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Reproductive and Developmental Genomics Retreat

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On June 14–15th, the Wiley journal *Molecular Reproduction and Development* partnered with the Cornell University's Center for Reproductive Genomics and Vertebrate Genomics, along with NICHD-funded training grants, to host a retreat on Cornell's Ithaca, New York campus. The event brought together Cornell scientists including NIH-trainees, and researchers from many other institutions, possessing varied expertise and utilizing diverse model systems. The goal was to facilitate multidisciplinary interaction amongst colleagues and foster a perspective of comparative biology towards a shared interest in genomics-based research.

Following a brief welcome address from MRD editor-in-chief Gary Wessel, the two-day retreat showcased a host of invited speakers, including visiting investigators and Cornell University faculty members. Presentations highlighted novel and recent findings from an impressive array of research efforts, spanning work in areas as diverse as meiosis and gametogenesis, ES cell differentiation and epigenetic regulation, to reproductive technologies in the pig and transgenic manipulation of gonadotropin signaling in mice. For example, Mariana Wolfner (Cornell), Harvey Florman (Univ. of MA, Medical School), and Pascal Gagneux (UCSD) underscored the important role sperm- and seminal fluid-associated proteins play in fertilization and mating behavior, while more developmentally oriented work from Natasza Kurpios (Cornell) highlighted both the cellular and genomic basis of left-right patterning and asymmetric organ morphogenesis.

A selection of the interesting findings presented at the retreat included those in the opening talk by Dr. Guang Hu from the National Institute of Environmental Health Sciences. He discussed the molecular basis of embryonic stem (ES) cell self-renewal and differentiation and described an siRNA-based genetic screen in which he and colleagues identified over 100 candidate self-renewal genes, including genes encoding the CCR4-Not (Cnot) complex previously demonstrated to be involved in transcriptional regulation and deadenylation (Hu *et al.* 2009; Zheng *et al.* 2012). Interestingly, these factors seem to operate independently of known self-renewal pathways involving Oct4/Sox/Stat factors, and are often upregulated in human cancers. Therefore, investigating the cellular function and mode of action of this complex will likely provide the necessary framework for our understanding of stem cell maintenance and differentiation. Amander Clark of University of California, Los Angeles, continued along the ES cell theme, describing her lab's studies of the chromatin landscape utilizing ES cells as a model to study germline cell establishment. Dr. Clark described a

transgene-free method of deriving primordial germ cells (PGC) from mouse ES cells, which is particularly desirable since it avoids some of the common pitfalls of various ES cell derivation methods. Importantly, the induced PGCs (iPGCs) undergo genomewide demethylation at CG dinucleotides and are Blimp1-positive, hallmarks of PGC identity (Vincent *et al.* 2011). Altogether, this novel iPGC model will now allow robust examination of early germline development.

Providing some insight into the mechanisms behind the re-establishment of chromatin marks during reprogramming, Paul Soloway from Cornell University presented his work on how epigenetic marks are regulated in the mammalian germline. The focus of his talk was on the role of a PIWI-interacting RNA (piRNA) during *de novo* methylation of the imprinted mouse *Rasgrf1* locus. A non-coding RNA transcribed from repeats found in the differentially methylated domain (DMR) of the *Rasgrf1* locus was found to be targeted by piRNAs (thus was termed pi-targeted-RNA; pit-RNA), and was originally thought to function only in *cis*, recruiting DNA methyltransferases and placing methyl groups at this site. Using mice that lack the repeated DMR region along with transgenic mice carrying the repeats at different sites, Dr. Soloway and his lab were able to show that this mechanism works in *trans* as well (Brideau *et al.* 2010; Watanabe *et al.* 2011). Interestingly, this result carried into the following generation of mice, in a manner reminiscent of the paramutation phenomenon observed in plants.

A number of talks provided an evolutionary perspective on the regulatory and organizational principles that underlie the structural complexity of genomes. Daniel Barbash of Cornell University spoke on the interaction of transposable DNA elements (TPEs), a major influence on genome size and complexity, with the host genome response in the context of hybrid incompatibility of divergent *Drosophila* species. The Barbash lab's work suggests that rapid adaptive evolution within the piRNA pathway functions to maintain epigenetic silencing of TPEs in a lineage-specific manner. A consequence of these rapid adaptive changes is that the incompatibility of piRNA pathway components between diverged species impacts piRNA production, leading to de-repression of TPEs and subsequent loss of female fertility in hybrid offspring.

Since the publication of the sequenced human genome over a decade ago, we have gained tremendous insight into the composition and structure of our genome as well as the molecular signatures of evolution itself. The organization and functional content of the X chromosome however has remained inscrutable, largely due to the high density of repetitive sequence and regional duplications that has hampered accurate sequencing and assembly efforts. Jacob Mueller, a postdoctoral fellow in David Page's lab at the Whitehead Institute is working to address this knowledge gap through targeted re-sequencing of the X chromosome. In the absence of accurate sequence data, it has long been believed that the mammalian X chromosome follows what is known as Ohno's law, having a high degree of conserved structure and content as well as enrichment for female-specific genes. Mueller presented interesting data that challenge these assumptions, finding instead that a significant number of genes (10–15%) are in fact not conserved, but rather appear to be recently acquired genes predominantly expressed in the testis. The significance of a potentially "masculinized," or at least "nonfeminized," X chromosome awaits further studies.

Throughout the sessions, we were delighted to hear talks from a variety of up-and-coming trainees, who spoke on a range of topics. Andrew Modzelewski (Cohen lab, Cornell) presented his findings on the role of small RNAs in gene silencing during meiosis, while the characterization of mouse models of pre-eclampsia and testicular cancer were presented by Jenny Sones (Davisson lab, Cornell) and Dr. Amy Lyndaker (Weiss lab, Cornell), respectively. One particularly compelling trainee presentation by Fernando Migone (Quirk lab, Cornell) reported on the combined application of genetic manipulation and *in vivo* multi-photon confocal imaging to study Hedgehog signaling regulation of vascular changes during ovulation. Other excellent trainee talks included Marcia Manterola (Wolgemuth lab, Dana-Farber Cancer Institute) on the role E-type cyclins in regulating meiotic chromosome stability, and Caroline Sartain (Wolfner lab, Cornell) discussing how cytoplasmic polyadenylation regulates gametogenesis and the egg-to-embryo transition. In addition to these selected abstract talks, the retreat also hosted a poster session where trainees were able to interact with the invited speakers and discuss their current research in a relaxed and supportive atmosphere.

A highlight of the retreat was the captivating keynote speech by Dr. Jun Wang, Executive Director of the BGI (previously known as the Beijing Genomics Institute). In addition to reporting the whole genome sequences of Asian and African humans (Wang *et al.* 2008; Li *et al.* 2010a), BGI has also fully sequenced “good-tasting species,” “useful species,” and “cute species,” including those of the foxtail millet (Zhang *et al.* 2012) and giant panda (Li *et al.* 2010b). However, Dr. Wang stressed that we should move from having a single genome sequence for a particular species, often called a reference sequence, to a set of genome sequences, as the amount of structural and sequence variation amongst human genomes is quite notable, with significant variation both within and between populations. An example of this variation is evident in a recent study in which Wang and colleagues examined structural variation between just two human genomes mapped at single-nucleotide resolution by whole-genome *de novo* assembly and found nearly 300 thousand structural variants (insertions, deletions, and inversions) ranging in length from 1–23kb (Li *et al.* 2011).

The central theme of Dr. Wang’s keynote address, “Life is a game of evolution,” was evident throughout the many varied topics that he covered. In work addressing the monoclonal versus polyclonal origins of tumors, he discussed how cancer is really a disease of cell evolution. Whether tumor cells are genetically clonal or more diverse and polyclonal has major implications for the success of cancer therapies, though this question of clonality has been difficult to assess within human tumors due to their heterogeneity. Under his direction, the BGI recently published studies in which they utilized single-cell exome sequencing to follow the cellular evolution of a hematopoietic tumor (Hou *et al.* 2012), which exhibited monoclonal evolution, and clear cell renal cell carcinoma (Xu *et al.* 2012), which interestingly did not contain significant clonal populations. In other technical feats, the BGI team has also utilized single-sperm genome sequencing to measure the total number of recombination events that occur during human meiosis, sequenced thousands of bird genomes, assessed the landscape of gene fusions in human prostate cancers (Ren *et al.* 2012), and analyzed genome-wide methylation patterns across generations and in 8,000 sets

of twins. Perhaps most intriguingly, Dr. Wang described the human gut bacterial genome as our “other” genome, which he and colleagues characterized by metagenomic sequencing in 2010 (Qin *et al.* 2010). In fact, the group has recently shown that special functions can be encoded by our own unique gut genome, where gut bacterial metagenomic markers are more indicative of type 2 diabetes than the sequence of our own genome. This type of study would not be feasible without current genomic technologies, including recent advances in whole-genome sequencing and sequence assembly and analysis. All in all, Dr. Wang gave the attendees an inspiring glimpse into the power of genomic technologies and the types of questions we can begin address with high-throughput sequencing.

Jun Wang’s keynote address appropriately summarized the overarching theme of this year’s retreat. From stem cell proliferation and maintenance to the terminally differentiated sperm, and everything in between, it is clear that evolution has played a complex game throughout our genetic history, one that only a decade ago we could have never imagined to be so intricate, and which we are only just now beginning to appreciate. This retreat provided the participants a unique opportunity to gather a wide range of experts, spanning many disciplines, in a single location to convene on these cutting edge topics. Enthusiastic feedback from attendees, faculty, and trainees alike indicates that the retreat was a resounding success. We look forward to equally rewarding Reproductive and Developmental Genomics Retreats in future years.

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