

Genetic Editing Research literatures

Dr. Ma Hongbao, Margaret Young

Queens, New York 11418, USA, ma8080@gmail.com

Abstract: Genome editing, or genome engineering is a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism. In 2018, the common methods for such editing use engineered nucleases, or molecular scissors. These nucleases create site-specific double-strand breaks (DSBs) at desired locations in the genome. The induced double-strand breaks are repaired through nonhomologous end-joining (NHEJ) or homologous recombination (HR), resulting in targeted mutations. As of 2015 four families of engineered nucleases were used: meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector-based nucleases (TALEN), and the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system.

[Ma H, Young M. **Genetic Editing Research literatures.** *Researcher* 2018;10(3):67-93]. ISSN 1553-9865 (print); ISSN 2163-8950 (online). <http://www.sciencepub.net/researcher>. 9. doi:[10.7537/marsrj100318.09](https://doi.org/10.7537/marsrj100318.09).

Keywords: genetic editing; research; literature; DNA; life;

1. Introduction

Genome editing, or genome engineering is a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism. In 2018, the common methods for such editing use engineered nucleases, or molecular scissors. These nucleases create site-specific double-strand breaks (DSBs) at desired locations in the genome. The induced double-strand breaks are repaired through nonhomologous end-joining (NHEJ) or homologous recombination (HR), resulting in targeted mutations. As of 2015 four families of engineered nucleases were used: meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector-based nucleases (TALEN), and the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system.

2. Literatures

The following gives some recent reference papers on.

Alapati, D. and E. E. Morrissey (2017). "Gene Editing and Genetic Lung Disease. Basic Research Meets Therapeutic Application." *Am J Respir Cell Mol Biol* **56**(3): 283-290.

Although our understanding of the genetics and pathology of congenital lung diseases such as surfactant protein deficiency, cystic fibrosis, and alpha-1 antitrypsin deficiency is extensive, treatment options are lacking. Because the lung is a barrier organ in direct communication with the external environment, targeted delivery of gene corrective technologies to the respiratory system via intratracheal or intranasal routes is an attractive option for therapy. CRISPR/Cas9 gene-editing technology is a promising approach to repairing or inactivating disease-causing mutations. Recent reports have provided proof of concept by using CRISPR/Cas9 to successfully repair

or inactivate mutations in animal models of monogenic human diseases. Potential pulmonary applications of CRISPR/Cas9 gene editing include gene correction of monogenic diseases in pre- or postnatal lungs and ex vivo gene editing of patient-specific airway stem cells followed by autologous cell transplant. Strategies to enhance gene-editing efficiency and eliminate off-target effects by targeting pulmonary stem/progenitor cells and the assessment of short-term and long-term effects of gene editing are important considerations as the field advances. If methods continue to advance rapidly, CRISPR/Cas9-mediated gene editing may provide a novel opportunity to correct monogenic diseases of the respiratory system.

Alfson, K. J., et al. (2015). "Genetic Changes at the Glycoprotein Editing Site Associated With Serial Passage of Sudan Virus." *J Infect Dis* **212** Suppl 2: S295-304.

Sudan virus (SUDV), like the closely related Ebola virus (EBOV), is a filovirus that causes severe hemorrhagic disease. They both contain an RNA editing site in the glycoprotein gene that controls expression of soluble and full-length protein. We tested the consequences of cell culture passage on the genome sequence at the SUDV editing site locus and determined whether this affected virulence. Passage resulted in expansion of the SUDV editing site, similar to that observed with EBOV. We compared viruses possessing either the wild-type or expanded editing site, using a nonhuman primate model of disease. Despite differences in virus serum titer at one time point, there were no significant differences in time to death or any other measured parameter. These data imply that changes at this locus were not important for SUDV lethality.

Anant, S. and N. O. Davidson (2003). "Hydrolytic nucleoside and nucleotide deamination, and genetic instability: a possible link between RNA-editing enzymes and cancer?" *Trends Mol Med* **9**(4): 147-152.

Post-transcriptional RNA editing generates novel gene products by changing the coding sequence of the transcript from that in the genome. Two classes of RNA editing exist in mammals, each of which involves an enzymatic deamination. These reactions have stringent sequence and structural requirements for their target RNAs, and each requires distinctive enzymatic machinery. Alterations in the expression or abundance of RNA-editing factors produce unanticipated alterations in the processing or expression of RNAs, in some cases outside their physiological targets. Recent findings suggest that unregulated expression of the cytidine-deaminase gene family might lead to deamination of deoxycytidine nucleotides in DNA. Aberrant or dysregulated RNA editing, or altered expression of editing factors, might contribute to genomic instability in cancer.

Azad, M. T. A., et al. (2017). "Site-directed RNA editing by adenosine deaminase acting on RNA for correction of the genetic code in gene therapy." *Gene Ther* **24**(12): 779-786.

Site-directed RNA editing is an important technique for correcting gene sequences and ultimately tuning protein function. In this study, we engineered the deaminase domain of adenosine deaminase acting on RNA (ADAR1) and the MS2 system to target-specific adenosines, with the goal of correcting G-to-A mutations at the RNA level. For this purpose, the ADAR1 deaminase domain was fused downstream of the RNA-binding protein MS2, which has affinity for the MS2 RNA. To direct editing to specific targets, we designed guide RNAs complementary to target RNAs. The guide RNAs directed the ADAR1 deaminase to the desired editing site, where it converted adenosine to inosine. To provide proof of principle, we used an allele of enhanced green fluorescent protein (EGFP) bearing a mutation at the 58th amino acid (TGG), encoding Trp, into an amber (TAG) or ochre (TAA) stop codon. In HEK-293 cells, our system could convert stop codons to read-through codons, thereby turning on fluorescence. We confirmed the specificity of editing at the DNA level by restriction fragment length polymorphism analysis and sequencing, and at the protein level by western blotting. The editing efficiency of this enzyme system was ~5%. We believe that this system could be used to treat genetic diseases resulting from G-to-A point mutations.

Bankamp, B., et al. (2008). "Genetic variability and mRNA editing frequencies of the phosphoprotein

genes of wild-type measles viruses." *Virus Res* **135**(2): 298-306.

The sequences of the nucleoprotein (N) and hemagglutinin (H) genes are routinely used for molecular epidemiologic studies of measles virus (MV). However, the amount of genetic diversity contained in other genes of MV has not been thoroughly evaluated. In this report, the nucleotide sequences of the phosphoprotein (P) genes from 34 wild-type strains representing 15 genotypes of MV were analyzed and found to be almost as variable as the H genes but less variable than the N genes. Deduced amino acid sequences of the three proteins encoded by the P gene, P, V and C, demonstrated considerably higher variability than the H proteins. Phylogenetic analysis showed the same tree topography for the P gene sequences as previously seen for the N and H genes. RNA editing of P gene transcripts affects the relative ratios of P and V proteins, which may have consequences for pathogenicity. Wild-type isolates produced more transcripts with more than one G insertion; however, there was no significant difference in the use of P and V open reading frames, suggesting that the relative amounts of P and V proteins in infected cells would be similar for both vaccine and wild-type strains.

Bentolila, S., et al. (2008). "Genetic architecture of mitochondrial editing in *Arabidopsis thaliana*." *Genetics* **178**(3): 1693-1708.

We have analyzed the mitochondrial editing behavior of two *Arabidopsis thaliana* accessions, Landsberg erecta (Ler) and Columbia (Col). A survey of 362 C-to-U editing sites in 33 mitochondrial genes was conducted on RNA extracted from rosette leaves. We detected 67 new editing events in *A. thaliana* rosette leaves that had not been observed in a prior study of mitochondrial editing in suspension cultures. Furthermore, 37 of the 441 C-to-U editing events reported in *A. thaliana* suspension cultures were not observed in rosette leaves. Forty editing sites that are polymorphic in extent of editing were detected between Col and Ler. Silent editing sites, which do not change the encoded amino acid, were found in a large excess compared to nonsilent sites among the editing events that differed between accessions and between tissue types. Dominance relationships were assessed for 15 of the most polymorphic sites by evaluating the editing values of the reciprocal hybrids. Dominance is more common in nonsilent sites than in silent sites, while additivity was observed only in silent sites. A maternal effect was detected for 8 sites. QTL mapping with recombinant inbred lines detected 12 major QTL for 11 of the 13 editing traits analyzed, demonstrating that efficiency of editing of individual mitochondrial C targets is generally governed by a major factor.

Bock, R. (2000). "Sense from nonsense: how the genetic information of chloroplasts is altered by RNA editing." *Biochimie* **82**(6-7): 549-557.

Plastid transcripts can be subject to an RNA processing mechanism changing the identity of individual nucleotides and thus altering the information content of the mRNA. This processing step was termed RNA editing and adds a novel mechanism to the multitude of RNA maturation events required before mRNAs can serve as faithful templates in plastid protein biosynthesis. RNA editing in chloroplasts proceeds by the conversion of individual cytidine residues to uridine and, in some bryophytes, also by the reverse event, uridine-to-cytidine transitions. The discovery of RNA editing in chloroplasts has provided researchers with a wealth of molecular and evolutionary puzzles, many of which are not yet solved. However, recent work employing chloroplast transformation technologies has shed some light on the molecular mechanisms by which RNA editing sites are recognized with extraordinarily high precision. Also, extensive phylogenetic studies have provided intriguing insights in the evolutionary dynamics with which editing sites may come and go. This review summarizes the state-of-the-art in the field of chloroplast RNA editing, discusses mechanistic and evolutionary aspects of editing and points out some of the important open questions surrounding this enigmatic RNA processing step.

Borel, F., et al. (2018). "Editing out five Serpina1 paralogs to create a mouse model of genetic emphysema." *Proc Natl Acad Sci U S A*.

Chronic obstructive pulmonary disease affects 10% of the worldwide population, and the leading genetic cause is alpha-1 antitrypsin (AAT) deficiency. Due to the complexity of the murine locus, which includes up to six Serpina1 paralogs, no genetic animal model of the disease has been successfully generated until now. Here we create a quintuple Serpina1a-e knockout using CRISPR/Cas9-mediated genome editing. The phenotype recapitulates the human disease phenotype, i.e., absence of hepatic and circulating AAT translates functionally to a reduced capacity to inhibit neutrophil elastase. With age, Serpina1 null mice develop emphysema spontaneously, which can be induced in younger mice by a lipopolysaccharide challenge. This mouse models not only AAT deficiency but also emphysema and is a relevant genetic model and not one based on developmental impairment of alveolarization or elastase administration. We anticipate that this unique model will be highly relevant not only to the preclinical development of therapeutics for AAT

deficiency, but also to emphysema and smoking research.

Bui, D. K., et al. (2015). "Genetic ME-a visualization application for merging and editing pedigrees for genetic studies." *BMC Res Notes* **8**: 241.

BACKGROUND: In order to study the genetics of diseases more accurately and effectively, one often collects large families. Different members of a large family may provide differing information about the structure and make-up of their pedigree. Thus, software is needed to facilitate reconciliation of pedigrees collected independently from multiple informants from a single large family to create a unified pedigree that is based on the best composite information available. **FINDINGS:** Our implementation demonstrates that Genetic ME performs merging in terms of adding, replacing and combining information from two pedigrees. Through a tracking process, all of the changes made to the data set for the individuals can be traced back to their original source material. A new pedigree structure can be easily visualized while reconciling disparate information from multiple pedigrees. **METHODS:** We developed the Genetic Merging & Editing (Genetic ME) program, an open source Java application built on top of CraneFoot and Ghostscript, to support comparing, editing and merging of pedigrees collected from multiple sources in a visually-oriented manner. **CONCLUSIONS:** Genetic ME constitutes an ideal addition to software packages for reconciling pedigree information from multiple sources. Genetic ME provides a friendly graphical user interface, traces the changes made by users, and produces viewable merged pedigree structures able to be further used by other popular analysis programs.

Bundo, M., et al. (2010). "Mutation screening and assessment of the effect of genetic variations on expression and RNA editing of serotonin receptor 2C in the human brain." *Psychiatry Clin Neurosci* **64**(1): 57-61.

AIM: Serotonin receptor 2C (HTR2C) has been postulated as being involved in the etiology or pathophysiology of mental disorders such as bipolar disorder, major depression and schizophrenia. We previously revealed the altered mRNA expression and RNA editing of HTR2C in the postmortem brains of patients with mental disorders. Here we examined the relationship between genetic variations and expression level or RNA editing level of HTR2C in the human brain. **METHODS:** We performed mutation screening of the HTR2C gene by sequencing all exons, exon-intron boundaries, and promoter region in the same cohort used for expression and RNA editing studies (n = 58). Using the detected genetic variations, we

examined the relationship between genetic variations and expression or RNA editing level. RESULTS AND CONCLUSION: We did not find novel mutations or single nucleotide polymorphisms that were specific to patients. Genotype and haplotype-based analyses revealed that genetic variations of HTR2C did not account for observed altered expression or RNA editing level of HTR2C in the brain.

Canal, C. E., et al. (2009). "RNA editing of the serotonin 2C receptor and expression of Galpha (q) protein: genetic mouse models do not support a role for regulation or compensation." *J Neurochem* **108**(5): 1136-1142.

The serotonin 2C (5-HT (2C)) receptor undergoes RNA editing at five bases in a region of the pre-mRNA encoding the second intracellular loop, generating many unique 5-HT (2C) receptor isoforms. Mechanisms regulating in vivo expression of different edited 5-HT (2C) receptor isoforms are poorly understood, as are the adaptive consequences of variation in editing profiles. Recent findings suggest a putative relationship between expression levels of Galpha (q/11) protein and the degree of editing of 5-HT (2C) receptor transcripts. To elucidate the potential regulatory or adaptive role of Galpha (q/11) protein levels, we quantified editing of 5-HT (2C) receptor RNA transcripts in Galpha (q) null mice and protein levels of Galpha (q) and Galpha (11) in transgenic male mice solely expressing either the non-edited (INI) or the fully edited (VGV) isoforms of the 5-HT (2C) receptor. Pyrosequencing of RNA isolated from amygdaloid cortex in Galpha (q) null and wild-type mice revealed no significant differences in 5-HT (2C) receptor mRNA editing profiles. Cortical tissue from INI/y, VGV/y, and wild-type mice was assayed for expression of Galpha (q) and Galpha (11) subunits by Western blotting. No differences in signal density between wild-type and INI/y or VGV/y groups were found, indicating equivalent levels of Galpha (q) and Galpha (11) protein. Together, these data do not support a causal or compensatory relationship between 5-HT (2C) receptor RNA editing and G (q) protein levels.

Cattaneo, R. (1990). "Messenger RNA editing and the genetic code." *Experientia* **46**(11-12): 1142-1148.

Messenger RNA editing is defined as a process leading to predetermined modifications of the coding region of a primary gene transcript. By this definition, splicing processes are special forms of editing; however, they are not dealt with in this review. Editing processes different from splicing have been defined in mammalian cells, in RNA viruses, and in mitochondria of trypanosomes, higher plants and vertebrates. These

post- or co-transcriptional processes involve addition, deletion, or modification-substitution of nucleotides, and represent previously unrecognized mechanisms for altering the coding potential of a gene and for modulating gene expression.

Chakravarti, D., et al. (2016). "Synthetic biology approaches in cancer immunotherapy, genetic network engineering, and genome editing." *Integr Biol (Camb)* **8**(4): 504-517.

Investigations into cells and their contents have provided evolving insight into the emergence of complex biological behaviors. Capitalizing on this knowledge, synthetic biology seeks to manipulate the cellular machinery towards novel purposes, extending discoveries from basic science to new applications. While these developments have demonstrated the potential of building with biological parts, the complexity of cells can pose numerous challenges. In this review, we will highlight the broad and vital role that the synthetic biology approach has played in applying fundamental biological discoveries in receptors, genetic circuits, and genome-editing systems towards translation in the fields of immunotherapy, biosensors, disease models and gene therapy. These examples are evidence of the strength of synthetic approaches, while also illustrating considerations that must be addressed when developing systems around living cells.

Collin, J. and M. Lako (2011). "Concise review: putting a finger on stem cell biology: zinc finger nuclease-driven targeted genetic editing in human pluripotent stem cells." *Stem Cells* **29**(7): 1021-1033.

Human pluripotent stem cells (hPSCs) encompassing human embryonic stem cells and human induced pluripotent stem cells (hiPSCs) have a wide appeal for numerous basic biology studies and for therapeutic applications because of their potential to give rise to almost any cell type in the human body and immense ability to self-renew. Much attention in the stem cell field is focused toward the study of gene-based anomalies relating to the causative affects of human disease and their correction with the potential for patient-specific therapies using gene corrected hiPSCs. Therefore, the genetic manipulation of stem cells is clearly important for the development of future medicine. Although successful targeted genetic engineering in hPSCs has been reported, these cases are surprisingly few because of inherent technical limitations with the methods used. The development of more robust and efficient means by which to achieve specific genomic modifications in hPSCs has far reaching implications for stem cell research and its applications. Recent proof-of-principle reports have shown that genetic alterations with minimal toxicity

are now possible through the use of zinc finger nucleases (ZFNs) and the inherent DNA repair mechanisms within the cell. In light of recent comprehensive reviews that highlight the applications, methodologies, and prospects of ZFNs, this article focuses on the application of ZFNs to stem cell biology, discussing the published work to date, potential problems, and future uses for this technology both experimentally and therapeutically.

Danilenko, N. G. (2001). "[RNA-editing: genetic information is corrected after transcription]." *Genetika* **37**(3): 294-316.

RNA editing (posttranscriptional alterations to messenger RNAs) is revealed in different genetic systems, primarily, in mitochondria and chloroplasts. In this review, we discuss the prevalence of the process, characteristic features in different taxonomic groups, specificity, biological implication, and possible evolutionary origin of this process. This is the first review of RNA editing in Russian.

Di Giulio, M., et al. (2014). "RNA editing and modifications of RNAs might have favoured the evolution of the triplet genetic code from an ennuplet code." *J Theor Biol* **359**: 1-5.

Here we suggest that the origin of the genetic code, that is to say, the birth of first mRNAs has been triggered by means of a widespread modification of all RNAs (proto-mRNAs and proto-tRNAs), as today observed in the RNA editing and in post-transcriptional modifications of RNAs, which are considered as fossils of this evolutionary stage of the genetic code origin. We consider also that other mechanisms, such as the trans-translation and ribosome frameshifting, could have favoured the transition from an ennuplet code to a triplet code. Therefore, according to our hypothesis all these mechanisms would be reflexive of this period of the evolutionary history of the genetic code.

Gonen, S., et al. (2017). "Potential of gene drives with genome editing to increase genetic gain in livestock breeding programs." *Genet Sel Evol* **49**(1): 3.

BACKGROUND: This paper uses simulation to explore how gene drives can increase genetic gain in livestock breeding programs. Gene drives are naturally occurring phenomena that cause a mutation on one chromosome to copy itself onto its homologous chromosome. **METHODS:** We simulated nine different breeding and editing scenarios with a common overall structure. Each scenario began with 21 generations of selection, followed by 20 generations of selection based on true breeding values where the breeder used selection alone, selection in combination with genome editing, or selection with

genome editing and gene drives. In the scenarios that used gene drives, we varied the probability of successfully incorporating the gene drive. For each scenario, we evaluated genetic gain, genetic variance [Formula: see text], rate of change in inbreeding ([Formula: see text]), number of distinct quantitative trait nucleotides (QTN) edited, rate of increase in favourable allele frequencies of edited QTN and the time to fix favourable alleles. **RESULTS:** Gene drives enhanced the benefits of genome editing in seven ways: (1) they amplified the increase in genetic gain brought about by genome editing; (2) they amplified the rate of increase in the frequency of favourable alleles and reduced the time it took to fix them; (3) they enabled more rapid targeting of QTN with lesser effect for genome editing; (4) they distributed fixed editing resources across a larger number of distinct QTN across generations; (5) they focussed editing on a smaller number of QTN within a given generation; (6) they reduced the level of inbreeding when editing a subset of the sires; and (7) they increased the efficiency of converting genetic variation into genetic gain. **CONCLUSIONS:** Genome editing in livestock breeding results in short-, medium- and long-term increases in genetic gain. The increase in genetic gain occurs because editing increases the frequency of favourable alleles in the population. Gene drives accelerate the increase in allele frequency caused by editing, which results in even higher genetic gain over a shorter period of time with no impact on inbreeding.

Gu, T., et al. (2016). "Genetic Architectures of Quantitative Variation in RNA Editing Pathways." *Genetics* **202**(2): 787-798.

RNA editing refers to post-transcriptional processes that alter the base sequence of RNA. Recently, hundreds of new RNA editing targets have been reported. However, the mechanisms that determine the specificity and degree of editing are not well understood. We examined quantitative variation of site-specific editing in a genetically diverse multiparent population, Diversity Outbred mice, and mapped polymorphic loci that alter editing ratios globally for C-to-U editing and at specific sites for A-to-I editing. An allelic series in the C-to-U editing enzyme Apobec1 influences the editing efficiency of Apob and 58 additional C-to-U editing targets. We identified 49 A-to-I editing sites with polymorphisms in the edited transcript that alter editing efficiency. In contrast to the shared genetic control of C-to-U editing, most of the variable A-to-I editing sites were determined by local nucleotide polymorphisms in proximity to the editing site in the RNA secondary structure. Our results indicate that RNA editing is a quantitative trait subject to genetic variation and that evolutionary constraints have given rise to distinct

genetic architectures in the two canonical types of RNA editing.

Gupta, R. M. and K. Musunuru (2014). "Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9." J Clin Invest **124**(10): 4154-4161.

The past decade has been one of rapid innovation in genome-editing technology. The opportunity now exists for investigators to manipulate virtually any gene in a diverse range of cell types and organisms with targeted nucleases designed with sequence-specific DNA-binding domains. The rapid development of the field has allowed for highly efficient, precise, and now cost-effective means by which to generate human and animal models of disease using these technologies. This review will outline the recent development of genome-editing technology, culminating with the use of CRISPR-Cas9 to generate novel mammalian models of disease. While the road to using this same technology for treatment of human disease is long, the pace of innovation over the past five years and early successes in model systems build anticipation for this prospect.

Hajjar, R. J., et al. (2003). "Genetic editing of dysfunctional myocardium." Med Clin North Am **87**(2): 553-567.

Ongoing advances in vector technology, cardiac gene delivery, and, most importantly, our understanding of HF pathogenesis, encourage consideration of gene therapy for HF at this time. At the present time, strategies that enhance sarcoplasmic calcium transport are supported by substantial evidence in both cardiomyocytes derived from patients with HF and in animal models. In addition, efforts to promote cardiomyocyte survival and function through modulation of antiapoptotic signaling appear quite promising. In ongoing efforts to target cardiac dysfunction, gene transfer provides an important tool to improve our understanding of the relative contribution of specific pathways. Through such experiments, molecular targets can be validated for therapeutic intervention, whether pharmacologic or genetic. Translating these basic investigations into clinical gene therapy for HF, however, remains a formidable challenge. Further development of concepts established in rodent models will be required in large animal models with clinical grade vectors and delivery systems to evaluate both efficacy and safety of these approaches. Nevertheless, practical advances and our growing understanding of the molecular pathogenesis of HF provide reason for cautious optimism.

Hassan, M. A., et al. (2014). "The genetic basis for individual differences in mRNA splicing and APOBEC1 editing activity in murine macrophages." Genome Res **24**(3): 377-389.

Alternative splicing and mRNA editing are known to contribute to transcriptome diversity. Although alternative splicing is pervasive and contributes to a variety of pathologies, including cancer, the genetic context for individual differences in isoform usage is still evolving. Similarly, although mRNA editing is ubiquitous and associated with important biological processes such as intracellular viral replication and cancer development, individual variations in mRNA editing and the genetic transmissibility of mRNA editing are equivocal. Here, we have used linkage analysis to show that both mRNA editing and alternative splicing are regulated by the macrophage genetic background and environmental cues. We show that distinct loci, potentially harboring variable splice factors, regulate the splicing of multiple transcripts. Additionally, we show that individual genetic variability at the Apobec1 locus results in differential rates of C-to-U (T) editing in murine macrophages; with mouse strains expressing mostly a truncated alternative transcript isoform of Apobec1 exhibiting lower rates of editing. As a proof of concept, we have used linkage analysis to identify 36 high-confidence novel edited sites. These results provide a novel and complementary method that can be used to identify C-to-U editing sites in individuals segregating at specific loci and show that, beyond DNA sequence and structural changes, differential isoform usage and mRNA editing can contribute to intra-species genomic and phenotypic diversity.

Hassan, M. A. and J. P. Saeij (2014). "Incorporating alternative splicing and mRNA editing into the genetic analysis of complex traits." Bioessays **36**(11): 1032-1040.

The nomination of candidate genes underlying complex traits is often focused on genetic variations that alter mRNA abundance or result in non-conservative changes in amino acids. Although inconspicuous in complex trait analysis, genetic variants that affect splicing or RNA editing can also generate proteomic diversity and impact genetic traits. Indeed, it is known that splicing and RNA editing modulate several traits in humans and model organisms. Using high-throughput RNA sequencing (RNA-seq) analysis, it is now possible to integrate the genetics of transcript abundance, alternative splicing (AS) and editing with the analysis of complex traits. We recently demonstrated that both AS and mRNA editing are modulated by genetic and environmental factors, and potentially engender phenotypic diversity in a genetically segregating mouse population.

Therefore, the analysis of splicing and RNA editing can expand not only the regulatory landscape of transcriptome and proteome complexity, but also the repertoire of candidate genes for complex traits.

Heckl, D., et al. (2014). "Generation of mouse models of myeloid malignancy with combinatorial genetic lesions using CRISPR-Cas9 genome editing." *Nat Biotechnol* **32**(9): 941-946.

Genome sequencing studies have shown that human malignancies often bear mutations in four or more driver genes, but it is difficult to recapitulate this degree of genetic complexity in mouse models using conventional breeding. Here we use the CRISPR-Cas9 system of genome editing to overcome this limitation. By delivering combinations of small guide RNAs (sgRNAs) and Cas9 with a lentiviral vector, we modified up to five genes in a single mouse hematopoietic stem cell (HSC), leading to clonal outgrowth and myeloid malignancy. We thereby generated models of acute myeloid leukemia (AML) with cooperating mutations in genes encoding epigenetic modifiers, transcription factors and mediators of cytokine signaling, recapitulating the combinations of mutations observed in patients. Our results suggest that lentivirus-delivered sgRNA:Cas9 genome editing should be useful to engineer a broad array of in vivo cancer models that better reflect the complexity of human disease.

Henry, M., et al. (2009). "Genetic editing of HBV DNA by monodomain human APOBEC3 cytidine deaminases and the recombinant nature of APOBEC3G." *PLoS One* **4**(1): e4277.

Hepatitis B virus (HBV) DNA is vulnerable to editing by human cytidine deaminases of the APOBEC3 (A3A-H) family albeit to much lower levels than HIV cDNA. We have analyzed and compared HBV editing by all seven enzymes in a quail cell line that does not produce any endogenous DNA cytidine deaminase activity. Using 3DPCR it was possible to show that all but A3DE were able to deaminate HBV DNA at levels from 10⁽⁻²⁾ to 10⁽⁻⁵⁾ in vitro, with A3A proving to be the most efficient editor. The amino terminal domain of A3G alone was completely devoid of deaminase activity to within the sensitivity of 3DPCR (approximately 10⁽⁻⁴⁾ to 10⁽⁻⁵⁾). Detailed analysis of the dinucleotide editing context showed that only A3G and A3H have strong preferences, notably CpC and TpC. A phylogenetic analysis of A3 exons revealed that A3G is in fact a chimera with the first two exons being derived from the A3F gene. This might allow co-expression of the two genes that are able to restrict HIV-1 Deltavir efficiently.

Huang, J. J., et al. (2017). "Genome editing technologies drive the development of pig genetic improvement." *Yi Chuan* **39**(11): 1078-1089.

Nuclease-mediated genome editing technologies contribute to the rapid advances in life sciences via the ability to edit the genomes within living cells, and present a new era for porcine genetic improvement. In this review, we introduce the development of various genomic editing technologies, particularly CRISPR/Cas9 strategies and characteristics of various naturally occurring and artificially engineered CRISPR enzymes. Also, we summarize progress in pig genetic improvement mediated by genome editing, especially those associated with meat quality traits and anti-virus resistance. We highlight the challenges in the implementation of pig genetic improvement and the prospects of pig genetic breeding based on genome editing technologies.

Iwamoto, K., et al. (2009). "Serotonin receptor 2C and mental disorders: genetic, expression and RNA editing studies." *RNA Biol* **6**(3): 248-253.

Serotonin receptor 2C (HTR2C) is one of the attractive candidate genes for studying pathophysiology of mental disorders. Here we overviewed the genetic, expression and RNA editing studies suggesting the close relationship between HTR2C and major mental disorders including schizophrenia, bipolar disorder and major depression. We especially focused on the human studies as well as with reference to relevant cellular and animal models. Possible significance of genetic variations affecting expression and RNA editing and appropriate animal models that mimic human mental disorders were discussed.

Jepson, J. E. and R. A. Reenan (2007). "Genetic approaches to studying adenosine-to-inosine RNA editing." *Methods Enzymol* **424**: 265-287.

Increasing proteomic diversity via the hydrolytic deamination of adenosine to inosine (A-to-I) in select mRNA templates appears crucial to the correct functioning of the nervous system in several model organisms, including *Drosophila*, *Caenorhabditis elegans*, and mice. The genome of the fruitfly, *Drosophila melanogaster*, contains a single gene encoding the enzyme responsible for deamination, termed ADAR (for adenosine deaminase acting on RNA). The mRNAs that form the substrates for ADAR primarily function in neuronal signaling, and, correspondingly, deletion of ADAR leads to severe nervous system defects. While several ADAR enzymes are present in mice, the presence of a single ADAR in *Drosophila*, combined with the diverse genetic toolkit available to researchers and the wide range of ADAR target mRNAs identified to date,

make *Drosophila* an ideal organism to study the genetic basis of A-to-I RNA editing. This chapter describes a variety of methods for genetically manipulating *Drosophila* A-to-I editing both in time and space, as well as techniques to study the molecular basis of ADAR-mRNA interactions. A prerequisite for experiments in this field is the ability to quantify the levels of editing in a given mRNA. Therefore, several commonly used methods for the quantification of editing levels will also be described.

Jobson, R. W. and Y. L. Qiu (2008). "Did RNA editing in plant organellar genomes originate under natural selection or through genetic drift?" *Biol Direct* 3: 43.

BACKGROUND: The C \leftrightarrow U substitution types of RNA editing have been observed frequently in organellar genomes of land plants. Although various attempts have been made to explain why such a seemingly inefficient genetic mechanism would have evolved, no satisfactory explanation exists in our view. In this study, we examined editing patterns in chloroplast genomes of the hornwort *Anthoceros formosae* and the fern *Adiantum capillus-veneris* and in mitochondrial genomes of the angiosperms *Arabidopsis thaliana*, *Beta vulgaris* and *Oryza sativa*, to gain an understanding of the question of how RNA editing originated. **RESULTS:** We found that 1) most editing sites were distributed at the 2nd and 1st codon positions, 2) editing affected codons that resulted in larger hydrophobicity and molecular size changes much more frequently than those with little change involved, 3) editing uniformly increased protein hydrophobicity, 4) editing occurred more frequently in ancestrally T-rich sequences, which were more abundant in genes encoding membrane-bound proteins with many hydrophobic amino acids than in genes encoding soluble proteins, and 5) editing occurred most often in genes found to be under strong selective constraint. **CONCLUSION:** These analyses show that editing mostly affects functionally important and evolutionarily conserved codon positions, codons and genes encoding membrane-bound proteins. In particular, abundance of RNA editing in plant organellar genomes may be associated with disproportionately large percentages of genes in these two genomes that encode membrane-bound proteins, which are rich in hydrophobic amino acids and selectively constrained. These data support a hypothesis that natural selection imposed by protein functional constraints has contributed to selective fixation of certain editing sites and maintenance of the editing activity in plant organelles over a period of more than four hundred millions years. The retention of genes encoding RNA editing activity may be driven by forces that shape nucleotide composition

equilibrium in two organellar genomes of these plants. Nevertheless, the causes of lineage-specific occurrence of a large portion of RNA editing sites remain to be determined.

Kang, X., et al. (2016). "Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing." *J Assist Reprod Genet* 33(5): 581-588.

PURPOSE: As a powerful technology for genome engineering, the CRISPR/Cas system has been successfully applied to modify the genomes of various species. The purpose of this study was to evaluate the technology and establish principles for the introduction of precise genetic modifications in early human embryos. **METHODS:** 3PN zygotes were injected with Cas9 messenger RNA (mRNA) (100 ng/mul) and guide RNA (gRNA) (50 ng/mul). For oligo-injections, donor oligo-1 (99 bp) or oligo-2 (99 bp) (100 ng/mul) or dsDonor (1 kb) was mixed with Cas9 mRNA (100 ng/mul) and gRNA (50 ng/mul) and injected into the embryos. **RESULTS:** By co-injecting Cas9 mRNA, gRNAs, and donor DNA, we successfully introduced the naturally occurring CCR5Delta32 allele into early human 3PN embryos. In the embryos containing the engineered CCR5Delta32 allele, however, the other alleles at the same locus could not be fully controlled because they either remained wild type or contained indel mutations. **CONCLUSIONS:** This work has implications for the development of therapeutic treatments of genetic disorders, and it demonstrates that significant technical issues remain to be addressed. We advocate preventing any application of genome editing on the human germline until after a rigorous and thorough evaluation and discussion are undertaken by the global research and ethics communities.

Kankowski, S., et al. (2017). "A Novel RNA Editing Sensor Tool and a Specific Agonist Determine Neuronal Protein Expression of RNA-Edited Glycine Receptors and Identify a Genomic APOBEC1 Dimorphism as a New Genetic Risk Factor of Epilepsy." *Front Mol Neurosci* 10: 439.

C-to-U RNA editing of glycine receptors (GlyR) can play an important role in disease progression of temporal lobe epilepsy (TLE) as it may contribute in a neuron type-specific way to neuropsychiatric symptoms of the disease. It is therefore necessary to develop tools that allow identification of neuron types that express RNA-edited GlyR protein. In this study, we identify NH4 as agonist of C-to-U RNA edited GlyRs. Furthermore, we generated a new molecular C-to-U RNA editing sensor tool that detects Apobec-1-dependent RNA editing in HEPG2 cells and rat

primary hippocampal neurons. Using this sensor combined with NH₄ application, we were able to identify C-to-U RNA editing-competent neurons and expression of C-to-U RNA-edited GlyR protein in neurons. Bioinformatic analysis of 1,000 Genome Project Phase 3 allele frequencies coding for human Apobec-1 80M and 80I variants showed differences between populations, and the results revealed a preference of the 80I variant to generate RNA-edited GlyR protein. Finally, we established a new PCR-based restriction fragment length polymorphism (RFLP) approach to profile mRNA expression with regard to the genetic APOBEC1 dimorphism of patients with intractable temporal lobe epilepsy (iTLE) and found that the patients fall into two groups. Patients with expression of the Apobec-1 80I variant mostly suffered from simple or complex partial seizures, whereas patients with 80M expression exhibited secondarily generalized seizure activity. Thus, our method allows the characterization of Apobec-1 80M and 80I variants in the brain and provides a new way to epidemiologically and semiologically classify iTLE according to the two different APOBEC1 alleles. Together, these results demonstrate Apobec-1-dependent expression of RNA-edited GlyR protein in neurons and identify the APOBEC1 80I/M-coding alleles as new genetic risk factors for iTLE patients.

Kobayashi, M., et al. (1999). "Genetic analysis of cytochrome b5 from arachidonic acid-producing fungus, *Mortierella alpina* 1S-4: cloning, RNA editing and expression of the gene in *Escherichia coli*, and purification and characterization of the gene product." *J Biochem* **125**(6): 1094-1103.

Information on the amino acid sequences of the internal peptide fragments of cytochrome b5 from *Mortierella hygrophila* was used to prepare synthetic oligonucleotides as primers for the polymerase chain reaction. A 100-base DNA fragment was thus amplified, by using a genomic gene from *Mortierella alpina* 1S-4 as a template, which produced polyunsaturated fatty acids such as arachidonic acid. The amplified DNA fragment was used as the probe to clone both a 523-base cDNA fragment and a 2.1-kilobase *SalI*-*NruI* genomic fragment coding for the whole *M. alpina* 1S-4 cytochrome b5. On the basis of nucleotide sequences of both cytochrome b5 genomic gene and cDNA, the genomic cytochrome b5 gene was found to consist of four exons and three introns. A novel type of RNA editing, in which the cDNA included either guanine insertion or adenine-->guanine substitution at one base upstream of poly (A), was interestingly observed. The deduced amino acid sequence of *M. alpina* 1S-4 cytochrome b5 showed significant similarities with those of cytochrome b5s

from other organisms such as rat, chicken, and yeast. The soluble form of the cytochrome b5 gene was expressed to 16% of the total soluble protein in *Escherichia coli*. The holo-cytochrome b5 accounted for 8% of the total cytochrome b5 in the transformants. The purified cytochrome b5 showed the oxidized and reduced absorbance spectra characteristic of fungal microsomal cytochrome b5.

Kobayashi, M., et al. (1998). "Lactone-ring-cleaving enzyme: genetic analysis, novel RNA editing, and evolutionary implications." *Proc Natl Acad Sci U S A* **95**(22): 12787-12792.

A lactonohydrolase from *Fusarium oxysporum* AKU 3702 is an enzyme catalyzing the hydrolysis of aldionate lactones to the corresponding aldonic acids. The amino acid sequences of the NH₂ terminus and internal peptide fragments of the enzyme were determined to prepare synthetic oligonucleotides as primers for the PCR. An approximate 1, 000-base genomic DNA fragment thus amplified was used as the probe to clone both genomic DNA and cDNA for the enzyme. The lactonohydrolase genomic gene consists of six exons separated by five short introns. A novel type of RNA editing, in which lactonohydrolase mRNA included the insertion of guanosine and cytidine residues, was observed. The predicted amino acid sequence of the cloned lactonohydrolase cDNA showed significant similarity to those of the gluconolactonase from *Zymomonas mobilis*, and paraoxonases from human and rabbit, forming a unique superfamily consisting of C-O cleaving enzymes and P-O cleaving enzymes. Lactonohydrolase was expressed under the control of the lac promoter in *Escherichia coli*.

Kurmangaliyev, Y. Z., et al. (2015). "Genetic Determinants of RNA Editing Levels of ADAR Targets in *Drosophila melanogaster*." *G3 (Bethesda)* **6**(2): 391-396.

RNA editing usually affects only a fraction of expressed transcripts and there is a vast amount of variation in editing levels of ADAR (adenosine deaminase, RNA-specific) targets. Here we explore natural genetic variation affecting editing levels of particular sites in 81 natural strains of *Drosophila melanogaster*. The analysis of associations between editing levels and single-nucleotide polymorphisms allows us to map putative cis-regulatory regions affecting editing of 16 A-to-I editing sites (cis-RNA editing quantitative trait loci or cis-edQTLs, $P < 10(-8)$). The observed changes in editing levels are validated by independent molecular technique. All identified regulatory variants are located in close proximity of modulated editing sites. Moreover, colocalized editing sites are often regulated by same

loci. Similar to expression and splicing QTL studies, the characterization of edQTLs will greatly expand our understanding of cis-regulatory evolution of gene expression.

Lander, N., et al. (2016). "Genome Editing by CRISPR/Cas9: A Game Change in the Genetic Manipulation of Protists." *J Eukaryot Microbiol* **63**(5): 679-690.

Genome editing by CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated gene 9) system has been transformative in biology. Originally discovered as an adaptive prokaryotic immune system, CRISPR/Cas9 has been repurposed for genome editing in a broad range of model organisms, from yeast to mammalian cells. Protist parasites are unicellular organisms producing important human diseases that affect millions of people around the world. For many of these diseases, such as malaria, Chagas disease, leishmaniasis and cryptosporidiosis, there are no effective treatments or vaccines available. The recent adaptation of the CRISPR/Cas9 technology to several protist models will be playing a key role in the functional study of their proteins, in the characterization of their metabolic pathways, and in the understanding of their biology, and will facilitate the search for new chemotherapeutic targets. In this work we review recent studies where the CRISPR/Cas9 system was adapted to protist parasites, particularly to Apicomplexans and trypanosomatids, emphasizing the different molecular strategies used for genome editing of each organism, as well as their advantages. We also discuss the potential usefulness of this technology in the green alga *Chlamydomonas reinhardtii*.

Landweber, L. F. and W. Gilbert (1993). "RNA editing as a source of genetic variation." *Nature* **363**(6425): 179-182.

Kinetoplastid RNA editing alters mitochondrial RNA transcripts by addition and deletion of uridine residues, producing open reading frames that may be twice as long as the original RNA. Although the COIII gene encoding cytochrome c oxidase subunit III in *Trypanosoma brucei* is edited along its entire length, the presumably homologous genes in two related trypanosomes, *Leishmania tarentolae* and *Crithidia fasciculata*, are only modestly edited at their 5' ends. We used a comparative approach to investigate the evolution of an edited gene and to determine how well editing creates conserved protein sequences. As RNA editing probably involves the pairing of several guide RNA molecules with the messenger RNA, we expected the edited proteins to be resistant to evolutionary change. Here we report that RNA editing is extensive in the mitochondria of four species of the

insect parasite *Herpetomonas*, which is possibly an evolutionary precursor of *T. brucei* and *L. tarentolae*, and the discovery that RNA editing is a novel source of frameshift mutations over evolutionary time. The edited proteins accumulate mutations nearly twice as rapidly as the unedited versions.

Landweber, L. F. and W. Gilbert (1994). "Phylogenetic analysis of RNA editing: a primitive genetic phenomenon." *Proc Natl Acad Sci U S A* **91**(3): 918-921.

RNA editing by extensive uridine addition and deletion creates over 90% of the amino acid codons in the cytochrome-c oxidase subunit III (COIII) transcript in *Trypanosoma brucei* and *Herpetomonas*, whereas editing of the COIII transcripts in *Leishmania tarentolae* and *Crithidia fasciculata* generates only 6% of the amino acid codons and is limited to the 5' ends. Is extensive RNA editing a primitive or derived character? We constructed a phylogenetic tree based on nuclear small-subunit and mitochondrial large- and small-subunit ribosomal RNA sequences for nine species of kinetoplastid protozoa. Our results suggest that extensive editing is a primitive genetic phenomenon that has disappeared in recent evolutionary time and also that there have been multiple losses of the digenetic lifestyle by loss of the vertebrate host in parasite evolution.

Lang, J., et al. (2016). "Receptor editing and genetic variability in human autoreactive B cells." *J Exp Med* **213**(1): 93-108.

The mechanisms by which B cells undergo tolerance, such as receptor editing, clonal deletion, and anergy, have been established in mice. However, corroborating these mechanisms in humans remains challenging. To study how autoreactive human B cells undergo tolerance, we developed a novel humanized mouse model. Mice expressing an anti-human Igkappa membrane protein to serve as a ubiquitous neo self-antigen (Ag) were transplanted with a human immune system. By following the fate of self-reactive human kappa (+) B cells relative to nonautoreactive lambda (+) cells, we show that tolerance of human B cells occurs at the first site of self-Ag encounter, the bone marrow, via a combination of receptor editing and clonal deletion. Moreover, the amount of available self-Ag and the genetics of the cord blood donor dictate the levels of central tolerance and autoreactive B cells in the periphery. Thus, this model can be useful for studying specific mechanisms of human B cell tolerance and to reveal differences in the extent of this process among human populations.

Lavrov, D. V., et al. (2013). "Mitochondrial DNA of *Clathrina clathrus* (Calcarea, Calcinea): six

linear chromosomes, fragmented rRNAs, tRNA editing, and a novel genetic code." *Mol Biol Evol* **30**(4): 865-880.

Sponges (phylum Porifera) are a large and ancient group of morphologically simple but ecologically important aquatic animals. Although their body plan and lifestyle are relatively uniform, sponges show extensive molecular and genetic diversity. In particular, mitochondrial genomes from three of the four previously studied classes of Porifera (Demospongiae, Hexactinellida, and Homoscleromorpha) have distinct gene contents, genome organizations, and evolutionary rates. Here, we report the mitochondrial genome of *Clathrina clathrus* (Calcinea, Clathrinidae), a representative of the fourth poriferan class, the Calcarea, which proves to be the most unusual. *Clathrina clathrus* mitochondrial DNA (mtDNA) consists of six linear chromosomes 7.6-9.4 kb in size and encodes at least 37 genes: 13 protein codings, 2 ribosomal RNAs (rRNAs), and 24 transfer RNAs (tRNAs). Protein genes include *atp9*, which has now been found in all major sponge lineages, but no *atp8*. Our analyses further reveal the presence of a novel genetic code that involves unique reassignments of the UAG codons from termination to tyrosine and of the CGN codons from arginine to glycine. *Clathrina clathrus* mitochondrial rRNAs are encoded in three (srRNA) and ≥ 6 (lrRNA) fragments distributed out of order and on several chromosomes. The encoded tRNAs contain multiple mismatches in the aminoacyl acceptor stems that are repaired posttranscriptionally by 3'-end RNA editing. Although our analysis does not resolve the phylogenetic position of calcareous sponges, likely due to their high rates of mitochondrial sequence evolution, it confirms mtDNA as a promising marker for population studies in this group. The combination of unusual mitochondrial features in *C. clathrus* redefines the extremes of mtDNA evolution in animals and further argues against the idea of a "typical animal mtDNA."

Li, K., et al. (2016). "Transcriptome, genetic editing, and microRNA divergence substantiate sympatric speciation of blind mole rat, *Spalax*." *Proc Natl Acad Sci U S A* **113**(27): 7584-7589.

Incipient sympatric speciation in blind mole rat, *Spalax galili*, in Israel, caused by sharp ecological divergence of abutting chalk-basalt ecologies, has been proposed previously based on mitochondrial and whole-genome nuclear DNA. Here, we present new evidence, including transcriptome, DNA editing, microRNA, and codon usage, substantiating earlier evidence for adaptive divergence in the abutting chalk and basalt populations. Genetic divergence, based on the previous and new evidence, is ongoing despite

restricted gene flow between the two populations. The principal component analysis, neighbor-joining tree, and genetic structure analysis of the transcriptome clearly show the clustered divergent two mole rat populations. Gene-expression level analysis indicates that the population transcriptome divergence is displayed not only by soil divergence but also by sex. Gene ontology enrichment of the differentially expressed genes from the two abutting soil populations highlights reproductive isolation. Alternative splicing variation of the two abutting soil populations displays two distinct splicing patterns. L-shaped *F_{ST}* distribution indicates that the two populations have undergone divergence with gene flow. Transcriptome divergent genes highlight neurogenetics and nutrition characterizing the chalk population, and energetics, metabolism, musculature, and sensory perception characterizing the abutting basalt population. Remarkably, microRNAs also display divergence between the two populations. The GC content is significantly higher in chalk than in basalt, and stress-response genes mostly prefer nonoptimal codons. The multiple lines of evidence of ecological-genomic and genetic divergence highlight that natural selection overrules the gene flow between the two abutting populations, substantiating the sharp ecological chalk-basalt divergence driving sympatric speciation.

Liu, L. F., et al. (1998). "Genetic characterization of the mRNAs encoding alpha-bungarotoxin: isoforms and RNA editing in *Bungarus multicinctus* gland cells." *Nucleic Acids Res* **26**(24): 5624-5629.

The mRNA encoding alpha-bungarotoxin (alpha-Butx) was prepared from the venom glands of *Bungarus multicinctus* by Cap-finder cDNA synthesis. The sequences of the 3'- and 5'-flanking regions including a signal peptide of alpha-Butx were almost identical with those of Elapidae and Hydrophiidae toxins, suggesting that they may have the same origin. Sixteen polymorphic mRNA sequences of alpha-Butx were detected from *B. multicinctus* gland cells. Analysis of the genomic DNA of alpha-Butx indicated that the polymorphic mRNA originated from one DNA sequence. Most of the mutations in alpha-Butx mRNA were silent and the hot-spot variations occurred at 78, 107, 129, 198 and 201 nt in alpha-Butx mRNA. Ten distinct protein sequences of alpha-Butx could be deduced from the polymorphic mRNA and one of the isoforms has already been isolated. Since alpha-Butx DNA is a single copy in the genome, the RNA polymorphism might result from post-transcriptional editing. These results indicate that the authentic alpha-Butx is in fact derived from edited mRNAs. RNA editing may contribute a common mechanism toward the diversity of alpha-neurotoxins in snake glands.

Louzoun, Y., et al. (2006). "Copying nodes versus editing links: the source of the difference between genetic regulatory networks and the WWW." *Bioinformatics* **22**(5): 581-588.

UNLABELLED: We study two kinds of networks: genetic regulatory networks and the World Wide Web. We systematically test microscopic mechanisms to find the set of such mechanisms that optimally explain each networks' specific properties. In the first case we formulate a model including mainly random unbiased gene duplications and mutations. In the second case, the basic moves are website generation and rapid surf-induced link creation (/destruction). The different types of mechanisms reproduce the appropriate observed network properties. We use those to show that different kinds of networks have strongly system-dependent macroscopic experimental features. The diverging properties result from dissimilar node and link basic dynamics. The main non-uniform properties include the clustering coefficient, small-scale motifs frequency, time correlations, centrality and the connectivity of outgoing links. Some other features are generic such as the large-scale connectivity distribution of incoming links (scale-free) and the network diameter (small-worlds). The common properties are just the general hallmark of autocatalysis (self-enhancing processes), while the specific properties hinge on the specific elementary mechanisms. SUPPLEMENTARY INFORMATION: Supplementary data are available at Bioinformatics Online.

Maas, S. and A. Rich (2000). "Changing genetic information through RNA editing." *Bioessays* **22**(9): 790-802.

RNA editing, the post-transcriptional alteration of a gene-encoded sequence, is a widespread phenomenon in eukaryotes. As a consequence of RNA editing, functionally distinct proteins can be produced from a single gene. The molecular mechanisms involved include single or multiple base insertions or deletions as well as base substitutions. In mammals, one type of substitutional RNA editing, characterized by site-specific base-modification, was shown to modulate important physiological processes. The underlying reaction mechanism of substitutional RNA editing involves hydrolytic deamination of cytosine or adenosine bases to uracil or inosine, respectively. Protein factors have been characterized that are able to induce RNA editing in vitro. A supergene family of RNA-dependent deaminases has emerged with the recent addition of adenosine deaminases specific for tRNA. Here we review the developments that have substantially increased our understanding of base-modification RNA editing over the past few years,

with an emphasis on mechanistic differences, evolutionary aspects and the first insights into the regulation of editing activity.

Maier, R. M., et al. (1995). "Complete sequence of the maize chloroplast genome: gene content, hotspots of divergence and fine tuning of genetic information by transcript editing." *J Mol Biol* **251**(5): 614-628.

The nucleotide sequence of the chloroplast (cp) DNA from maize (*Zea mays*) has been completed. The circular double-stranded DNA, which consists of 140,387 base-pairs, contains a pair of inverted repeat regions (IRA and IRB) with 22,748 base-pairs each, which are separated by a small and a large single copy region (SSC and LSC) of 12,536 and 82,355 base-pairs, respectively. The gene content and the relative positions of a total of 104 genes (70 peptide-encoding genes, 30 tRNA genes and four rRNA genes) are identical with the chloroplast DNA of the closely related species rice (*Oryza sativa*). A detailed analysis of the two graminean plastomes allows the identification of hotspots of divergence which predominate in one region containing a cluster of tRNA genes and in two regions containing degenerated reading frames. One of these length differences is thought to reflect a gene transfer event from the plastome to the nucleus, which is followed by progressive degradation of the respective chloroplast gene resulting in gene fragments. The other divergent plastome region seems to be due to the complete loss of a plastid gene and its functional substitution by a nuclear encoded eukaryotic homologue. The rate of neutral nucleotide substitutions is significantly reduced for protein coding genes located in the inverted repeat regions. This indicates that the existence of inverted repeat regions confers increased genetic stability of the genes positioned in these regions as compared to genes located in the two single copy regions. Editing events cause the primary structures of several transcripts to deviate from the corresponding genomic sequences by C to U transitions. The unambiguous deduction of amino acid sequences from the nucleotide sequences of the corresponding genes is, therefore, not possible. A survey of the 25 editing positions identified in 13 different transcripts of the maize plastome shows that representatives of all protein coding gene classes are subject to editing. A strong bias exists for the second codon position and for certain codon transitions. Based on the number and the codon transition types, and taking into account the frequency of putative editing sites in all peptide encoding genes and unidentified reading frames, a total number of only few more than the experimentally verified 25 editing sites encoded in the maize plastome is estimated. This corresponds to 0.13% of amino acid positions which cannot be

derived from the corresponding codons present in the corresponding genes.

Martinez-Garcia, E. and V. de Lorenzo (2012). "Transposon-based and plasmid-based genetic tools for editing genomes of gram-negative bacteria." *Methods Mol Biol* **813**: 267-283.

A good part of the contemporary synthetic biology agenda aims at reprogramming microorganisms to enhance existing functions and/or perform new tasks. Moreover, the functioning of complex regulatory networks, or even a single gene, is revealed only when perturbations are entered in the corresponding dynamic systems and the outcome monitored. These endeavors rely on the availability of genetic tools to successfully modify a la carte the chromosome of target bacteria. Key aspects to this end include the removal of undesired genomic segments, systems for the production of directed mutants and allelic replacements, random mutant libraries to discover new functions, and means to stably implant larger genetic networks into the genome of specific hosts. The list of gram-negative species that are appealing for such genetic refactoring operations is growingly expanding. However, the repertoire of available molecular techniques to do so is very limited beyond *Escherichia coli*. In this chapter, utilization of novel tools is described (exemplified in two plasmids systems: pBAM1 and pEMG) tailored for facilitating chromosomal engineering procedures in a wide variety of gram-negative microorganisms.

Midic, U., et al. (2017). "Quantitative assessment of timing, efficiency, specificity and genetic mosaicism of CRISPR/Cas9-mediated gene editing of hemoglobin beta gene in rhesus monkey embryos." *Hum Mol Genet* **26**(14): 2678-2689.

Gene editing technologies offer new options for developing novel biomedical research models and for gene and stem cell based therapies. However, applications in many species demand high efficiencies, specificity, and a thorough understanding of likely editing outcomes. To date, overall efficiencies, rates of off-targeting and degree of genetic mosaicism have not been well-characterized for most species, limiting our ability to optimize methods. As a model gene for measuring these parameters of the CRISPR/Cas9 application in a primate species (rhesus monkey), we selected the beta-hemoglobin gene (HBB), which also has high relevance to the potential application of gene editing and stem-cell technologies for treating human disease. Our data demonstrate an ability to achieve a high efficiency of gene editing in rhesus monkey zygotes, with no detected off-target effects at selected off-target loci. Considerable genetic mosaicism and variation in the fraction of embryonic cells bearing

targeted alleles are observed, and the timing of editing events is revealed using a new model. The uses of Cas9-WT protein combined with optimized concentrations of sgRNAs are two likely areas for further refinement to enhance efficiency while limiting unfavorable outcomes that can be exceedingly costly for application of gene editing in primate species.

Mitzelfelt, K. A., et al. (2017). "Efficient Precision Genome Editing in iPSCs via Genetic Co-targeting with Selection." *Stem Cell Reports* **8**(3): 491-499.

Genome editing in induced pluripotent stem cells is currently hampered by the laborious and expensive nature of identifying homology-directed repair (HDR)-modified cells. We present an approach where isolation of cells bearing a selectable, HDR-mediated editing event at one locus enriches for HDR-mediated edits at additional loci. This strategy, called co-targeting with selection, improves the probability of isolating cells bearing HDR-mediated variants and accelerates the production of disease models.

Morrison, D. A., et al. (2015). "Genome editing by natural genetic transformation in *Streptococcus mutans*." *J Microbiol Methods* **119**: 134-141.

Classical mutagenesis strategies using selective markers linked to designed mutations are powerful and widely applicable tools for targeted mutagenesis via natural genetic transformation in bacteria and archaea. However, the markers that confer power are also potentially problematic as they can be cumbersome, risk phenotypic effects of the inserted genes, and accumulate as unwanted genes during successive mutagenesis cycles. Alternative mutagenesis strategies use temporary plasmid or cassette insertions and can in principle achieve equally flexible mutation designs, but design of suitable counter-selected markers can be complex. All these drawbacks are eased by use of direct genome editing. Here we describe a strategy for directly editing the genome of *S. mutans*, which is applied to the widely studied reference strain UA159 (ATCC 700610) and has the advantage of extreme simplicity, requiring construction of only one synthetic donor amplicon and a single transformation step, followed by a simple PCR screen among a few dozen clones to identify the desired mutant. The donor amplicon carries the mutant sequence and extensive flanking segments of homology, which ensure efficient and precise integration by the recombination machinery specific to competent cells. The recipients are highly competent cells, in a state achieved by treatment with a synthetic competence pheromone.

Nakamuta, M., et al. (1996). "Complete phenotypic characterization of apobec-1 knockout

mice with a wild-type genetic background and a human apolipoprotein B transgenic background, and restoration of apolipoprotein B mRNA editing by somatic gene transfer of Apobec-1." *J Biol Chem* **271**(42): 25981-25988.

We have produced gene knockout mice by targeted disruption of the apobec-1 gene. As recently reported by Hirano et al. (Hirano, K.-I., Young, S. G., Farese, R. V., Jr., Ng, J., Sande, E., Warburton, C., Powell-Braxton, L. M., and Davidson, N. O. (1996) *J. Biol. Chem.* **271**, 9887-9890), these animals do not edit apolipoprotein (apo) B mRNA or produce apoB-48. In this study we have performed a detailed analysis of the lipoprotein phenotypic effects of apobec-1 gene disruption that were not examined in the previous study. We first analyzed the plasma lipoproteins in knockout animals with a wild-type genetic background. Although there was no difference in plasma cholesterol between apobec-1(-/-), +/-, or +/+ mice, there was a marked (176%) increase in plasma apoB-100, from 1.8 +/- 1.2 mg/dl in apobec-1(+/+) mice to 2.7 +/- 0.6 mg/dl in apobec-1(+/-) and 5.0 +/- 1.4 mg/dl in apobec-1(-/-) mice. Plasma apoE was similar in these animals. By fast protein liquid chromatography (FPLC) analysis, there was a significant decrease in plasma high density lipoprotein (HDL) cholesterol in apobec-1(-/-) mice. We further fractionated the plasma lipoproteins into d < 1.006, 1.006-1.02, 1.02-1.05, 1.05-1.08, 1.08-1.10, and 1.10-1.21 g/ml classes, and found a marked (30-40%) reduction in the cholesterol and protein content in the (d 1.08-1.10 and 1.10-1.21) HDL fractions, corroborating the FPLC data. SDS-gel analysis revealed an absence of apoB-48, an increase in apoB-100 in the very low density lipoprotein (VLDL) and low density lipoprotein (LDL) fractions, and a small decrease in apoA-I in the HDL fractions in the apobec-1(-/-) samples. We next raised the basal plasma apoB levels in the apobec-1(-/-) animals by cross-breeding them with human apoB transgenic (TgB) mice. The plasma apoB-100 was 3-fold higher in apobec-1(-/-)/TgB+/- mice (26.6 +/- 18.3 mg/dl) than in apobec-1(+/+)/TgB+/- mice (9.8 +/- 3.9 mg/dl, p < 0.05). The apobec-1(-/-)/TgB+/- mice had a plasma cholesterol levels of 170 +/- 28 mg/dl and triglyceride levels of 106 +/- 31 mg/dl, which are 80% and 58% higher, respectively, than the corresponding values of 94 +/- 21 mg/dl and 67 +/- 11 mg/dl in apobec+//TgB+/- mice. By FPLC, the apobec-1(-/-)/TgB+/- animals developed markedly elevated plasma LDL cholesterol (518.5 +/- 329.5 microg/ml) that is 373% that of apobec1(+/+)/TgB+/- mice (139.0 +/- 87.0 microg/ml) (p < 0.05). The elevated plasma triglyceride was accounted for mainly by a 97% increase in VLDL triglyceride in the apobec1(-/-)/TgB+/- mice. We conclude that apobec-1(-/-) animals have a distinctive

lipoprotein phenotype characterized by significant hyperapoB-100 and HDL deficiency in mice with a wild-type genetic background. Furthermore, the abolition of apoB mRNA editing elevates plasma total cholesterol and LDL cholesterol in apobec-1(-/-) animals with a TgB background. Finally, to exclude the possibility that absence of apoB mRNA editing was a secondary effect of chronic Apobec-1 deficiency, we treated apobec-1(-/-) mice with a replication-defective mouse Apobec-1 adenoviral vector and found that we could acutely restore apoB mRNA editing in the liver. These experiments indicate that Apobec-1 is an essential component of the apoB mRNA editing machinery and absence of editing in the knockout animals is a direct consequence of the absence of functional Apobec-1.

Nangle, L. A., et al. (2002). "Genetic code ambiguity. Cell viability related to the severity of editing defects in mutant tRNA synthetases." *J Biol Chem* **277**(48): 45729-45733.

The rules of the genetic code are established in reactions that aminoacylate tRNAs with specific amino acids. Ambiguity in the code is prevented by editing activities whereby incorrect aminoacylations are cleared by specialized hydrolytic reactions of aminoacyl tRNA synthetases. Whereas editing reactions have long been known, their significance for cell viability is still poorly understood. Here we investigated in vitro and in vivo four different mutations in the center for editing that diminish the proofreading activity of valyl-tRNA synthetase (ValRS). The four mutant enzymes were shown to differ quantitatively in the severity of the defect in their ability to clear mischarged tRNA in vitro. Strikingly, in the presence of excess concentrations of alpha-aminobutyrate, one of the amino acids that is misactivated by ValRS, growth of bacterial strains bearing these mutant alleles is arrested. The concentration of misactivated amino acid required for growth arrest correlates inversely in a rank order with the degree of the editing defect seen in vitro. Thus, cell viability depends directly on the suppression of genetic code ambiguity by these specific editing reactions and is finely tuned to any perturbation of these reactions.

Novoa, E. M., et al. (2015). "Ancestral AlaX editing enzymes for control of genetic code fidelity are not tRNA-specific." *J Biol Chem* **290**(16): 10495-10503.

Accurate protein synthesis requires the hydrolytic editing of tRNAs incorrectly aminoacylated by aminoacyl-tRNA synthetases (ARSs). Recognition of cognate tRNAs by ARS is less error-prone than amino acid recognition, and, consequently, editing domains are generally believed to act only on the tRNAs

cognate to their related ARSs. For example, the AlaX family of editing domains, including the editing domain of alanyl-tRNA synthetase and the related free-standing trans-editing AlaX enzymes, are thought to specifically act on tRNA (Ala), whereas the editing domains of threonyl-tRNA synthetases are specific for tRNA (Thr). Here we show that, contrary to this belief, AlaX-S, the smallest of the extant AlaX enzymes, deacylates Ser-tRNA (Thr) in addition to Ser-tRNA (Ala) and that a single residue is important to determine this behavior. Our data indicate that promiscuous forms of AlaX are ancestral to tRNA-specific AlaXs. We propose that former AlaX domains were used to maintain translational fidelity in earlier stages of genetic code evolution when mis-serylation of several tRNAs was possible.

Oseni, S., et al. (2004). "Genetic parameters for days open and pregnancy rates in US Holsteins using different editing criteria." *J Dairy Sci* **87**(12): 4327-4333.

The influence of various editing criteria for days open (DO) records on genetic parameter estimates of DO and pregnancy rates (PR) in US Holsteins was investigated. Data included first parity 305-d milk yield and DO records from 8 states: Georgia (GA), Florida (FL), North Carolina (NC), Texas (TX), Arizona (AZ), California (CA), New York (NY), and Wisconsin (WI). The pregnancy rate was computed as $1/[(DO - VWP)/HI + 1]$, where VWP was the approximate voluntary waiting period and HI was the heat interval set as 21 d. The upper limit for PR was set to 1.0. A bivariate animal model for DO (or PR) and 305-d milk yield was fit separately for each state. The model included fixed effects of herd-year, month of calving, and age of cow, as well as random animal and residual effects. In separate analyses, maximum DO records were limited to 150, 200, 250, 300, and 365 d. Analyses for PR used values of 50, 80, and 120 d for the VWP. Genetic and residual variances for DO were strongly dependent on the upper limit; both variances were 8 times larger as the upper bound increased from 150 to 365 d. Estimates of heritability for DO varied between 0.03 and 0.06. There was a 30% increase in the heritability estimate as the upper limit increased from 150 to 250 d for FL and NC, and small or no increases for the other states. The increase of the upper limit from 250 to 365 d resulted in little change. The genetic correlation between milk and DO was the highest for FL (0.6) and the lowest for GA (0.12 to 0.23). For PR with VWP=50, the heritability was higher than the corresponding estimate for DO in GA, equal to that in AZ, and lower in the remaining states. Heritabilities of PR also varied by the length of VWP; highest heritabilities were obtained at VWP=50 d for GA and AZ; at VWP=80 d for NY and WI; at

VWP=120 d for FL, NC, and CA. Increase of genetic variation for records of DO < 250 d was small. Days open and PR are strongly influenced by differences in management protocols among states.

Pring, D., et al. (1993). "RNA editing gives a new meaning to the genetic information in mitochondria and chloroplasts." *Plant Mol Biol* **21**(6): 1163-1170.

RNA editing in plant mitochondria and chloroplasts alters mRNA sequences to code for different proteins than the DNA. Most of these C-to-U transitions occur in open reading frames, but a few are observed in intron sequences. Influences of the nuclear genome on editing patterns suggest that cytoplasmic factors participate in this process.

Ramaswami, G., et al. (2015). "Genetic mapping uncovers cis-regulatory landscape of RNA editing." *Nat Commun* **6**: 8194.

Adenosine-to-inosine (A-to-I) RNA editing, catalysed by ADAR enzymes conserved in metazoans, plays an important role in neurological functions. Although the fine-tuning mechanism provided by A-to-I RNA editing is important, the underlying rules governing ADAR substrate recognition are not well understood. We apply a quantitative trait loci (QTL) mapping approach to identify genetic variants associated with variability in RNA editing. With very accurate measurement of RNA editing levels at 789 sites in 131 *Drosophila melanogaster* strains, here we identify 545 editing QTLs (edQTLs) associated with differences in RNA editing. We demonstrate that many edQTLs can act through changes in the local secondary structure for edited dsRNAs. Furthermore, we find that edQTLs located outside of the edited dsRNA duplex are enriched in secondary structure, suggesting that distal dsRNA structure beyond the editing site duplex affects RNA editing efficiency. Our work will facilitate the understanding of the cis-regulatory code of RNA editing.

Retter, M. W. and D. Nemazee (1999). "Receptor editing: genetic reprogramming of autoreactive lymphocytes." *Cell Biochem Biophys* **31**(1): 81-88.

The clonal selection theory postulates that immune tolerance mediated selection occurs at the level of the cell. The receptor editing model, instead, suggests that selection occurs at the level of the B-cell receptor, so that self-reactive receptors that encounter autoantigen in the bone marrow are altered through secondary rearrangement. Recent studies in transgenic model systems and normal B cells, both in vivo and in vitro, have demonstrated that receptor editing is a major mechanism for inducing B-cell tolerance.

Rivera-Torres, N. and E. B. Kmiec (2016). "Genetic spell-checking: gene editing using single-stranded DNA oligonucleotides." Plant Biotechnol J **14**(2): 463-470.

Single-stranded oligonucleotides (ssODNs) can be used to direct the exchange of a single nucleotide or the repair of a single base within the coding region of a gene in a process that is known, generically, as gene editing. These molecules are composed of either all DNA residues or a mixture of RNA and DNA bases and utilize inherent metabolic functions to execute the genetic alteration within the context of a chromosome. The mechanism of action of gene editing is now being elucidated as well as an understanding of its regulatory circuitry, work that has been particularly important in establishing a foundation for designing effective gene editing strategies in plants. Double-strand DNA breakage and the activation of the DNA damage response pathway play key roles in determining the frequency with which gene editing activity takes place. Cellular regulators respond to such damage and their action impacts the success or failure of a particular nucleotide exchange reaction. A consequence of such activation is the natural slowing of replication fork progression, which naturally creates a more open chromatin configuration, thereby increasing access of the oligonucleotide to the DNA template. Herein, how critical reaction parameters influence the effectiveness of gene editing is discussed. Functional interrelationships between DNA damage, the activation of DNA response pathways and the stalling of replication forks are presented in detail as potential targets for increasing the frequency of gene editing by ssODNs in plants and plant cells.

Roesch, E. A. and M. L. Drumm (2017). "Powerful tools for genetic modification: Advances in gene editing." Pediatr Pulmonol **52**(S48): S15-S20.

Recent discoveries and technical advances in genetic engineering, methods called gene or genome editing, provide hope for repairing genes that cause diseases like cystic fibrosis (CF) or otherwise altering a gene for therapeutic benefit. There are both hopes and hurdles with these technologies, with new ideas emerging almost daily. Initial studies using intestinal organoid cultures carrying the common, F508del mutation have shown that gene editing by CRISPR/Cas9 can convert cells lacking CFTR function to cells with normal channel function, providing a precedent that this technology can be harnessed for CF. While this is an important precedent, the challenges that remain are not trivial. A logistical issue for this and many other genetic diseases is genetic heterogeneity. Approximately, 2000 mutations associated with CF have been found in CFTR, the gene responsible for CF, and thus a feasible

strategy that would encompass all individuals affected by the disease is particularly difficult to envision. However, single strategies that would be applicable to all subjects affected by CF have been conceived and are being investigated. With all of these approaches, efficiency (the proportion of cells edited), accuracy (how often other sites in the genome are affected), and delivery of the gene editing components to the desired cells are perhaps the most significant, impending hurdles. Our understanding of each of these areas is increasing rapidly, and while it is impossible to predict when a successful strategy will reach the clinic, there is every reason to believe it is a question of "when" and not "if."

Sanchez-Rivera, F. J., et al. (2014). "Rapid modelling of cooperating genetic events in cancer through somatic genome editing." Nature **516**(7531): 428-431.

Cancer is a multistep process that involves mutations and other alterations in oncogenes and tumour suppressor genes. Genome sequencing studies have identified a large collection of genetic alterations that occur in human cancers. However, the determination of which mutations are causally related to tumorigenesis remains a major challenge. Here we describe a novel CRISPR/Cas9-based approach for rapid functional investigation of candidate genes in well-established autochthonous mouse models of cancer. Using a Kras (G12D)-driven lung cancer model, we performed functional characterization of a panel of tumour suppressor genes with known loss-of-function alterations in human lung cancer. Cre-dependent somatic activation of oncogenic Kras (G12D) combined with CRISPR/Cas9-mediated genome editing of tumour suppressor genes resulted in lung adenocarcinomas with distinct histopathological and molecular features. This rapid somatic genome engineering approach enables functional characterization of putative cancer genes in the lung and other tissues using autochthonous mouse models. We anticipate that this approach can be used to systematically dissect the complex catalogue of mutations identified in cancer genome sequencing studies.

Scott, D. A. and F. Zhang (2017). "Implications of human genetic variation in CRISPR-based therapeutic genome editing." Nat Med **23**(9): 1095-1101.

CRISPR-Cas genome-editing methods hold immense potential as therapeutic tools to fix disease-causing mutations at the level of DNA. In contrast to typical drug development strategies aimed at targets that are highly conserved among individual patients, treatment at the genomic level must contend with

substantial inter-individual natural genetic variation. Here we analyze the recently released ExAC and 1000 Genomes data sets to determine how human genetic variation impacts target choice for Cas endonucleases in the context of therapeutic genome editing. We find that this genetic variation confounds the target sites of certain Cas endonucleases more than others, and we provide a compendium of guide RNAs predicted to have high efficacy in diverse patient populations. For further analysis, we focus on 12 therapeutically relevant genes and consider how genetic variation affects off-target candidates for these loci. Our analysis suggests that, in large populations of individuals, most candidate off-target sites will be rare, underscoring the need for prescreening of patients through whole-genome sequencing to ensure safety. This information can be integrated with empirical methods for guide RNA selection into a framework for designing CRISPR-based therapeutics that maximizes efficacy and safety across patient populations.

Seiwert, S. D. and K. Stuart (1994). "RNA editing: transfer of genetic information from gRNA to precursor mRNA in vitro." *Science* **266**(5182): 114-117.

RNA editing in the mitochondrion of *Trypanosoma brucei* extensively alters the adenosine triphosphate synthase (ATPase) subunit 6 precursor messenger RNA (pre-mRNA) by addition of 447 uridines and removal of 28 uridines. In vivo, the guide RNA gA6[14] is thought to specify the deletion of two uridines from the editing site closest to the 3' end. In this study, an in vitro system was developed that accurately removed uridines from this editing site in synthetic ATPase 6 pre-mRNA when gA6[14] and ATP were added. Mutations in both the guide RNA and the pre-mRNA editing site suggest that base-pairing interactions control the number of uridines deleted in vitro. Thus, guide RNAs are required for RNA editing and for the transfer of genetic information to pre-mRNAs.

Shen, L., et al. (2017). "Rapid generation of genetic diversity by multiplex CRISPR/Cas9 genome editing in rice." *Sci China Life Sci* **60**(5): 506-515.

The clustered regularly interspaced short palindromic repeats (CRISPR)-associated endonuclease 9 (CRISPR/Cas9) system has emerged as a promising technology for specific genome editing in many species. Here we constructed one vector targeting eight agronomic genes in rice using the CRISPR/Cas9 multiplex genome editing system. By subsequent genetic transformation and DNA sequencing, we found that the eight target genes have high mutation efficiencies in the T0 generation. Both heterozygous and homozygous mutations of all editing

genes were obtained in T0 plants. In addition, homozygous sextuple, septuple, and octuple mutants were identified. As the abundant genotypes in T0 transgenic plants, various phenotypes related to the editing genes were observed. The findings demonstrate the potential of the CRISPR/Cas9 system for rapid introduction of genetic diversity during crop breeding.

Shi, Z. D., et al. (2017). "Genome Editing in hPSCs Reveals GATA6 Haploinsufficiency and a Genetic Interaction with GATA4 in Human Pancreatic Development." *Cell Stem Cell* **20**(5): 675-688 e676.

Human disease phenotypes associated with haploinsufficient gene requirements are often not recapitulated well in animal models. Here, we have investigated the association between human GATA6 haploinsufficiency and a wide range of clinical phenotypes that include neonatal and adult-onset diabetes using CRISPR (clustered regularly interspaced short palindromic repeat)/Cas9-mediated genome editing coupled with human pluripotent stem cell (hPSC) directed differentiation. We found that loss of one GATA6 allele specifically affects the differentiation of human pancreatic progenitors from the early PDX1+ stage to the more mature PDX1+NKX6.1+ stage, leading to impaired formation of glucose-responsive beta-like cells. In addition to this GATA6 haploinsufficiency, we also identified dosage-sensitive requirements for GATA6 and GATA4 in the formation of both definitive endoderm and pancreatic progenitor cells. Our work expands the application of hPSCs from studying the impact of individual gene loci to investigation of multigenic human traits, and it establishes an approach for identifying genetic modifiers of human disease.

Slattery, S. S., et al. (2018). "An Expanded Plasmid-Based Genetic Toolbox Enables Cas9 Genome Editing and Stable Maintenance of Synthetic Pathways in *Phaeodactylum tricornutum*." *ACS Synth Biol* **7**(2): 328-338.

With the completion of the genome sequence, and development of an efficient conjugation-based transformation system allowing the introduction of stable episomes, *Phaeodactylum tricornutum* has become an ideal platform for the study of diatom biology and synthetic biology applications. The development of plasmid-based genetic tools is the next step to improve manipulation of this species. Here, we report the identification of endogenous *P. tricornutum* promoters and terminators allowing selective expression of antibiotic resistance markers from stably replicating plasmids in *P. tricornutum*. Significantly, we developed a protocol for sequential conjugation of plasmids from *Escherichia coli* to *P. tricornutum* and demonstrated simultaneous replication of two plasmids

in *P. tricornutum*. We developed a simple and robust conjugative system for Cas9 editing that yielded up to 60% editing efficiency of the urease gene. Finally, we constructed a plasmid encoding eight genes involved in vanillin biosynthesis that was propagated in *P. tricornutum* over four months with no evidence of rearrangements, with whole-plasmid sequencing indicating that the majority of mutations occurred after plasmid assembly and initial conjugation rather than during long-term propagation. The plasmid-based tools described here will facilitate investigation of the basic biology of *P. tricornutum* and enable synthetic biology applications.

Stuart, K. (1989). "RNA editing: new insights into the storage and expression of genetic information." *Parasitol Today* **5**(1): 5-8.

Kinetoplastid flagellates, and possibly other parasites and viruses, have evolved a novel form of gene regulation at different phases of their life cycle by 'editing' their own RNA transcripts. This article discusses the significance of the process and proposes a hypothesis to explain how it may be done.

Stuart, K. (1998). "RNA editing: trypanosomes rewrite the genetic code." *Verh K Acad Geneesk Belg* **60**(1): 63-74.

The understanding of how genetic information is stored and expressed has advanced considerably since the "central dogma" asserted that genetic information flows from the nucleotide sequence of DNA to that of messenger RNA (mRNA) which in turn specifies the amino acid sequence of a protein. It was found that genetic information can be stored as RNA (e.g. in RNA viruses) and can flow from RNA to DNA by reverse transcriptase enzyme activity. In addition, some genes contain introns, nucleotide sequences that are removed from their RNA (by RNA splicing) and thus are not represented in the resultant protein. Furthermore, alternative splicing was found to produce variant proteins from a single gene. More recently, the study of trypanosome parasites revealed an unexpected and indeed counter-intuitive genetic complexity. Genetic information for a single protein can be dispersed among several (DNA) genes in these organisms. One of these genes specifies an encrypted precursor mRNA that is converted to a functional mRNA by a process called RNA editing that inserts and deletes uridylylate nucleotides. The sequence of the edited mRNA is specified by multiple small RNAs, named guide RNAs, (gRNAs) each of which is encoded in a separate gene. Thus, edited mRNA sequences are assembled from multiple genes by the transfer of information from one type of RNA to another. The existence of editing was surprising but has stimulated the discovery of other types of RNA

editing. The Stuart laboratory has been exploring RNA editing in trypanosomes from the time of its discovery. They found dramatic differences between the mitochondrial gene sequences and those of the corresponding mRNAs, which indicated editing by the insertion and deletion of uridylates. Some editing was modest; simply eliminating shifts in sequence register of minimally extending the protein coding sequence. However, editing of many mRNAs was startlingly extensive. The RNA sequence was essentially entirely remodeled with its sequence more the result of editing than the gene sequence. The identities of genes for such extensively edited RNA were not recognizable from the DNA sequence but they were readily identifiable from the edited mRNA sequence. Thus, despite the complex and extensive editing the resultant mRNA sequence is precise. Characterization of partially edited RNAs indicated that editing proceeds in the direction opposite to that used to specify the protein which reflects the use of the gRNAs. The numerous gRNAs that are used for editing are encoded in the DNA molecules whose role was previously a mystery. Using information gained in our earlier studies, the Stuart group developed an in vitro system that reproduces the fundamental process of editing in order to resolve the mechanism by which it occurs. They determined that editing entails a series of enzymatic steps rather than the mechanism used in RNA splicing. They also showed that chimeric gRNA-mRNA molecules are aberrant by-products of editing rather than intermediates in the process as had been proposed. Additional studies are exploring precisely how the number of added and deleted uridylates is specified by the gRNA. The Stuart laboratory showed that editing is performed by an aggregation of enzymes that catalyze the separate steps of editing. It also developed a method to purify this multimolecule complex that contains several, perhaps tens of, proteins. This will allow the study of its composition and the functions of its component parts. Indeed, the gene for one component has been identified and its detailed characterization begun. These studies are developing tools to explore related processes. An early finding in the lab was that the various mRNAs are differentially edited during the life cycle of the parasite. The pattern of this editing indicates that editing serves to regulate the alternation between two modes of energy generation. This regulation is coordinated with other events that are occurring during the life cycle.

Suspene, R., et al. (2011). "Genetic editing of herpes simplex virus 1 and Epstein-Barr herpesvirus genomes by human APOBEC3 cytidine deaminases in culture and in vivo." *J Virol* **85**(15): 7594-7602.

Human APOBEC3 cytidine deaminases target and edit single-stranded DNA, which can be of viral, mitochondrial, or nuclear origin. Retrovirus genomes, such as human immunodeficiency virus (HIV) genomes deficient in the *vif* gene and the hepatitis B virus genome, are particularly vulnerable. The genomes of some DNA viruses, such as human papillomaviruses, can be edited *in vivo* and in transfection experiments. Accordingly, herpesviruses should be no exception. This is indeed the case for herpes simplex virus 1 (HSV-1) in tissue culture, where APOBEC3C (A3C) overexpression can reduce virus titers and the particle/PFU ratio approximately 10-fold. Nonetheless, A3A, A3G, and AICDA can edit what is presumably a small fraction of HSV genomes in an experimental setting without seriously impacting the viral titer. Hyperediting was found in HSV genomes recovered from 4/8 uncultured buccal lesions. The phenomenon is not restricted to HSV, since hyperedited Epstein-Barr virus (EBV) genomes were readily recovered from 4/5 established cell lines, indicating that episomes are vulnerable to editing. These findings suggest that the widely expressed A3C cytidine deaminase can function as a restriction factor for some human herpesviruses. That the A3C gene is not induced by type I interferons begs the question whether some herpesviruses encode A3C antagonists.

Tajaddod, M., et al. (2016). "The dynamic epitranscriptome: A to I editing modulates genetic information." *Chromosoma* **125**(1): 51-63.

Adenosine to inosine editing (A to I editing) is a cotranscriptional process that contributes to transcriptome complexity by deamination of adenosines to inosines. Initially, the impact of A to I editing has been described for coding targets in the nervous system. Here, A to I editing leads to recoding and changes of single amino acids since inosine is normally interpreted as guanosine by cellular machines. However, more recently, new roles for A to I editing have emerged: Editing was shown to influence splicing and is found massively in Alu elements. Moreover, A to I editing is required to modulate innate immunity. We summarize the multiple ways in which A to I editing generates transcriptome variability and highlight recent findings in the field.

Takenaka, M., et al. (2010). "Reverse genetic screening identifies five E-class PPR proteins involved in RNA editing in mitochondria of *Arabidopsis thaliana*." *J Biol Chem* **285**(35): 27122-27129.

RNA editing in flowering plant mitochondria post-transcriptionally alters several hundred nucleotides from C to U, mostly in mRNAs. Several factors required for specific RNA-editing events in

plant mitochondria and plastids have been identified, all of them PPR proteins of the PLS subclass with a C-terminal E-domain and about half also with an additional DYW domain. Based on this information, we here probe the connection between E-PPR proteins and RNA editing in plant mitochondria. We initiated a reverse genetics screen of T-DNA insertion lines in *Arabidopsis thaliana* and investigated 58 of the 150 E-PPR-coding genes for a function in RNA editing. Six genes were identified to be involved in mitochondrial RNA editing at specific sites. Homozygous mutants of the five genes MEF18-MEF22 display no gross disturbance in their growth or development patterns, suggesting that the editing sites affected are not crucial at least in the greenhouse. These results show that a considerable percentage of the E-PPR proteins are involved in the functional processing of site-specific RNA editing in plant mitochondria.

Tauson, E. L. (2004). "[RNA editing in different genetic systems]." *Zh Obshch Biol* **65**(1): 52-73.

One of the significant amendments to the central dogma of the molecular biology was the discovery of the RNA editing process in different genetic systems. Whereas other forms of co- and posttranscriptional modifications of messenger RNA (mRNA) (capping, polyadenylation, splicing) retain the correspondence of the primary structure of exon and final transcript, RNA editing disturbs this correspondence changing the primary structure of mRNA after its transcription. The variants of RNA-editing mechanisms are various and include site-specific insertions and deletions of one or several nucleotides (insertion-deletion editing) as well as specific modifications of nucleosides such as C→U and A→I deamination or U→C transamination (conversion editing). These mechanisms differ greatly in different genetic systems but they always result in synthesis of functionally valuable proteins from "incorrect" genes and, correspondingly, can play a significant role in regulation of their expression.

Telugu, B. P., et al. (2017). "Genome editing and genetic engineering in livestock for advancing agricultural and biomedical applications." *Mamm Genome* **28**(7-8): 338-347.

Genetic modification of livestock has a longstanding and successful history, starting with domestication several thousand years ago. Modern animal breeding strategies predominantly based on marker-assisted and genomic selection, artificial insemination, and embryo transfer have led to significant improvement in the performance of domestic animals, and are the basis for regular supply of high quality animal derived food. However, the current strategy of breeding animals over multiple generations to introduce novel traits is not realistic in

responding to the unprecedented challenges such as changing climate, pandemic diseases, and feeding an anticipated 3 billion increase in global population in the next three decades. Consequently, sophisticated genetic modifications that allow for seamless introgression of novel alleles or traits and introduction of precise modifications without affecting the overall genetic merit of the animal are required for addressing these pressing challenges. The requirement for precise modifications is especially important in the context of modeling human diseases for the development of therapeutic interventions. The animal science community envisions the genome editors as essential tools in addressing these critical priorities in agriculture and biomedicine, and for advancing livestock genetic engineering for agriculture, biomedical as well as "dual purpose" applications.

Thompson, J. and S. Gopal (2006). "Correction: genetic algorithm learning as a robust approach to RNA editing site prediction." BMC Bioinformatics 7: 406.

After the publication of [1], we were alerted to an error in our data. The error was an one-off miscalculation in the extraction of position information for our set of true negatives. Our data set should have used randomly selected non-edited cytosines (C) as true negatives, but the data generation phase resulted in a set of nucleotides that were each one nucleotide downstream of known, unedited cytosines. The consequences of this error are reflected in changes to our results, although the general conclusions presented in our original publication remain largely unchanged.

Thompson, J. and S. Gopal (2006). "Genetic algorithm learning as a robust approach to RNA editing site prediction." BMC Bioinformatics 7: 145.

BACKGROUND: RNA editing is one of several post-transcriptional modifications that may contribute to organismal complexity in the face of limited gene complement in a genome. One form, known as C → U editing, appears to exist in a wide range of organisms, but most instances of this form of RNA editing have been discovered serendipitously. With the large amount of genomic and transcriptomic data now available, a computational analysis could provide a more rapid means of identifying novel sites of C → U RNA editing. Previous efforts have had some success but also some limitations. We present a computational method for identifying C → U RNA editing sites in genomic sequences that is both robust and generalizable. We evaluate its potential use on the best data set available for these purposes: C → U editing sites in plant mitochondrial genomes. **RESULTS:** Our method is derived from a machine learning approach

known as a genetic algorithm. REGAL (RNA Editing site prediction by Genetic Algorithm Learning) is 87% accurate when tested on three mitochondrial genomes, with an overall sensitivity of 82% and an overall specificity of 91%. REGAL's performance significantly improves on other ab initio approaches to predicting RNA editing sites in this data set. REGAL has a comparable sensitivity and higher specificity than approaches which rely on sequence homology, and it has the advantage that strong sequence conservation is not required for reliable prediction of edit sites. **CONCLUSION:** Our results suggest that ab initio methods can generate robust classifiers of putative edit sites, and we highlight the value of combinatorial approaches as embodied by genetic algorithms. We present REGAL as one approach with the potential to be generalized to other organisms exhibiting C → U RNA editing.

Tidball, A. M., et al. (2017). "Rapid Generation of Human Genetic Loss-of-Function iPSC Lines by Simultaneous Reprogramming and Gene Editing." Stem Cell Reports 9(3): 725-731.

Specifically ablating genes in human induced pluripotent stem cells (iPSCs) allows for studies of gene function as well as disease mechanisms in disorders caused by loss-of-function (LOF) mutations. While techniques exist for engineering such lines, we have developed and rigorously validated a method of simultaneous iPSC reprogramming while generating CRISPR/Cas9-dependent insertions/deletions (indels). This approach allows for the efficient and rapid formation of genetic LOF human disease cell models with isogenic controls. The rate of mutagenized lines was strikingly consistent across experiments targeting four different human epileptic encephalopathy genes and a metabolic enzyme-encoding gene, and was more efficient and consistent than using CRISPR gene editing of established iPSC lines. The ability of our streamlined method to reproducibly generate heterozygous and homozygous LOF iPSC lines with passage-matched isogenic controls in a single step provides for the rapid development of LOF disease models with ideal control lines, even in the absence of patient tissue.

Torikai, H., et al. (2016). "Genetic editing of HLA expression in hematopoietic stem cells to broaden their human application." Sci Rep 6: 21757.

Mismatch of human leukocyte antigens (HLA) adversely impacts the outcome of patients after allogeneic hematopoietic stem-cell transplantation (alloHSCT). This translates into the clinical requirement to timely identify suitable HLA-matched donors which in turn curtails the chances of recipients, especially those from a racial minority, to successfully

undergo alloHCT. We thus sought to broaden the existing pool of registered unrelated donors based on analysis that eliminating the expression of the HLA-A increases the chance for finding a donor matched at HLA-B, -C, and -DRB1 regardless of a patient's race. Elimination of HLA-A expression in HSC was achieved using artificial zinc finger nucleases designed to target HLA-A alleles. Significantly, these engineered HSCs maintain their ability to engraft and reconstitute hematopoiesis in immunocompromised mice. This introduced loss of HLA-A expression decreases the need to recruit large number of donors to match with potential recipients and has particular importance for patients whose HLA repertoire is under-represented in the current donor pool. Furthermore, the genetic engineering of stem cells provides a translational approach to HLA-match a limited number of third-party donors with a wide number of recipients.

Traxler, E. A., et al. (2016). "A genome-editing strategy to treat beta-hemoglobinopathies that recapitulates a mutation associated with a benign genetic condition." *Nat Med* **22**(9): 987-990.

Disorders resulting from mutations in the hemoglobin subunit beta gene (HBB; which encodes beta-globin), mainly sickle cell disease (SCD) and beta-thalassemia, become symptomatic postnatally as fetal gamma-globin expression from two paralogous genes, hemoglobin subunit gamma 1 (HBG1) and HBG2, decreases and adult beta-globin expression increases, thereby shifting red blood cell (RBC) hemoglobin from the fetal (referred to as HbF or alpha2gamma2) to adult (referred to as HbA or alpha2beta2) form. These disorders are alleviated when postnatal expression of fetal gamma-globin is maintained. For example, in hereditary persistence of fetal hemoglobin (HPFH), a benign genetic condition, mutations attenuate gamma-globin-to-beta-globin switching, causing high-level HbF expression throughout life. Co-inheritance of HPFH with beta-thalassemia- or SCD-associated gene mutations alleviates their clinical manifestations. Here we performed CRISPR-Cas9-mediated genome editing of human blood progenitors to mutate a 13-nt sequence that is present in the promoters of the HBG1 and HBG2 genes, thereby recapitulating a naturally occurring HPFH-associated mutation. Edited progenitors produced RBCs with increased HbF levels that were sufficient to inhibit the pathological hypoxia-induced RBC morphology found in SCD. Our findings identify a potential DNA target for genome-editing-mediated therapy of beta-hemoglobinopathies.

Vu, L. T., et al. (2015). "Changing blue fluorescent protein to green fluorescent protein using

chemical RNA editing as a novel strategy in genetic restoration." *Chem Biol Drug Des* **86**(5): 1242-1252.

Using the transition from cytosine of BFP (blue fluorescent protein) gene to uridine of GFP (green fluorescent protein) gene at position 199 as a model, we successfully controlled photochemical RNA editing to effect site-directed deamination of cytidine (C) to uridine (U). Oligodeoxynucleotides (ODNs) containing 5'-carboxyvinyl-2'-deoxyuridine ((CV) U) were used for reversible photoligation, and single-stranded 100-nt BFP DNA and in vitro-transcribed full-length BFP mRNA were the targets. Photo-cross-linking with the responsive ODNs was performed using UV (366 nm) irradiation, which was followed by heat treatment, and the cross-linked nucleotide was cleaved through photosplitting (UV, 312 nm). The products were analyzed using restriction fragment length polymorphism (RFLP) and fluorescence measurements. Western blotting and fluorescence-analysis results revealed that in vitro-translated proteins were synthesized from mRNAs after site-directed RNA editing. We detected substantial amounts of the target-base-substituted fragment using RFLP and observed highly reproducible spectra of the transition-GFP signal using fluorescence spectroscopy, which indicated protein stability.

Vu, L. T., et al. (2016). "Chemical RNA Editing for Genetic Restoration: The Relationship between the Structure and Deamination Efficiency of Carboxyvinyldeoxyuridine Oligodeoxynucleotides." *Chem Biol Drug Des* **87**(4): 583-593.

Oligodeoxynucleotides containing 5-carboxyvinyl-2'-deoxyuridine ((CV) U-containing ODNs) for successful site-specific transition of cytosine to uridine by photo-cross-linking have three parts: the complementary sequence, hairpin loop and the 5'-terminal photoresponsive nucleobase (CV) U. Photo-cross-linking with (CV) U-containing ODNs was performed using UV (366 nm) irradiation, followed by heat treatment for deamination. The cross-linked nucleotide was cleaved by photosplitting (UV, 312 nm). The products were analyzed using restriction fragment length polymorphism and fluorescence measurements. In previous studies, we have successfully performed site-directed photochemical base substitution toward a synthetic single-stranded 100-mer ODN target (ss100-nt) and in vitro-synthesized full-length blue fluorescent protein mRNA as targets. Although the efficiency of C-to-U site-specific transition strongly depends on the sequence and structure of (CV) U-containing ODNs, the relationship between (CV) U-containing ODNs and the deamination efficiency of targeted editing remains unclear. Therefore, in this study, we attempted to identify the optimal sequence and primary structure of

(CV) U-containing ODNs for site-directed specific transition. To evaluate the structure-deamination efficiency relationship, a series of eight (CV) U-containing ODNs were designed and studied. We showed that the optimal deamination efficiency was achieved with ODNs having a complementary sequence length slightly more than 14 nt and a hairpin length of 9 nt.

Vu, L. T. and T. Tsukahara (2017). "C-to-U editing and site-directed RNA editing for the correction of genetic mutations." *Biosci Trends* **11**(3): 243-253.

Cytidine to uridine (C-to-U) editing is one type of substitutional RNA editing. It occurs in both mammals and plants. The molecular mechanism of C-to-U editing involves the hydrolytic deamination of a cytosine to a uracil base. C-to-U editing is mediated by RNA-specific cytidine deaminases and several complementation factors, which have not been completely identified. Here, we review recent findings related to the regulation and enzymatic basis of C-to-U RNA editing. More importantly, when C-to-U editing occurs in coding regions, it has the power to reprogram genetic information on the RNA level, therefore it has great potential for applications in transcript repair (diseases related to thymidine to cytidine (T>C) or adenosine to guanosine (A>G) point mutations). If it is possible to manipulate or mimic C-to-U editing, T>C or A>G genetic mutation-related diseases could be treated. Enzymatic and non-enzymatic site-directed RNA editing are two different approaches for mimicking C-to-U editing. For enzymatic site-directed RNA editing, C-to-U editing has not yet been successfully performed, and in theory, adenosine to inosine (A-to-I) editing involves the same strategy as C-to-U editing. Therefore, in this review, for applications in transcript repair, we will provide a detailed overview of enzymatic site-directed RNA editing, with a focus on A-to-I editing and non-enzymatic site-directed C-to-U editing.

Wakui, M., et al. (2004). "Genetic dissection of lupus pathogenesis: Sle3/5 impacts IgH CDR3 sequences, somatic mutations, and receptor editing." *J Immunol* **173**(12): 7368-7376.

Sle3/5 is a lupus susceptibility locus identified on mouse chromosome 7 of the New Zealand Black/New Zealand White (NZB/NZW)-derived NZM2410 strain. Based on previous observations, this locus appears to contribute to lupus pathogenesis through its impact on diversification of immune responses. To understand how Sle3/5 affects somatic diversification of humoral responses, we analyzed IgH rearrangements preferentially encoding hapten-reactive IgG1 repertoires after immunization and assessed peripheral

IgH VDJ recombination activities in C57BL/6 (B6) mice congenic for Sle3/5 (B6.Sle3/5). In addition to altered somatic V (H) mutation profiles, sequences from B6.Sle3/5 mice exhibited atypical IgH CDR3 structures characteristic of autoreactive B cells and consistent with peripheral B cells bearing putatively edited receptors. Significant expression of Rag genes and circular V (H)D gene excision products were detected in splenic mature B cells of B6.Sle3/5 but not B6 mice, showing that peripheral IgH rearrangements occurred beyond allelic exclusion. Taken together, on the nonautoimmune background, Sle3/5 affected V (H)DJ (H) junctional diversity and V (H) mutational diversity and led to recombinational activation of allelically excluded IgH genes in the periphery. Such impact on somatic IgH diversification may contribute to the development of autoreactive B cell repertoires.

Wang, L., et al. (2014). "Editing genomic DNA in cancer cells with high genetic variance: benefit or risk?" *Oncol Rep* **31**(5): 2079-2084.

The generation of stably-transfected cell lines is a common and very important technology in cancer science. Considerable knowledge in the field of life sciences has been gained through the modification of the genetic code. However, there is a risk in evaluating exogenous gene function through editing genomic DNA in a cancer cell with high genetic variance. In the present study, we showed that genomic DNA status should be considered when evaluating the exogenous gene function in a cancer cell line with high variant genome through stable transfection technology, immunostaining, wound healing assay, transwell invasion assay, real-time PCR, western blot and karyotyping analysis. Our results showed that the S100P expression level was not related to the migration and invasion abilities in these stably transfected cell lines derived from a human salivary adenoid cystic carcinoma cell line SACC-83. The MMP expression pattern was detected by western blot analysis which matched the biological behaviors in these cells. The genomic analysis showed that SACC-83 presented hypotetraploid karyotyping with high variance. Our data indicated that establishment of stable transgenic cancer cell lines should consider the status of genetic variance in a cancer cell to avoid any biased conclusion.

Wang, M., et al. (2018). "From Genetic Stock to Genome Editing: Gene Exploitation in Wheat." *Trends Biotechnol* **36**(2): 160-172.

Bread wheat (*Triticum aestivum*) ranks as one of our most important staple crops. However, its hexaploid nature has complicated our understanding of the genetic bases underlying many of its traits. Historically, functional genetic studies in wheat have

focused on identifying natural variations and have contributed to assembling and enriching its genetic stock. Recently, mold-breaking advances in whole genome sequencing, exome-capture based mutant libraries, and genome editing have revolutionized strategies for genetic research in wheat. We review new trends in wheat functional genetic studies along with germplasm conservation and innovation, including the relevance of genetic stocks, and the application of sequencing-based mutagenesis and genome editing. We also highlight the potential of multiplex genome editing toolkits in addressing species-specific challenges in wheat.

Wu, Y., et al. (2015). "Correction of a genetic disease by CRISPR-Cas9-mediated gene editing in mouse spermatogonial stem cells." *Cell Res* **25**(1): 67-79.

Spermatogonial stem cells (SSCs) can produce numerous male gametes after transplantation into recipient testes, presenting a valuable approach for gene therapy and continuous production of gene-modified animals. However, successful genetic manipulation of SSCs has been limited, partially due to complexity and low efficiency of currently available genetic editing techniques. Here, we show that efficient genetic modifications can be introduced into SSCs using the CRISPR-Cas9 system. We used the CRISPR-Cas9 system to mutate an EGFP transgene or the endogenous *Crygc* gene in SSCs. The mutated SSCs underwent spermatogenesis after transplantation into the seminiferous tubules of infertile mouse testes. Round spermatids were generated and, after injection into mature oocytes, supported the production of heterozygous offspring displaying the corresponding mutant phenotypes. Furthermore, a disease-causing mutation in *Crygc* (*Crygc* (-/-)) that pre-existed in SSCs could be readily repaired by CRISPR-Cas9-induced nonhomologous end joining (NHEJ) or homology-directed repair (HDR), resulting in SSC lines carrying the corrected gene with no evidence of off-target modifications as shown by whole-genome sequencing. Fertilization using round spermatids generated from these lines gave rise to offspring with the corrected phenotype at an efficiency of 100%.

Yadav, R., et al. (2017). "Gene editing and genetic engineering approaches for advanced probiotics: A review." *Crit Rev Food Sci Nutr*: 1-12.

The applications of probiotics are significant and thus resulted in need of genome analysis of probiotic strains. Various omics methods and systems biology approaches enables us to understand and optimize the metabolic processes. These techniques have increased the researcher's attention towards gut microbiome and provided a new source for the revelation of

uncharacterized biosynthetic pathways which enables novel metabolic engineering approaches. In recent years, the broad and quantitative analysis of modified strains relies on systems biology tools such as in silico design which are commonly used methods for improving strain performance. The genetic manipulation of probiotic microorganisms is crucial for defining their role in intestinal microbiota and exploring their beneficial properties. This review describes an overview of gene editing and systems biology approaches, highlighting the advent of omics methods which allows the study of new routes for studying probiotic bacteria. We have also summarized gene editing tools like TALEN, ZFNs and CRISPR-Cas that edits or cleave the specific target DNA. Furthermore, in this review an overview of proposed design of advanced customized probiotic is also hypothesized to improvise the probiotics.

Yamamoto, T. (2017). "[Genome editing ~Principle and possibility of a novel genetic engineering technology. Basic principles of genome editing.]." *Clin Calcium* **27**(11): 1638-1644.

Programmable site-specific nuclease mediated-genome editing is an emerging biotechnology for precise manipulation of target genes. In genome editing, gene-knockout as well as gene-knockin are possible in various organisms and cultured cells. CRISPR-Cas9, which was developed in 2012, is a convenient and efficient programmable site-specific nuclease and the use spreads around the world rapidly. For this, it is important for the progress of life science research to introduce the genome editing technology.

Yang, F., et al. (2017). "CRISPR/Cas9-loxP-Mediated Gene Editing as a Novel Site-Specific Genetic Manipulation Tool." *Mol Ther Nucleic Acids* **7**: 378-386.

Cre-loxP, as one of the site-specific genetic manipulation tools, offers a method to study the spatial and temporal regulation of gene expression/inactivation in order to decipher gene function. CRISPR/Cas9-mediated targeted genome engineering technologies are sparking a new revolution in biological research. Whether the traditional site-specific genetic manipulation tool and CRISPR/Cas9 could be combined to create a novel genetic tool for highly specific gene editing is not clear. Here, we successfully generated a CRISPR/Cas9-loxP system to perform gene editing in human cells, providing the proof of principle that these two technologies can be used together for the first time. We also showed that distinct non-homologous end-joining (NHEJ) patterns from CRISPR/Cas9-mediated gene editing of the targeting sequence locates at the level of plasmids (episomal) and

chromosomes. Specially, the CRISPR/Cas9-mediated NHEJ pattern in the nuclear genome favors deletions (64%-68% at the human AAVS1 locus versus 4%-28% plasmid DNA). CRISPR/Cas9-loxP, a novel site-specific genetic manipulation tool, offers a platform for the dissection of gene function and molecular insights into DNA-repair pathways.

Yong, L., et al. (2017). "Genetic editing of the androgen receptor contributes to impaired male courtship behavior in zebrafish." *J Exp Biol* **220**(Pt 17): 3017-3021.

Elucidating the genes that contribute to behavioral variation has become an important endeavor in behavioral studies. While advances in genomics have narrowed down the list of candidate genes, functional validation of them has lagged behind, partly because of challenges associated with rapid gene manipulations. Consequently, few studies have demonstrated causal genetic changes linked to behaviors. The 'gene editing revolution' has offered unprecedented opportunities to investigate candidate genes responsible for critical behaviors. Here, we edited the androgen receptor gene (AR), which is associated with male reproductive behavior in zebrafish, using TAL effector nucleases (TALENs), and tested whether modifications at the AR impacted courtship during mating trials. We reveal that males lacking AR courted females significantly less, showing reduced levels of stereotypic behaviors. Consistent with previous studies, disrupting androgen mechanisms can lead to behavioral changes with potential fitness consequences. Our study highlights the possibility of genetically altering a reproductive behavior, further solidifying the link between genotype and behavior.

Zentner, G. E. and M. J. Wade (2017). "The promise and peril of CRISPR gene drives: Genetic variation and inbreeding may impede the propagation of gene drives based on the CRISPR genome editing technology." *Bioessays* **39**(10).

Gene drives are selfish genetic elements that use a variety of mechanisms to ensure they are transmitted to subsequent generations at greater than expected frequencies. Synthetic gene drives based on the clustered regularly interspersed palindromic repeats (CRISPR) genome editing system have been proposed as a way to alter the genetic characteristics of natural populations of organisms relevant to the goals of public health, conservation, and agriculture. Here, we review the principles and potential applications of CRISPR drives, as well as means proposed to prevent their uncontrolled spread. We also focus on recent work suggesting that factors such as natural genetic

variation and inbreeding may represent substantial impediments to the propagation of CRISPR drives.

References

- Alapati, D. and E. E. Morrisey (2017). "Gene Editing and Genetic Lung Disease. Basic Research Meets Therapeutic Application." *Am J Respir Cell Mol Biol* **56**(3): 283-290.
- Alfson, K. J., et al. (2015). "Genetic Changes at the Glycoprotein Editing Site Associated With Serial Passage of Sudan Virus." *J Infect Dis* **212** Suppl 2: S295-304.
- Anant, S. and N. O. Davidson (2003). "Hydrolytic nucleoside and nucleotide deamination, and genetic instability: a possible link between RNA-editing enzymes and cancer?" *Trends Mol Med* **9**(4): 147-152.
- Azad, M. T. A., et al. (2017). "Site-directed RNA editing by adenosine deaminase acting on RNA for correction of the genetic code in gene therapy." *Gene Ther* **24**(12): 779-786.
- Baidu. <http://www.baidu.com>. 2018.
- Bankamp, B., et al. (2008). "Genetic variability and mRNA editing frequencies of the phosphoprotein genes of wild-type measles viruses." *Virus Res* **135**(2): 298-306.
- Bentolila, S., et al. (2008). "Genetic architecture of mitochondrial editing in Arabidopsis thaliana." *Genetics* **178**(3): 1693-1708.
- Bock, R. (2000). "Sense from nonsense: how the genetic information of chloroplasts is altered by RNA editing." *Biochimie* **82**(6-7): 549-557.
- Borel, F., et al. (2018). "Editing out five Serpina1 paralogs to create a mouse model of genetic emphysema." *Proc Natl Acad Sci U S A*.
- Bui, D. K., et al. (2015). "Genetic ME-a visualization application for merging and editing pedigrees for genetic studies." *BMC Res Notes* **8**: 241.
- Bundo, M., et al. (2010). "Mutation screening and assessment of the effect of genetic variations on expression and RNA editing of serotonin receptor 2C in the human brain." *Psychiatry Clin Neurosci* **64**(1): 57-61.
- Canal, C. E., et al. (2009). "RNA editing of the serotonin 2C receptor and expression of Galpha (q) protein: genetic mouse models do not support a role for regulation or compensation." *J Neurochem* **108**(5): 1136-1142.
- Cattaneo, R. (1990). "Messenger RNA editing and the genetic code." *Experientia* **46**(11-12): 1142-1148.
- Chakravarti, D., et al. (2016). "Synthetic biology approaches in cancer immunotherapy, genetic network engineering, and genome editing." *Integr Biol (Camb)* **8**(4): 504-517.

15. Collin, J. and M. Lako (2011). "Concise review: putting a finger on stem cell biology: zinc finger nuclease-driven targeted genetic editing in human pluripotent stem cells." Stem Cells 29(7): 1021-1033.
16. Danilenko, N. G. (2001). "[RNA-editing: genetic information is corrected after transcription]." Genetika 37(3): 294-316.
17. Di Giulio, M., et al. (2014). "RNA editing and modifications of RNAs might have favoured the evolution of the triplet genetic code from an ennuplet code." J Theor Biol 359: 1-5.
18. Gonen, S., et al. (2017). "Potential of gene drives with genome editing to increase genetic gain in livestock breeding programs." Genet Sel Evol 49(1): 3.
19. Google. <http://www.google.com>. 2018.
20. Gu, T., et al. (2016). "Genetic Architectures of Quantitative Variation in RNA Editing Pathways." Genetics 202(2): 787-798.
21. Gupta, R. M. and K. Musunuru (2014). "Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9." J Clin Invest 124(10): 4154-4161.
22. Hajjar, R. J., et al. (2003). "Genetic editing of dysfunctional myocardium." Med Clin North Am 87(2): 553-567.
23. Hassan, M. A. and J. P. Saeij (2014). "Incorporating alternative splicing and mRNA editing into the genetic analysis of complex traits." Bioessays 36(11): 1032-1040.
24. Hassan, M. A., et al. (2014). "The genetic basis for individual differences in mRNA splicing and APOBEC1 editing activity in murine macrophages." Genome Res 24(3): 377-389.
25. Heckl, D., et al. (2014). "Generation of mouse models of myeloid malignancy with combinatorial genetic lesions using CRISPR-Cas9 genome editing." Nat Biotechnol 32(9): 941-946.
26. Henry, M., et al. (2009). "Genetic editing of HBV DNA by monodomain human APOBEC3 cytidine deaminases and the recombinant nature of APOBEC3G." PLoS One 4(1): e4277.
27. Huang, J. J., et al. (2017). "Genome editing technologies drive the development of pig genetic improvement." Yi Chuan 39(11): 1078-1089.
28. Iwamoto, K., et al. (2009). "Serotonin receptor 2C and mental disorders: genetic, expression and RNA editing studies." RNA Biol 6(3): 248-253.
29. Jepson, J. E. and R. A. Reenan (2007). "Genetic approaches to studying adenosine-to-inosine RNA editing." Methods Enzymol 424: 265-287.
30. Jobson, R. W. and Y. L. Qiu (2008). "Did RNA editing in plant organellar genomes originate under natural selection or through genetic drift?" Biol Direct 3: 43.
31. Kang, X., et al. (2016). "Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing." J Assist Reprod Genet 33(5): 581-588.
32. Kankowski, S., et al. (2017). "A Novel RNA Editing Sensor Tool and a Specific Agonist Determine Neuronal Protein Expression of RNA-Edited Glycine Receptors and Identify a Genomic APOBEC1 Dimorphism as a New Genetic Risk Factor of Epilepsy." Front Mol Neurosci 10: 439.
33. Kobayashi, M., et al. (1998). "Lactone-ring-cleaving enzyme: genetic analysis, novel RNA editing, and evolutionary implications." Proc Natl Acad Sci U S A 95(22): 12787-12792.
34. Kobayashi, M., et al. (1999). "Genetic analysis of cytochrome b5 from arachidonic acid-producing fungus, *Mortierella alpina* 1S-4: cloning, RNA editing and expression of the gene in *Escherichia coli*, and purification and characterization of the gene product." J Biochem 125(6): 1094-1103.
35. Kurmangaliyev, Y. Z., et al. (2015). "Genetic Determinants of RNA Editing Levels of ADAR Targets in *Drosophila melanogaster*." G3 (Bethesda) 6(2): 391-396.
36. Lander, N., et al. (2016). "Genome Editing by CRISPR/Cas9: A Game Change in the Genetic Manipulation of Protists." J Eukaryot Microbiol 63(5): 679-690.
37. Landweber, L. F. and W. Gilbert (1993). "RNA editing as a source of genetic variation." Nature 363(6425): 179-182.
38. Landweber, L. F. and W. Gilbert (1994). "Phylogenetic analysis of RNA editing: a primitive genetic phenomenon." Proc Natl Acad Sci U S A 91(3): 918-921.
39. Lang, J., et al. (2016). "Receptor editing and genetic variability in human autoreactive B cells." J Exp Med 213(1): 93-108.
40. Lavrov, D. V., et al. (2013). "Mitochondrial DNA of *Clathrina clathrus* (Calcarea, Calcinea): six linear chromosomes, fragmented rRNAs, tRNA editing, and a novel genetic code." Mol Biol Evol 30(4): 865-880.
41. Li, K., et al. (2016). "Transcriptome, genetic editing, and microRNA divergence substantiate sympatric speciation of blind mole rat, *Spalax*." Proc Natl Acad Sci U S A 113(27): 7584-7589.
42. Liu, L. F., et al. (1998). "Genetic characterization of the mRNAs encoding alpha-bungarotoxin: isoforms and RNA editing in *Bungarus multicinctus* gland cells." Nucleic Acids Res 26(24): 5624-5629.

43. Louzoun, Y., et al. (2006). "Copying nodes versus editing links: the source of the difference between genetic regulatory networks and the WWW." *Bioinformatics* 22(5): 581-588.
44. Ma H, Chen G. Stem cell. *The Journal of American Science* 2005;1(2):90-92.
45. Ma H, Cherg S. Eternal Life and Stem Cell. *Nature and Science*. 2007;5(1):81-96.
46. Ma H, Cherg S. Nature of Life. *Life Science Journal* 2005;2(1):7-15.
47. Ma H, Yang Y. *Turritopsis nutricula*. *Nature and Science* 2010;8(2):15-20. http://www.sciencepub.net/nature/ns0802/03_1279_hongbao_turritopsis_ns0802_15_20.pdf.
48. Ma H. The Nature of Time and Space. *Nature and science* 2003;1(1):1-11. *Nature and science* 2007;5(1):81-96.
49. Maas, S. and A. Rich (2000). "Changing genetic information through RNA editing." *Bioessays* 22(9): 790-802.
50. Maier, R. M., et al. (1995). "Complete sequence of the maize chloroplast genome: gene content, hotspots of divergence and fine tuning of genetic information by transcript editing." *J Mol Biol* 251(5): 614-628.
51. Marsland Press. <http://www.sciencepub.net>. 2018.
52. Martinez-Garcia, E. and V. de Lorenzo (2012). "Transposon-based and plasmid-based genetic tools for editing genomes of gram-negative bacteria." *Methods Mol Biol* 813: 267-283.
53. Mitzelfelt, K. A., et al. (2017). "Efficient Precision Genome Editing in iPSCs via Genetic Co-targeting with Selection." *Stem Cell Reports* 8(3): 491-499.
54. Morrison, D. A., et al. (2015). "Genome editing by natural genetic transformation in *Streptococcus mutans*." *J Microbiol Methods* 119: 134-141.
55. Nakamuta, M., et al. (1996). "Complete phenotypic characterization of apobec-1 knockout mice with a wild-type genetic background and a human apolipoprotein B transgenic background, and restoration of apolipoprotein B mRNA editing by somatic gene transfer of Apobec-1." *J Biol Chem* 271(42): 25981-25988.
56. Nangle, L. A., et al. (2002). "Genetic code ambiguity. Cell viability related to the severity of editing defects in mutant tRNA synthetases." *J Biol Chem* 277(48): 45729-45733.
57. National Center for Biotechnology Information, U.S. National Library of Medicine. <http://www.ncbi.nlm.nih.gov/pubmed>. 2018.
58. Novoa, E. M., et al. (2015). "Ancestral AlaX editing enzymes for control of genetic code fidelity are not tRNA-specific." *J Biol Chem* 290(16): 10495-10503.
59. Oseni, S., et al. (2004). "Genetic parameters for days open and pregnancy rates in US Holsteins using different editing criteria." *J Dairy Sci* 87(12): 4327-4333.
60. Pring, D., et al. (1993). "RNA editing gives a new meaning to the genetic information in mitochondria and chloroplasts." *Plant Mol Biol* 21(6): 1163-1170.
61. Ramaswami, G., et al. (2015). "Genetic mapping uncovers cis-regulatory landscape of RNA editing." *Nat Commun* 6: 8194.
62. Retter, M. W. and D. Nemazee (1999). "Receptor editing: genetic reprogramming of autoreactive lymphocytes." *Cell Biochem Biophys* 31(1): 81-88.
63. Rivera-Torres, N. and E. B. Kmiec (2016). "Genetic spell-checking: gene editing using single-stranded DNA oligonucleotides." *Plant Biotechnol J* 14(2): 463-470.
64. Roesch, E. A. and M. L. Drumm (2017). "Powerful tools for genetic modification: Advances in gene editing." *Pediatr Pulmonol* 52(S48): S15-S20.
65. Sanchez-Rivera, F. J., et al. (2014). "Rapid modelling of cooperating genetic events in cancer through somatic genome editing." *Nature* 516(7531): 428-431.
66. Scott, D. A. and F. Zhang (2017). "Implications of human genetic variation in CRISPR-based therapeutic genome editing." *Nat Med* 23(9): 1095-1101.
67. Seiwert, S. D. and K. Stuart (1994). "RNA editing: transfer of genetic information from gRNA to precursor mRNA in vitro." *Science* 266(5182): 114-117.
68. Shen, L., et al. (2017). "Rapid generation of genetic diversity by multiplex CRISPR/Cas9 genome editing in rice." *Sci China Life Sci* 60(5): 506-515.
69. Shi, Z. D., et al. (2017). "Genome Editing in hPSCs Reveals GATA6 Haploinsufficiency and a Genetic Interaction with GATA4 in Human Pancreatic Development." *Cell Stem Cell* 20(5): 675-688 e676.
70. Slattery, S. S., et al. (2018). "An Expanded Plasmid-Based Genetic Toolbox Enables Cas9 Genome Editing and Stable Maintenance of Synthetic Pathways in *Phaeodactylum tricorutum*." *ACS Synth Biol* 7(2): 328-338.
71. Stuart, K. (1989). "RNA editing: new insights into the storage and expression of genetic information." *Parasitol Today* 5(1): 5-8.

72. Stuart, K. (1998). "RNA editing: trypanosomes rewrite the genetic code." Verh K Acad Geneeskde Belg 60(1): 63-74.
73. Suspene, R., et al. (2011). "Genetic editing of herpes simplex virus 1 and Epstein-Barr herpesvirus genomes by human APOBEC3 cytidine deaminases in culture and in vivo." J Virol 85(15): 7594-7602.
74. Tajaddod, M., et al. (2016). "The dynamic epitranscriptome: A to I editing modulates genetic information." Chromosoma 125(1): 51-63.
75. Takenaka, M., et al. (2010). "Reverse genetic screening identifies five E-class PPR proteins involved in RNA editing in mitochondria of *Arabidopsis thaliana*." J Biol Chem 285(35): 27122-27129.
76. Tauson, E. L. (2004). "[RNA editing in different genetic systems]." Zh Obshch Biol 65(1): 52-73.
77. Telugu, B. P., et al. (2017). "Genome editing and genetic engineering in livestock for advancing agricultural and biomedical applications." Mamm Genome 28(7-8): 338-347.
78. Thompson, J. and S. Gopal (2006). "Correction: genetic algorithm learning as a robust approach to RNA editing site prediction." BMC Bioinformatics 7: 406.
79. Thompson, J. and S. Gopal (2006). "Genetic algorithm learning as a robust approach to RNA editing site prediction." BMC Bioinformatics 7: 145.
80. Tidball, A. M., et al. (2017). "Rapid Generation of Human Genetic Loss-of-Function iPSC Lines by Simultaneous Reprogramming and Gene Editing." Stem Cell Reports 9(3): 725-731.
81. Torikai, H., et al. (2016). "Genetic editing of HLA expression in hematopoietic stem cells to broaden their human application." Sci Rep 6: 21757.
82. Traxler, E. A., et al. (2016). "A genome-editing strategy to treat beta-hemoglobinopathies that recapitulates a mutation associated with a benign genetic condition." Nat Med 22(9): 987-990.
83. Vu, L. T. and T. Tsukahara (2017). "C-to-U editing and site-directed RNA editing for the correction of genetic mutations." Biosci Trends 11(3): 243-253.
84. Vu, L. T., et al. (2015). "Changing blue fluorescent protein to green fluorescent protein using chemical RNA editing as a novel strategy in genetic restoration." Chem Biol Drug Des 86(5): 1242-1252.
85. Vu, L. T., et al. (2016). "Chemical RNA Editing for Genetic Restoration: The Relationship between the Structure and Deamination Efficiency of Carboxyvinyldeoxyuridine Oligodeoxynucleotides." Chem Biol Drug Des 87(4): 583-593.
86. Wakui, M., et al. (2004). "Genetic dissection of lupus pathogenesis: Sle3/5 impacts IgH CDR3 sequences, somatic mutations, and receptor editing." J Immunol 173(12): 7368-7376.
87. Wang, L., et al. (2014). "Editing genomic DNA in cancer cells with high genetic variance: benefit or risk?" Oncol Rep 31(5): 2079-2084.
88. Wang, M., et al. (2018). "From Genetic Stock to Genome Editing: Gene Exploitation in Wheat." Trends Biotechnol 36(2): 160-172.
89. Wikipedia. The free encyclopedia. <http://en.wikipedia.org>. 2018.
90. Wu, Y., et al. (2015). "Correction of a genetic disease by CRISPR-Cas9-mediated gene editing in mouse spermatogonial stem cells." Cell Res 25(1): 67-79.
91. Yadav, R., et al. (2017). "Gene editing and genetic engineering approaches for advanced probiotics: A review." Crit Rev Food Sci Nutr: 1-12.
92. Yamamoto, T. (2017). "[Genome editing ~Principle and possibility of a novel genetic engineering technology. Basic principles of genome editing.]" Clin Calcium 27(11): 1638-1644.
93. Yang, F., et al. (2017). "CRISPR/Cas9-loxP-Mediated Gene Editing as a Novel Site-Specific Genetic Manipulation Tool." Mol Ther Nucleic Acids 7: 378-386.
94. Yong, L., et al. (2017). "Genetic editing of the androgen receptor contributes to impaired male courtship behavior in zebrafish." J Exp Biol 220(Pt 17): 3017-3021.
95. Zentner, G. E. and M. J. Wade (2017). "The promise and peril of CRISPR gene drives: Genetic variation and inbreeding may impede the propagation of gene drives based on the CRISPR genome editing technology." Bioessays 39(10).