Alec Pokrandt

CRISPR

Outline

- Liu Lab
- What the CRISPR system is and how it works
- Why CRISPR is important
- Advanced editing techniques enabled by The Holy Grail
- Applications and real-world results
- Our unique opportunity in time
- Deep future thoughts

- Liu's lab aims:
  - illuminate biological processes
  - enable next-generation therapeutics
  - advance genetic medicine and biotechnology

Synthetic Molecules:
discovering bioactive synthetic small molecules and polymers through DNA-templated organic synthesis (drug discovery)

Protein Evolution:
phage-assisted continuous evolution (PACE) to evolve proteins with novel therapeutic potentials

Genome Editing:
engineering and delivering genome editing proteins, such as base editors and prime editors

DNA-Templated Synthesis

Protein Evolution & Delivery

Genome Editing

- CRISPR is a **bacterial immune system**
- The bacterial system **integrates fragments of viral sequences into its own genome**
- The system includes an **enzyme** (Cas-CRISPR associated protein)
  - It has helicase and nuclease activity
  - It is guided by a **gRNA** (guide RNA)
- Integrated sequences can be transcribed, complexed with Cas, and used to base pair with that viral sequence if it is infected again
- The result is cleavage of viral sequences: **viral immunity**

- The sequence of the system eventually named CRISPR was first observed in Japan in 1987 by Ishino et al.
  - They did not know it, but they had identified something that decades later would change the future of the human race

An unusual structure was found in the 3′-end flanking region of *iap* (Fig. 5). Five highly homologous sequences of 29 nucleotides were arranged as direct repeats with 32 nucleotides as spacing. The first sequence was included in the putative transcriptional termination site and had less homology than the others. Well-conserved nucleotide sequences containing a dyad symmetry, named REP sequences, have been found in *E. coli* and *Salmonella typhimurium* (28) and may act to stabilize mRNA (18). A dyad symmetry with 14 nucleotide pairs was also found in the middle of these sequences (underlining, Fig. 5), but no homology was found between these sequences and the REP sequence. So far, no sequence homologous to these has been found elsewhere in procaryotes, and the biological significance of these sequences is not known.

Dozens of Cas homologues have been discovered.

https://doi.org/10.1126/science.1247997

https://doi.org/10.1126/science.1247997

https://doi.org/10.2210/pdb7S4X/pdb

Thought Experiment

CRISPR system integrates viral sequences into its genome

Cas9 protein complexes with a transcript of the viral DNA sequence library

Cas9 protein then cuts DNA sequences complementary to its complexed gRNA (library transcript)

What is the implication of these facts?

PAM (Proto Spacer Adjacent Motif)

- PAM is a portion of the Cas9 enzyme that recognizes and binds a 3 base DNA sequence (NGG)
  - The CRISPR system has evolved to not integrate PAM binding sequences into its own genome!!!

Why the hell does all this matter?

- We can complex Cas9 with a synthetic gRNA to cleave any DNA

Article | Published: November 1998

A specific monovalent metal ion integral to the AA platform of the RNA tetraloop receptor

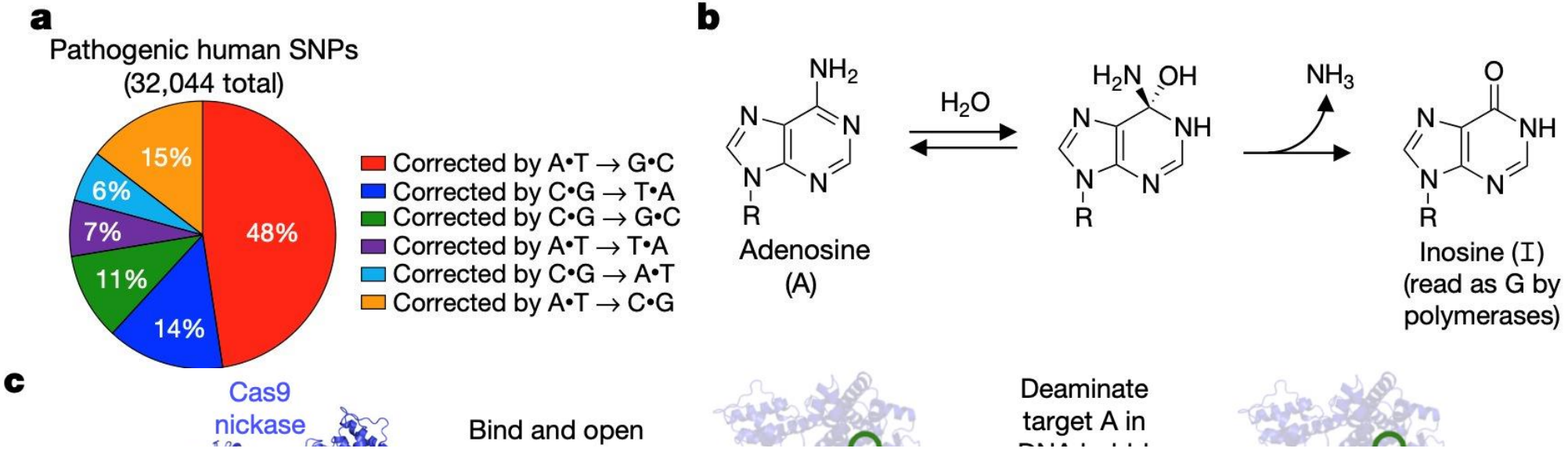
Soumitra Basu, Robert P. Rambo, Juliane Strauss-Soukup, Jamie H.Cate, Adrian R. Ferré-D´Amaré, Scott A. Strobel & Jennifer A. Doudna

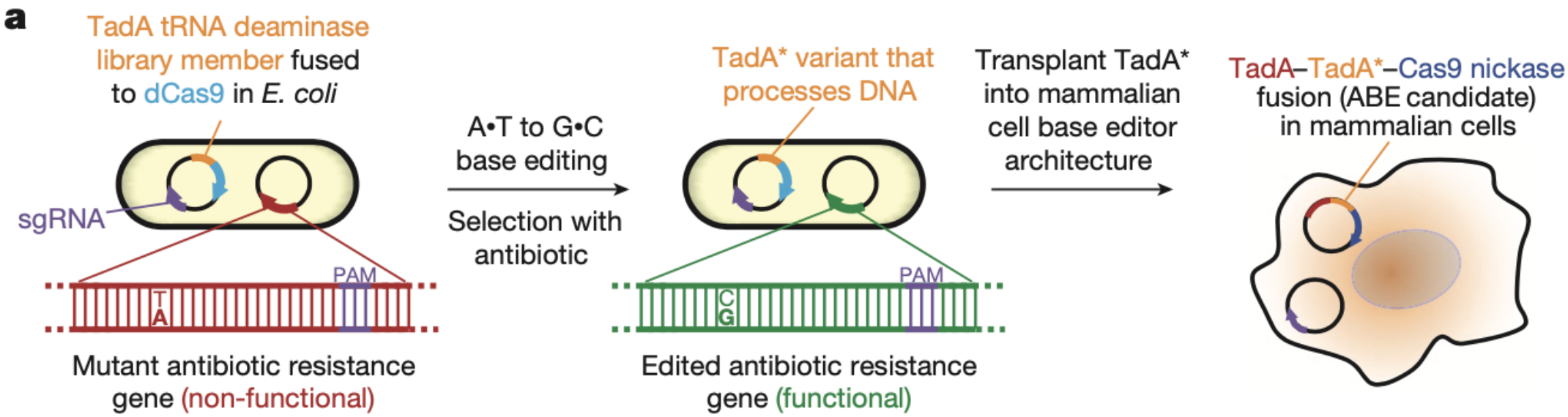
  - TALENs

gRNA- The Holy Grail

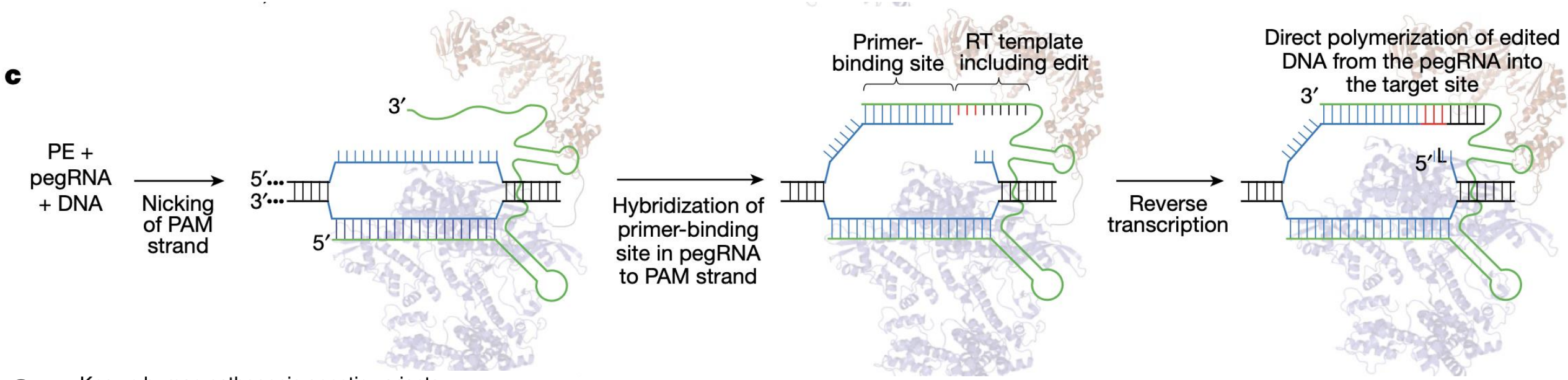
- The critical implication of Cas9 is that you can **deliver something to DNA at a specific sequence**
- What if one day we could redesign the Cas9 enzyme to do more complex and advanced modifications beyond cleavage?

Base Editing

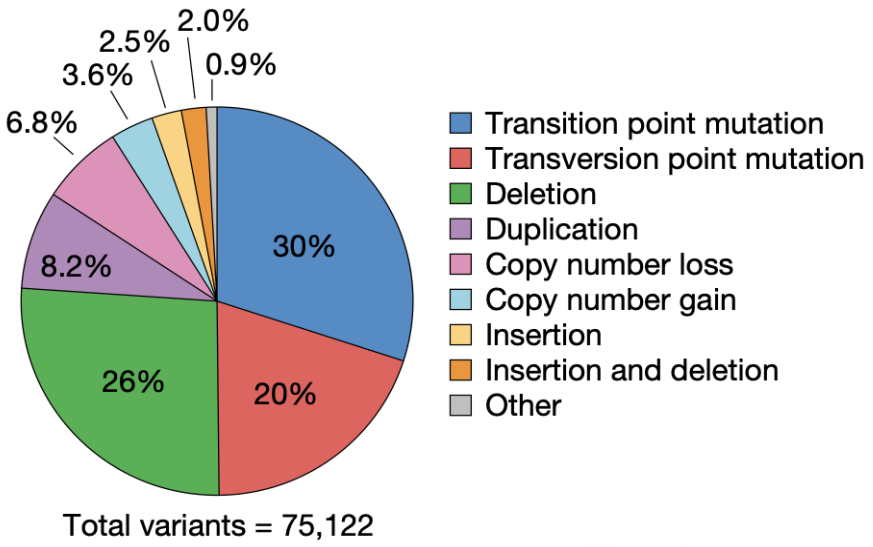




Prime Editing



a Known human pathogenic genetic variants

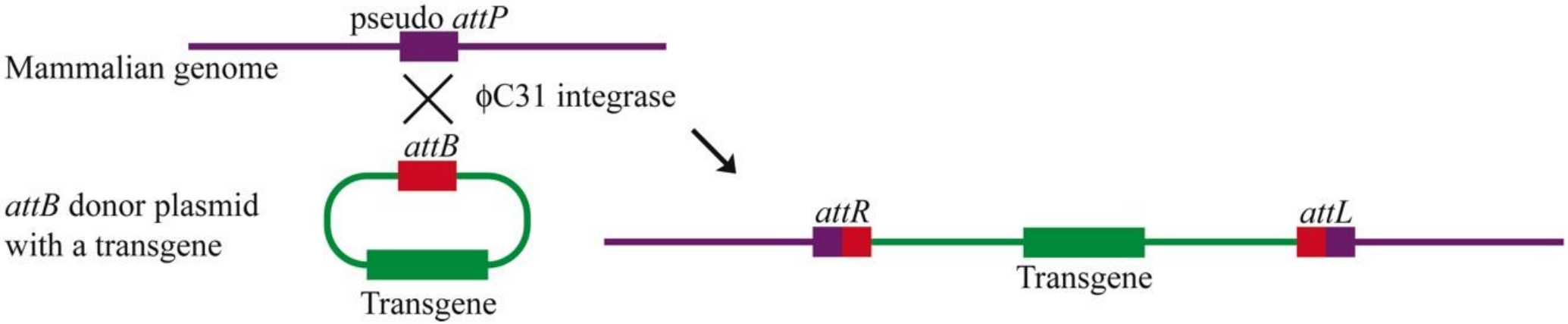


Prime editing scope:

- All 4 transition point mutations
- All 8 transversion point mutations
- Insertions (1 bp to ≥ 44 bp)
- Deletions (1 bp to ≥ 80 bp)
- Combinations of the above

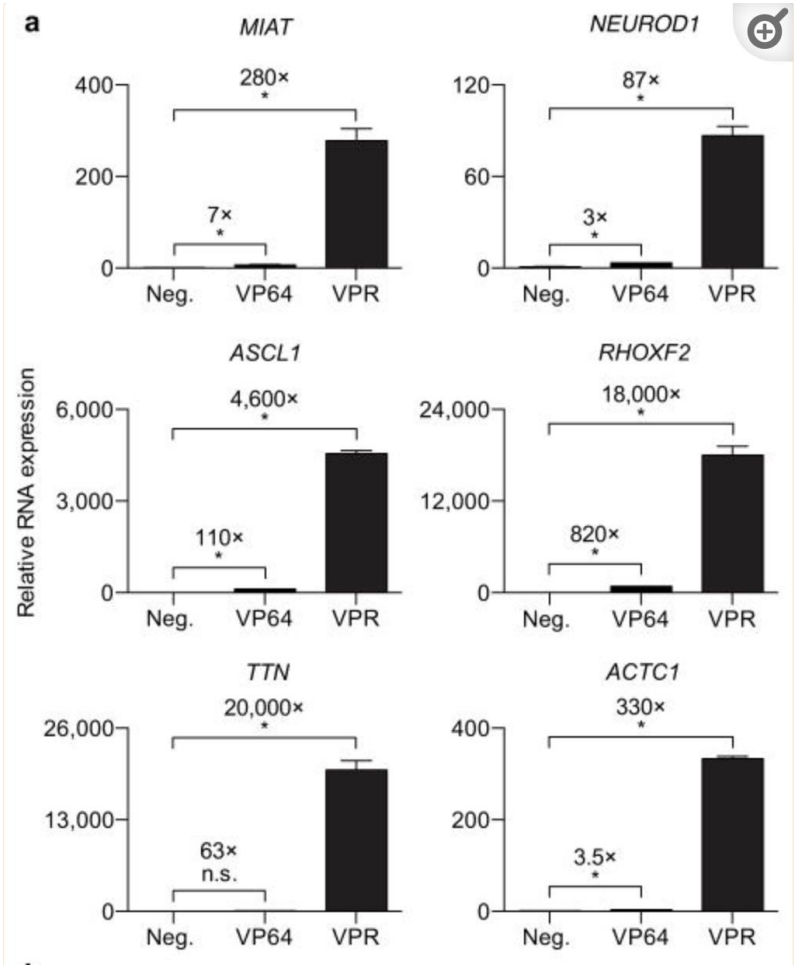
Large Payload Integration

- Bxb1 is a site specific integrase that will insert a DNA payload
  - **Payload** has **attP** sequence
  - **Target** site has **attB** sequence

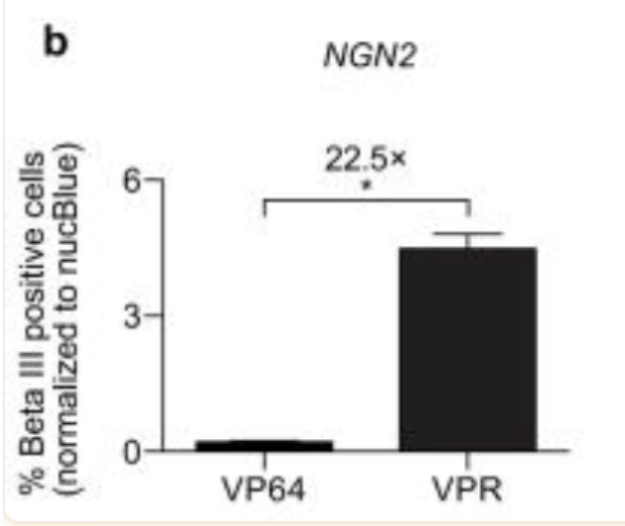


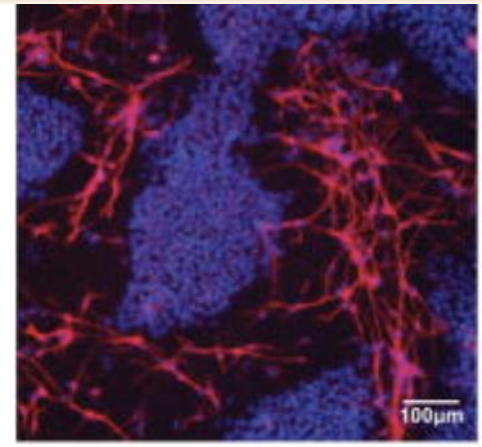
CRISPRa/i

- CRISPRi
  - i is for **inhibits** transcription
- CRISPRa
  - a is for **activation**
- Church et al. show impressive activation of target genes



VPR is their improved activation system.





"We demonstrate its utility in activating endogenous coding and non-coding genes, targeting several genes simultaneously and **stimulating neuronal differentiation** of hiPSCs"

Applications of the suite

- Research
  - Change expression - protein or RNAs
  - Change 1 or more bases in a protein to understand functions
  - complex genetically encoded systems (for example, CRISPR-recorder)
- Therapy
  - Change expression to control cells for wound healing, regeneration, differentiation
  - Repairing deleterious SNP or mutations
  - Replacing and repairing
  - Immune cell therapies
  - Allogenic cell therapies (T1D cure)
- Engineering
  - Synthetic Bio
  - Crops/Livestock:
    - High yield
    - Drought/Disease Resistance
    - Nutritious
- Living Biosensors

CRISPR Therapeutics AG | Ticker:CRSP

- The FDA approved the cure of SCD this year: Casgevy
  - Simple technique: Cas9 cleavage
    - Indels in silencer protein of fHb
    - fHb is now turned on
    - SCD is cured

"Jimi Olaghere on the summit of Kilimanjaro, Sept. 16, 2024. Olaghere, 39, was functionally cured of sickle cell disease by an infusion of Casgevy in September 2020."

Xenotransplantation

- 69 Edits in total (nice)
  - Remove genes that cause immune rejection
  - Disable retrovirus
  - Add human genes that **modulate immune responses, prevent clotting, and reduce rejection**
  - Hearts and kidneys: success in humans
    - **Heart:** The pig was infected with a herpes virus and the patient died months later
    - **Kidney:** took place March, 2024. As of now, the patient is healthy and dialysis free

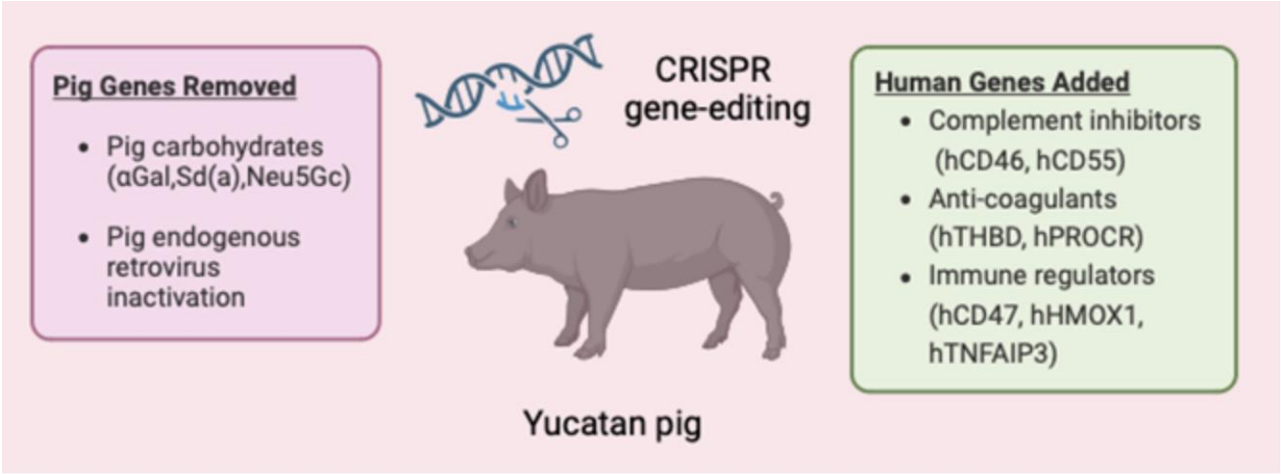
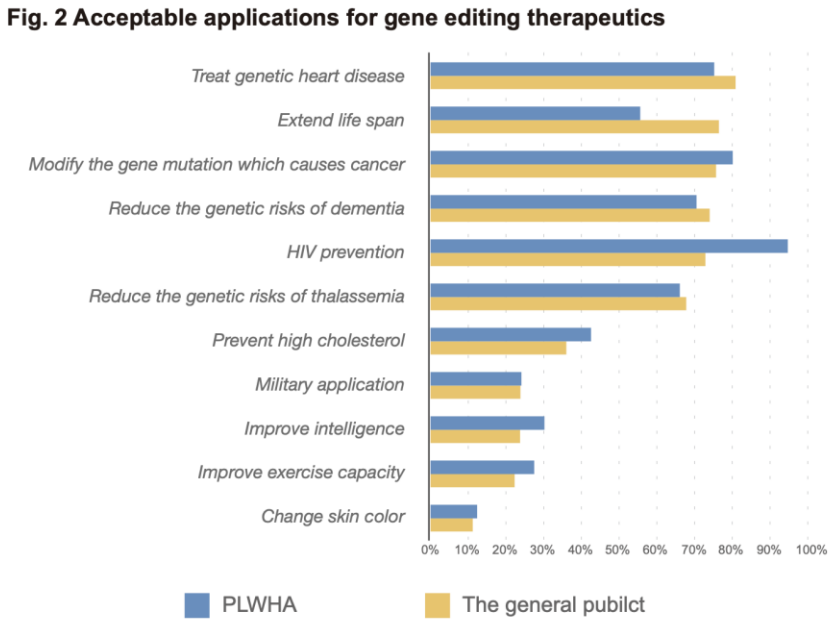


Illustration of the gene edits made to the donor pig. Image: Mass General

Embryo Editing

- In China, HIV burdens people and children with astounding social stigma and exile. Even with **undetectable viral load**
  - "attitudes toward HIV infected individuals take forms of **denial, indifference, labeling, separation, rejection, status loss, shame, hopelessness, and fear**"



Dr. Jiankui used Cas9 to **disrupt the CCR5 gene** from 2 embryos, removing the attachment point for HIV

- There is no evidence the mother has HIV- the father supposedly does
- Dr. Jiankui was sentences to 3 years in prison, fined $427,000
  - Convicted of "illegal medical practices"
  - Forged ethical review data and misled doctors https://www.science.org/content/article/chinese-scientist-who-produced-genetically-altered-babies-sentenced-3-years-jail
  - In a deleted tweet, he claimed that the CCP seized all his stocks

"Get in luddite, we're going gene editing." -Dr. He Jiankui

- He is now proposing introducing the APP A673T mutation to mouse and human embryos in vitro
  - This will effectively test the beta amyloid plaque hypothesis

Research Proposal

Human embryo gene editing to protect against Alzheimer's disease

Jiankui He, Ph.D.

The ageing population is of grave importance as both a socioeconomic issue and a strain on the medical system. More than 5% of the population above 60 years old is effected by dementia; of these, two thirds are the result of Alzheimer's disease (AD). After the age of 65, the prevalence of Alzheimer's disease almost doubles every five years. Currently, there is no effective drug treatment for Alzheimer's disease.

The APP gene point mutation A673T was discovered to decrease the incidence of this disease. In 2012, Jonsson et al. published a study in which they searched for APP coding variants in a sample of 1,795 Icelanders using whole-genome sequencing. They found a point mutation in the APP gene wherein the alanine at position 673 was substituted for a threonine (A673T), and this mutation protects against Alzheimer's disease. The A673T mutation was shown to reduce the formation of β-amyloid peptides by about 40%. Moreover, the A673T mutation may also help to prolong the lifespan of its carriers. Indeed, individuals with this mutation were reported to have 1.47 times greater chances of reaching the age of 85 when compared to non-carriers. Jonsson et al. concluded that the A673T mutation confers a strong protection against Alzheimer's disease. Later, Kero et al. found the A673T variant in a deceased individual aged 104.8 years whose brain presented little β-amyloid pathology. This report supports the hypothesis that A673T protects the brain against β-amyloid accumulation and Alzheimer's disease.

We propose to introduce the APP(A673T) mutation into embryos, and test whether this mutation confers protection against Alzheimer's disease.

Recently, there have been reports that classical CRISPR-Cas9 gene editing technologies may have unwanted and potentially dangerous consequences if they are applied to human embryos. In our study we will use base editing technologies. Compared to classical CRISPR-Cas9, base editing can avoid a double-strand DNA break, and therefore is safer for human embryo editing.

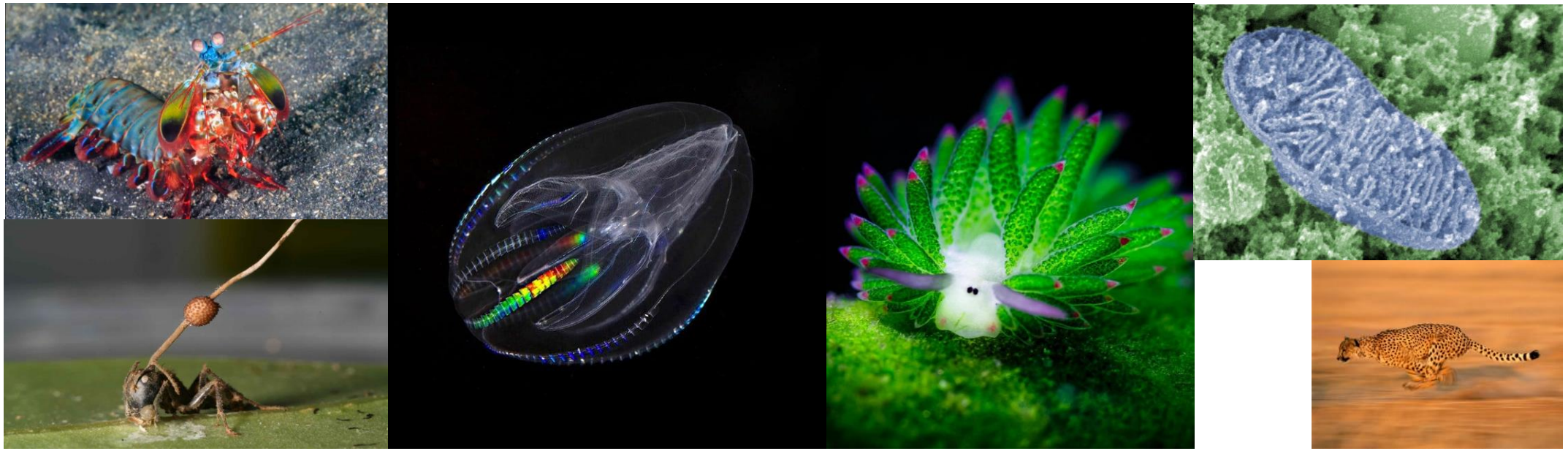
We will first perform an experiment using a mouse model. By introducing the APP A673T mutation in mouse embryo, a variety of outcome measures will be applied to APP A673T mouse, including both neuropathological and functional test.

We then will introduce APP A673T mutation into human tripronuclear zygotes. Extensive studies have shown that polyspermic zygotes such as tripronuclear (3PN) zygotes, discarded in clinics, may serve as an alternative for studies of normal human zygotes. Polyspermic zygotes, which occur in ~2%–5% of zygotes during in vitro fertilization (IVF) clinical trials, may generate blastocysts in vitro but invariably fail to develop normally in vivo, providing an ideal model system to examine the targeting efficiency and off-target effects of base editing APP A673T during early human embryonic development.

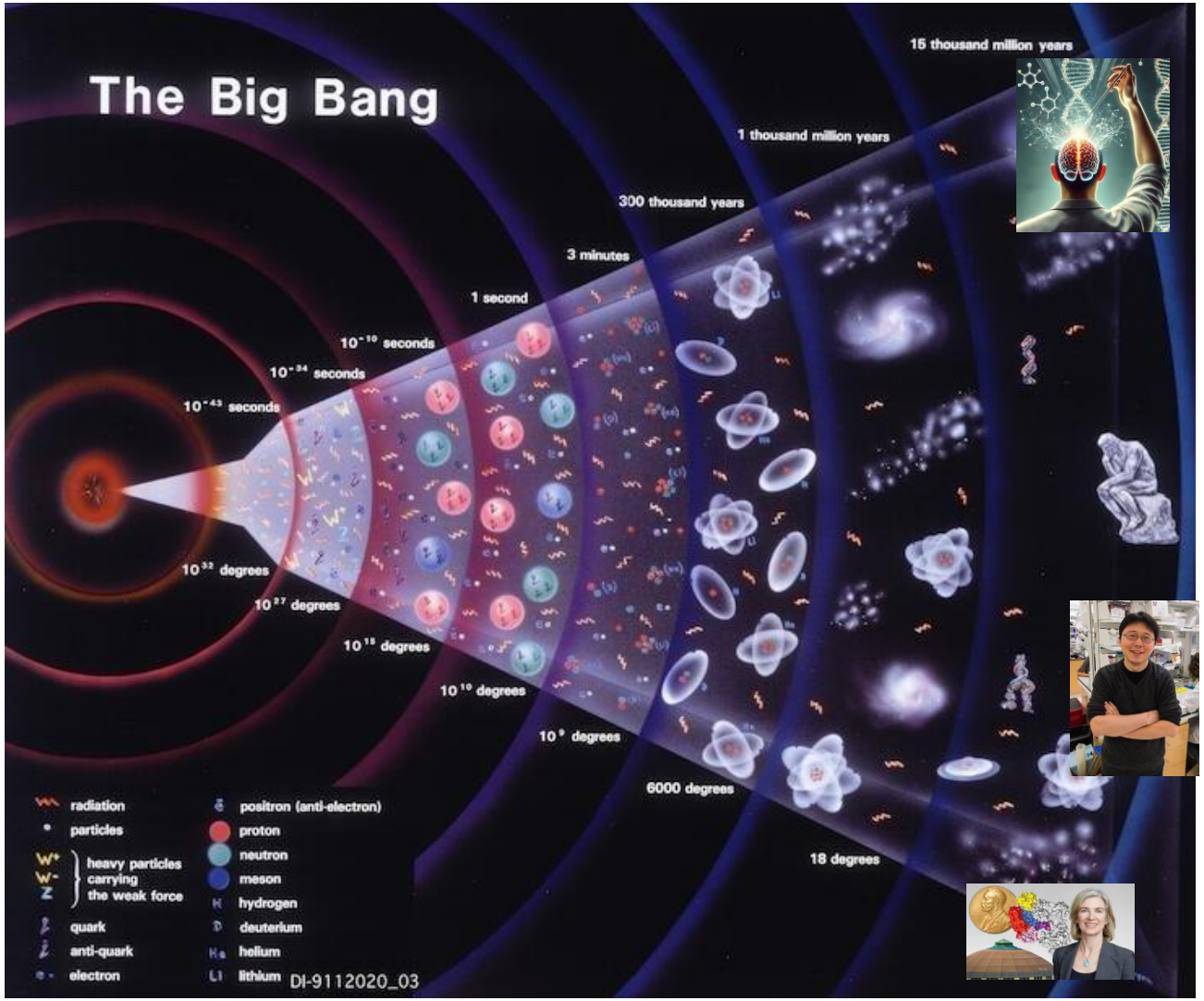
*Note: no human embryo will be implanted for pregnancy in this study.
*Note: government permit and ethical approval are required before any experiment is conducted.

Biology is Technology –not magic

- Any given organism on Earth is more complex and ingenious then any technology ever invented by man



- All biology is hardware encoded by software. That hardware protects, gathers resources and duplicate the software.



- **Big Bang** — The beginning of the universe, around 13.8 billion years ago, where all matter and energy emerged from a singularity.
- **Stars** — After millions of years, hydrogen and helium gases coalesced to form the first stars, illuminating the universe.
- **Planets** — Planets formed around stars, creating systems like our solar system where Earth emerged as a habitable planet.
- **Biology** — Life began on Earth about 3.8 billion years ago, starting with simple single-celled organisms evolving into complex multicellular life.
- **Consciousness & Intelligence** — Human beings and other

We live in a unique and transformational time: for the first time in the known history of the universe, it has the ability to observe itself, pear into its own functions, and manipulate itself.

How to Get Rich
(without getting lucky)

WEALTH, MONEY, STATUS. Seek wealth, not money or status. Wealth is having assets that earn while you sleep. Money is how we transfer time and wealth. Status is your place in the social hierarchy. Understand that ethical wealth creation is possible. If you secretly despise wealth, it will elude you. Ignore people playing status games. They gain status by attacking people playing wealth creation games.

EQUITY. You're not going to get rich renting out your time. You must own equity - a piece of a business - to gain your financial freedom. You will get rich by giving society what it wants but does not yet know how to get. At scale. Pick an industry where you can play long term games with long term people. The Internet has massively broadened the possible space of careers. Most people haven't figured this out yet.

TACTICS. Play iterated games. All the returns in life, whether in wealth, relationships, or knowledge, come from compound interest. Pick business partners with high intelligence, energy, and, above all, integrity. Don't partner with cynics and pessimists. Their beliefs are self-fulfilling. Learn to sell. Learn to build. If you can do both, you will be unstoppable.

SPECIFIC KNOWLEDGE. Arm yourself with specific knowledge, accountability, and leverage. Specific knowledge is knowledge that you cannot be trained for. If society can train you, it can train someone else, and replace you. Specific knowledge is found by pursuing your genuine curiosity and passion rather than whatever is hot right now. Building specific knowledge will feel like play to you but will look like work to others. When specific knowledge is taught, it's through apprenticeships, not schools. Specific knowledge is often highly technical or creative. It cannot be outsourced or automated.

ACCOUNTABILITY. Embrace accountability, and take business risks under your own name. Society will reward you with responsibility, equity, and leverage. The most accountable people have singular, public, and risky brands: Oprah, Trump, Kanye, Elon.

LEVERAGE. *"Give me a lever long enough, and a place to stand, and I will move the earth." - Archimedes* Fortunes require leverage. Business leverage comes from capital, people, and products with no marginal cost of replication (code and media). Capital means money. To raise money, apply your specific knowledge, with accountability, and show resulting good judgment. Labor means people working for you. It's the oldest and most fought-over form of leverage. Labor leverage will impress your parents, but don't waste your life chasing it. Capital and labor are permissioned leverage. Everyone is chasing capital, but someone has to give it to you. Everyone is trying to lead, but someone has to follow you. Code and media are permissionless leverage. They're the leverage behind the newly rich. You can create software and media that works for you while you sleep. An army of robots is freely available - it's just packed in data centers for heat and space efficiency. Use it. If you can't code, write books and blogs, record videos and podcasts. Leverage is a force multiplier for your judgement. Judgement requires experience, but can be built faster by learning foundational skills.

SKILLS. There is no skill called "business." Avoid business magazines and business classes. Study microeconomics, game theory, psychology, persuasion, ethics, mathematics, and computers. Reading is faster than listening. Doing is faster than watching.

YOU. You should be too busy to "do coffee," while still keeping an uncluttered calendar. Set and enforce an aspirational personal hourly rate. If fixing a problem will save less than your hourly rate, ignore it. If outsourcing a task will cost less than your hourly rate, outsource it. Work as hard as you can. Even though who you work with and what you work on are more important than how hard you work. Become the best in the world at what you do. Keep redefining what you do until this is true.

PARTING WORDS. There are no get rich quick schemes. That's just someone else getting rich off you. Apply specific knowledge, with leverage, and eventually you will get what you deserve. When you're finally wealthy, you'll realize that it wasn't what you were seeking in the first place. But that's for another day.

Naval Ravikant. @naval

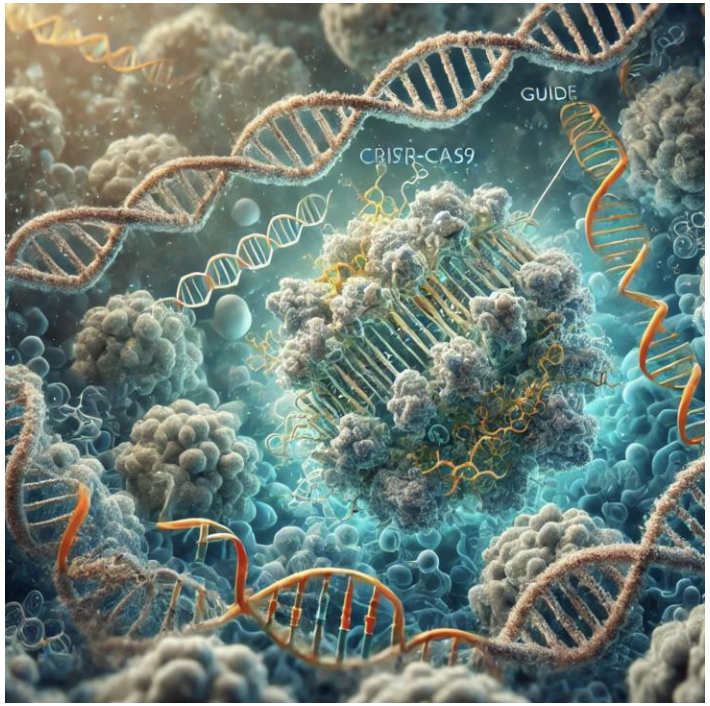
Software

DNA is too!

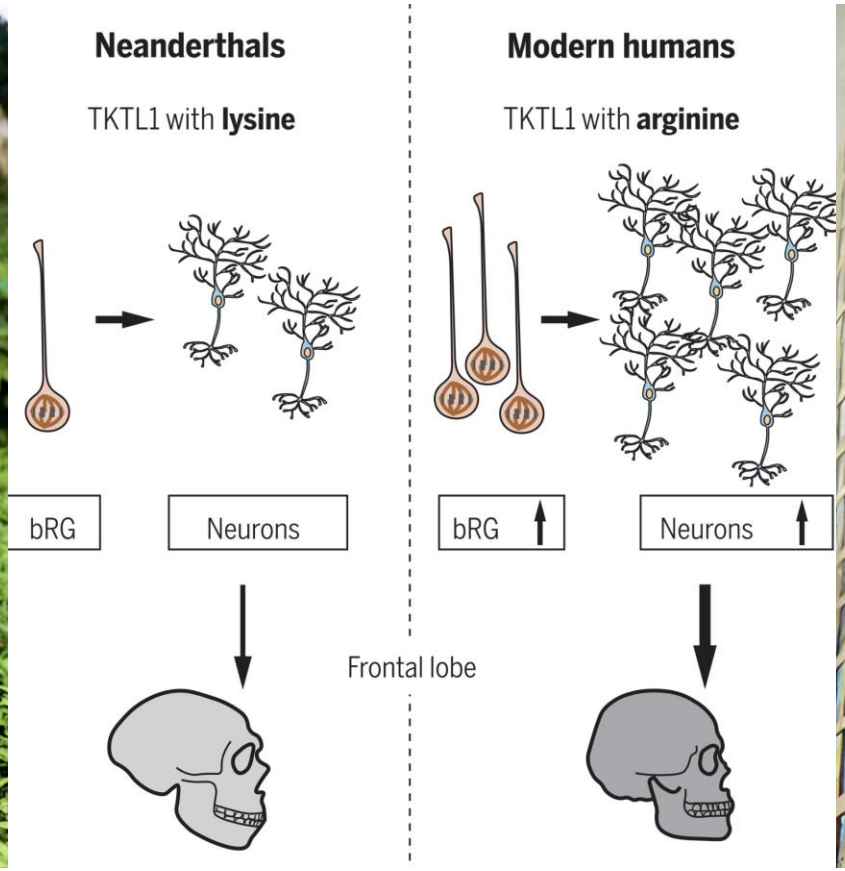
- Computer code enable humans to store, manipulate, transport, and analyze **data**

- CRISPR enables you and me to write DNA code.
- The limit: the laws of physics
- We are starting our careers during the emergence of this capability

Deep future thoughts – BSI & BCI



Deep future thoughts – Assimilation



Deep future thoughts- Terraforming

Deep future thoughts -Panspermia

More life=good

Citations

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Slide 5:
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