BIOLOGY OF REPRODUCTION (2012) **86**(2):42, 1–4
Published online before print 16 November 2011.
DOI 10.1095/biolreprod.111.097436

Commentary

Meiotic Genetics Moves Forward with SPATA22 (*repro42*)

Gregory M. Buchold[1]

Gamete Biology Group, Laboratory of Reproduction and Developmental Toxicology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina

A number of genes with conserved roles in reproduction were first discovered in yeast, flies, and worms using a forward genetics approach, such as a mutagenesis screen. Aside from occasional rare mutations spontaneously arising in mice, rats, sheep, and dogs, identification of reproduction-related genes in mammals has depended historically upon a reverse genetic approach, targeting candidate genes for disruption in the mouse, based on similarity to simpler model organisms or by identifying interesting expression or functional characteristics. Because the majority of male infertility is expected to arise spontaneously, mouse models that disrupt genes with male reproductive roles by point mutations may provide a more bona fide model of human male infertility than large gene deletions; the *AZF* deletions on the Y chromosome represent an exception to this general rule. The most significant forward genetics screen to identify mammal-specific fertility genes is the Reproductive Genomics ethylnitrosourea mutagenesis screen initiated at The Jackson Laboratory. To date this effort has identified roughly 30 mouse lines with various gonadal and extragonadal defects in both the male and female reproductive systems, with male germ cell mutant phenotypes ranging from sperm motility defects to Sertoli cell-only testes [1, 2]. The causative mutations for six of the *repro* mutants were previously identified, including *repro4*/*Mtap2*, *repro5*/*Brwd1*, *repro8*/*Eif4g3*, *repro9*/*Mybl1*, *repro32*/*Capza3*, and *repro34*/*Stx2* [3–8]. In this issue of *Biology of Reproduction*, the link has been made between *repro42 and Spata22* by La Salle and colleagues [9]. The phenotype of several other *repro* mutants has been characterized, but the causative mutations are still unknown [10–12]. Collectively these mutations have identified multiple autosomal fertility genes in a variety of pathways (Table 1 and Supplemental Table S1 [available online at www.biolreprod.org]).

Vertebrate genomes typically display increasing spatiotemporal compartmentalization of function compared to their fungal and invertebrate counterparts. For example, SPO11 has a broadly conserved role in meiotic recombination initiation, but among its yeast cofactors only MEI4 has a mammalian ortholog [13] (n.b., forkhead transcription factor *mei4* in *Schizosaccharomyces pombe* [14] and the *mei4* allele of CCNB1IP1 [15] are unrelated to mouse *Mei4*). Similarly, a subset of proteins in the Fanconi anemia complex, including FANCD2 and FANCI and their monoubiquinase UBE2T-FANCL, is broadly conserved with invertebrate orthologs and functions in response to DNA cross-linking agents and during meiosis. Yet mutations in multiple vertebrate-specific members of the Fanconi complex also result in defects in primordial, mitotic, and meiotic germ cells [16–22]. Moreover, reduction of BRCA2 (Fanconi protein J) in the germline causes meiotic arrest in the mouse, contrasting to the elevation of breast cancer risk associated with missense mutations in the same gene in humans [23]. This illustrates that human diseases may be specific to the type of mutation induced and to whether the mutant allele is dominant or recessive—a caveat to predicting the nature of mutations to expect in human male infertility based solely on mouse models of deleted alleles. Therefore, forward genetics combined with targeted knock-in of mutated alleles may be critical to the identification of other meiotic proteins essential in vertebrates.

The phenotype of the *repro42* mutant allele indicates a potential role for SPATA22 in meiotic DNA repair or recombination. Recent findings indicate a role for ataxia telangiectasia mutated (ATM) kinase in fine-tuning the number of meiotic crossovers by inhibiting the number of SPO11-induced double-strand breaks [24]. Another component of the Fanconi anemia complex, BTBD12/SLX4 (BTB/POZ domain containing 12/structure-specific endonuclease subunit homolog 4), is stabilized by ATM phosphorylation and has been recently shown to be required for male and female fertility, blocking meiotic progression at the diplotene stage during spermatogenesis with some cells displaying relatively normal synapsis [25]. So it is intriguing that SPATA22 has a putative site for phosphorylation by ATM/ATR kinases, yet its phenotype is arrested earlier in meiotic prophase with synaptic defects. These findings imply that these two proteins have distinct actions during meiotic prophase. Many of the Fanconi complex components, including BTBD12/SLX4, share a common null phenotype—ocular deficits, anemia, and germ cell defects—in contrast to targeted inactivation of mismatch repair (MMR) processes, homologous recombination (HR) pathways, or SPATA22. Disruption of the HR pathway (e.g., *Mre11* and *Nbn*) causes sex-specific reproductive defects while also altering other somatic tissues [26–28]. With the exception of *Pms2*, which has no effect on female fertility, deletions of most MMR proteins (e.g., *Msh4*, *Msh5*, *Mlh1*, and *Mlh3*) affect gametes in both sexes, with arrest points in spermatogenesis ranging from the zygonema to the meiotic division stage [29–34]. Synaptonemal complex (SC) protein deficits (*Sycp1-3*, *Syce1-3*, *Tex11*, *Tex12*, and *Fkbp6*) cause infertility in both sexes but in a sexually dimorphic fashion—arrest of meiotic

[1]Correspondence: Gregory M. Buchold, Gamete Biology Group, Laboratory of Reproduction and Developmental Toxicology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709.
E-mail: greg.buchold@nih.gov

eISSN: 1529-7268 http://www.biolreprod.org
ISSN: 0006-3363

TABLE 1. A partial list of reproduction-defective (*repro*) mouse lines affecting germ cells with identified mutations and divided into early and late categories consistent with IVF classification of male infertility.[a]

Line[b]	Map position	Gene	Mutation	Spermatogenesis Phenotype	Reference
Early					
repro42	chr11:73143243–73159546	*Spata22*	T>A; Y275X (nonsense)	Meiotic arrest, zygonema[c]	[9]
repro9	chr1:9658916–9690290	*Mybl1*	C>A; A213E (missense)	Meiotic arrest, pachynema	[6]
repro8	chr4:137549385–137762386	*Eif4g3*	G>C; A1572P (missense)	Meiotic arrest, diplonema	[5]
repro4	chr1:66368572–66489157	*Mtap2*	T>A (intronic)	Meiotic arrest, diplonema-MI division	[8]
Late					
repro34	chr5:129490436–129514439	*Stx2*	C>T; R41X (nonsense)	Spermiogenesis arrest, azoospermic	[7]
repro5	chr16:96213699–96304035	*Brwd1*	C>T (splicing defect)	Spermiogenesis defect, oligoasthenoteratospermic[c]	[3]
repro32	chr6:139990046–139991307	*Capza3*	T>A; M44K (missense)	Spermiogenesis defect, oligoasthenoteratospermic	[4]

[a] See Supplemental Table S1 (available online at www.biolreprod.org) for more mouse lines. A complete list and detailed description of mutant phenotypes and current map intervals can be found on the Reproductive Genomics website (http://reproductivegenomics.jax.org).
[b] Within the early and late categories, mutants are listed with increasing attainment of maturation during spermatogenesis.
[c] Mutants that also show female infertility.

prophase in spermatocytes but defects in the first meiotic division in oocytes [35–43]. Although SPATA22 could affect recombination or synapsis indirectly, such as by altering meiotic chromatin, putative phosphorylation sites in SPATA22 recognized by the BRCT (BRCA1 C-terminal) interaction domain offer the intriguing possibility that the protein could act in concert with BRCA1 (Fig. 1). TEX15 has been proposed to interact with BRCA1, and its loss prevents synapsis, but, in

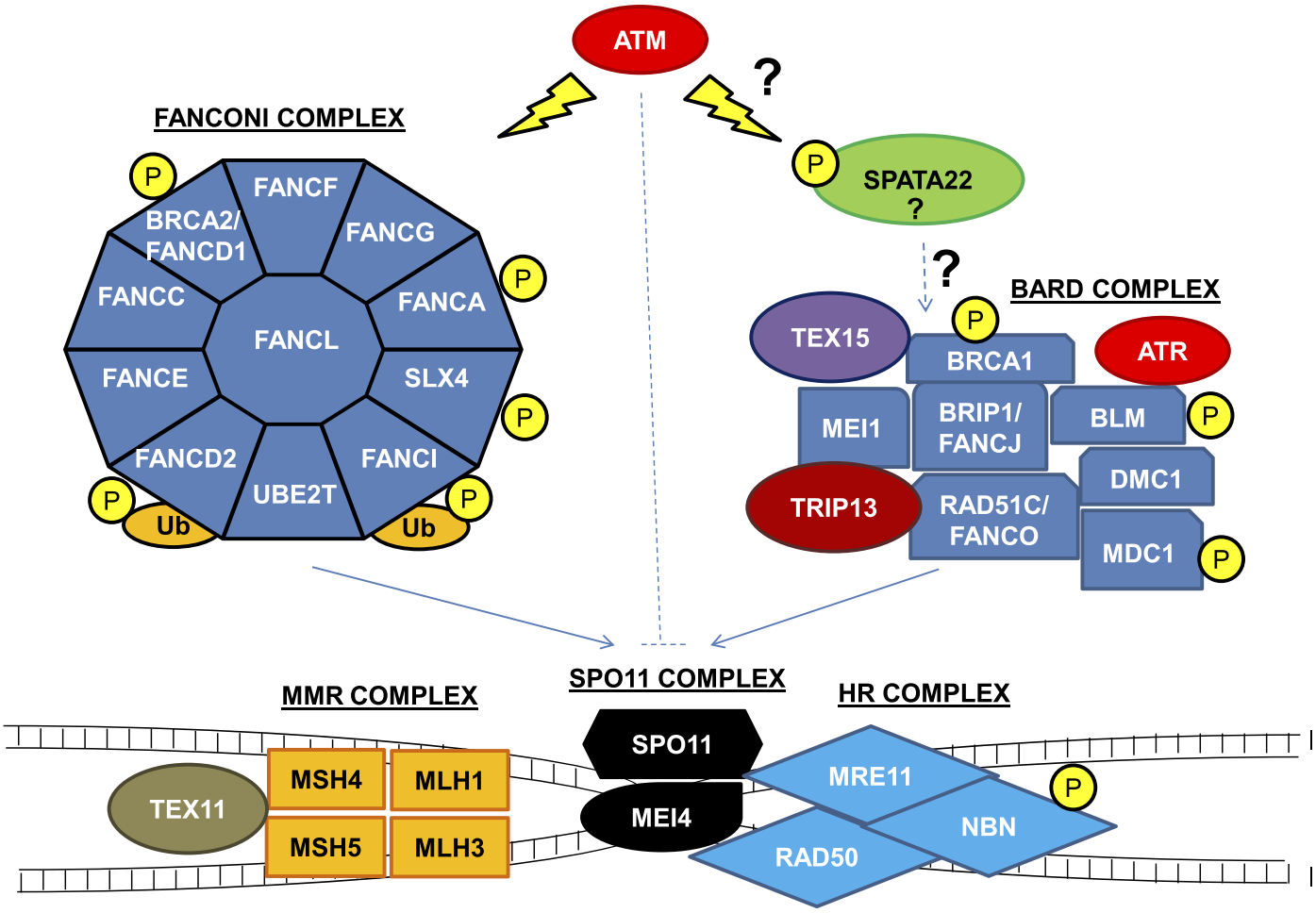

FIG. 1. A speculative model for pathways converging on mammalian meiotic recombination. ATM, through multiple phosphorylation events, targets the Fanconi complex to SPO11 positive double-strand breaks through the action of UBE2T (E2) and FANCL (E3) monoubiquitination of FANCD2/FANCI. Multiple components of complexes involved in HR, MMR, and BRCA1 (BARD) that affect processing of SPO11-induced double strand breaks are also ATM targets. The precise role for SPATA22 in meiotic recombination is unclear but appears distinct from BTBD12/SLX4, despite potential mutual targeting by ATM phosphorylation. In addition to stimulating DNA break processing, ATM also has a distinct inhibitory action in limiting the number of new breaks by an unknown mechanism inhibiting the SPO11 complex. The substructure of the complexes shown is diagrammatic and not intended to represent the known direct protein-protein interactions. This biochemical model is temporo-spatially compressed to emphasize interactions between complexes and common posttranslational modifications, simplifying the subunit complexity likely present at distinct sites on DNA.

contrast to SPATA22, it is required only for male fertility, and the *Tex15* mutant arrest is at pachynema [44]. BRCT domain-containing *Mei1* in *Arabidopsis* is required for male pollen development [45], and its mouse ortholog *Topbp1* localizes to meiotic chromatin, but its somatic requirement for DNA repair confers embryonic lethality upon its mutant [46]. However, mouse MEI1 (which is unrelated to the *Arabidopsis* gene) lacks these domains that could allow direct binding to SPATA22, and *Mei1* mutant mice have a phenotype similar to that described above for SC mutants [47]. Collectively there is an impression that assembly of the SC and processing of meiotic recombination intermediates is an admixture of sex-specific processes and checkpoints superimposed upon common components. This may account for some reported sex-specific differences in the distribution of crossover events. Two criteria—the lack of a sex bias and the arrest point of the *repro42* phenotype—are insufficient alone for a strong correlation between this mutant and specific classes of other meiotic mutants affecting synapsis and recombination.

Identification of novel meiotic mutants in the mouse provides tools to determine the stepwise processes within meiotic recombination. Spermatocyte-specific TEX11 appears to promote crossover processing of Holliday junctions in males [48], whereas the more broadly expressed TRIP13 acts to favor a noncrossover outcome [49]. Significant recent progress has been made in identifying the collaborations between repair and SC proteins in mammals necessary to create a milieu that biases strand invasion or resolution favoring greater interhomolog versus intersister exchange during meiosis than in somatic cells [50]. Future efforts aimed at determining the mechanism of SPATA22 action are likely to advance our understanding of the current model of mammalian meiosis, as will identification of mutations in other *repro* mutant lines with meiotic arrests.

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