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Mitochondrial Replacement Techniques: Divergence in Global Policy

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In 2015, the UK became the first country permitting the clinical application of mitochondrial replacement techniques (MRT). Here, we explore how MRT have led to diverging international policy. In response, we recommend focused regulatory efforts coupled with United Nations (UN) leadership to build international consensus on the future of MRT.

Introduction

Mutant mitochondrial DNA (mtDNA) is associated with a variety of clinically heritable syndromes resulting in a range of debilitating clinical conditions [1–4]. Over 250 mtDNA mutations have been identified to be associated with a broad phenotypic spectrum, ranging from mild myopathy to devastating multisystem syndromes [5,6].

Therapeutic interventions are limited. However, recent scientific advances now carry the potential to reduce disease transmission using germline engineering techniques applied to assisted reproductive technology (ART). One such process utilizes a specialized technique where the nucleus from the intended parent's egg is extracted and inserted into the cytoplasm of a donor's egg with healthy mitochondria (with its nucleus removed), producing a hybrid egg with healthy mitochondria for fertilization, known as MRT [1,7].

Concerns about MRT

While the goal of MRT is therapeutic, the social and market forces that drive adoption could result in misuse or abuse of MRT for nontherapeutic reproductive uses beyond its intended use. The field of mitochondrial genetics is also characterized by intricacies that make predicting behavior of mtDNA difficult and uncertain. Inherent complexities associated with mitochondrial genetics include: heteroplasmy (threshold effect and mitotic segregation); mtDNA bottleneck; and mtDNA evolutionary theory [mtDNA–nuclear (n)DNA mismatch] [8,9].

In addition, there are several uncertainties in establishing the safety and efficacy of MRT before human use, such as: limitations of current disease animal and *in vitro* model studies; lack of clinical data; and the potential adverse effects of reagents and manipulations used in MRT on the future child [8,10–12]. Collectively, these factors make it hard to predict how MRT will work in humans and their offspring [8].

Despite myriad risks and uncertainties, MRT represents a promising option that could mitigate the risk of mtDNA disease transmission for parents who seek a healthy, genetically related child. Hence, the multigenerational impact of MRT and its precedent as a form of human germline modification, albeit narrow in application and with the specific purpose of preventing potentially life-threatening diseases, has sparked international debate [13].

Policy Responses

UK

In 2008, the Human Fertilisation and Embryology Act 1990 (HFE Act) of the UK was amended, allowing for regulations permitting techniques altering mtDNA of an egg and/or embryo used in conception, to prevent the transmission of serious mitochondrial diseases [14]. Subsequently, in 2014, the Human Fertilization & Embryology Authority (HFEA), which oversees the use of gametes and embryos in fertility treatment and/or research in the UK, concluded consultation on MRT and advised the introduction of new mitochondrial donation regulations [15]. In October 2015, the UK Parliament approved regulations permitting the licensed clinical use of spindle transfer and pronuclear transfer, two techniques used in MRT [16,17].

The decision by the UK to enact legislation permitting mitochondrial donation was based on the prospect of reducing harm to offspring born with mitochondrial disease and mitigating the devastating effects on families, including premature death of children, painful debilitating and disabling conditions, long-term ill-health, and lower quality of life [18]. However, before MRT can transition from lab to clinic, HFEA will need to implement a licensing framework that will assess MRT clinics on a case-by-case basis based upon the applicant's staff, skill and/or experience, equipment, and general clinical environment, with special consideration given to clinics conducting follow-up of offspring [15].

Furthermore, new applications have to await satisfactory results based on three outstanding safety and efficacy reports before receiving approval from the HFEA Board and an independent expert panel [19]. Following approval, it is estimated that the first MRT services, within the context of a clinical study, will enroll ten eligible applicants per year to generate the observational data necessary to assess safety and efficacy [13].

Hence, despite the fact that MRT has yet to be proven safe and effective in humans and concerns remain regarding its safety, risks, and benefits, the UK has nevertheless decided to move forward on MRT, given its potential to prevent the transmission of mitochondrial diseases.

USA

To date, the USA has no federal legislation specifically addressing human genetic modification (including germline and/or somatic cells). In the USA, both the FDA

and Recombinant DNA Advisory Committee (RAC) of the National Institute of Health (NIH) have a key role in current federal oversight of gene transfer research. Although the NIH provides oversight of gene-transfer technologies and funding, as a matter of policy, they do not review any research proposals that seek to modify gametes or embryos [20].

Relatedly, the remit of the FDA is to monitor the safety and effectiveness of gene-transfer products. However, its authority is limited to regulating claims for germline therapy products used on human subjects, not the product themselves. In the case of MRT, the 'product' is the gamete and early embryo; therefore, MRT does not specifically fall under the current regulatory scope of the FDA, which is focused on market approval for drugs, devices, and biologics.

In February 2014, the Cell, Tissue, and Gene Therapies Advisory Committee of the FDA held its first workshop to assess existing evidence in support of human clinical trials to prevent mitochondrial disease [21]. However, the advisory committee was only charged with assessing the level of necessary preclinical data for possible clinical approval, potential health risks for trial participants, and how to mitigate patient safety issues associated with mitochondrial manipulation. The FDA expert panel concluded that it was best to further delay MRT approval until more preclinical data were available [1,21].

At the request of the FDA, the National Academies of Sciences (NAS) assembled an expert committee to consider the ethical, social, and policy issues associated with MRT. The committee issued its final report in February 2016, concluding that MRT is ethically permissible if conditions are met to minimize the risk of harm to MRT offspring [22]. It recommended that MRT should only be allowed in women who carry a pregnancy and are specifically at risk of transmitting a severe mitochondrial genetic disease that could lead to early death or substantial impairment. It also concluded that MRT should only create male embryos to prevent the inheritance of modified mitochondria, and that there should be standardization of MRT protocols and long-term collection and disclosure of data from children born from MRT [22].

To date, the FDA is reviewing the NAS report and will not comment on future MRT plans [23]. This approach differs from the UK, reflecting divergent policy prioritization and a different risk tolerance when confronted by 'dual-use' technology. Additionally, there is no Federal or State legislation pending for formal MRT approval. Instead, the FDA is taking a cautionary stance by first generating information on ethical, scientific, and health-related ramifications of MRT, which could lead to future approval in clinical trials.

International Responses

From an international perspective, there is general consensus that procedures resulting in inheritable genetic modification should be prohibited and could constitute unethical human experimentation or human rights abuse [19,24]. To date, most developed countries prohibit germline modification procedures based on existing legislation [25].

Several international instruments attempt to address germline modification, including the UNESCO Universal Declaration on the Human Genome and Human Rights, (Article 24 states that 'germline interventions' could be 'contrary to human dignity'), which questions the use of germline modification but does not explicitly call for a ban. This position is also reflected in a recent report issued by UNESCO's International Bioethics Committee that called for an international moratorium on genome editing and argued that interventions should be limited to 'preventive, diagnostic, and therapeutic reasons and without enacting modifications for descendants'.

For European countries, the 1999 Oviedo Convention is the first legally binding international text designed to preserve human dignity, rights, and freedoms, through a series of principles and prohibitions against the misuse of biological and medical advances. The treaty, signed by most European states, only allows genetic engineering for preventive, diagnostic, or therapeutic reasons and only if it does not change the genetic make-up of a person's descendants [26]. The 2001 EU Directive on clinical trials also states: 'No gene therapy trials may be carried out which result in modifications to the subject's germline genetic identity' [27].

Several members of the Parliamentary Assembly of the Council of Europe (PACE), which is an international body charged with upholding human rights and ethical standards, have also signed Written Declaration No. 557 that criticizes the decision of the UK to allow MRT [28]. Signatories of the Declaration believe that this experimental technique constitutes a violation of human dignity based on international human rights law [28].

Hence, the range of responses at different national and regional levels indicates that there is policy divergence around MRT. The UK is moving forward with approval of MRT, the USA is taking a more cautionary approach, and most of Europe supports a ban of germline modification and/or germline engineering in humans, which includes the use of MRT. Importantly, divergence indicates that needed international consensus on the future use and regulation of MRT remains underdeveloped.

Future Directions

The 'transgressive' qualities of MRT have resulted in different policy, legal, and regulatory responses at national, regional, and international levels of governance [1,29]. In fact, a recent study that surveyed 39 countries found that 29 banned germline gene modification through either legislation or guidelines, with the remaining nine countries categorized as 'ambiguous' or 'restrictive' [25]. Relatedly, a separate study found that, even in countries

Table 1. Potential Advantages of Establishing Dedicated Regulatory Agencies and UN Engagement on MRT

Description	Potential Benefits	Possible Limitations
Establishment of dedicated domestic regulatory agencies and/or structures whose specific remit is to oversee genetic engineering technologies that impact human health	**Centralize:** centralize decision-making and regulatory policy development in one single agency instead of across multiple agencies	Challenges to establishing dedicated regulatory include: lack of sufficient resources, absence of a legal mandate and/or framework in certain countries, issues of regulatory maturity, and conflict between existing laws and/or legislation addressing genetic engineering. Furthermore, even if such dedicated agencies exist, policy-making may nevertheless diverge as national regulatory bodies pursue development of domestic regulations tailored to meet their own national interests, leading to lack of international harmonization, similar to challenges faced by ART and even in the pharmaceutical industry
	Policy coherence: establish domestic policy coherence across other national health, clinical, and science agencies (such as the NIH and FDA)	
	Single point of contact: provide a single and dedicated single point-of-contact for regulatory guidance, research, funding issues, and monitoring and evaluation of mtDNA transfer techniques either in experimental or clinical settings	
	Expertise: by staffing agency with the appropriate mix of experts who can dynamically address the attendant social, ethical, and philosophical issues posed by these technologies, and promote a national conversation about what constitutes acceptable use of mitochondrial transfer with patients, clinicians, and the broader scientific community	
Greater involvement, coordination and support by the UN	**International coordination:** establish broader cooperation and coordination across a larger group of stakeholders (member states, international organizations, private sector, civil society, and patient advocates), including cooperation among UN specialized agencies, programs, and instruments active on cross-cutting issues of biotechnology and human health issues (e.g., WHO, ICGEB, UNESCO, CBD, and WIPO)	International coordination through UN bodies such as the BIP may require significant resource allocation to set up meetings, workshops, summits, follow-up, and other dialog, that may not necessarily translate to policy making or formal international negotiations. Deliberations may also be 'member state' centric, focusing on issues of interest to state parties versus interests of the private or academic sectors. Furthermore, even in the event that BIP deliberations result in international negotiations, treaty instruments are expensive to negotiate and may not lead to state adoption (e.g., the 2005 UN Declaration on Human Cloning is nonbinding and was not supported by all Member States, although it has been translated into national legislation by several countries). Finally, the BIP would likely require remobilization and broader stakeholder support and/or financing to represent a viable forum for addressing MRT and other issues related to biotechnology and human health
	Multi-stakeholder consensus: establish a forum to build multi-stakeholder consensus on the development of international norms, rules, and principles related to MRT and other dual-use biomedical technologies	
	Technical assistance and policy formulation: clarify the applicability of existing international laws and governance mechanisms in the context of development of future MRT policies by countries and act as a consensus-building body to support international negotiations or policy development at the national level	
	Capacity building: aid individual countries in developing capacity for regulatory and legal systems to address MRT in a way that is responsive to their domestic and societal needs, but that also adheres to certain agreed upon international principles	
	Preliminary agenda: as a first step towards building international cooperation, the BIP could pursue an agenda that: (i) convenes a panel of experts to collate scientific evidence to develop evidence-based multi-stakeholder consensus on the future clinical application of MRT co-chaired by WHO and UNESCO IBC; (ii) conducts a detailed comparative assessment of different domestic, regional, and international laws impacting MRT; and (iii) identifies critical global policy issues (such as the risks of MRT fertility tourism) that require special attention to ensure the integrity of reproductive science, human rights, and patient safety	

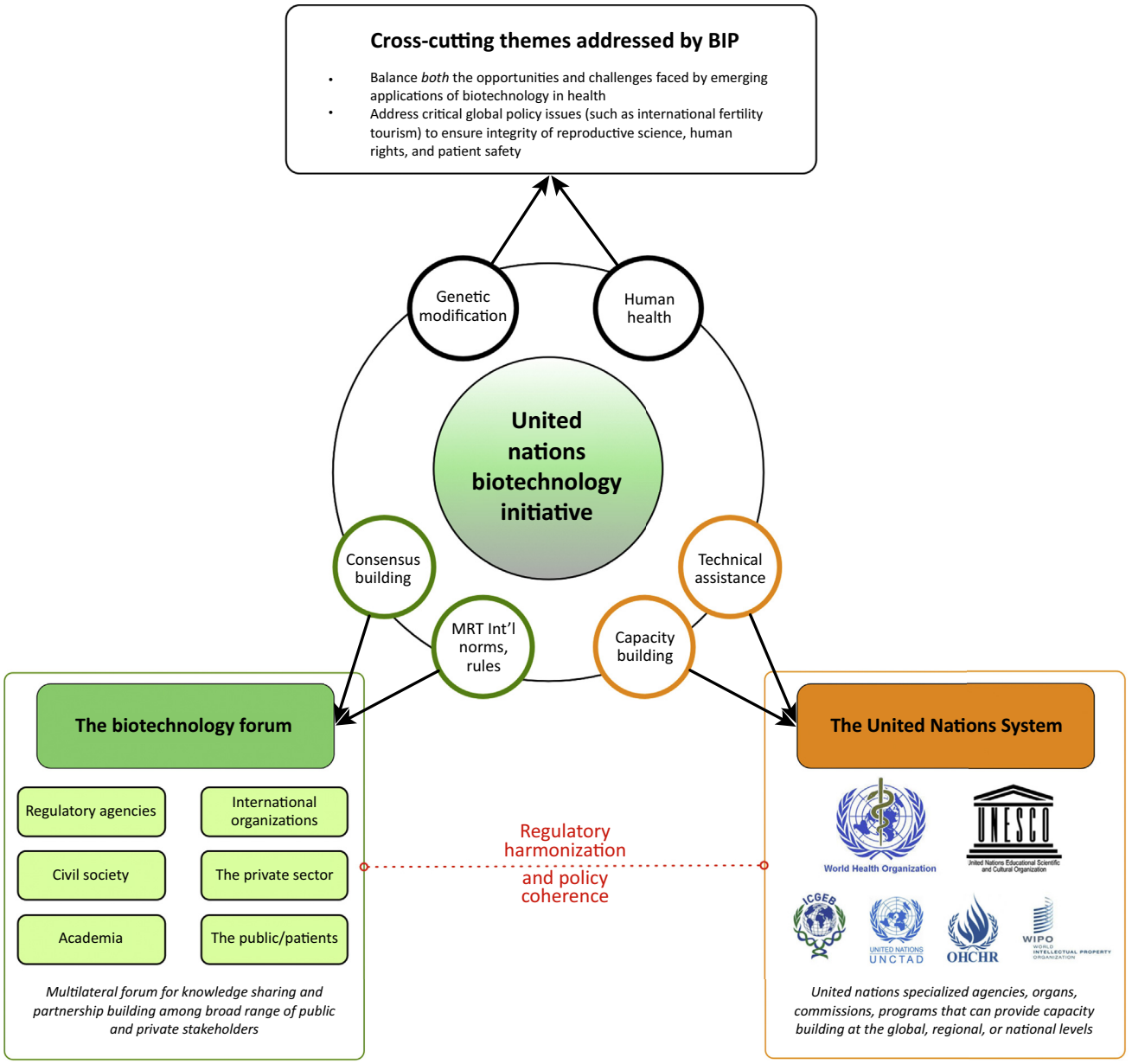

Figure 1. Illustration of the Biotechnology Initiative Program (BIP)-Led Policy Proposal on Mitochondrial Replacement Techniques (MRT).

that permit human embryonic stem cell research, human germline modifications remained largely forbidden [17]. This indicates that most countries prohibit genetic modification of the human germline for reproductive purposes, although may do so for different policy reasons (e.g., risks to offspring and/or future generations, concerns regarding use for eugenics and genetic enhancement, and breaching natural and human rights law) [30].

Policy divergence on MRT could also have broader ethical and safety implications for the unregulated growth of medical tourism internationally [31]. Approval of MRT by the UK could incentivize less-regulated markets to develop their own MRT industries to meet demand of candidates who do not qualify under HFEA. In this sense, MRT legalization could further exacerbate current regulatory, legal, and ethical challenges faced by the globalization of 'fertility tourism', which in some cases has led to the exploitation of donors, surrogates, and the offspring themselves [32].

In response, promoting the establishment of dedicated regulatory agencies whose specific remit is to oversee genetic engineering technologies that impact human health may be a viable response [1]. These agencies exist in countries such as the UK and Canada, and could be extended to other jurisdictions, but also face their own challenges [33]. Organizations such as the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, could also work to harmonize regulations on gene therapy products in partnership with national regulatory agencies [34].

As an example, HFEA is required to screen MRT candidates and reconvene its panel of experts to continually review the latest evidence on the safety and efficacy of MRT [35]. Hence, HFEA represents a dedicated regulatory structure with targeted expertise and technical capacity on the issue, and one that should have the capability to re-evaluate the impact of evidence on policy, allowing for 'adaptive' scientific policymaking given the changing and unknown nature of this technology [1].

More broadly, MRT and the advent of other dual-use biomedical technologies, such as CRISPR/Cas genome-editing technology, stem cell therapy, direct-to-consumer genetic testing, and other emerging innovations (including synthetic genomics and biology, and its potential as a 'dual-use research of concern'), arguably requires greater involvement by intergovernmental organizations to support needed capacity building and promote regulatory harmonization [36–39]. A possible forum for international coordination on human health and genetic engineering is the Biotechnology Initiative Program (BIP).

The BIP is coordinated by the Strategic Planning Unit of the Executive Office of the UN Secretary-General, and already has the appropriate remit (charged with exploring the specific themes of genetic modification, synthetic biology, and human health) to tackle the complex policy, ethics, and technology diffusion issues characterized by MRT. The BIP also operates a suitable multi-stakeholder forum (through its Biotechnology Forum, a multilateral program for discussion, knowledge sharing, and partnership building) to frame future international discussions regarding the clinical application of genetic modification techniques.

Hence, with its operational mandate to balance both the opportunities and challenges faced by emerging applications of biotechnology in human health, the BIP may be well situated to build policy coherence across key UN stakeholders (e.g., WHO, International Centre for Genetic Engineering and Biotechnology, UNESCO, and the Office of the High Commissioner for Human Rights) as well as foster cooperation among broader public and private sector actors.

We outline the strengths and weaknesses of the targeted regulatory and BIP approach in Table 1 and also describe the general structure of the BIP in Figure 1.

Concluding Remarks

With the UK now fully embarking on its policy experiment of supporting MRT for clinical use, other countries will now be forced to confront how they regulate this controversial form of ART. Hence, the ongoing MRT debate represents a potential catalyst for needed international dialog on human germline engineering technologies that impact human health. Critically, appropriately addressing MRT represents an opportunity for the international community to develop governance structures desperately needed to determine the future role of this technology and a host of other genetic and biomedical innovations looming on the horizon.

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References

1. Cohen, I.G. *et al.* (2015) Medicine. Transatlantic lessons in regulation of mitochondrial replacement therapy. *Science* 348, 178–180
2. Liu, C.-S. *et al.* (2014) Delivering healthy mitochondria for the therapy of mitochondrial diseases and beyond. *Int. J. Biochem. Cell Biol.* 53, 141–146
3. Reinhardt, K. *et al.* (2013) Medicine. Mitochondrial replacement, evolution, and the clinic. *Science* 341, 1345–1346
4. Tanaka, A.J. *et al.* (2013) Harnessing the stem cell potential: the path to prevent mitochondrial disease. *Nat. Med.* 19, 1578–1579
5. Dhillon, V.S. and Fenech, M. (2014) Mutations that affect mitochondrial functions and their association with neurodegenerative diseases. *Mutat. Res. Rev. Mutat. Res.* 759, 1–13
6. Grady, J.P. *et al.* (2014) Disease progression in patients with single, large-scale mitochondrial DNA deletions. *Brain* 137, 323–334
7. Chiaratti, M.R. *et al.* (2011) Therapeutic treatments of mtDNA diseases at the earliest stages of human development. *Mitochondrion* 11, 820–828
8. Claiborne, A.B. *et al.* (2016) Finding an ethical path forward for mitochondrial replacement. *Science* 351, 668–670
9. Wolf, D.P. *et al.* (2015) Mitochondrial replacement therapy in reproductive medicine. *Trends Mol. Med.* 21, 68–76
10. Morrow, E.H. *et al.* (2015) Risks inherent to mitochondrial replacement. *EMBO Rep.* 16, 541–544
11. He, Y. *et al.* (2010) Heteroplasmic mitochondrial DNA mutations in normal and tumour cells. *Nature* 464, 610–614
12. Sato, A. *et al.* (2005) Gene therapy for progeny of mito-mice carrying pathogenic mtDNA by nuclear transplantation. *Proc. Natl. Acad. Sci. U.S.A.* 102, 16765–16770
13. Klitzman, R. *et al.* (2015) Controversies concerning mitochondrial replacement therapy. *Fertil. Steril.* 103, 344–346
14. HFEA (2013) *Mitochondria Replacement Consultation: Advice to Government,* HFEA
15. HFEA (2015) *World First as Mitochondrial Donation Regulations Come into Force,* HFEA
16. Isasi, R.M. *et al.* (2006) *National Regulatory Frameworks Regarding Human Genetic Modification Technologies,* Université de Montréal CP
17. Ishii, T. (2014) Potential impact of human mitochondrial replacement on global policy regarding germline gene modification. *Reprod. Biomed. Online* 29, 150–155
18. Department of Health (2014) *Serious Mitochondrial Disease: New Techniques to Prevent Transmission,* Department of Health
19. Center for Genetics and Society (2015) *Human Germline Modification Summary of National and International Policies,* Center for Genetics and Society
20. Baruch, S. *et al.* (2005) *Human Germline Genetic Modification: Issues and Options for Policymakers,* Pew Charitable Trusts
21. FDA (2014) *Oocyte Modification in Assisted Reproduction for the Prevention of Transmission of Mitochondrial Disease or Treatment of Infertility,* FDA
22. Anon (2016) *Ethical and Social Policy Considerations of Novel Techniques for Prevention of Maternal Transmission of Mitochondrial DNA Diseases,* Institute of Medicine
23. Reardon, S. (2016) US panel greenlights creation of male 'three-person' embryos. *Nature* 530, 142
24. Frankel, M.S. and Hagen, B.T. (2011) *Germline Therapies: Background Paper,* Nuffield Bioethics
25. Araki, M. and Ishii, T. (2014) International regulatory landscape and integration of corrective genome editing into in vitro fertilization. *Reprod. Biol. Endocrino.* 12, 108
26. Hendriks, A. (1997) Convention for the Protection of Human Rights and Dignity of the Human Being with Regard to the

Application of Biology and Medicine. *Eur. J. Health L.* 4, 89–100

27. European Parliament (2001) *Directive 2001/20/EC of the European Parliament and of the Council,* European Parliament
28. European Dignity Watch (2013) *Parliamentarians from across Europe against UK Project to Create 3-Parent-Embryo,* European Dignity Watch
29. Darnovsky, M. (2013) A slippery slope to human germline modification. *Nat. News* 499, 127
30. Ishii, T. (2015) Germ line genome editing in clinics: the approaches, objectives and global society. *Brief. Funct. Genomics*
31. Bergmann, S. (2011) Fertility tourism: circumventive routes that enable access to reproductive technologies and substances. *Signs (Chic)* 36, 280–288
32. Ramskold, L.A.H. and Posner, M.P. (2013) Commercial surrogacy: how provisions of monetary remuneration and powers of international law can prevent exploitation of gestational surrogates. *J. Med. Ethics* 39, 397–402
33. Kelly, G. (2013) Choosing the genetics of our children: options for framing public policy. *Santa. Clara. High Tech. LJ.* 30, 303–347
34. Takefman, D. and Bryan, W. (2012) The state of gene therapies: the FDA perspective. *Mol. Ther.* 20, 877–878
35. Anon (2014) Mitochondrial manipulations. *Nat. Med.* 20, 451–452
36. Liang, B.A. and Mackey, T.K. (2012) Stem cells, dot-com. *Sci. Transl. Med.* 4, 151cm9
37. König, H. *et al.* (2013) Synthetic genomics and synthetic biology applications between hopes and concerns. *Curr. Genomics* 14, 11–24
38. Darnovsky, M. and Cussins, J. (2014) FDA halts 23andMe personal genetic tests. What might this mean for the future of direct-to-consumer testing? *MLO Med. Lab. Obs.* 46, 33
39. Charo, R.A. (2016) On the road (to a cure?) — stem-cell tourism and lessons for gene editing. *N. Engl. J. Med.* 374, 901–903

Spotlight

NORAD: Defender of the Genome

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Long non-coding RNAs (lncRNAs) are a fascinating, but still largely uncharacterized, class of genes. A recent paper by the Mendell group identifies NORAD, a novel lncRNA that is regulated in response to DNA damage and plays a key role in maintaining genome integrity by modulating the activity the RNA binding proteins PUM2 and PUM1.

The non-coding fraction of the human genome has been the subject of intense scrutiny over the past two decades. This interest was ignited by the discovery of miRNAs and fueled by the identification of other classes of short non-coding RNAs, including piRNAs, endogenous siRNAs, and circular RNAs.

Members of another class of non-coding RNAs – long non-coding RNAs (lncRNAs) – have proven more difficult to study, in part because of their heterogeneity, relatively poor conservation, and generally low abundance [1]. In fact, only a few lncRNAs have been extensively characterized, and the functional relevance of the vast majority of annotated mammalian lncRNAs, if any, remains unclear.

A recent paper by the Mendell group now provides strong evidence that lncRNAs can act as regulators of genome stability, substantially raising the bar for future studies [2]. To discover lncRNAs implicated in the DNA damage response, Lee and colleagues mined a previously published dataset of murine lncRNAs induced by doxorubicin in a p53-dependent manner [3]. One of them, a 4.9-kb transcript annotated as *2900097C17Rik*, caught their attention because of its unusually high abundance (>300 copies per cell) and conservation (~65% nucleotides identity with its human ortholog, *LINC00657*).

The finding that the human ortholog, renamed *NORAD* by Lee and colleagues, is induced in response to DNA-damaging agents prompted the authors to further investigate its functions. To do so, they took advantage of genome-editing methods to generate a NORAD-deficient human cancer cell line. Creating true loss-of-function alleles of lncRNAs is not a trivial task because, by contrast to protein coding genes, there is no reading frame that can be disrupted. Simply deleting the entire locus is not an ideal alternative as it might result in the disruption of DNA regulatory elements that can affect nearby genes. Lee and colleagues overcame these issues by using TALEN-based genome editing to place a floxed transcriptional STOP cassette within the first 300 nt of NORAD. This allowed them not only to abolish transcription of NORAD without deleting any genomic sequence but also to subsequently revert to a wild-type configuration using the Cre recombinase.

So what did Lee and colleagues find? Although the canonical p53-dependent response was not affected in NORAD$^{-/-}$ cells, these cells exhibited a marked chromosomal instability (CIN), characterized by a tendency to lose and gain chromosomes and an increased frequency of spontaneous tetraploidization. Lee and colleagues went to great lengths to ensure that the phenotype was not a byproduct of the genome-editing tools used to generate the mutant alleles, or an intrinsic feature of the mismatch repair deficient HCT116 cells used in the initial experiment. For example, they inactivated NORAD in a different, non-transformed cell line and showed that in this case CIN ensued as well. But perhaps the most convincing result, made possible by the clever experimental design chosen by Lee and colleagues, was to show that the CIN phenotype could be reverted by restoring endogenous NORAD expression.

Lee and colleagues also suggest an appealing model for how NORAD works and how its loss can result in genomic instability. Figuring out how lncRNAs work poses unique challenges because they comprise a highly heterogeneous group of RNAs, whose members have different subcellular localization and multiple possible mechanisms of action. The NORAD RNA is found almost exclusively in the cytoplasm, suggesting a mechanism of action that does not involve direct regulation of transcription. In fact, multiple lines of evidence indicate that NORAD affects genomic stability through its direct interaction with Pumilio2 (PUM2) and possibly Pumilio 1 (PUM1), two RNA-binding proteins belonging to the Pumilio–Fem3-binding factor (PUF) family. PUM1 and PUM2 are known to bind to