ART’S brave new world: The cat’s out of the bag…

Newest unexpected discoveries in fundamental molecular, cell and reproductive biology

Nothing to declare
stem cells, grown under the right conditions in vitro, have a remarkable ability to undergo differentiation self-assembly to form complex, three-dimensional organoids, similar in structural and functional organization to the developing brain, kidney, gut and other tissues (1). On page 153 of this issue, Harrison et al. (2) show that when mouse embryonic stem cells are cultured together with trophoblast stem cells (which give rise to part of the placenta), the resulting constructs develop into structures that bear a striking resemblance to the mouse embryo after it has implanted into the womb. The finding raises the possibility that by using advanced cell-culture techniques, including coculture of multiple cell types, and engineering the appropriate culture microenvironment, it might be possible to model human embryogenesis in a petri dish.

..."it might be possible to model human embryogenesis in a petri dish"

Human embryo culture and embryonic stem cell culture have opened up a previously inaccessible phase of the human life cycle to experimental study. Until recently, in vitro growth of mouse or human embryos has mainly been limited to cultures that reach the blastocyst stage, or the equivalent of about 5 or 6 days of embryonic development. Many of the most important events in embryogenesis, including those involved in developmental disorders, occur in a critical interval shortly after this stage. In 2016, the culture of human embryos was extended out to 13 days of development, a point at which many of the key structures that will support the growth of the embryo have formed, at this stage, the embryo is preparing to implant.

The Jackson Laboratory, Bar Harbor, ME, USA.
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"We are at an early stage of developing embryos from stem cells in culture"

STEM CELLS

Embryogenesis in a dish

"It might be possible to model human embryogenesis in a petri dish"
CHINA’S PUSH FOR BETTER BABIES

A campaign to increase preimplantation genetic diagnosis could put the country on the path towards eliminating certain diseases.

BY DAVID CYRANSKI

Opening time with Qiao Jie is not easy. At 7:30 a.m., she is coming out of the fertility clinic that she runs. Blocks the dense city extends some 80 metres down the street, she asks, about 25 physicians on her team are discussing recent findings, but Qiao, a fertility specialist and president of Peking University Third Hospital in Beijing, is still on an early-morning consult.

When she finally emerges, she jumps to the topic at hand: spreading awareness of preimplantation genetic diagnosis (PGD), a procedure that helps couples undergoing in vitro fertilization (IVF) avoid passing on genetic mutations that could cause disease or disability in their children. Qiao typically refuse interview requests, but she’s concerned that people aren’t getting the message about PGD fast enough. “Now, more and more diseases can be stopped — if not immediately, in the next generation,” she says.

Early experiments are beginning to show how genome-editing technologies such as CRISPR might one day fix disease-causing mutations before embryos are implanted. But refining the techniques and getting regulatory approval will take years. PGD has already helped thousands of couples. And whereas the expansion of PGD around the world has generally been slow, in China, it is starting to explode.

The conditions that are ripe: genetic diseases carry heavy stigma, people with disabilities get very little support and religious and ethical push-back against PGD is almost non-existent. China has also lifted some restrictions on family size and seen a subsequent rise in fertility treatments among older couples. Genetic screening during pregnancy for chromosomal abnormalities linked to maternal age has taken off throughout the country, and many see this as a precursor to wider adoption of PGD.

Although Chinese fertility doctors were slow to the game in adopting the procedure, they have been pursuing a more aggressive, comprehensive and systematic path towards it — more than anywhere else. The country’s central government, known for its long-term thinking, has only this past decade stepped up efforts to bring high-quality health care to the people, and its current 5-year plan has made reproductive medicine, including PGD, a priority, an effort that Qiao is leading. Researchers are hunting down various mutations in the Chinese population that might be screened for in PGD. And well-equipped and powerful clinical research groups, including Qiao’s, are stepping up efforts to improve the technology, increase awareness and bring down costs.

Comprehensive figures are difficult to come by, but estimates from leading PGD providers show that China’s use of the technique already outpaces that in the United States, and it is growing up to five times faster. Qiao’s clinic alone now performs more procedures with PGD each year than all of the United Kingdom.

“Looking over the development in China over the last 10 years, they might start to think it’s possible to get rid of these diseases,” says Kangpu Xu, a Chinese-born reproductive biologist at Weill Cornell Medical College in New York City.

Such systematic efforts raise thorny questions for biologists. Some worry that it pushes to eliminate disabilities devalue the lives of those who already have them. The cost and accessibility of the procedure raise concerns about genetic traits further widening the divide between rich and poor people. There are concerns about
Designer Babies?
Gene correction in S-phase-injected human embryos

A

paternal
maternal

MYBPC3

sid mutants

Cas9

Inter-homologue HDR (non-crossover)

Inter-homologue HDR (crossover)

NHEJ (long deletion)

no PCR product

WT

WT

WT

C

maternal only

m

m

m

PB

NEBD

Interphase

Metaphase

Telophase

G1

Interphase

Prometaphase

Metaphase

0-30min

100 min

3.5h

day 1

day 1
FOXP2 Gene

- FOXP2 has been called the "language gene."

- Several cases of developmental verbal dyspraxia in humans have been linked to mutations in the FOXP2 gene. In humans, mutations of FOXP2 cause a severe speech and language disorder.

- fMRI analysis of these individuals shows underactivation of Broca's area and the putamen, brain centers thought to be involved in language.

- Scientists have also looked for associations between FOXP2 and autism.
Paternal Influence

Researchers analyzed genetic material from 78 Icelandic children and their parents, including 44 children with autism spectrum disorder. Children of older fathers tended to have a higher number of mutations that are not inherited from either parent.
“You’re healthy enough for sexual activity but not attractive enough.”
Out of Africa 55-65 kyr ago

Migration of click-language speakers 50 kyr ago

Neolithic expansion 10 kyr ago

Sintashta expansion 2.5-3.5 kyr ago

Yamnaya expansion 4.5 kyr ago

Palaeolithic Eurasians 45-55 kyr ago

Migration into Sahul 47.5-55 kyr ago

Paleo-Eskimo expansion 4-5 kyr ago

Inuit expansion 3-4 kyr ago

Peopling of America across Beringia 15-23 kyr ago

Northern-southern split 13 kyr ago

Alternative route to America

Polynesian expansion 3-5 kyr ago

Possible pre-Columbian contact

Kyr ago

-60

-40

-20

0

20

40

60
Between 1650 and 1860, approximately 10 to 15 million enslaved people were transported from western Africa to the Americas. Most were shipped to the West Indies, Central America, and South America.
Maternal Haplogroup

You descend from a long line of women that can be traced back to eastern Africa over 150,000 years ago. These are the women of your maternal line, and your maternal haplogroup sheds light on their story.

Gerald, your maternal haplogroup is K1a1b1a.

As our ancestors ventured out of eastern Africa, they branched off in diverse groups that crossed and recrossed the globe over tens of thousands of years. Some of their migrations can be traced through haplogroups, families of lineages that descend from a common ancestor. Your maternal haplogroup can reveal the path followed by the women of your maternal line.

Migrations of Your Maternal Line

https://you.23andme.com/reports/maternal_haplogroup/print/

180,000 Years Ago
Haplogroup L
If every person living today could trace his or her maternal line back over thousands of generations, all of our lines would meet at a single woman who lived in eastern Africa between 150,000 and 200,000 years ago. Though she was one of perhaps thousands of women alive at the time, only the diverse branches of her haplogroup have survived to today. The story of your maternal line begins with her.

65,000 Years Ago
Haplogroup L3
Your branch of L3 haplogroup L3, which arose from a woman who likely lived in eastern Africa between 60,000 and 70,000 years ago. While many of her descendants remained in Africa, one small group ventured east across the Red Sea, likely across the narrow Bab-el-Mandeb into the tip of the Arabian Peninsula.

59,000 Years Ago
Haplogroup N
Massive genetic study shows how humans are evolving

Analysis of 215,000 people's DNA suggests variants that shorten life are being selected against.

Bruno Martin
06 September 2017

Human populations are evolving to improve fitness in unexpected ways.

A huge genetic study that sought to pinpoint how the human genome is evolving suggests that natural selection is getting rid of harmful genetic mutations that shorten people's lives. The work, published in *PLoS Biology*¹, analysed DNA from 215,000 people and is one of the first attempts to probe directly how humans are evolving over one or two generations.

To identify which bits of the human genome might be evolving, researchers scoured large US and UK genetic databases for mutations whose prevalence changed across different age groups. For each person, the parents' age of death was recorded as a measure of longevity, or their own age in some cases.
Estimating the human mutation rate from autozygous segments reveals population differences in human mutational processes

Vagheesh M. Narasimhan1, Raheleh Rahbari1, Aylwyn Scally2, Arthur Wuster1-3, Dan Mason4, Yali Xue5, John Wright4, Richard C. Trembath5, Eamonn R. Maher6,7, David A. van Heel8, Adam Auton9, Matthew E. Hurles1, Chris Tyler-Smith1 & Richard Durbin1

Heterozygous mutations within homozygous sequences descended from a recent common ancestor offer a way to ascertain de novo mutations across multiple generations. Using exome sequences from 3222 British-Pakistani individuals with high parental relatedness, we estimate a mutation rate of 1.45 ± 0.05 × 10⁻⁸ per base pair per generation in autosomal coding sequence, with a corresponding non-crossover gene conversion rate of 8.75 ± 0.05 × 10⁻⁶ per base pair per generation. This is at the lower end of exome mutation rates previously estimated in parent–offspring trios, suggesting that post-zygotic mutations contribute little to the human germ-line mutation rate. We find frequent recurrence of mutations at polymorphic CpG sites, and an increase in C to T mutations in a 5′ CCG 3′ to 5′ CTG 3′ context in the Pakistani population compared to Europeans, suggesting that mutational processes have evolved rapidly between human populations.
EPIGENETICS

A mechanism for regulating gene activity independent of DNA sequence that determines which genes are turned on or off:
- in a particular cell type
- in different disease states
- in response to a physiological stimulus
• ~5.4% of pregnant females age 15-44 were actively using illicit drugs.
• ~9.4% of pregnant women reported that they were active users of alcohol.

In 2012, the total number of OPR prescriptions rose to 259 million, enough for every American adult to have one bottle.

The incidence of NAS in the United States nearly doubled during our study period and has grown nearly 5-fold since 2000.

During the study period aggregate hospital charges for NAS nearly doubled, from an estimated total of $731,841,300 in 2009 to $1,449,389,600 in 2012. Through all study years the majority of hospital charges were attributed to state Medicaid programs, growing from $563,809,300 to $1,170,206,600: Patrick et al. 2016 February 01.

2013 National Survey on Drug Use and Health (NSDUH) reports the following statistics on substance abuse in pregnant women:
Replication

DNA

mRNA

Translation (protein synthesis)

Ribosome

Protein

Transcription (RNA synthesis)
Figure 1. Mechanisms involved in X-chromosome inactivation (XCI).
Pseudouridine (Ψ) and N6-methyladenosine (m6A) are the two most abundant mRNA modifications in eukaryotes.

Pavanapuresan P. Vaidyanathan et al. RNA 2017;23:611-618
<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Affected modification</th>
<th>Disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTO*</td>
<td>Polymorphism</td>
<td>m^6^A</td>
<td>Obesity, type 2 diabetes</td>
<td>Dina et al., 2007; Frayling et al., 2007; Scott et al., 2007; Scuteri et al., 2007</td>
</tr>
<tr>
<td>FTO</td>
<td>Inactivating mutation</td>
<td>m^6^A</td>
<td>Autosomal-recessive lethal syndrome</td>
<td>Boissel et al., 2009</td>
</tr>
<tr>
<td>DKC1</td>
<td>Mutation</td>
<td>ψ</td>
<td>X-linked form of dyskeratosis congenita</td>
<td>Heiss et al., 1998</td>
</tr>
<tr>
<td>DKC1</td>
<td>Mutation</td>
<td>ψ</td>
<td>Hoyeraal-Hreidarsson syndrome</td>
<td>Knight et al., 1999</td>
</tr>
<tr>
<td>NSUN2</td>
<td>Inactivating mutation</td>
<td>m^5^C</td>
<td>Intellectual disability and Dubowitz-like syndrome</td>
<td>Abbasi-Moheb et al., 2012; Blanco et al., 2014; Khan et al., 2012; Komara et al., 2015; Martinez et al., 2012</td>
</tr>
<tr>
<td>NSUN3</td>
<td>Inactivating mutation</td>
<td>m^5^C, f^5^C</td>
<td>Mitochondrial disease</td>
<td>Van Haute et al., 2016</td>
</tr>
<tr>
<td>NSUN7</td>
<td>Loss-of-function mutation</td>
<td>m^5^C</td>
<td>Infertility in males</td>
<td>Khosronezhad et al., 2014</td>
</tr>
<tr>
<td>IKBKAP</td>
<td>Inactivating mutation</td>
<td>mcm^5^s^2^U</td>
<td>Familial dysautonomia (recessive neurodegenerative disorder)</td>
<td>Karlsborn et al., 2014</td>
</tr>
<tr>
<td>WDR4</td>
<td>Inactivating mutation</td>
<td>m^7^G</td>
<td>Microcephalic primordial dwarfism</td>
<td>Shaheen et al., 2015</td>
</tr>
<tr>
<td>FTSJ1</td>
<td>Mutations and copy-number variation</td>
<td>2'-O- methylation</td>
<td>Non-syndromic X-linked intellectual disability</td>
<td>Freude et al., 2004; Honda et al., 2010</td>
</tr>
<tr>
<td>TRIT1</td>
<td>Inactivating mutation</td>
<td>i^6^A</td>
<td>Encephalopathy and myoclonic epilepsy</td>
<td>Yarham et al., 2014</td>
</tr>
<tr>
<td>ADAT3</td>
<td>Mutation</td>
<td>I</td>
<td>Intellectual disability and strabismus</td>
<td>Alazami et al., 2013</td>
</tr>
<tr>
<td>TRMT10A</td>
<td>Mutation</td>
<td>m^1^G</td>
<td>Young onset diabetes, short stature and microcephaly with intellectual disability</td>
<td>Igoillo-Esteve et al., 2013</td>
</tr>
<tr>
<td>TRMT1</td>
<td>Frameshift alteration</td>
<td>m^2^G and m^2^G</td>
<td>Intellectual disability</td>
<td>Davarniya et al., 2015</td>
</tr>
</tbody>
</table>

m^6^A, N^6^-methyladenosine; ψ, pseudouridine; m^5^C, 5-methylcytosine; mcm^5^s^2^U, 5-methoxycarbonylmethyl-2-thiouridine; m^7^G, 7-methylguanosine; i^6^A, N^6^-isopentenyl-adenosine; I, inosine; m^1^G, 1-methylguanosine; m^2^G and m^2^G.N2-methylguanosine and N2,N2-dimethylguanosine; f^5^C, 5-formylcytosine.

*Recent genome-wide association studies showed that single-nucleotide polymorphisms within the FTO region directly interact with IRX3 expression regulation, which may be directly influencing body mass and composition (Smemo et al., 2014).
Simplified schematic of eukaryotic ribosome biogenesis.

Anupama Narla, and Benjamin L. Ebert Blood
2010;115:3196-3205

©2010 by American Society of Hematology
Selected physical abnormalities seen in ribosomopathies.

Diamond Blackfan Anemia

Dyskeratosis Congenita

Treacher Collins

Anupama Narla, and Benjamin L. Ebert Blood
2010;115:3196-3205
Mislocalized proteins that have been associated with human diseases.

Table 1. Mislocalized proteins that have been associated with human diseases

<table>
<thead>
<tr>
<th>Protein</th>
<th>Disease</th>
<th>Mechanism</th>
<th>Mislocalization</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRY</td>
<td>Swyer syndrome</td>
<td>Mutation of NLS</td>
<td>Loss of nuclear localization</td>
<td>(McLane and Corbett, 2009)</td>
</tr>
<tr>
<td>SHOX</td>
<td>Léri–Weill dyschondrostosis</td>
<td>Mutation of NLS</td>
<td>Cytoplasmic retention</td>
<td>(Sahnerwal et al., 2004)</td>
</tr>
<tr>
<td>TRPS1</td>
<td>TRPS</td>
<td>Mutation of NLS</td>
<td>Loss of nuclear localization</td>
<td>(Kaiser et al., 2004)</td>
</tr>
<tr>
<td>ARX</td>
<td>XLAG</td>
<td>Mutation of NLS</td>
<td>Loss of nuclear localization</td>
<td>(Shoubridge et al., 2010)</td>
</tr>
<tr>
<td>FOXP2</td>
<td>Speech-language disorder</td>
<td>Mutation of NLS</td>
<td>Loss of nuclear localization</td>
<td>(Mizutani et al., 2007)</td>
</tr>
<tr>
<td>AIRE</td>
<td>APECEED</td>
<td>Mutation of ZFD</td>
<td>Cytoplasmic retention</td>
<td>(Bjorres et al., 2000)</td>
</tr>
<tr>
<td>RPS19</td>
<td>Diamond-Blackfan anemia</td>
<td>Mutation of NoS</td>
<td>Loss of nucleolar localization</td>
<td>(Da Costa et al., 2003b)</td>
</tr>
<tr>
<td>AGT</td>
<td>Primary hyperoxaluria type 1</td>
<td>Polymorphism and/or mutation</td>
<td>Mitochondrial mislocalization</td>
<td>(Djordjevic et al., 2010)</td>
</tr>
<tr>
<td>hsmMOK2</td>
<td>Laminopathy</td>
<td>Mutation of lamin A/C</td>
<td>Formation of nuclear aggregates</td>
<td>(Dreuilhet et al., 2008)</td>
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<tr>
<td>SHOC2</td>
<td>Noonan-like syndrome</td>
<td>Acquired N-myristoylation</td>
<td>Mislocalization to the plasma membrane</td>
<td>(Cordes et al., 2009)</td>
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<tr>
<td>Rhodopsin</td>
<td>Retinitis pigmentosa</td>
<td>Mutations</td>
<td>ER retention</td>
<td>(Mendes et al., 2005)</td>
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<td>AVPR2</td>
<td>Nephrogenic diabetes insipidus</td>
<td>Mutations</td>
<td>ER retention</td>
<td>(Robben et al., 2006)</td>
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<td>ATP7B</td>
<td>Wilson disease</td>
<td>H1069Q mutation</td>
<td>ER retention</td>
<td>(Payne et al., 1998)</td>
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<td>ABCA1</td>
<td>Tangier disease</td>
<td>Mutations</td>
<td>Loss of plasma membrane localization</td>
<td>(Tanaka et al., 2003)</td>
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<td>Tau</td>
<td>Neurodegenerative diseases</td>
<td>Hyperphosphorylation</td>
<td>Mislocalization to dendritic spines</td>
<td>(Hoover et al., 2010)</td>
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<td>TARDBP</td>
<td>ALS and FTLD</td>
<td>Unknown</td>
<td>Cytoplasmic mislocalization</td>
<td>(Winton et al., 2008)</td>
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<td>FUS</td>
<td>FTLD</td>
<td>Mutations</td>
<td>Cytoplasmic mislocalization</td>
<td>(Vance et al., 2009)</td>
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<tr>
<td>FOXO</td>
<td>Various types of cancer</td>
<td>Post-translational modifications</td>
<td>Cytoplasmic mislocalization</td>
<td>(Dassen and Burgering, 2008)</td>
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<tr>
<td>p53</td>
<td>Various types of cancer</td>
<td>Mutations, post-translational modifications</td>
<td>Cytoplasm</td>
<td>(Fabbro and Henderson, 2003)</td>
</tr>
</tbody>
</table>

APECEED, autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy; ALS, amyotrophic lateral sclerosis; FTLD, frontotemporal lobar degeneration; TRPS, trichorhinophalangeal syndrome; XLAG, X-linked lissencephaly with absent corpus callosum and ambiguous genitalia.

Mien-Chie Hung, and Wolfgang Link J Cell Sci
2011;124:3381-3392
Despite progress in human reproductive biology, the cause of male infertility often remains unknown, due to the lack of appropriate and convenient *in vitro* models of meiosis. Induced pluripotent stem cells (iPSCs) derived from the cells of infertile patients could provide a gold standard model for generating primordial germ cells and studying their development and the process of spermatogenesis. We report the characterization of a complex chromosomal rearrangement (CCR) in an azoospermic patient, and the successful generation of specific-iPSCs from PBMC-derived erythroblasts. The CCR was characterized by karyotype, fluorescence *in situ* hybridization and oligonucleotide-based array- comparative genomic hybridization. The CCR included five breakpoints and was caused by the inverted insertion of a chromosome 12 segment into the short arm of one chromosome 7 and a pericentric inversion of the structurally rearranged chromosome 12. Gene mapping of the breakpoints led to the identification of a candidate gene, *SYCP3*. Erythroblasts from the patient were reprogrammed with Sendai virus vectors to generate iPSCs. We assessed iPSC pluripotency by RT-PCR, immunofluorescence staining and teratoma induction. The generation of specific-iPSCs from patients with a CCR provides a valuable *in vitro* genetic model for studying the mechanisms by which chromosomal abnormalities alter meiosis and germ cell development.
In vitro differentiation of human embryonic stem cells into ovarian follicle-like cells

Dajung Jung1*, Jie Xiong1*, Min Ye1*, Xunsi Qin2*, Lin Li1,2, Shunfeng Cheng2, Mengyuan Luo1, Ji Peng3, Ji Dong6, Fuchou Tang6, Wei Shen2, Martin M. Matzuk4,5,7,8,9 & Kehkooi Kee1,3,10

Understanding the unique mechanisms of human oogenesis necessitates the development of an in vitro system of stem cell differentiation into oocytes. Specialized cell types and organoids have been derived from human pluripotent stem cells in vitro, but generating a human ovarian follicle remains a challenge. Here we report that human embryonic stem cells can be induced to differentiate into ovarian follicle-like cells (FLCs) in vitro. First, we find that two RNA-binding proteins specifically expressed in germ cells, DAZL and BOULE, regulate the exit from pluripotency and entry into meiosis. By expressing DAZL and BOULE with recombinant human GDF9 and BMP15, these meiotic germ cells are further induced to form ovarian FLCs, including oocytes and granulosa cells. This robust in vitro differentiation system will allow the study of the unique molecular mechanisms underlying human pluripotent stem cell differentiation into late primordial germ cells, meiotic germ cells and ovarian follicles.
Reprogrammed cells relieve Parkinson's symptoms in trials

Monkeys implanted with neurons derived from stem cells showed sustained improvement after two years.

Ewen Callaway 30 August 2017

Japanese researchers report promising results from an experimental therapy for Parkinson's disease that involves implanting neurons made from 'reprogrammed' stem cells into the brain. A trial conducted in monkeys with a version of the disease showed that the treatment improved their symptoms and seemed to be safe, according to a report published on 30 August in Nature.¹

The study's key finding — that the implanted cells survived in the brain for at least two years without causing any dangerous effects in the body — provides a major boost to researchers' hopes of testing stem-cell treatments for Parkinson's in humans, say scientists.

Jun Takahashi, a stem-cell scientist at Kyoto University in Japan who led the study, says that his team plans to begin transplanting neurons made from induced pluripotent stem (iPS) cells into people with Parkinson's in clinical trials soon.

A depletion of brain cells that produce dopamine is responsible for the mobility problems seen in people with Parkinson's disease.
“We tell no one.”