5] Xin C. Li,[6] John Schimenti,[6] Christer Höög,[3] and Yoshinori Watanabe[1,2,7]

[1]Laboratory of Chromosome Dynamics, Institute of Molecular and Cellular Biosciences, [2]Graduate School of Agricultural and Life Science, University of Tokyo, Tokyo 113-0032, Japan; [3]Department of Cell and Molecular Biology, Karolinska Institute, Stockholm S171 77, Sweden; [4]Department of Molecular Genetics, National Institute of Genetics, the Graduate University for Advanced Studies, Mishima, Shizuoka 411-8540, Japan; [5]Laboratory for Animal Resources and Genetic Engineering, RIKEN Center for Developmental Biology (CDB), Kobe 650-0047, Japan; [6]Department of Biomedical Sciences, Center for Vertebrate Genomics, Cornell University College of Veterinary Medicine, Ithaca, New York 14853, USA

During meiosis, homologous chromosome (homolog) pairing is promoted by several layers of regulation that include dynamic chromosome movement and meiotic recombination. However, the way in which homologs recognize each other remains a fundamental issue in chromosome biology. Here, we show that homolog recognition or association initiates upon entry into meiotic prophase before axis assembly and double-strand break (DSB) formation. This homolog association develops into tight pairing only during or after axis formation. Intriguingly, the ability to recognize homologs is retained in *Sun1* knockout spermatocytes, in which telomere-directed chromosome movement is abolished, and this is the case even in *Spo11* knockout spermatocytes, in which DSB-dependent DNA homology search is absent. Disruption of meiosis-specific cohesin RAD21L precludes the initial association of homologs as well as the subsequent pairing in spermatocytes. These findings suggest the intriguing possibility that homolog recognition is achieved primarily by searching for homology in the chromosome architecture as defined by meiosis-specific cohesin rather than in the DNA sequence itself.

[*Keywords*: homolog pairing; cohesin; DSB; bouquet]

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Meiosis generates haploid gametes from a diploid parental cell. This process is initiated by the pairing of homologous chromosomes (homologs) and subsequent double-strand break (DSB)-mediated recombination (Neale and Keeney 2006; Baudat and de Massy 2007). A number of mechanisms are involved in chromosome pairing and alignment. The first step is the attachment of telomeres (or pairing centers in *Caenorhabditis elegans*) to the nuclear envelope (NE) (Scherthan 2001; Hiraoka and Dernburg 2009). The following telomere-led nuclear movement and polarized chromosome arrangement bouquet facilitate chromosome alignment and homolog pairing/synapsis (Zickler and Kleckner 1999; Scherthan 2001; Page and Hawley 2004). The physical recognition of homologs might be driven by DSB-dependent recombination machinery that involves a homology search on the basis of DNA sequence. However, since repetitive elements comprise 30%–50% of mammalian genomes, wide-range homology search, rather than regional DNA sequence identity, might be important to avoid nonallelic pairing and recombination in meiosis (Zickler and Kleckner 1999; Page and Hawley 2004; Sasaki et al. 2010). In fact, in some organisms undergoing recombinationless meiosis, homologs are properly paired and even synapsed in a DSB-independent manner (Dernburg et al. 1998; McKim et al. 1998). Also, in fungi (yeasts and *Sordaria*), while a tight association is established by

[7]**Corresponding author**
E-mail ywatanab@iam.u-tokyo.ac.jp
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nature cell biology

The TRF1-binding protein TERB1 promotes chromosome movement and telomere rigidity in meiosis

Hiroki Shibuya[1,2], Kei-ichiro Ishiguro[1] and Yoshinori Watanabe[1,2,3]

During meiotic prophase, telomere-mediated chromosomal movement along the nuclear envelope is crucial for homologue pairing and synapsis. However, how telomeres are modified to mediate chromosome movement is largely elusive. Here we show that mammalian meiotic telomeres are fundamentally modified by a meiosis-specific Myb-domain protein, TERB1, that localizes at telomeres in mouse germ cells. TERB1 forms a heterocomplex with the canonical telomeric protein TRF1 and binds telomere repeat DNA. Disruption of *Terb1* in mice abolishes meiotic chromosomal movement and impairs homologous pairing and synapsis, causing infertility in both sexes. TERB1 promotes telomere association with the nuclear envelope and deposition of the SUN–KASH complex, which recruits cytoplasmic motor complexes. TERB1 also binds and recruits cohesin to telomeres to develop structural rigidity, strikingly reminiscent of centromeres. Our study suggests that TERB1 acts as a central hub for the assembly of a conserved meiotic telomere complex required for chromosome movements.

Meiosis is a specialized cell division for gametogenesis. This process is initiated by the pairing of homologous chromosomes (homologues), subsequent homologous recombination and synapsis in prophase I. In most organisms, homologue pairing/synapsis is promoted by meiosis-specific chromosome movement. During chromosome segregation in mitosis, or even in meiosis, the key site for chromosome movement localizes to the centromere, where a macromolecular structure called the kinetochore is assembled. In meiotic prophase, when chromosomes search for a homologous partner, telomeres rather than centromeres are used to assemble an apparatus that binds to the nuclear envelope and its associated force-generating machinery[1–3]. Notably, *Caenorhabditis elegans*, which has holocentric chromosomes, also uses specialized chromosomal sites called pairing centres, but not kinetochores, for meiotic prophase chromosome movement[4,5]. Despite the presumed conservation of meiosis-specific chromosome movement and a polarized chromosomal arrangement (bouquet) mediated by non-centromeric chromosome sites among eukaryotes[1,2], the molecular identity of the apparatus is largely elusive compared with that of the canonical kinetochore.

Studies in yeasts and nematodes have suggested that the nuclear membrane protein complex, SUN–KASH, contributes to the meiosis-specific chromosomal movement along the nuclear envelope[2]. The KASH-domain protein localizes to the outer nuclear membrane and is connected to cytoplasmic motors, whereas its partner, the SUN-domain protein, is located at the inner nuclear membrane. The SUN protein associates with the KASH protein in the nuclear lumen through its carboxy-terminal SUN domain and with the chromosomal part through amino-terminal nuclear extrusion[2]. This SUN–KASH linkage transmits cytoplasmic forces to the nuclear chromatin[5–12]. In yeasts and nematodes, meiosis-specific chromosomal proteins link chromosomes and SUN–KASH proteins, although these proteins are evolutionarily diverged even between fission yeast and budding yeast[4,13–15]. In contrast, there is little information about the molecules required for telomere movement in higher eukaryotes. In mammals, the SUN1–KASH5 complex has recently been identified[10,16,17], although its requirement for chromosome movement is not directly shown. Further, it is totally unknown how telomeres are modified to mediate chromosome movement during meiosis.

Meiotic telomere protein TERB1

With the aim of identifying meiosis-specific telomere proteins, we focused on the ovarian genes, identified by a micro-array-based study in mice[18]. We performed PCR with reverse transcription using complementary DNAs prepared from several organs to screen for

[1]Laboratory of Chromosome Dynamics, Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1 Yayoi, Tokyo 113-0032, Japan. [2]Graduate School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Yayoi, Tokyo 113-0032, Japan.
[3]Correspondence should be addressed to Y.W. (e-mail: ywatanab@iam.u-tokyo.ac.jp)

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RESEARCH ARTICLE

p600 regulates spindle orientation in apical neural progenitors and contributes to neurogenesis in the developing neocortex

Camille Belzil[1], Naoyuki Asada[2], Kei-ichiro Ishiguro[3,5,*], Takeo Nakaya[3,*], Kari Parsons[1], Valentina Pendolino[4], Gernot Neumayer[1], Marina Mapelli[4], Yoshihiro Nakatani[3], Kamon Sanada[2,‡] and Minh Dang Nguyen[1,‡]

ABSTRACT

Apical neural progenitors (aNPs) drive neurogenesis by means of a program consisting of self-proliferative and neurogenic divisions. The balance between these two manners of division sustains the pool of apical progenitors into late neurogenesis, thereby ensuring their availability to populate the brain with terminal cell types. Using knockout and *in utero* electroporation mouse models, we report a key role for the microtubule-associated protein 600 (p600) in the regulation of spindle orientation in aNPs, a cellular event that has been associated with cell fate and neurogenesis. We find that p600 interacts directly with the neurogenic protein Ndel1 and that aNPs knockout for p600, depleted of p600 by shRNA or expressing a Ndel1-binding p600 fragment all display randomized spindle orientation. Depletion of p600 by shRNA or expression of the Ndel1-binding p600 fragment also results in a decreased number of Pax6-positive aNPs and an increased number of Tbr2-positive basal progenitors destined to become neurons. These Pax6-positive aNPs display a tilted mitotic spindle. In mice wherein *p600* is ablated in progenitors, the production of neurons is significantly impaired and this defect is associated with microcephaly. We propose a working model in which p600 controls spindle orientation in aNPs and discuss its implication for neurogenesis.

KEY WORDS: p600, UBR4, Ndel1, Neurogenesis, Apical neural progenitors, Spindle orientation

INTRODUCTION

In the developing neocortex, neurogenesis requires the survival, renewal and differentiation of apical neural progenitors (aNPs). Composed of neuroepithelial stem cells (NESCs) and their derivative, the radial glia cells (RGCs), aNPs give rise directly to neurons populating the layers of the cortex or indirectly through the generation of basal progenitors (BPs) in the subventricular zone (Götz and Huttner, 2005; Kriegstein and Alvarez-Buylla, 2009; Rakic et al., 2009; Sessa et al., 2010; Postiglione et al., 2011). During early phases of mammalian corticogenesis, aNPs divide symmetrically to expand the progenitor pool. As corticogenesis proceeds, they then divide asymmetrically to generate either one neuron and one aNP, or one neuron and one BP that will produce two neurons (Götz and Huttner, 2005).

The orientation of the mitotic spindle, perpendicular to the cleavage furrow, is highly linked to the manner of cell division in aNPs (Fietz and Huttner, 2011; Götz and Huttner, 2005; Huttner and Kosodo, 2005; Buchman and Tsai, 2007; Kriegstein and Alvarez-Buylla, 2009; Lancaster and Knoblich, 2012). During the early expansion phase, the spindle is precisely oriented horizontally relative to the apical surface, resulting in a vertical cleavage plane. During the neurogenic phase, the fraction of aNPs with obliquely/vertically-oriented spindle increases (Götz and Huttner, 2005; Kriegstein and Alvarez-Buylla, 2009; Rakic et al., 2009; Sessa et al., 2010; Postiglione et al., 2011). Such plan of division is often associated with an unequal segregation of fate determinant signaling molecules (Par3/Par6/aPKC, numb/numb-like, Neuregulin/APC, Pals1) (Hur and Zhou, 2010; Kim et al., 2010; Petersen et al., 2002; Kim and Walsh, 2007; Bultje et al., 2009; Yokota et al., 2009), the apical/basal membrane domain and/or organelles (primary cilium, centrosome) (Wang et al., 2009), thereby implicating oblique/vertical spindle orientation in asymmetric outcome of daughter cell fates. Though the correlation between spindle orientation and cell fate is demonstrably imperfect and thus not exclusively causal, the close link between spindle orientation, mitotic delay, and severe neurogenic failure warrants study.

The formation and orientation of the mitotic spindle depends on the polymerization, stability and capture of microtubules (MTs) at the plus-end (Wynshaw-Boris et al., 2010). In the neocortex around embryonic day (E) 12, Ndel1 and its homolog Nde1 promote symmetric proliferative division of aNPs. Via association to Lis1 and Dynein, they regulate the formation of aster MTs, their capture at the cell cortex and stabilize the horizontally-aligned spindle (Alkuraya et al., 2011; Pramparo et al., 2011; Feng and Walsh, 2004; Yingling et al., 2008; Moon et al., 2014). Depletion of Ndel1 or Lis1 causes randomization of the spindle orientation, an event that could trigger apoptosis or precocious neuronal differentiation of aNPs, thereby resulting in depletion of progenitor pools and an overall marked decrease in neuronal production (Yingling et al., 2008). Thus, spindle

[1]Hotchkiss Brain Institute, University of Calgary, Departments of Clinical Neurosciences, Cell Biology and Anatomy, and Biochemistry and Molecular Biology, 3330 Hospital Drive NW, Heritage Medical Research Building, Calgary, AB T2N 4N1, Canada. [2]Molecular Genetics Research Laboratory, Graduate School of Science, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo, 113-0033, Japan. [3]Dana Farber Cancer Institute, 44 Binney Street, Smith Building 836, Boston, MA 02115, USA. [4]European Institute of Oncology, Department of Experimental Oncology, Via Adamello, 16-20139 Milan, Italy. [5]Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1 Yayoi, Tokyo 113-0032, Japan.
*These authors contributed equally to this work

‡Authors for correspondence (mdnguyen@ucalgary.ca; kamon_sanada@gen.s.u-tokyo.ac.jp)

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p600 Plays Essential Roles in Fetal Development

Takeo Nakaya[1,2¶], Kei-ichiro Ishiguro[1,3¶], Camille Belzil[4], Anna M. Rietsch[1¤], Qunyan Yu[1], Shin-ichi Mizuno[5,6], Roderick T. Bronson[7], Yan Geng[1], Minh Dang Nguyen[4], Koichi Akashi[5,6], Piotr Sicinski[1], Yoshihiro Nakatani[1]*

1 Department of Cancer Biology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts, United States of America, **2** Translational Research Unit and Department of Molecular Pathology, Tokyo Medical University, Shinjuku, Tokyo, Japan, **3** Institute of Molecular and Cellular Biosciences, University of Tokyo, Bunkyo, Tokyo, Japan, **4** Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta, Canada, **5** Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute and Harvard Medical School, Boston, Massachusetts, United States of America, **6** Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Science, Fukuoka, Japan, **7** Dana-Farber/Harvard Cancer Center, Harvard Medical School, Boston, Massachusetts, United States of America

Abstract

p600 is a multifunctional protein implicated in cytoskeletal organization, integrin-mediated survival signaling, calcium-calmodulin signaling and the N-end rule pathway of ubiquitin-proteasome-mediated proteolysis. While *push*, the *Drosophila* counterpart of *p600*, is dispensable for development up to adult stage, the role of *p600* has not been studied during mouse development. Here we generated *p600* knockout mice to investigate the in vivo functions of p600. Interestingly, we found that homozygous deletion of *p600* results in lethality between embryonic days 11.5 and 13.5 with severe defects in both embryo and placenta. Since *p600* is required for placental development, we performed conditional disruption of *p600*, which deletes selectively *p600* in the embryo but not in the placenta. The conditional mutant embryos survive longer than knockout embryos but ultimately die before embryonic day 14.5. The mutant embryos display severe cardiac problems characterized by ventricular septal defects and thin ventricular walls. These anomalies are associated with reduced activation of FAK and decreased expression of MEF2, which is regulated by FAK and plays a crucial role in cardiac development. Moreover, we observed pleiotropic defects in the liver and brain. In sum, our study sheds light on the essential roles of p600 in fetal development.

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* E-mail: yoshihiro_nakatani@dfci.harvard.edu

¤ Current address: Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio, United States of America

¶ These authors contributed equally to this work.

Introduction

p600, also known as UBR4, is a 600 kDa cellular protein that is ubiquitously expressed and plays various roles depending on cell type [1,2]. One obvious structural motif of p600 is the UBR box of the N-end rule pathway ubiquitin ligases. The UBR box is responsible for recognition of the N-terminus of their substrate proteins that are produced by protein processing and/or modifications in response to environmental conditions [3–6]. Additionally, p600 has a non-canonical calmodulin-binding domain and binds to calmodulin in a calcium-dependent manner [1]. Moreover, p600 associates with the cytoskeleton to impact cell morphology and intracellular transport [1,7].

Regarding its biological functions, p600 is involved in activation of integrin-mediated survival signaling pathways in adherent cells. Suppression of p600 expression by short hairpin RNA (shRNA) abrogates formation of integrin-mediated ruffled membranes and cellular polarity. These phenotypes in the knockdown cells are associated with reduced activation of focal adhesion kinase (FAK), which plays a role in integrin-mediated survival signaling pathways [1]. p600 functions not only in anchorage-dependent growth but also in anchorage-independent growth. Suppression of p600 expression prevents anchorage-dependent growth in various cancer cells including osteocarcinoma, cervical cancers, and gastric cancers [8–10]. Moreover, knockdown of p600 suppresses growth of gastric cancer cells in SCID mice [10]. Although the exact molecular mechanisms whereby p600 contributes to cancer growth still remain unclear, p600 has been shown to be a direct target for viral oncoproteins, namely, human and bovine papillomavirus E7 [8,9,11]. Importantly, experiments with papillomavirus E7 mutants demonstrated a relation between p600-binding activity and transforming activity [8,9]. Moreover, suppression of either E7 or p600 leads to loss of ability in anchorage-independent growth [8,9,12]. Thus, formation of the E7-p600 complex in transformed cells could be crucial for anchorage-independent growth presumably by inhibiting apoptosis. Likewise, association of p600 with cellular factors may be disorganized in nonvirus-mediated cancers, although such factors have not been identified yet.

Chapter 3

Studying Meiosis-Specific Cohesins in Mouse Embryonic Oocytes

Ji-hye Kim, Kei-ichiro Ishiguro, Nobuaki Kudo, and Yoshinori Watanabe

Abstract

Distinct meiotic cohesin complexes play fundamental roles in various meiosis-specific chromosomal events in spatiotemporally different manners during mammalian meiotic prophase. Immunostaining is one of the essential methods to study meiotic cohesin dynamics. For the study of cohesins in the meiotic prophase of oocytes, ovaries should be taken from the embryos during a very limited period before birth. Here we focus on some technical tips concerning the preparation of oocyte chromosome spreads for immunostaining. Further, we describe a method for chromosome fluorescence in situ hybridization (FISH) against immunostained oocytes.

Key words: Fetal oocyte, Embryonic ovary, Meiosis, Cohesin, Prophase, Axial element, Homolog synapsis, Synaptonemal complex, Chromosome spread, FISH

1. Introduction

Cohesin is essential for faithful chromosome segregation to establish cohesion between sister chromatids (1). The meiotic cohesin complex differs from that of mitosis since the mitotic RAD21/SCC1 subunit of the cohesin complex is largely replaced by meiotic counterparts, REC8 and RAD21L (2–5) in mammals. Also other meiosis-specific cohesin subunits, SA3 and SMC1β, are known to be expressed (6, 7). During meiotic prophase I, sister chromatids are organized into proteinaceous structures of axial elements (AEs) on which the synaptonemal complex (SC) is assembled to promote interhomolog recombination, a process yielding chiasmata between homologues (8, 9). The meiotic cohesin complexes, which interact with the SC components and localize along AEs, might act as a basis for SC assembly (10–14). Thus the cohesin complex is crucial

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A conserved KASH domain protein associates with telomeres, SUN1, and dynactin during mammalian meiosis

Akihiro Morimoto,[1] Hiroki Shibuya,[1,2] Xiaoqiang Zhu,[3,4] Jihye Kim,[1,2] Kei-ichiro Ishiguro,[1] Min Han,[5,6] and Yoshinori Watanabe[1,2]

[1]Laboratory of Chromosome Dynamics, Institute of Molecular and Cellular Biosciences, and [2]Graduate School of Agricultural and Life Science, University of Tokyo, Tokyo 113-0032, Japan
[3]Institute of Developmental Biology and Molecular Medicine and [4]School of Life Sciences, Fudan University, Shanghai 200433, China
[5]Howard Hughes Medical Institute and [6]Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309

In yeasts and worms, KASH (Klarsicht/ANC-1/Syne/homology) domain and SUN (Sad-1/UNC-84) domain nuclear envelope (NE) proteins play a crucial role in meiotic chromosome movement and homologue pairing. However, although the vertebrate SUN domain protein SUN1 is involved in these processes, its partner has remained identified. Based on subcellular localization screening in mouse spermatocytes, we identified a novel germ cell–specific protein, KASH5, that localized exclusively at telomeres from the leptotene to diplotene stages in both spermatocytes and oocytes. KASH5 possesses hitherto unknown KASH-related sequences that directly interacted with SUN1 and mediated telomere localization. Thus, KASH5 is a mammalian meiosis-specific KASH domain protein. We show that meiotic chromosome movement depended on microtubules and that KASH5 interacted with the microtubule-associated dynein–dynactin complex. These results suggest that KASH5 connects the telomere-associated SUN1 protein to the cytoplasmic force–generating mechanism involved in meiotic chromosome movement. Our study strongly suggests that the meiotic homologue-pairing mechanism mediated by the SUN–KASH NE bridge is highly conserved among eukaryotes.

Introduction

Many cellular and developmental events, such as cell migration, cell division, and fertilization, occur depending on proper nuclear localization and movement. These processes are controlled by cytoplasmic microtubule and actin-based networks. The SUN (Sad-1/UNC-84) domain family of inner nuclear membrane (INM) proteins interacts with KASH (Klarsicht/ANC-1/Syne/homology) domain proteins, which are localized to the outer nuclear membrane (ONM). Thus, the SUN–KASH protein complexes bridge across the INM and ONM. Because cytoplasmic extensions of the KASH domain proteins tether the nucleus to the cytoskeleton, the SUN–KASH protein complexes play a crucial role in transferring the driving force generated by the cytoskeleton to the nuclear envelope (NE; Fridkin et al., 2009; Razafsky and Hodzic, 2009; Starr and Fridolfsson, 2010).

The pairing of homologous chromosomes during meiosis is a vital event for proper meiotic recombination and chromosome segregation, and this process largely depends on the dynamic chromosome movements specifically observed during meiotic prophase (Scherthan, 2001; Bhalla and Dernburg, 2008). In yeasts and worms, SUN domain proteins are tethered to telomeres and specific chromosomal loci (pairing centers), respectively, and SUN–KASH protein complexes connect the chromosomes to cytoskeleton, promoting chromosome movements and homologue pairing during meiosis (Hiraoka and Dernburg, 2009).

In mammalian spermatocytes, nuclear movements (nuclear rotation and chromosome movement) are observed from late leptotene toward zygotene, slowing down in early pachytene (Scherthan et al., 1996). In mice, SUN domain protein SUN1

A. Morimoto and H. Shibuya contributed equally to this paper.

Correspondence to Yoshinori Watanabe: ywatanab@iam.u-tokyo.ac.jp

Abbreviations used in this paper: INM, inner nuclear membrane; KO, knockout; LR, luminal region; NE, nuclear envelope; ONM, outer nuclear membrane.

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