A CRISPR Philosophy

How a Prokaryotic Immunity-Generating Mechanism Invaded the Center of Molecular Biology

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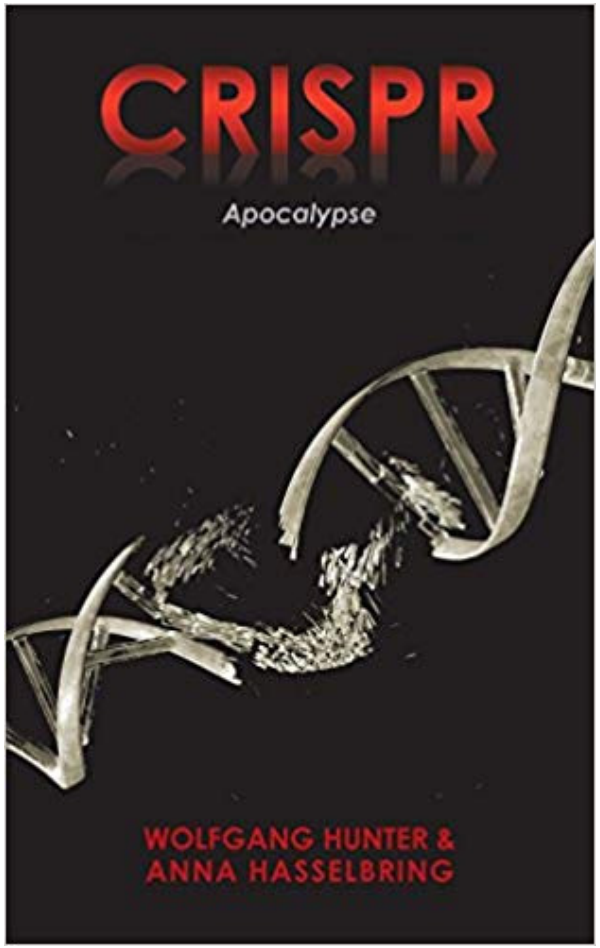
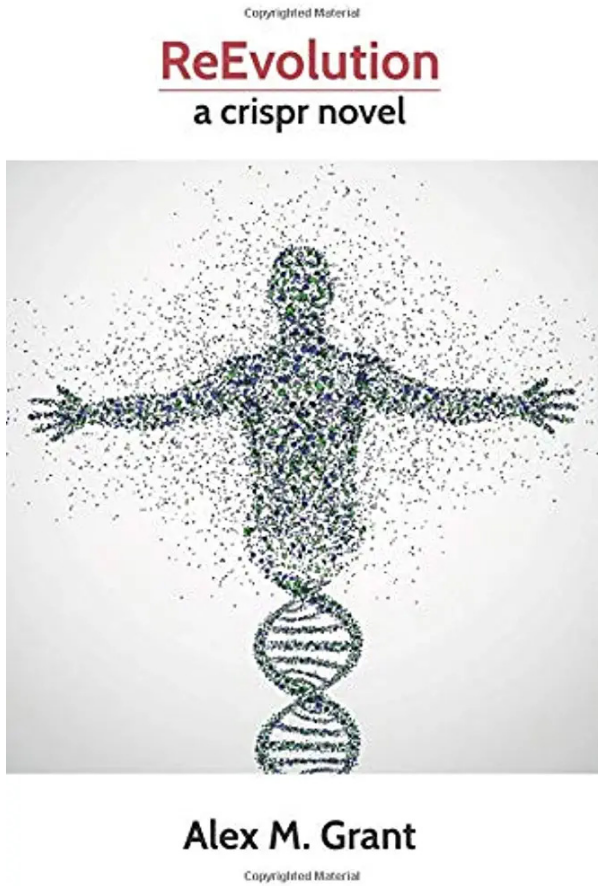
Outline

Section 1

Introduction

CRISPR as a Cultural Phenomenon





Why CRISPR?

- In November 2018 He Jiankui announced the birth of twin girls in China whose genomes had been edited using CRISPR/Cas9 -based methods with the goal of conferring resistance to HIV.
  - The furore that followed cost him his job—can take up this story during Q & A or later, informally; also told in my blog (`https://sahotra-sarkar.org/blog/`).
- He's work raised the possibility of a brave new eugenics and, of all horrors, one based on GM technology!
- We supposedly face a world filled with designer babies?
- This is important but not the only reason why CRISPR is important, especially philosophically important.

Why is CRISPR important?

- CRISPR is *biologically* interesting. The structure of the CRISPR system is unique and the mechanism sophisticated.
- CRISPR provides the most tangible challenge to the neo-Darwinian interpretation of evolution though only in prokaryotes (and I have been urging a broadening of that framework since 1990).
- CRISPR begs analysis to understand how and why immune sytems have so much variability across taxa.

Why is CRISPR important?, contd.

- CRISPR has social implications beyond designer babies.
- CRISPR-based gene editing is our best bet so far for somatic gene therapy.
- CRISPR-based gene drives could eradicate vector-borned diseases—but could also drive species extinct; environmental ethics becomes relevant.
- CRISPR has biosecurity impications, especially for food security.

Two Aspects of CRISPR

- The CRISPR array and associated loci constitute a unique prokaryotic immune system that is poorly understood.
  - Its origin and evolution present problems for the neo-Darwinian interpretation of evolution.
  - This story will occupy half the talk.
- In what is called CRISPR gene editing, a CRISPR associated protein (most often Cas9) is used with a guide RNA to edit genes with high accuracy.
  - This technology enables somatic gene and germ-line changes.
  - It also enables gene drives against vector-borne diseases as well as food security attacks.
  - This story will be discussed to the extent which time permits.

Section 2

Whence CRISPR?

Timeline: Biological Fundamentals I

1989 Yoshizumi Ishino notices a CRISPR sequence in *Escherichia coli* in Atsuo Nakata's laboratory in Osaka (Ishino et al. 1987).

1993 Francisco Mojica reports CRISPR sequences in archaea (Mojica et al. 1993).

2002 Term "CRISPR" for "clustered regularly interspaced short palindromic repeats" introduced (Jansen et al. 2002).

2005 Three groups independently recognize the similarity between CRISPR spacer sequences and those of bacteriophages, archaea viruses, plasmids, *etc* (Bolotin et al. 2005; Mojica et al. 2005; Pourcel et al. 2005).

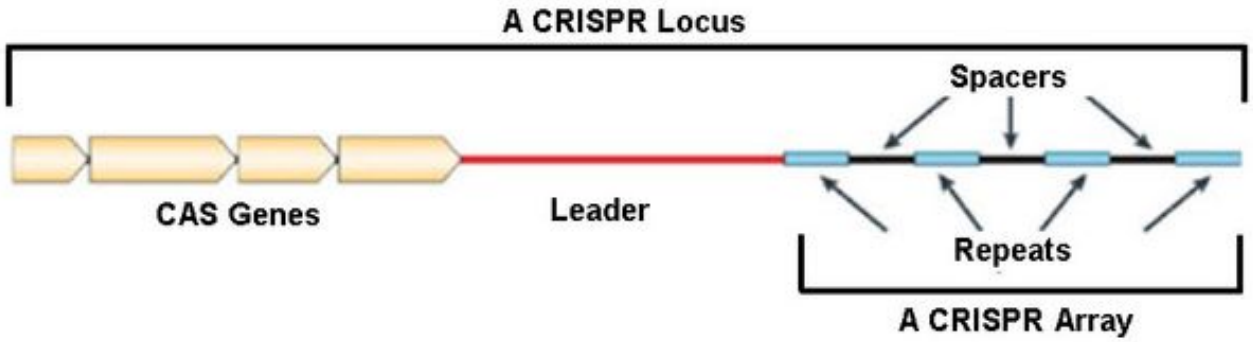
Timeline: Biological Fundamentals II

2006 CRISPR-Cas system hypothesized to function as an acquired immunity system (Makarova et al. 2006).

2007 Experimental demontration that CRISPR-Cas system provides immune resistance to viruses in bacteria (Barrangou et al. 2007).

2012 Doudna and Charpentier laboratories use Cas9 protein for targeted gene editing (Jinek et al. 2012).

CRISPR Arrays



- CRISPR sequence consists of a leader sequence followed by $2 - 10^2$ repeats (each 25 -35 base pairs) interspaced with spacers (25 -35 highly variable base pairs each).
- Repeats are partly palindromic by base pair complementarity:

CGGTTTATCCCCGCTT**CGCGGGGAACTC

CRISPR Immunity

- Spacer sequences are derived from invaders such as viruses and plasmids.
- They thus constitute an immune memory.
- New invasion triggers response: RNA derived from the spacers are used to recognize the invader's *protospacer*.
- Accomanying Cas proteins incapacitate the invader.
- In particular, Cas9 very effectively cleaves RNA.
- Three stages: *adaptation*; *expression/processing*; and *interference*.

First Stage: Adaptation

1. *Adaptation*:
   - Complex of Cas proteins binds to target DNA.
   - This requires self-nonself discrimination.
   - Complex migrates down the molecule until it enconters a 2 -4 base pair motif known as protospacer adjacent motif (PAM).
   - It cleaves out an adjacent portion of the target DNA.
   - Inserts it in the CRISPR array between two repeats, typically at the beginning of the array.
   - This is the most common mechanism; some CRISPR sytems acquire spacers through reverse transcription of RNA.
2. *Expression/processing*.
3. *Interference*.

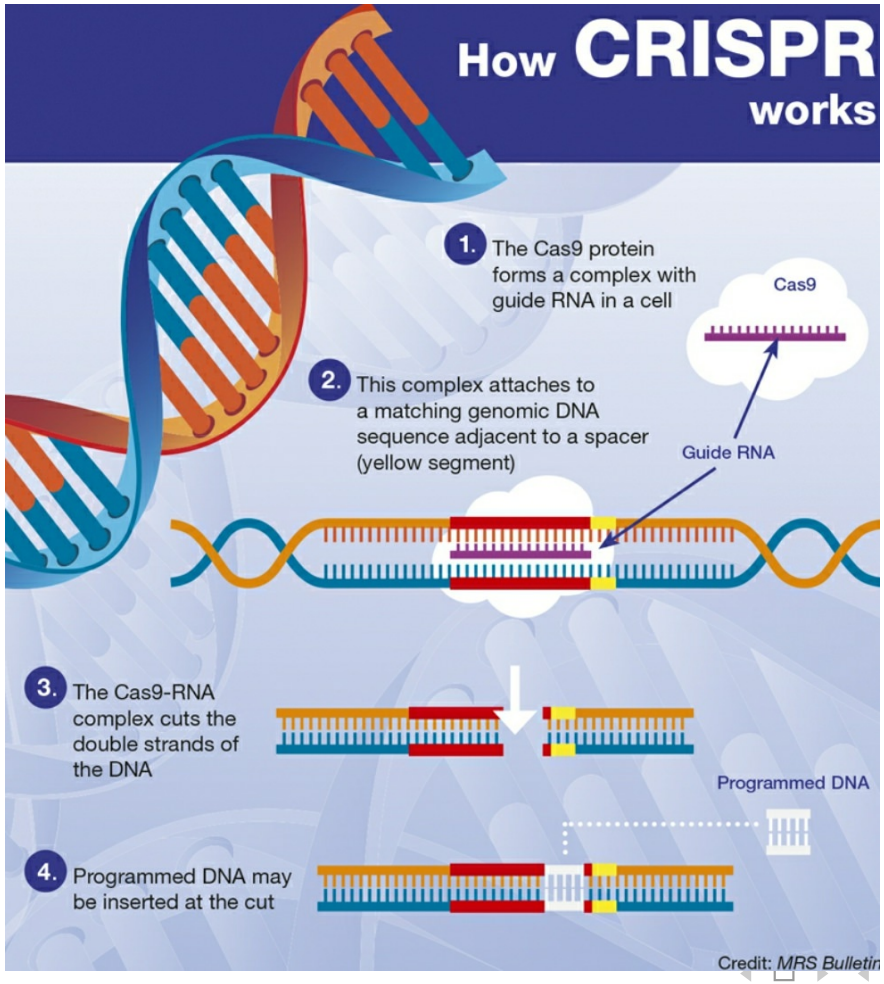
Second Stage: Expression/Processing

1. *Adaptation*.
2. *Expression/processing*:
   - CRISPR array is transcribed into a single long transcript, the pre-CRISPR RNA or pre-crRNA.
   - Proceesed into mature crRNAs, each consisting of a spacer and part of an adjacent repeat.
   - One of these typically remains bound to the processing complex (Cas proteins).
3. *Interference*.

Third Stage: Interference

1. *Adaptation*.
2. *Expression/processing*:
3. *Interference*.
   - Typically the crRNA bound to the processing complex is used as a guide to find a region of the DNA next to a PAM.
   - The PAM is required for proteins such as Cas9 to latch on and cleave DNA.
   - The invading DNA is thus cleaved and deactivated.
   - Descendent cells will continue to have this capacity.
   - The system is similar to the PIWI RNAi system in eukaryotes.

How CRISPR Gene Editing Works



Power of CRISPR-Based Gene Editing

- Accuracy unprecedented compared to earlier technologies.
- Low cost compared to other technologies.
- Unique flexibility: can be used to target any gene (exceptions theoretically possible but I know of no such exception).

Section 3

Biology of CRISPR

CRISPR and Fundamental Biology

- Two aspects are particularly important:
  1. Evolution: Does CRISPR challenge the neo-Darwinian interpretation of evolution?
  2. Immunity: How should the self-nonself discrimination in CRISPR systems be conceptualized?
- Will only focus on the first problem here.

Does CRISPR Challenge the Received View of Evolution?

- Reporting on joint work with **Joanna Masel** and **Arlin Stoltzfus**.
- Return to the acquisition of immunity through CRISPR-based mechanisms.
- Appears to be a clear case of IAAC: inheritance of acquired adaptive characters, which clearly challenges the receivd view (a.k.a. neo-Darwinism).
- Process consists of adaptation + expression/processing + interference.
  - **This takes time**.
  - Won't the bacterial cell have been killed by the virus before all this can happen?
  - Best estimate expert answer: **Yes**. So we don't have enough time.

Puzzle of CRISPR-Based Immunity Acquisition

- What follows is expert opinion only partly based on solid empirical results (but fully testable).
- Empirical facts:
  - Invading population of viruses have some defectives that cannot replicate successfully.
  - Fraction of bacteria that acquire immunity closely agree with fraction of defective viruses.

Model for CRISPR-Based Immunity Acquisition

- When viruses invade a bacterial population, most of the bacteria (or archaea) get killed.
- Sometimes, a lucky bacterium gets invaded by a defective virus that cannot kill it.
- It then begins the process of adaptation.
- After completion, if the "same" virus invades again, it can launch into expression/processing.
- But, typically, the only cells that will be in this environmental context are is descendents.

The Question of Adaptation

- But is this adaptation?
- If the bacterium encounters the "same" virus again, yes—but will problematize "safe" soon.
- If not, we must face up to the problem of the unit of adaptation:
  - If the unit is the individual, no.
  - If the unit is the lineage, yes.
  - Perhaps lineages are the proper units of evolution—leave further discussion to Q & A.

The Question of the Environment

- Yet another problem: what is the environment with respect to which it is an adaptation?
- If it is the defective virus, then it is not an adaptation to it because it was already completely fit with respect to that virus.
- If it is the intact virus, it was not exposed to that environment.
- So, we have a paradox here.
- Possible solution: perhaps the relevant environment must be the populatin of viruses?

The Question of "Lamarckian"

- Is CRISPR a neo-Lamarckian system?
- Should we stop asking this question?
- IAC versus IAAC:
  - IAC: Inherited Acquired Character.
  - IAAC: Inherited Acquired Adaptive Character.
- Throughout the twentieth century, Lamarckism was identified with IAC. But this is clearly inappropriate.
- IAAC was defended by Sarkar (1991) in the context of the Cairns-led controversy over directed mutations in bacteria.

Problems

- Is CRISPR-based immunity an IAAC?
- The problem with "adaptive" was noted earlier; but, for the sake of argument, let us shelve that.
- Would you be happy to call something so dependent on chance "Lamarckian"?
- Perhaps what really divides Lamarckian from Darwinian is the reliance on determinate versus stochastic mechanisms?
- Add that to the thesis that "blindness" of variation refers to the separation of the *processes* of adpatation and the generation of variation.
- However, should we simply stop worrying about "Lamarckian"?

Section 4

Ethics of CRISPR

Somatic Gene Editing: Non-Human Species

- No ethical problem here. It is akin to any ordinary veterinary intervention.

Germ-line Editing: Non-Human Species

- Standard use of CRISPR/Cas9 editing in agricultural contexts.
- Large class of problematic results from livestock:
  - *MSTN* (myostatin gene) modification in pigs leads to leaner meat but extra vertebrae.
  - *MSTN* (myostatin gene) modification in rabbits leads to more meat but enlarged tongues
  - *MSTN* (myostatin gene) modification in lambs leads to changed wool color (though not the predicted ones) and Caesarian births,
- From the animal welfare perspective, has been a disaster.

Human Somatic Gene Editing

- No ethical problem here. It is akin to any very intrusive medical interventions.
- Makes it likely that gene therapy (finally) will be routinely successful.
- If you are worried about foreign DNA being incorporated into your body, think of how this happens with ordinary organ transplants.

Human Germ-line Editing

- Finally!
- Raises the specter of eugenics. And it would be eugenics. But is all eugenics undesirable.
- Difficulty of defining eugenics is well known.
- Proposal:
  1. Target a human phenotypic trait that we wish to promote or discourage.
  2. Endorse conscious intervention in individual reproduction
  3. Purpose must be to change the future distribution of the trait in the population as a whole and not be limited to selected individuals.
  4. Liberal eugenics: achieving these traits must not endorse coercion.

Four Objections

- *Playing God*: if we are, what is wrong with it.
- *Human heritage*: relies on a bad analogy.
- *No informed consent*: but that is true of fetuses, embryos, infants, children . . .
- *Disability rights*: this is the most imortant objection: demands caution rather than exclusion.

Genetic Enhancement versus Disease Gene Elimination

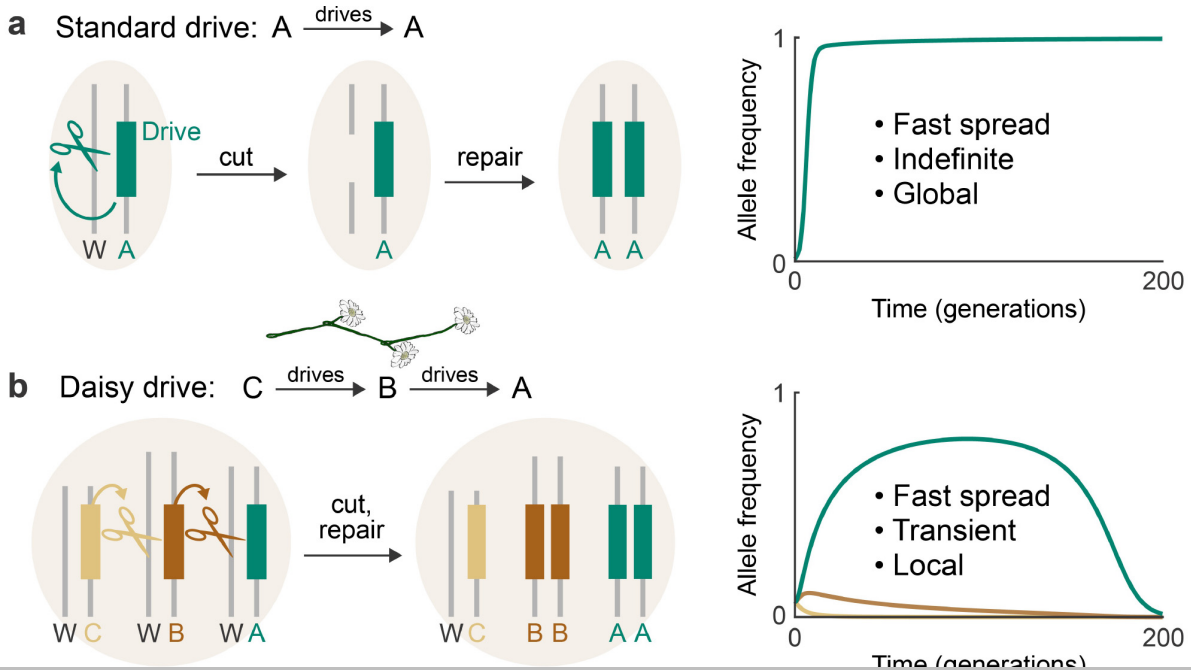
- Corresponds with the old disinction between positive and negative eugenics.
- Problems outweigh benefits of gene enhancement:
  - Biologically implausible: targeted genes cannot deliver the desired traits in almost all situations. Designer babies are science fiction.
  - Equally important: traits chosen for enhancement will only reflect social prejudice.
  - Problem made worse by likely very unequal access to intervention.
- Would justify a complete ban, but this does infringe on presumed reproductive freedom.
- But should there be complete reproductive freedom?

Disease Gene Elimination

- Restrict to diseases that are not perceived as only an alternative life culture by anyone who has the allele.
- Satisfy three criteria:
  - *Safety*: this is the same criterion as used for any new medical procedure.
  - *Accuracy*: targets the selected gene with precision; this criterion has been recognized for all gene editing technologies.
  - *Specificity*: **this is the elephant in the room**. Requires intended effect and *only* that effect at the phenotypic level; then subsumes complete *penetrance* and constrained *expressivity*.
- Most important limitation is delivery.

Gene Drives Using CRISPR

- CRISPR/Cas9 can be used to generate two copies of a gene, *e.g.*, of a gene on the X chromosome.
- If this gene reduces viability, can lead to *extinction* of all populations and thus *of a species*.



Ethics of Gene Drives

- Of greatest relevance for diseas vectors.
- However, gene drives may help control invasive or other "undesirable" species.
- For example, for *Aedes aegypti*, classical biological control (CBC) has not worked:
  - Pesticides are environmental and human health risk factors.
  - In Brazil and other countries inadequate water supply and poor sanitation generates open containers of water for mosquitoes to breed in.

Gene Drive Problems

- Practical (pragmatic) problems.
  - These arise mainly from the many uncertainties.
- *In principle* problems:
  - Invoke "deeper" issues about what should be done whether or not we have the ability to do something.
- Both are questions of ethics (*e.g.*, "ought implies can" principle shows that if practical issues of implementation cannot be resolved, there can be no moral oblgation).

Practical Problems

- *Effectiveness*: long-term stability; unique important of species; assumptions about dynamics.
- *Irreversibility*: what if it goes wrong?
- *Unintended consequences*: problem of specificity (as in the case of gene editing).

In principle Problems

- Logical conclusion of the program would be to eliminate the species.
  - Should we (intentionally) eliminate any species?
  - Extensive debated within environmental ethics since the early 1970s.
  - Probably conclude against some species.
- Also have the question of the wisdom of intentionally permanently removing a species.
- Lost evolutionary and other potential? But what does that mean?
- What if we proceed to eliminate scores of species?

Proceed with Caution

- Proceed with caution:
  - Ecological risk analysis must accompany risk analysis from molecular biology (for which protocols remain in flux).
  - Must face normative question of acceptable risk.
- Every time a species is lost, some unique aspect of biological heritage is lost.
  - True, but also true at every other level of the taxonomic hierarchy.
  - Don't the benefits in terms of human suffering outweigh this cost?

Proposal: Sufficient Criteria

- Following proposal consists of criteria that are *jointly* sufficient to alllow the possibiity of driving a species to extinction:
  - Disease vector.
  - Gene drive technology is demonstrably safe.
  - Will *significantly* reduce the disease burden for *at least one* disease.
  - Removal unlikely to disrupt ecological community (as shown by field experiments).
  - Absence of plausible alternatives.
- These criteria can be strengthened (*e.g.*, *all* diseases rather than at least one).

Section 5

Final Remarks

Conclusions

- Biological issues:
  - Challenge for the audience.
  - Philosophers have paid very little attention to CRISPR and have been very, very superficial—witness the recent *Biology and Philosophy* exchange with Koonin.
- Ethical issues:
  - Proceed with caution.
  - Need **extensive** and **immediate** public discussion.