Meeting report

Genomic and proteomic techniques applied to reproductive biology

John C Rockett

Address: Reproductive Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, United States Environmental Protection Agency, Research Triangle Park, NC 27711, USA. E-mail: rockett.john@epa.gov

Published: 24 August 2001

Genome ***Biology*** 2001, **2(9)**:reports4020.1–4020.3

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2001/2/9/reports/4020

A report on the Frontiers in Reproduction Symposium 2001 'Reproductive genetics, genomics and proteomics: advances in genetic, molecular and bioinformatics techniques', Cambridge, USA, 30 June to 1 July, 2001.

Specialty meetings focused on the application of new genomic and proteomic technologies are increasingly common. One might romantically suppose that the genomics bandwagon ought to find no better propulsion system than the very engine to which the genome owes its immortality. And yet, ironically, the field of reproductive biology has been slower to incorporate post-genomic era approaches than many other biological disciplines. Frontiers in Reproduction (FIR), established in 1998, is an international training course held annually at the Marine Biological Laboratory, Woods Hole, USA. The six-week course is focused on techniques and concepts of advanced reproductive-biology research, and includes a two or three day symposium that addresses a high-profile research area. This year's FIR symposium offered the participants a look at some of the latest developments in the application of genomic and proteomic technologies to reproductive biology.

Peter Schlegel (Weill Medical College of Cornell University, New York, USA) opened the symposium with an overview of male clinical infertility. There is a long list of abnormalities underlying infertility, the etiology of which is not always delineated before treatment with *in vitro* fertilization (IVF) techniques such as intra-cytoplasmic sperm injection (ICSI). One of the main problems, as later expressed by Dolores Lamb (Baylor College of Medicine, Houston, USA), is that the use of techniques such as ICSI allows conveyance of genetic deficiencies into the next generation, thus perpetuating infertility problems rather than treating them. Current genetic tests on infertile men are normally limited to karyotyping and Y-chromosome microdeletion analysis, both of which are relatively crude and do not evaluate the majority of genetic abnormalities. Hence, Schlegel argued that more thorough genetic testing should be carried out before all IVF treatments in order to assess the quality of the sperm.

For those who do not realize, or sometimes forget, that genomics is more than microarrays and real-time reverse transcriptase (RT)-coupled PCR, this meeting was a timely reminder that beyond gene-expression profiling there is still the requirement for fundamental research to be carried out to elucidate gene function. Knockout mice are probably the most versatile and widely used tool for such studies. Thus, Carolyn Smith (Baylor College of Medicine) described her attempts to elucidate molecular mechanisms of estrogen-receptor action using mice deficient in E6-associated protein (E6-AP/UBE3A). E6-AP coactivates the hormone-dependent transcriptional activities of several members of the nuclear-hormone-receptor superfamily, and Smith described how the knockout of the *E6-AP* gene reduced testis and prostate size in the male and compromised estradiol action in the female. Megerditch Kitedjian (Rutgers University, New Brunswick, USA) explained how he is investigating post-transcriptional pathways of gene expression, particularly the mechanisms by which RNA-binding proteins such as Daz (deleted in azoospermia) affect spermatogenesis. Microdeletions containing the *Daz* gene are the most frequently observed deletions in infertile men, with as many as 10% carrying the abnormality. Kitedjian found that the male gametes of Daz-deficient mice fail to progress past the spermatogonia stage, and that its expression pattern suggests that Daz acts on multiple target RNAs. He then described a strategy for the isolation of specific nucleic acids associated with proteins (SNAAPs) that facilitates the capture and subsequent identification of these mRNAs. Blanche Capel (Duke University, Durham, USA) shared how she is addressing the difficult task of elucidating Sry-directed formation of the testes. There are currently no known targets of Sry, but fibroblast growth factor 9 (Fgf9) appears to act downstream of Sry and stimulates mesenchymal proliferation, mesonephric-cell migration, and Sertoli-cell differentiation in the embryonic

testis. Capel has shown that the reproductive-system phenotypes in Fgf9 knockout mice range from testicular hypoplasia to complete sex reversal, with most XY reproductive systems appearing grossly female at birth.

Another use of mice as tools for investigating possible causes of infertility is the generation of mutant mouse lines to carry out functional genomic analysis. Infertile models can arise spontaneously, but accelerating the process using genome-wide or targeted mutagenesis is clearly a more attractive option. Monica Justice (Baylor College of Medicine) has targeted a gene-rich region of mouse chromosome 11 using *Cre*/*Lox* targeted recombination technology, and has isolated a number of dominant and recessive mutations with various infertile phenotypes. In contrast, John Schimenti (The Jackson Laboratory, Bar Harbor, USA) has isolated a number of mutant phenotypes created by random mutagenesis with ethyl methyl sulfonate (EMS). Colin Bishop (Baylor College of Medicine) has been using pre-existing mouse mutants to analyze the genes acting at specific stages of germ-cell development. He described how the transplantation of germ cells from spermatogenically arrested phenotypes into wild-type testis (and *vice versa)* can be used to assess whether problems in spermatogenesis lie with the germ cells themselves or in the testicular environment. In this way, when wild-type stem cells were transplanted into the tubules of mice with juvenile spermatogonial depletion (JSD), the cells developed, whereas in the reverse experiment (JSD stem cells to wild-type tubules), they did not. These experiments indicate that inhibition of maturation of the JSD stem cells is an inherent property of the cells themselves, and is not related to their environment.

Two other approaches to gene silencing, more accurately described as 'gene-knockdown' technologies, were also discussed. RNA interference (RNAi) and RNA antisense technologies are not new, but are proving useful tools for analyzing gene function. Richard Schultz (University of Pennsylvania, Philadelphia, USA) used RNAi to silence genes such as the protooncogene *c-mos* and *Plat*, a gene playing an important role in tissue remodeling and degradation, cell migration and other physiopathological processes. He found that RNAi probes injected into one-cell embryos persist at least through to the blastocyst stage, and are more effective than the single-stranded-antisense approach to gene knockdown. Scott Coonrod (University of Virginia, Charlottesville, USA) reported, however, that the use of 'morpholinos', antisense oligonucleotides that are not recognized by ribonucleases, also appears effective at knocking out *c-mos* expression.

Another approach towards *in vivo* studies of infertility was taken by Barry Hinton (University of Virginia), who reported on his successful attempts at electroporating plasmid constructs into live testis and epididymis of rats. In this way, specific regions of the epididymis can be targeted to explore the regulation of promoters of interest *in vivo* and to test the biological function of proteins encoded by transgenes.

Back in the realm of gene-expression profiling, David Dix (US Environmental Protection Agency (EPA), Research Triangle Park, USA) and Eli Adashi (University of Utah, Salt Lake City, USA) described their different applications of gene-expression-profiling techniques. Dix utilized various DNA arrays, including a custom-made array of 950 genes expressed in testis, to generate expression profiles from a number of fertile and infertile mouse and human models in the hope of identifying conserved patterns of gene expression. The characterization of sperm mRNA appears to be a promising, if somewhat controversial, approach to monitoring fertility status. Adashi opted for an 'open' expression-analysis system - suppression-subtractive hybridization (SSH) - to search for genes that constitute critical molecular determinants of ovarian function. Open systems such as SSH can be used to identify any genes, both known and unknown, which show altered gene expression, because - unlike arrays - they are not restricted to a particular set of pre-selected probes. For further, functional analysis, he looked for genes that are novel, ovary-specific, phase-specific and cell-type-specific, and detailed his experiences isolating and characterizing ovarian-specific acidic protein (OSAP). He found that OSAP is expressed in the ovary, corpus lutea, preovulatory follicles, granulosa cells and steroidogenic cells, and its expression increases dramatically in the post-ovulatory phase in a hormone-dependent manner. He later discovered, however, that OSAP is also expressed in the adrenal gland, spleen and testis, demonstrating that it probably has a role in steroidogenesis.

Julianne Mayne (Los Alamos National Laboratory, USA) later discussed the characterization of single-nucleotide polymorphisms (SNPs), an increasingly important genomic technique. Mayne revealed how flow-cytometry-based minisequencing was being pioneered at Los Alamos through the development of genomic analysis using multiplexed microsphere arrays (GAMMArrays). These are rapid and sensitive cytometry-based assays for the multiplexed analysis of SNPs based on polymerase-mediated primer extension that uses microspheres as solid supports. The method uses subnanomolar concentrations of sample in small volumes, which can be analyzed at rates of one sample per minute or faster and enables the simultaneous analysis of dozens, and potentially hundreds, of SNPs per sample.

Proteomics is being used increasingly as a tool for discovering new gene products, and Don Hunt (University of Virginia) provided an overview of proteomic technology and how tandem mass spectrometry can be used to rapidly profile protein expression, starting with as little as 1.5 attomol of sample. Hunt demonstrated how such analysis is used to probe signal-transduction pathways and protein-protein

interactions. Importantly for those struggling with apparent inconsistencies between proteomic and genomic data, he has found that, at least in *Escherichia coli*, 90% of protein-product changes correlate with gene-expression changes in the same sample.

One of the most common aims of applying proteomics to reproductive biology is to discover possible targets for contraceptive vaccination. By isolating proteins that are abundant, expressed at the cell surface, immunogenic, unique, testis-specific and conserved between species, it may be possible to develop a contraceptive vaccine for women. John Herr (University of Virginia) used a combination of proteomic and genomic approaches to isolate and perform early characterization of several candidate proteins, including C58, an expressed sequence tag (EST) expressed only in the testis. This EST was used to isolate a full-length gene from a testis cDNA library, and was later named sperm acrosomal membrane protein 14 (Sam14). It can first be detected at the round spermatid stage and, as the name implies, localizes primarily to the acrosomal compartment. Hamster-egg penetration assays using rat anti-Sam14 antibody demonstrated that it inhibits sperm binding of the egg by as much as 45%. Herr used the same overall approach to isolate other potential vaccine candidates, including equatorial segment protein (Esp), a testis-specific protein first seen in the developing acrosomal vesicles of round spermatids. Hamster-egg penetration assays showed a 42% decrease in sperm-egg binding in the presence of anti-Esp antibody. On the female side, Coonrod has used proteomics to characterize oocyte development, in the hope of discovering possible targets for drugs that will prevent oocyte maturation without affecting normal ovarian function and cycling. Phosphodiesterase 3 has previously been shown to have this effect, but unfortunately it is not specific to oocytes and therefore is not a suitable drug candidate.

Participants were interested to hear from Chris Hogue (University of Toronto, Canada) of a new online database, the Biomolecular Interaction Network Database (BIND) [http://www.bind.ca], designed to store all known and publicly available descriptions of biomolecular interactions and reactions. It currently lists over 5,800 such interactions, including protein, DNA, RNA and other small molecule data. Another database announced during the meeting was GermOnline [http://wwmeb.igh.cnrs.fr/], an information database designed for those working in reproductive biology. It holds information about gene expression during gametogenesis and is planned to include 11 model systems as well *Homo sapiens*.

The symposium showed clearly that the integration of large-scale genomic and proteomic approaches opens up new perspectives for reproductive-biology research and it is to be hoped that this will help push back the frontiers of clinical reproductive science in the future.

Acknowledgements

The information in this document has been subjected to review by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. Thanks to David Dix (US EPA) and Kary Thompson (University of Arizona, Tuscon, USA) for critically reviewing the manuscript.