A future task in good hands

Bundesamt für Naturschutz

Comments from a Risk Assessor's Perspective

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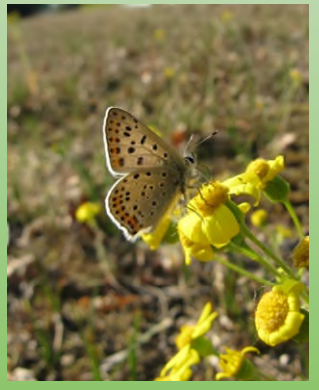

CRISPR/Cas is NEW

- Jinek et al. (2012) CRISPR/Cas application
- Tool constantly and rapidly evolving
- Yet no product on the market

A powerful molecular tool

- Alter genetically linked genes
- Alter multiple different or multiple identical DNA-sequences (multiplexing)
- Access *all* genes of a genome

Difficult to predict impacts at higher levels.

Such changes even without the insertion of foreign DNA do not/hardly occur naturally.

GMO definition

Directive 2001/18/EC, Art. 2 (2)

'genetically modified organism (GMO)' means an organism, with the exception of human beings, in ***which the genetic material has been altered in a way that does not occur naturally*** *by mating and/or natural recombination.*

Claiming naturalness

- *Similar to nature, close to nature, nature-like…*
- Descriptions refer to **S**NV and process identification
- Not justified, missing
  - CRISPR/Cas' potential
  - Application trends (multiplexing)
  - Unintended effects
  - Efforts to develop methods
- Besides, natural does not mean without risks.

SNV = single nucleotide variation

Range of unintended molecular effects

- OTEs
- Extent influenced by experimental parameters
  - Design, concentration, incubation time…
  - Trade-off specificity *vs* efficiency observed
- Various OnTEs: *range of molecular outcomes*
- OnTEs at OT sites!
- Impacts at higher levels to be determined.
- Causes for OnTEs?

OTEs = off-target effects; OnTEs = on-target effects; OT = off-target

Is CRISPR/Cas fully understood?

- Design of CRISPR/Cas (rational and screening)
- Sometimes no cuts at target site
- Origin as bacterial immune system

However, future work should be aimed at not only detecting these OnTEs but also ***understanding their biological roots and reasons for occurrence****, leading to strategies to avoid their formation in the first place.*

Weisheit et al. (2020) DOI: 10.1016/j.celrep.2020.107689.

More quotes from the literature

However, the use of Cas9 or other nucleases can yield unpredictable events at the target site or off target. To overcome these challenge, it ***is critical to understand and accurately predict the whole range of possible editing outcomes****.*[1)]

Additionally, applying new machine learning interpretability tools to our model ***may further illuminate the underlying biology of the cutting dynamics of CRISPR-Cas systems*** *captured by crispr2vec.* [2)]

Burgio and Teboul (2020) DOI: 10.1016/j.tig.2020.09.011
Trivedi et al. (2020) bioRxiv preprint doi: https://doi.org/10.1101/2020.10.28.359885

Calls for best practice

- *To ensure that the mutants created do not contain hidden surprises that could produce artifacts*
- *GE can be used with precision to engineer the genome, by following best practices*
- *Anticipating and verifying the result of GE essential for the success for all applications*

Sharpe et al. (2017) Unexpected consequences. Exon skipping caused by CRISPR-generated mutations. DOI: 10.1186/s13059-017-1240-0.

Thomas et al. (2019) Collateral damage and CRISPR genome editing. DOI: 10.1371/journal.pgen.1007994

Burgio and Teboul (2020) Anticipating and Identifying Collateral Damage in Genome Editing. DOI: 10.1016/j.tig.2020.09.011

Some thoughts on best practice

- In medicine, but also for agricultural use
- Strategies to prevent damage at molecular level

<u>In plants</u>

- Hybridisation and segregation; controls?
- Direct editing of elite lines possible
- Beyond molecular level:
  Assess impacts of unintended and deliberate changes (new traits and new environments)

Why it is sound to regulate GE under Directive 2001/18

- GE leads to GMOs (ECJ ruling)
- No other adequate law
- Technique new, evolving and not fully understood
- Outcomes vary and stakes are high (Health & ENV)

➢ Obligatory standards for MC under EU regulation

- Possibly new traits and environments

➢ Assessing impacts (ERA)

Regulation is common in FF sector and a chance.

MC = molecular characterisation; ERA = environmental risk assessment; FF = food and feed

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Thank you for your attention!