Minireview

Taking care of Dad's DNA

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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.

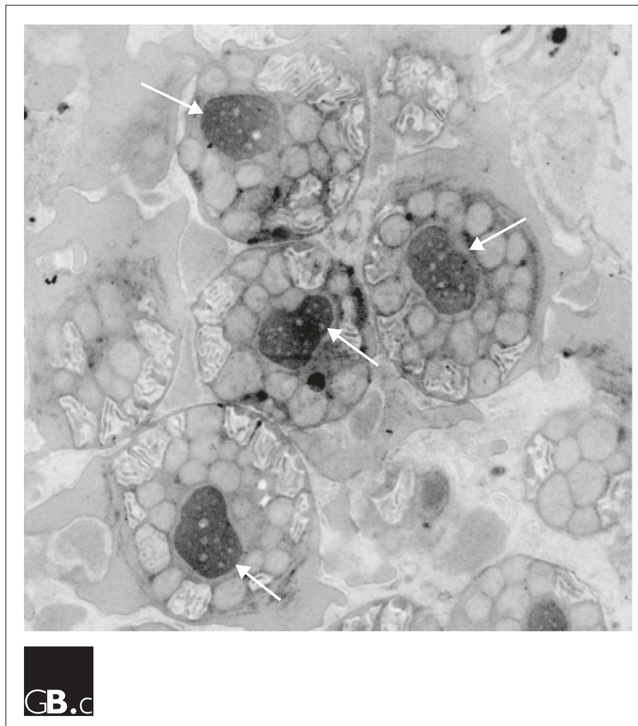

Figure 1
Electron micrograph of *C. elegans* spermatozoa. Arrows indicate sperm nuclei.

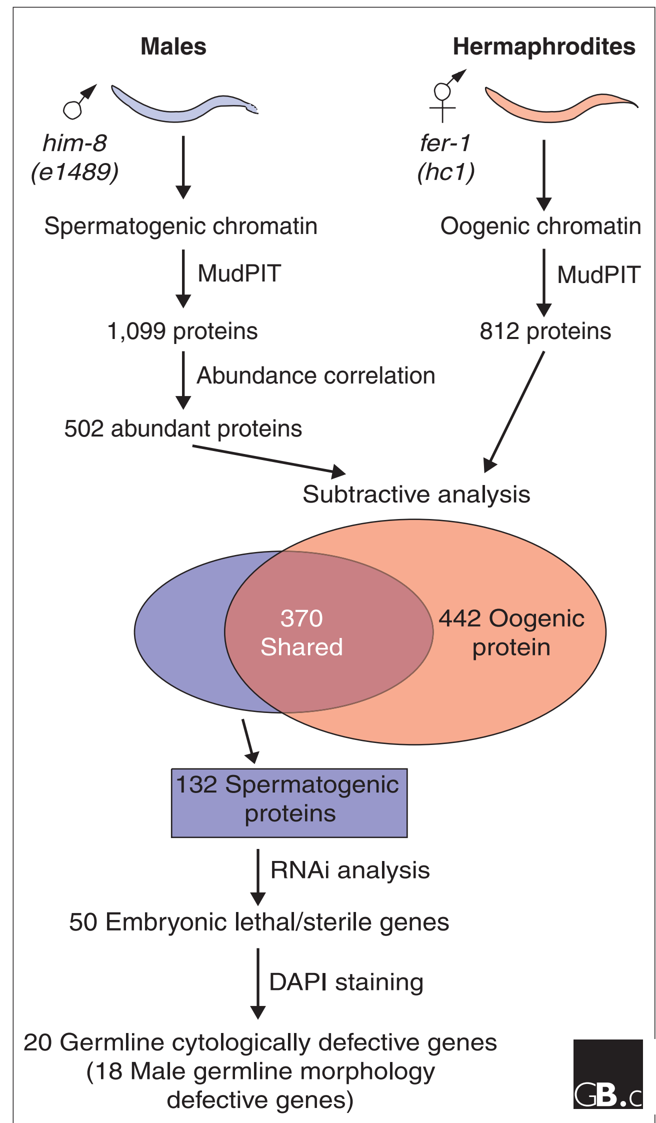

Figure 2
The proteomic strategy used to identify sperm chromatin factors. Spermatogenic chromatin from *him-8(e1489)* males and oogenic chromatin from *fer-1(hc1)* hermaphrodites was purified. Proteins that co-purified with chromatin were examined by multidimensional protein identification technology (MudPIT). As a result, 1,099 spermatogenic proteins and 812 oogeneic 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.

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.

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 observed in female germ cells. In *smz-1(RNAi)* and *smz-2(RNAi)* male germlines, meiotic chromosomes did not congress to the metaphase plate or segregate.

Category I also included *C. elegans* SNBP candidate proteins, which localized to male 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 identified for several previously studied proteins. For instance, one of the category II proteins found by Chu *et al.* [2] is topoisomerase I (TOP-1). This is a nucleolar protein in somatic cells and hermaphrodite germ cells [16] and TOP-1 localization to mature sperm chromatin and a function during spermatogenesis were previously unknown. RNAi of *top-1* caused abnormally large sperm nuclei and aberrant progression through male meiosis.

The study by Chu *et al.* [2] provides important clues to understanding mechanisms of male germline development that are conserved between worms and mammals. Of the 132 proteins detected by this approach in worms, 14 correspond to 7 mouse homologs whose knockout causes male infertility, and 70 *C. elegans* proteins have human homologs that have not yet been tested for roles in reproductive success. An approach similar to that established by this study in additional species, along with cross-species comparisons of sperm proteomes, will provide additional insights into the molecular basis of sperm evolution and male fertility.

It should be noted that many reproductive biologists consider true fertility factors to be molecules that are directly involved in gamete interactions or that function at fertilization. It is not yet clear whether this study has identified fertility factors by these criteria. It is, however, undeniable that the proper regulation and packaging of the paternal genome (the sperm's primary cargo) is critically important for reproductive success. Finally, we still do not have a comprehensive understanding of the molecular 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.

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