Minireview

Taking care of Dad’s DNA
Rika Maruyama and Andrew Singson

Address: Waksman Institute and Department of Genetics, Rutgers University, Piscataway, NJ 08854, USA.

Correspondence: Andrew Singson. Email: singson@waksman.rutgers.edu

Published: 1 December 2006

Genome Biology 2006, 7:124 (doi:10.1186/gb-2006-7-12-244)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2006/7/12/244

© 2006 BioMed Central Ltd

Abstract

Inheritance of paternal genetic information requires proper sperm development and DNA packaging. A proteomic analysis of sperm chromatin in Caenorhabditis elegans has identified conserved proteins that are important for the transmission of sperm DNA and for male fertility.

Sexually reproducing animal species need to make two complementary types of gametes - sperm and eggs. The role of sperm is to deliver paternal genetic information to the egg. This process is dependent on the execution of meiosis and the packaging of haploid DNA inside the small sperm head. Maturing sperm undergo chromatin remodeling, which typically includes a transition from a histone-dependent organization to an organization dependent on sperm nuclear basic protein (SNBP) [1]. For example, protamines are thought to be required for the compact morphology of mammalian sperm nuclei [1]. Using Caenorhabditis elegans as a model system, a recent study by Chu et al. [2] used proteomics to identify conserved proteins essential for male meiosis and for the chromatin structure of sperm (Figure 1).

Many genes in C. elegans that are essential for proper meiosis and germline development have been identified by genomic approaches. RNA interference (RNAi) induces the reduction of gene products and easily allows for the observation of loss-of-function phenotypes [3]. Several independent genome-wide RNAi analyses have identified a large number of genes associated with sterile phenotypes [4-8]. DNA microarray studies identified 1,343 sperm-enriched or sperm-specific genes, 1,652 oocyte-enriched or oocyte-specific genes and 3,144 germline-intrinsic genes [9,10]. Furthermore, to identify genes involved in chromosome morphogenesis and nuclear organization during meiosis, 192 germline-enriched genes whose expression patterns were similar to those of known meiosis genes were selected for an RNAi screen focusing on the germline phenotypes [11]. From this study 51 genes were identified for which RNAi-induced loss of function caused strong germline defects. Beyond microarray analysis [9,10], however, there were no gene profiles for function specifically in male fertility and sperm development. A proteomic approach to identifying the genes important for germline development was also lacking.

Chu et al. [2] chose to use proteomics to identify male-specific chromatin-associated proteins in C. elegans (Figure 2). Spermatogenic chromatin was purified from male germ nuclei and oogenic chromatin was purified from female germ nuclei. Proteins that co-purified with chromatin were examined by multidimensional protein identification technology (MudPIT), which is mass spectrometry combined with two-dimensional chromatography of peptides [12], similar to an approach used in previous studies [13,14]. As a result, 1,099 spermatogenic proteins and 812 oogenic proteins were identified. Of these, 502 spermatogenic proteins were then selected on the basis of their high abundance. For further analysis, 132 abundant spermatogenic proteins were chosen after subtracting oogenic proteins (Figure 2).

To help confirm the identification of sperm chromatin factors, immunostaining was used to evaluate the localization of 11 molecules. Of these, 8 proteins were localized specifically on male meiotic chromosomes and mature sperm chromatin; 3 proteins were also detected on the sperm chromosomes, although they were known also to function in somatic cells and/or the hermaphrodite germline. It was inferred that many more of the 132 candidate proteins would also localize to sperm chromatin.
For further validation of the study, the function of the 132 proteins was evaluated with RNAi in hermaphrodites and males (Figure 2); 50 of the 132 genes caused sterile or embryonic lethal phenotypes. These 50 genes were also examined for germline defects resulting from RNAi, and 20 had cytologically detectable germline alterations. RNAi of 18 of these 20 genes resulted in altered meiotic chromosome segregation and germline morphology in the male gonad. Therefore, at least 18 genes are required during spermatogenesis. Given that many sperm genes are known to be resistant to RNAi, it is possible that additional genes identified by this proteomic approach will prove to have important roles in spermatogenesis: future gene knockouts are likely to identify these functions.

Chu et al. [2] divided a selected set of the proteins they identified into three categories. Category I proteins (9 proteins) are localized specifically to male germ cells. Category II proteins (3 proteins) are known to function in other cell types but their roles in spermatogenesis were newly discovered by this study. Finally, category III proteins (27 proteins) were shown on the basis of RNAi to have roles in the hermaphrodite and male germline or only in the hermaphrodite germline.

Category I, germline-localized proteins, included the proteins GSP-3 and GSP-4, which are homologous to protein phosphatase 1 (PP1). These proteins localize to chromosomes during male meiosis and in mature sperm but were not detected on oocyte chromosomes. RNAi of their genes caused chromosome segregation defects during spermatogenesis.

Figure 1
Electron micrograph of C. elegans spermatozoa. Arrows indicate sperm nuclei.

Figure 2
The proteomic strategy used to identify sperm chromatin factors. Spermatogenic chromatin from him-8(e1489) males and oogenic chromatin from fer-1(hc1) hermaphrodites was purified. Proteins that co-purified with chromatin were examined by multidimensional protein identification technology (MudPIT). As a result, 1,099 spermatogenic proteins and 812 oogenic proteins were identified. This list was then cut down to 502 high-abundance spermatogenic proteins. Of the abundant spermatogenic proteins, 132 were further selected after subtracting oogenic proteins. For functional analysis, RNAi against the genes that encode the spermatogenic proteins was carried out, and 50 genes showed embryonic lethal or sterile phenotypes. For germline phenotypic analysis, RNAi-treated worms were stained with DAPI; 20 genes that caused germline cytological defects when knocked down were identified; of these, 18 showed morphological defects in the male germline after RNAi.
Disruption of PP1cγ (a specific PP1 family member) in mice results in males with defects in meiosis and spermiogenesis, whereas the females are fertile [15]. Some PP1 family members may therefore have important specific roles in male fertility in other species.

SMZ-1 and SMZ-2 contain PDZ domains and are also category I proteins with no clear homologs in other species. These proteins localized to male meiotic germ nuclei and sperm chromatin but were not found in female germ cells. In smz-1(RNAi) and smz-2(RNAi) male germ lines, meiotic chromosomes did not congress to the metaphase plate or segregate.

Category I also included C. elegans SNBP candidate proteins, which localized to meiotic DNA and mature sperm chromatin. RNAi of these genes induced no detectable phenotype or a very weak phenotype. But because sperm genes are typically refractory to RNAi, it remains possible that these genes might have essential roles during spermatogenesis. Thus, the category I gene data suggest that the proteomic approach by Chu et al. [2] successfully identified new genes that are important for male meiosis and sperm development.

In addition, new roles in spermatogenesis were previously unknown. RNAi of top-1 caused abnormally large sperm nuclei and aberrant progression through meiotic events required for reproductive success. Much still needs to be learned in order to treat specific cases of human infertility and develop alternative contraceptives that are as effective as those already available. The study by Chu et al. [2] is a significant advance, because of the broad significance of the underlying cell biology with regards to all aspects of fertilization, and its potential relevance to our own reproductive biology.

References

1. Kimmins S, Sassone-Corsi P: Chromatin remodelling and epigenetic features of germ cells. Nature 2005, 434:583-589.

2. Chu DS, Liu H, Nix P, Wu TF, Razakien E, Yates JR 3rd, Meyer BJ: Sperm chromatin proteome identifies evolutionarily conserved fertility factors. Nature 2006, 443:101-105.

3. Fire A, Xu S, Montgomery MK, Costas SA, Driver SE, Mello CC: Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 1998, 391:806-811.

4. Fraser AG, Kamath RS, Zipperlen P, Martinez-Campos M, Sohrmann M, Ahringer J: Functional genomic analysis of C. elegans chromosome I by systematic RNA interference. Nature 2000, 408:325-330.

5. Gonczy P, Echeverri C, Oegema K, Coulson A, Jones SJ, Copley RR, Duperon J, Oegema J, Brehm M, Cassin E et al: Functional genomic analysis of cell division in C. elegans using RNAi of genes on chromosome III. Nature 2000, 408:331-336.

6. Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, Kanapin A, Le Bot N, Moreno S, Sohrmann M, et al: Systematic functional analysis of the Caenorhabditis elegans genome using RNAi. Nature 2003, 421:231-237.

7. Maeda I, Kohara Y, Yamamoto M, Sugimoto A: Large-scale analysis of gene function in Caenorhabditis elegans by high-throughput RNAi. Curr Biol 2001, 11:171-176.

8. Sonnichsen B, Koski LB, Walsh A, Marschall P, Neumann B, Brehm M, Alleaume AM, Artelt J, Bettencourt P, Cassin E, et al: Full-genome RNAi profiling of early embryogenesis in Caenorhabditis elegans. Nature 2005, 434:462-469.

9. Reinke V, Gil I, Ward S, Kazmer K: Genome-wide germline-enriched and sex-biased expression profiles in Caenorhabditis elegans. Development 2004, 131:311-323.

10. Reinke V, Smith HE, Nance J, Wang J, Van Doren C, Begley R, Jones SJ, Davis EB, Scherer S, Ward S, et al: A global profile of germline gene expression in C. elegans. Mol Cell 2000, 6:605-616.

11. Colaiacovo MP, Stanfield GM, Reddy KC, Reinke V, Kim SK, Villeneuve AM: A targeted RNAi screen for genes involved in chromosome morphogenesis and nuclear organization in the Caenorhabditis elegans genome. Genetics 2002, 162:113-128.

12. Washburn MP: Utilization of proteomics datasets generated via multidimensional protein identification technology (MudPIT). Brief Funct Genomic Proteomic 2004, 3:280-286.

13. Schirmer EC, Floresn L, Guan T, Yates JR 3rd, Gerace L: Nuclear membrane proteins with potential disease links found by subtractive proteomics. Science 2003, 301:1380-1382.

14. Skop AR, Liu H, Yates JR 3rd, Meyer BJ, Heald R: Dissection of the mammalian midbody proteome reveals conserved cytokinesis mechanisms. Science 2004, 305:61-66.

15. Yamazaki S, Jurisicova A, Okano K, Hudson J, Boekelheide K, Shipp EB: Spermiogenesis is impaired in mice bearing a targeted mutation in the protein phosphatase 1 gamma gene. Dev Biol 1999, 205:98-110.

16. Lee PH, Park H, Shim G, Lee J, Koo HS: Regulation of gene expression, cellular localization, and in vivo function of Caenorhabditis elegans DNA topoisomerase I. Genes Cells 2001, 6:303-312.