Meeting report

The genome and the germ cell

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A report on the third biennial Cold Spring Harbor Laboratory meeting on Germ Cells, Cold Spring Harbor, USA, 9-13 October 2002.

Primordial germ cells (PGCs) are the embryonic precursors of the gametes. In most species, PGCs spend much of early development as nomadic residents within other developing tissues. Despite the changing terrain of the embryo, PGCs are able to remain pluripotent, with the potential to develop into many types of specialized cells, and they eventually home in on the position of the developing gonad. Once they reach the gonad, germ cells undergo a sexually dimorphic process of differentiation that eventually (after days, weeks or years, depending on the organism) culminates in reductive divisions (meiosis) giving rise to the haploid sperm or egg. The accuracy and efficiency of this process has profound effects on the health and fitness of future generations, so germ cells are of interest to the medical community.

Historically, most advances in germ cell biology have come from genetically tractable organisms such as *Drosophila* and *Caenorhabditis elegans* where 'forward', or traditional, genetic strategies were used to identify genes required at various stages of PGC development. Spontaneous or targeted mutations - 'reverse genetics' - in less genetically tractable organisms have also contributed to our understanding of how PGCs function in mammalian systems. To expand these strategies, John Schimenti (The Jackson Laboratory, Bar Harbor, USA) has recently spearheaded a move to apply forward genetics to studies of PGCs in the mouse. In his talk, Schimenti described mutagenesis strategies for generating animals with fertility defects. Chemical mutagenesis, either in males or embryonic stem cells, followed by the generation of chimeric mice can result in dominant or recessive mutations affecting fertility. Schimenti described two sets of mutants recovered from these screens, the spermatogonial depletion (*sgdp*) mutants that exhibit fewer or no germ cells in the testis, and the meiosis (*mei*) mutants that are defective in meiosis. Currently, few of these mutations have been mapped to specific genes, but Schimenti was able to describe the *mei1* mutation in detail. In *mei1* mutants, PGCs arrest during the prophase of meiosis I and have defects in chromosomal synapsis. The DNA-repair protein Rad51 is not recruited to meiotic chromosomes in these animals, indicating that single-strand breaks do not occur or that Rad51 binding is blocked in this strain. The mutation was mapped near the gene for disrupted meiotic cDNA 1 (*Dmc1*) on chromosome 15 and identified fortuitously when an expressed sequence tag (EST) was mapped to this region. The *Mei1* gene is a novel gene with no known functional domains; it has homologs in humans, chickens and zebrafish, however, indicating that it may have a conserved function in vertebrate meiosis.

In all systems, from flies to humans, genomic and bioinformatic approaches (including microarrays, differential display and whole-genome sequencing) were prominent, and this article highlights the most exciting discoveries stemming from these techniques. In the field of germ cell origins, Yasuhisa Matsui (Osaka Medical Center for Maternal and Child Health, Japan) and Azim Surani (Wellcome/CRC Institute, Cambridge, UK) gave back-to-back talks about how the PGC lineage is established in mammals. In the mouse, PGCs are induced to form during the first third of gestation at the junction between the embryo (epiblast) and the extraembryonic tissue. The process occurs in two steps: firstly, at embryonic day 6.5 (E6.5), bone morphogenic proteins (BMP4 and BMP8) induce the proximal epiblast cells to become competent to develop into PGCs; and secondly, competent cells become committed to the PGC lineage by E7.25, but this commitment process is poorly understood. By culturing isolated epiblasts, Matsui was able to determine the time at which epiblast cells become responsive to BMP4 and BMP8. He also found that the response of these cells correlates with

expression of Smad5, a known mediator of BMP signals. To identify new factors involved in restricting cells to the PGC lineage, Matsui and coworkers performed differential display analysis. They compared mRNAs from individual PGCs isolated from E8.5 embryos to those from individual blastocyst cells, which are obtained prior to gastrulation at E4-5. They identified 11 candidate genes that were enriched in PGCs, including two known interferon-inducible transcripts (*Mil-1/Fragilis* and *Mil-2*). On the basis of this observation, their current hypothesis is that an interferon-like signal may cooperate with BMP4 and BMP8 to specify PGC fate. Surani and colleagues took a similar approach to identify novel genes elevated in nascent PGCs. They used differential display and single-cell microarray analysis to compare the expression of genes in individual E8.5 PGCs with individual neighboring somatic cells. They also identified *Fragilis* and another gene, *Stella,* as interesting candidates. *Fragilis* encodes a transmembrane protein that has been shown to mediate adhesion between cells of the same type. The function of the *Stella* gene product is uncertain but it has domains characteristic of proteins involved in splicing. Additionally, from their differential screen, Surani and colleagues found that expression of the homeobox transcription factor gene *Hoxb1* is excluded from PGCs. They propose a model whereby the BMP4 and BMP8 signal leads to expression of *Fragilis*. As a result of differential adhesion, the highest expressers become segregated and begin to express markers such as *Stella*, indicating commitment to the PGC fate. Lower levels of Fragilis may correlate with *Hoxb1* expression, which Surani and colleagues propose blocks PGC fate and causes the cells to develop along a somatic path.

In discussing the embryonic germline, Erez Raz (Max Planck Institute for Biophysical Chemistry, Göttingen, Germany) described a new signaling pathway that guides migrating PGCs in zebrafish embryos. In this system, PGCs form as a result of the inheritance of maternal determinants. The maternally provided mRNA and proteins become partitioned into the zebrafish PGCs during the early cleavage stages of development of the zygote, resulting in the formation of four clusters of PGCs within the blastocyst-stage embryo. These widely separated clusters realign during gastrulation, and the PGCs accumulate at the junction between the head the trunk. The resulting line of PGCs then separates into two as the cells move to the left or right forming clusters at the sides of the embryo. Then, the two lateral groups of PGCs move posterially as the PGCs migrate to the developing gonads (marked by expression of the zebrafish homolog of the Wilm's Tumor gene). In order to identify genes that are involved in orchestrating this elaborate pattern of movements, Raz and colleagues, together with Mermaid Pharmaceuticals (Hamburg, Germany), conducted a screen based on morpholino-modified antisense oligonucleotides to target around 10,000 zebrafish cDNAs. Depletion of one gene, encoding the chemokine receptor CXCR4, caused PGCs to stray into inappropriate locations. In mammals, CXCR4 is the receptor for stromal-derived factor (SDF), a chemokine involved in many migratory processes including growth-cone guidance, leukocyte trafficking and tumor metastasis. Based on *in situ* hybridization analysis, Raz and coworkers found that zebrafish SDF was expressed at all sites in the embryo where PGCs normally accumulate, and that changes in its expression perfectly match the times at which PGCs realign. They then used morpholino oligonucleotides to deplete SDF and found that PGCs scatter, instead of accumulating within the gonads. They were also able to ectopically express SDF (together with a green fluorescent protein marker) in zebrafish embryos and found that PGCs would accumulate in these ectopic locations.

Steve Rozen (Whitehead Institute, Cambridge, USA) described some of the unusual properties of the human Y chromosome in his talk. Regions of the Y chromosome that lack a meiotic pairing partner are believed to be undergoing degradation on an evolutionary time scale. This 'Rotting Y' hypothesis is challenged by the finding that these non-pairing regions contain many genes necessary for male fertility and hence should be selected for evolutionarily. From the sequence of the human genome, Rozen and coworkers have identified unusual structural elements in the non-pairing arm of the Y chromosome, namely long palindromes with highly conserved arms. Six of these palindromes can be found on the Y chromosomes of chimpanzee and bonobo, suggesting that these structures predate the divergence of humans and chimps. The fact that the palindrome arms are so highly conserved within species indicates that there may be gene conversion events and selective pressure to maintain these structures. Additionally, Rozen and colleagues examined the distribution of eight Y-linked gene families that are expressed in the testis and found that all genes were present within the palindromes, whereas pseudogenes were more randomly distributed. Rozen suggests several mechanisms whereby these structures might become fixed during evolution. One possibility is that duplication of testis-specific genes within palindromes may confer a breeding advantage on males. Alternatively or in addition, palindromes could provide shelter from deleterious mutations that would otherwise be unmasked in an unpaired chromosome. Finally, intrachromosomal crossover events (gene conversions) could stabilize these regions during meiosis and effectively combat the rotting Y phenomenon.

In summary, the talks discussed here are examples of what can be done with microarray, differential display and sequence-based approaches. Novel genes have been identified that are involved in PGC development, functional screens have been devised using the massive amounts of new sequence information, and the completed human genome has been mined to glean novel insights into human evolution. There were many other talks and posters utilizing similar approaches, indicating that the advent of this new technology has had a clear impact on the field of germ cell biology.