SYM-21: Pairing Adverse Outcome Pathway Discovery with Advances in Gene Editing to Solve Toxicity Mechanisms

Leah Wehmas, ORD USEPA, Chair

Tamara Tal, UFZ, Co-Chair

March 16, 2021

SYM-21: Pairing Adverse Outcome Pathway Discovery with Advances in Gene Editing to Solve Toxicity Mechanisms

Dr. Leah Wehmas (US EPA)	The state of CRISPR-Cas9 gene editing technology and applications for toxicology research	20 min
Dr. Max Russo (UF Gainsville, formerly)	Functional pathway identification with CRISPR-Cas9 genome wide knockout screening in human dopaminergic neuronal cells following chronic treatment with dieldrin or iron	20 min
Dr. Luoping Zhang (UC Berkeley)	Mechanisms of formaldehyde hematotoxicity revealed by genome-wide functional screening	20 min
Dr. Dan Gorelick (Baylor College of Medicine)	Using CRISPR-Cas to identify how endocrine disruptors cause malformations and functional defects in the zebrafish heart	20 min
Dr. Tamara Tal (Helmholtz Centre for Environmental Research)	Gene editing reveals microbiome-host signaling mechanisms that are perturbed by chemical exposure	20 min
Panel	Live discussion with Q & A	65 min

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Please submit your questions via chat.
Stay for the live discussion at the end.

The state of CRISPR gene editing technology and applications for toxicology research

SYM-21: Pairing Adverse Outcome Pathway Discovery with Advances in Gene Editing to Solve Toxicity Mechanisms

Leah Wehmas, PhD

US EPA, ORD

March 16, 2021

Disclaimers

This presentation does not represent US EPA policy and any mention of commercial products does not constitute endorsement.

No conflicts to disclose.

The Good

THE CRISPR REVOLUTION

A Young Mississippi Woman's Journey Through A Pioneering Gene-Editing Experiment

December 25, 2019 · 7:00 AM ET
Heard on All Things Considered

ROB STEIN

22-Minute Listen + PLAYLIST

https://www.npr.org/sections/health-shots/2019/12/25/784395525/a-young-mississippi-womans-journey-through-a-pioneering-gene-editing-experiment

The Good, the Great

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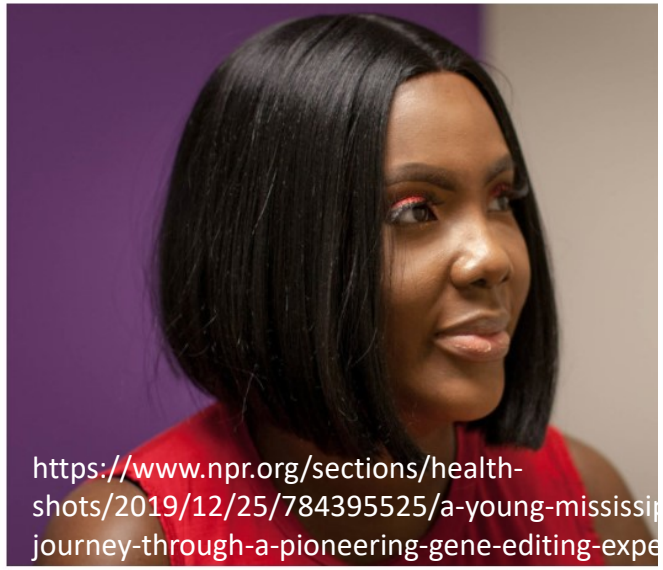

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Pioneers of revolutionary CRISPR gene editing win chemistry Nobel

Emmanuelle Charpentier and Jennifer Doudna share the award for developing the precise genome-editing technology.

Heidi Ledford & Ewen Callaway

https://www.nature.com/articles/d41586-020-02765-9

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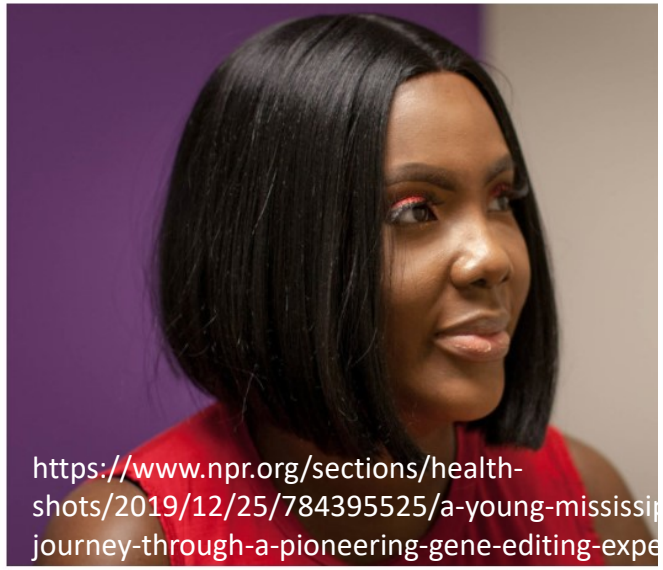

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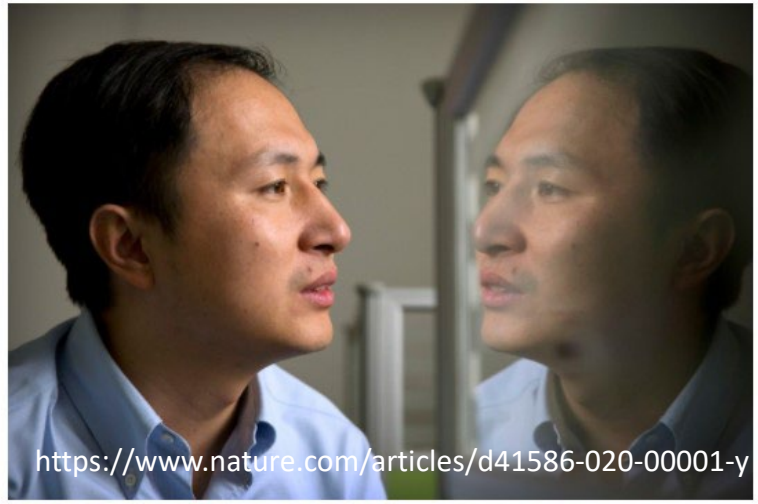
Chinese court sends strong signal by punishing He Jiankui and two colleagues.

David Cyranoski



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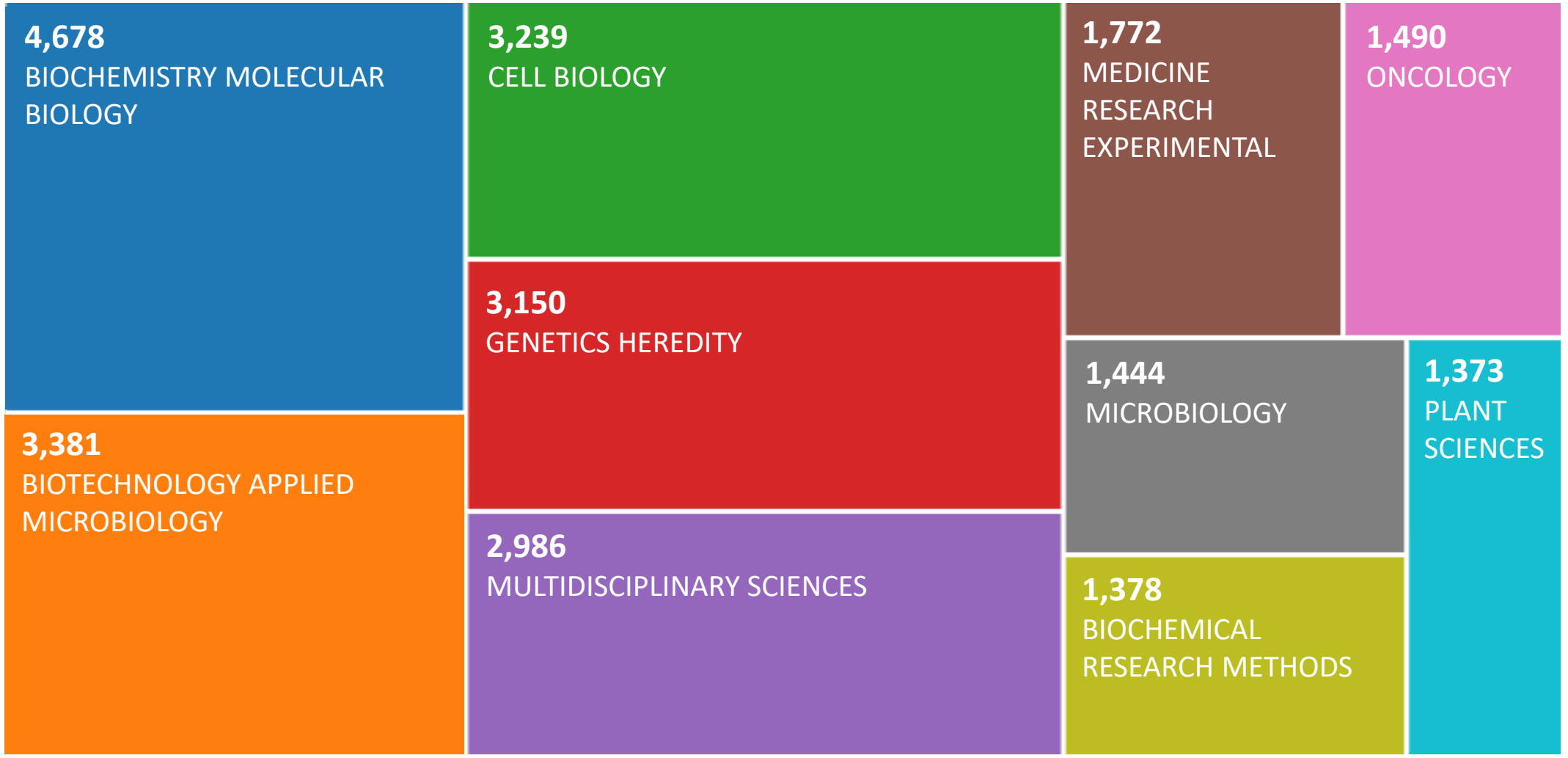
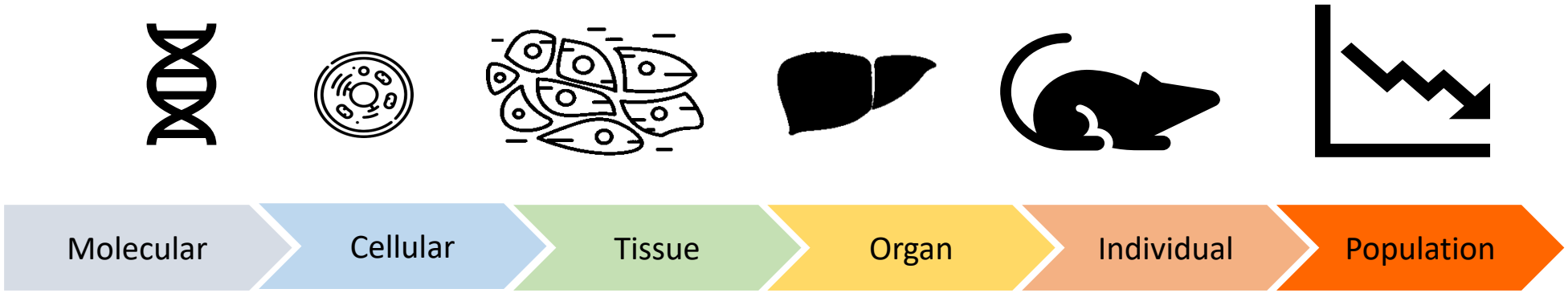
Growth in CRISPR use



Gene editing's promise for AOPs



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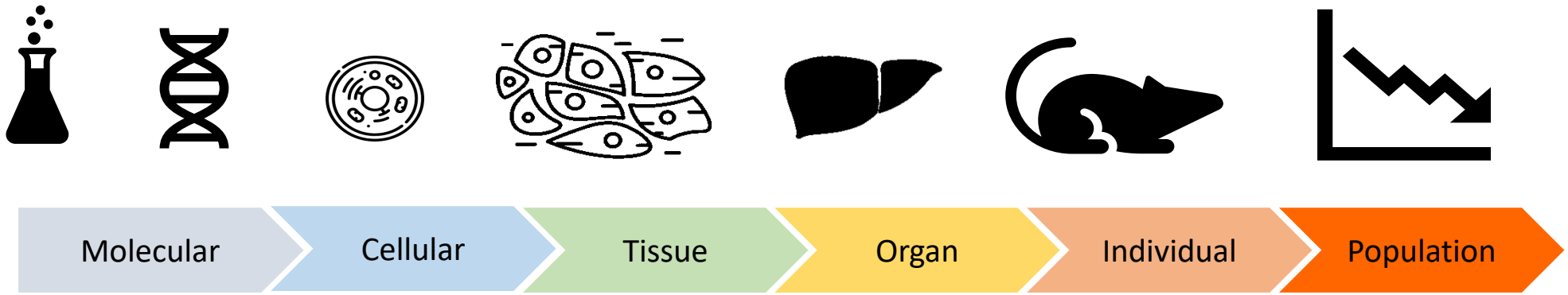

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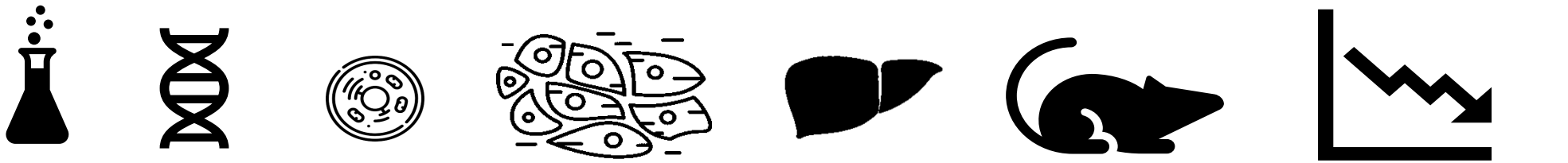


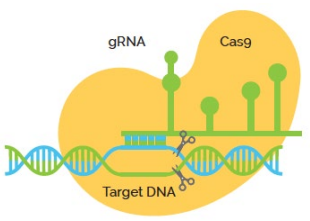
Molecular | Cellular | Tissue | Organ | Individual | Population

Molecular Endpoints
(identify relationships)

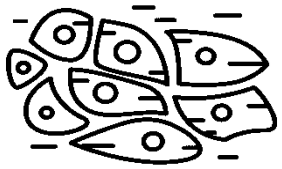
Traditional Endpoints
(associate with adverse effects)

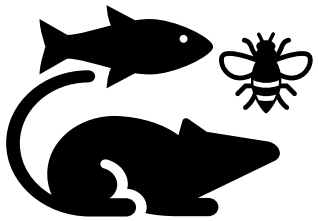
Molecular Initiating Event → Key Events → Adverse Outcome

Gene editing's promise for AOPs and NAMs



CRISPR System





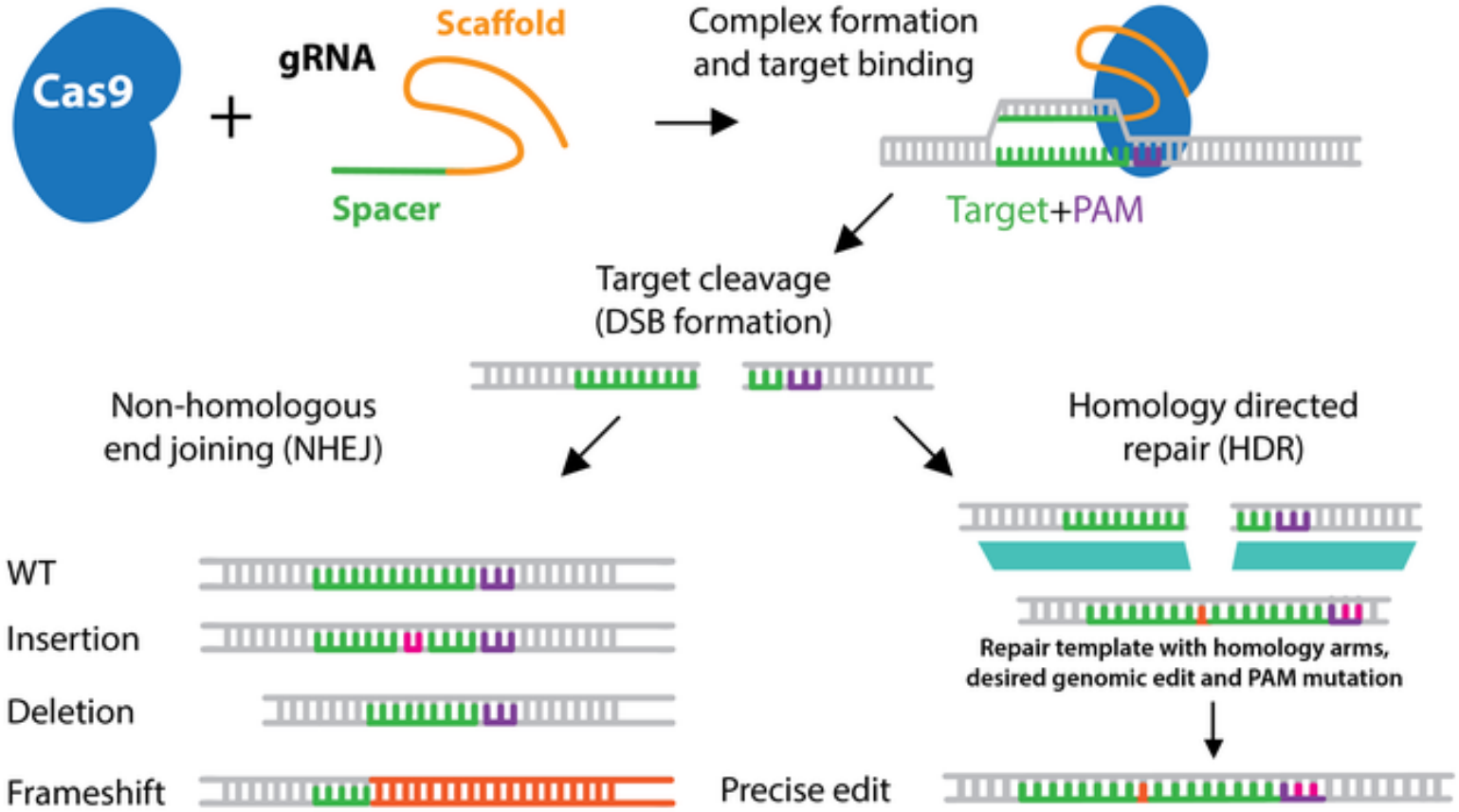
Molecular | Cellular | Tissue | Organ | Individual | Population

Molecular Endpoints/NAMs
(confirm relationships)

Traditional Endpoints/NAMs
(adverse effects)

~~Ini[illegible]nt~~ → Key Events → Adverse Outcome

Gene Editing with **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats or CRISPR



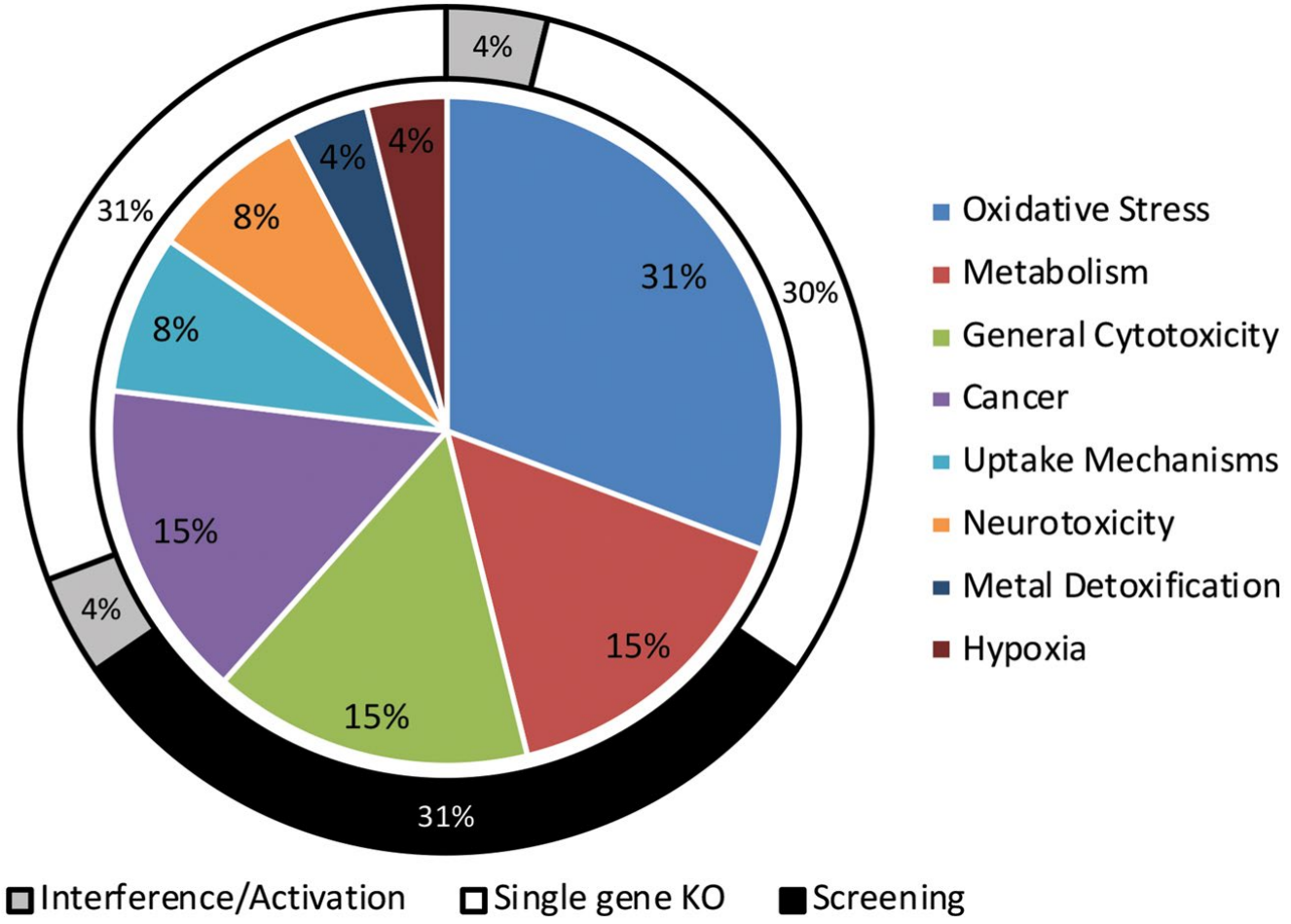
Components

- gRNA: Guide RNA
- Spacer or crRNA: defines target sequence
- Scaffold or tracrRNA: links target to Cas9
- PAM: protospacer adjacent motif

Traditional CRISPR systems for gain of function and loss of function edits

https://www.addgene.org/guides/crispr/

Examples of CRISPR-system use in toxicology growing



Lujen *et al.* 2020. *Tox Sci*

- Neurodegeneration - Russo
- Occupational health - Zhang
- Endocrine toxicity - Gorelick
- Behavior and microbiome - Tal

Rare and new uses for CRISPR system in mechanistic toxicology

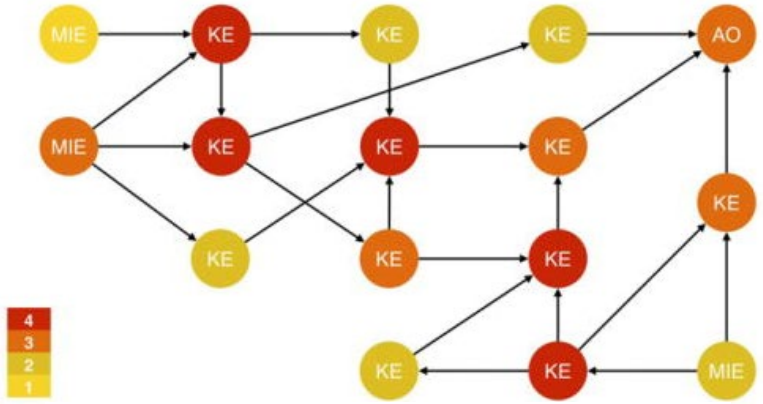
<u>Rare</u>

1. Epigenetic alterations*

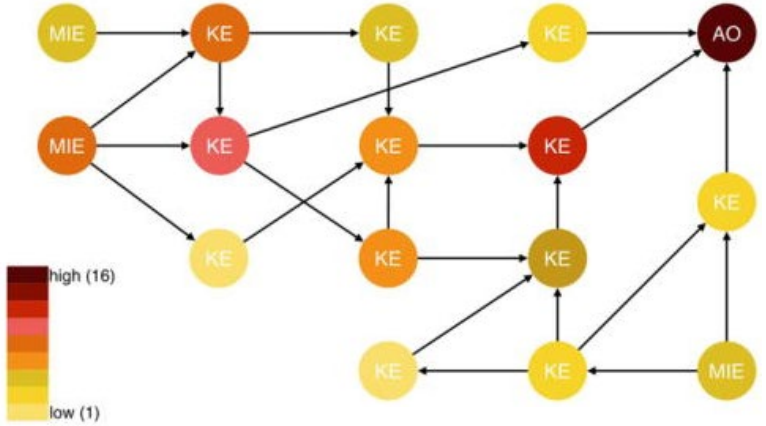
<u>New</u>

2. Single base modifications
3. Comparative functional toxicogenomics
4. AOP networks
5. Gene editing for biomarker detection and real time toxicodynamics

AOP Networks



Degree of KEs

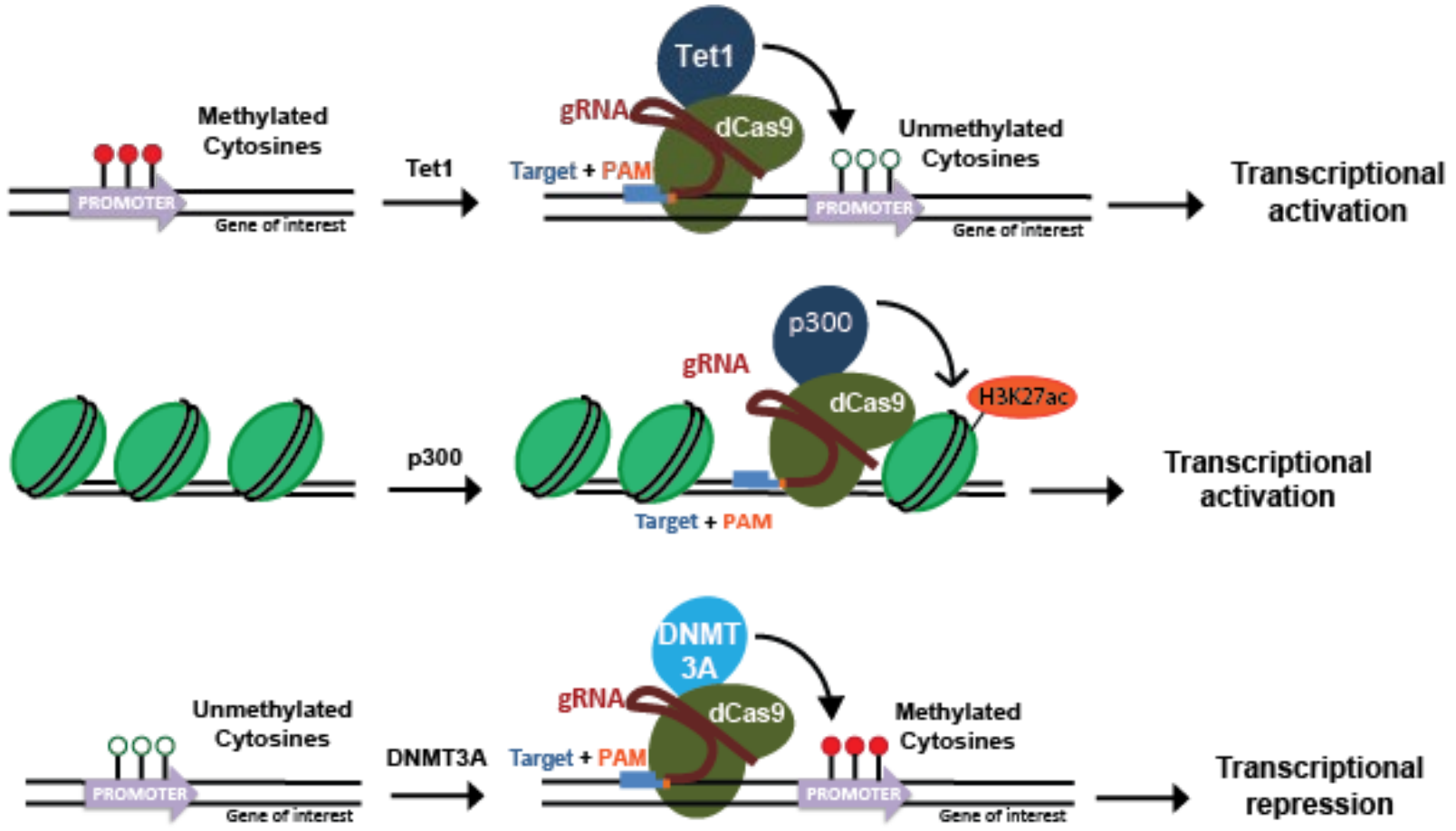


Path occurrence of KEs

Adapted from Dries *et al.* 2018, *ET&C*

Rare. Example 1. CRISPRa/CRISPRi can improve understanding of epigenetic mechanisms of toxicity

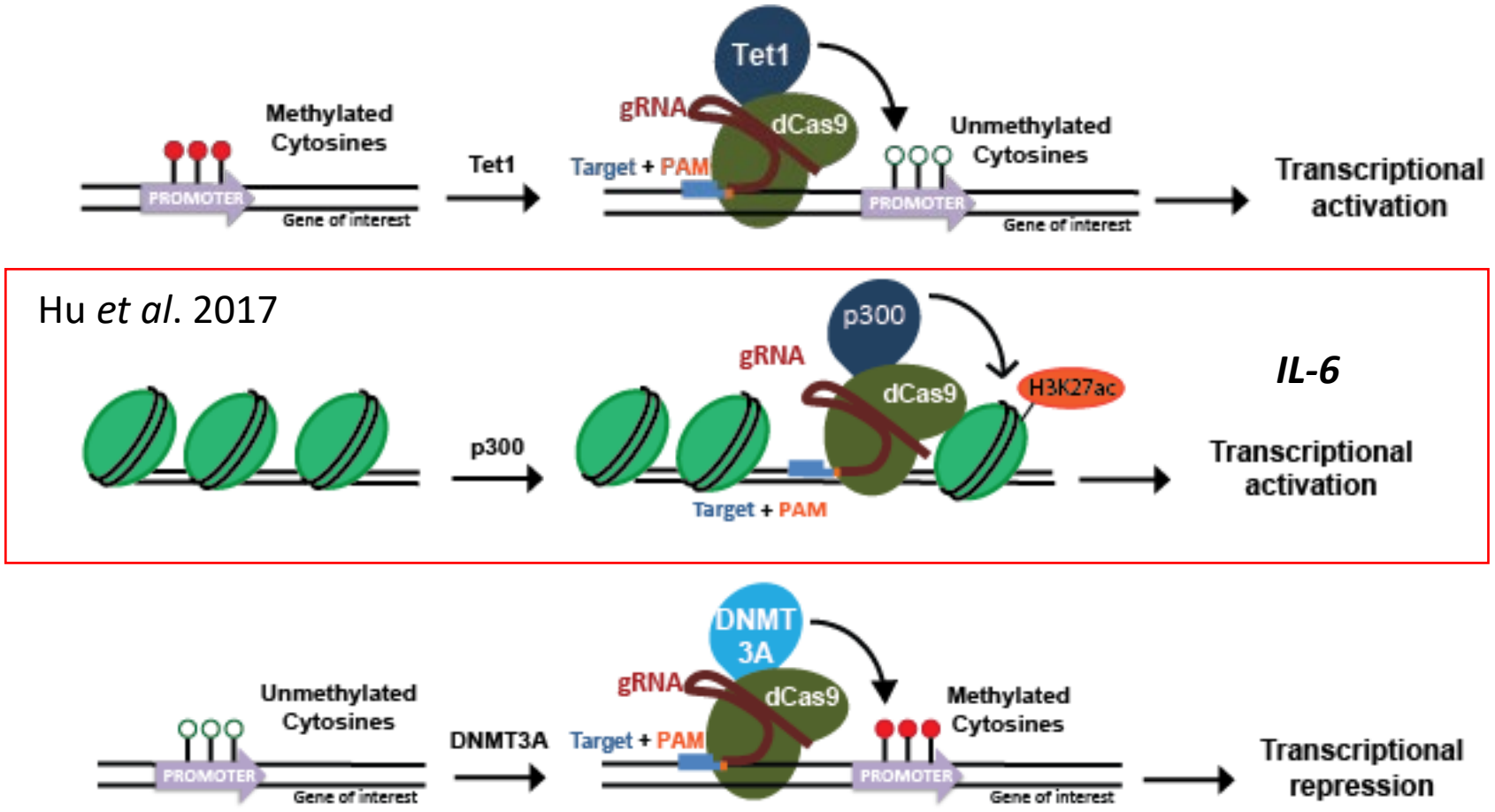
Epigenetic alteration using deactivated Cas9 fusion



https://blog.addgene.org/crispr-101-editing-the-epigenome

Rare. Example 1. Histone acetylation mediates *IL-6* activation and paraquat mediated lung fibrosis

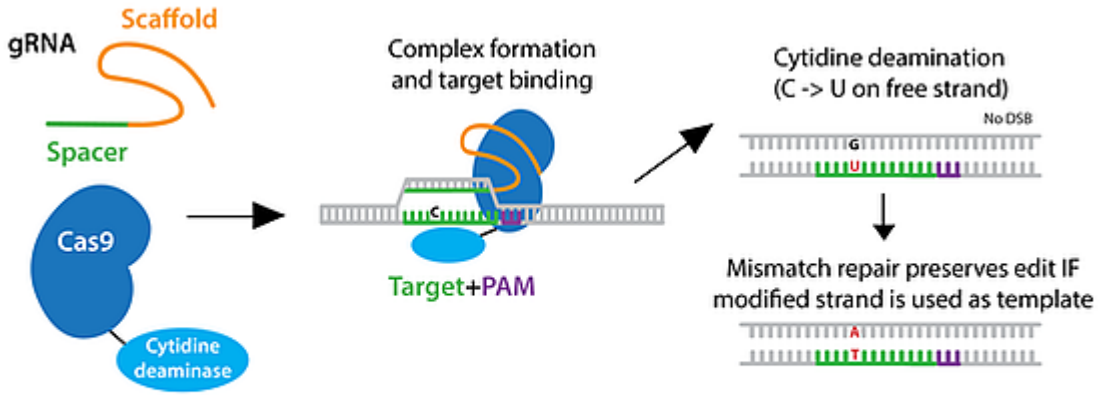
Epigenetic alteration using dCas9



https://blog.addgene.org/crispr-101-editing-the-epigenome

New. Example 2. Single base edits can improve understanding of gene-environment interactions

Single base editing

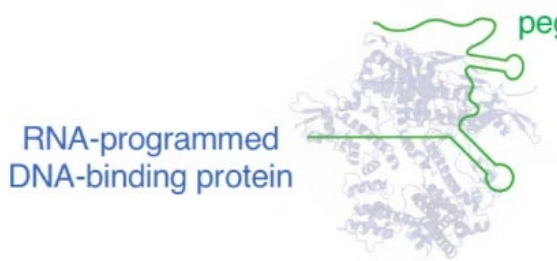


dCas9 or Cas9 nickase fused to a cytidine deaminase

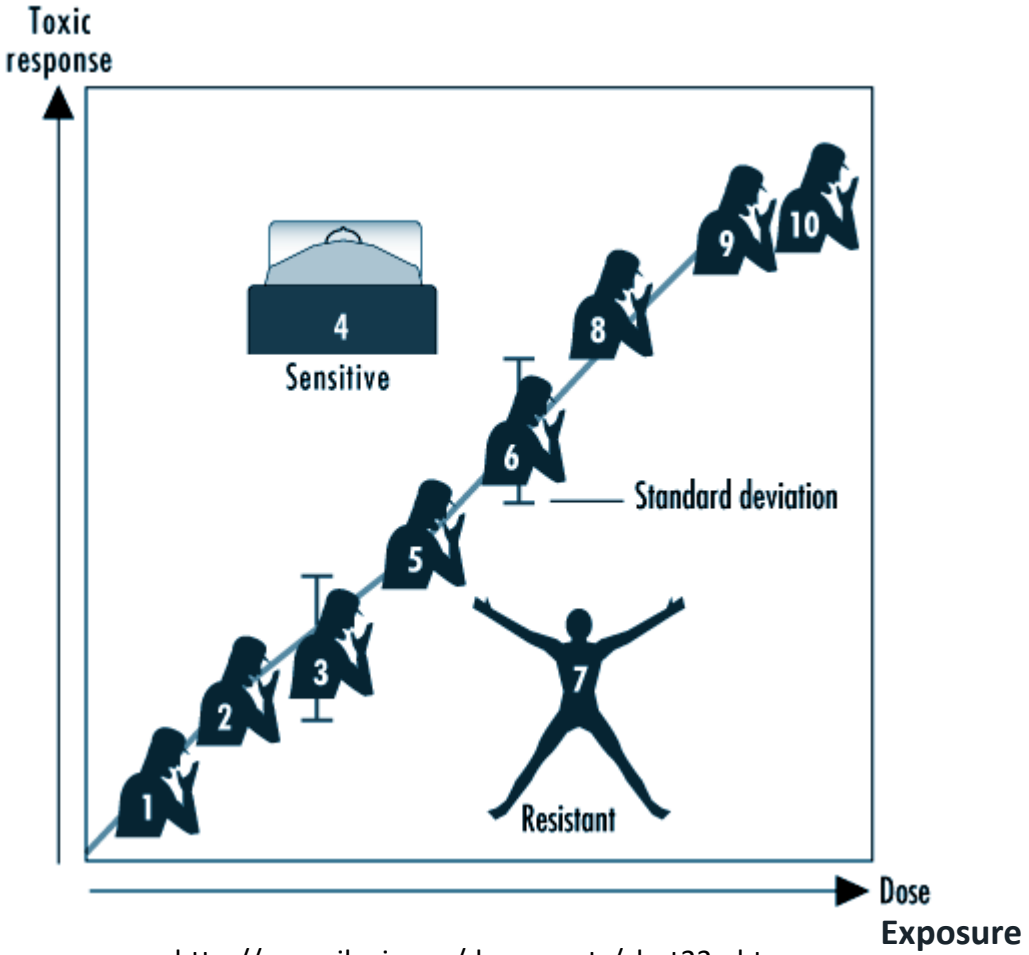
Base excision repair inhibitor is also present in fusion protein

https://blog.addgene.org/;
Anzalone *et al.* 2020, *Nat. Biotech*

Prime editing



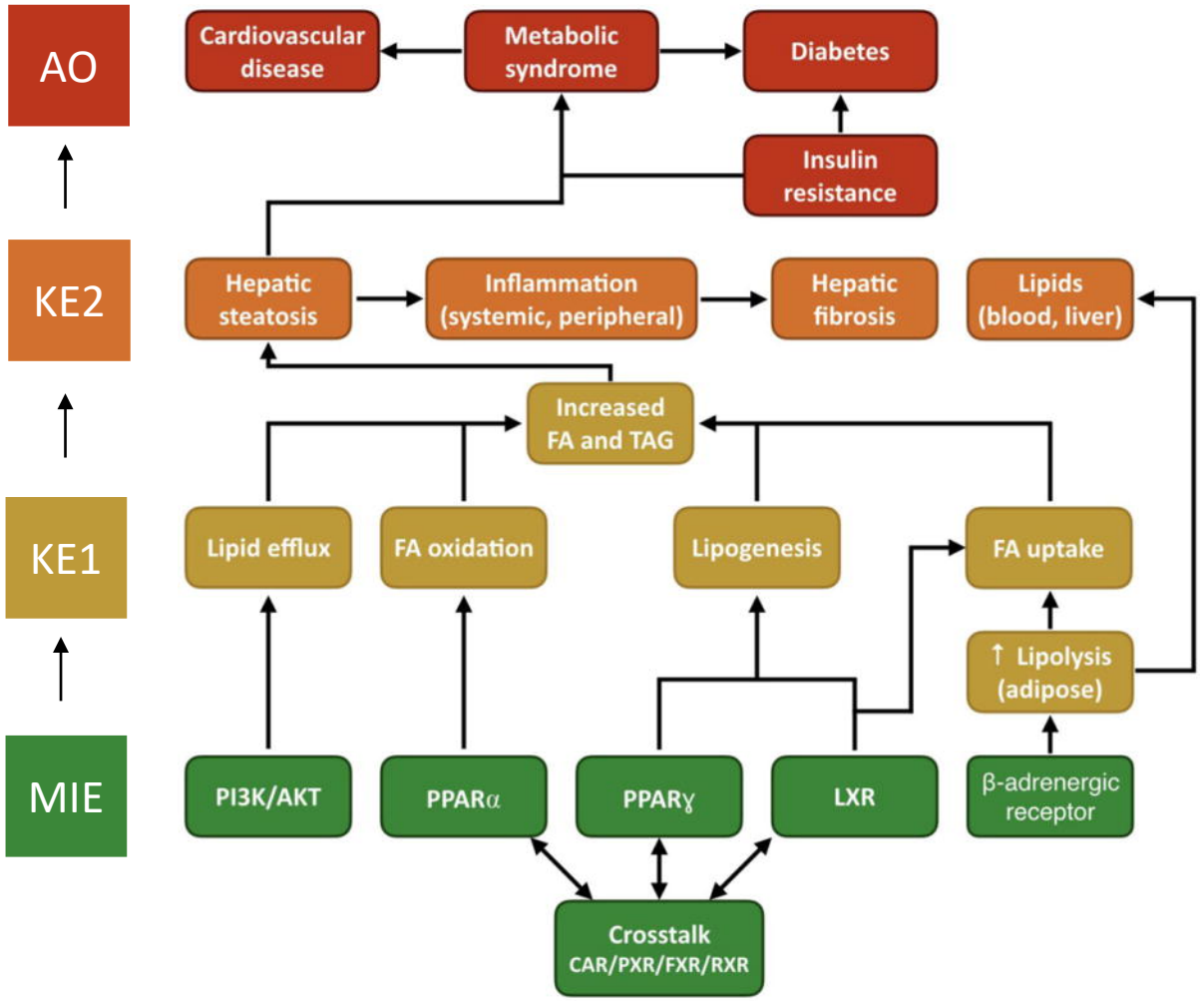
Cas9 nickase fused with reverse transcriptase to make Prime Editor



http://www.ilocis.org/documents/chpt33e.htm

Enhance risk assessment

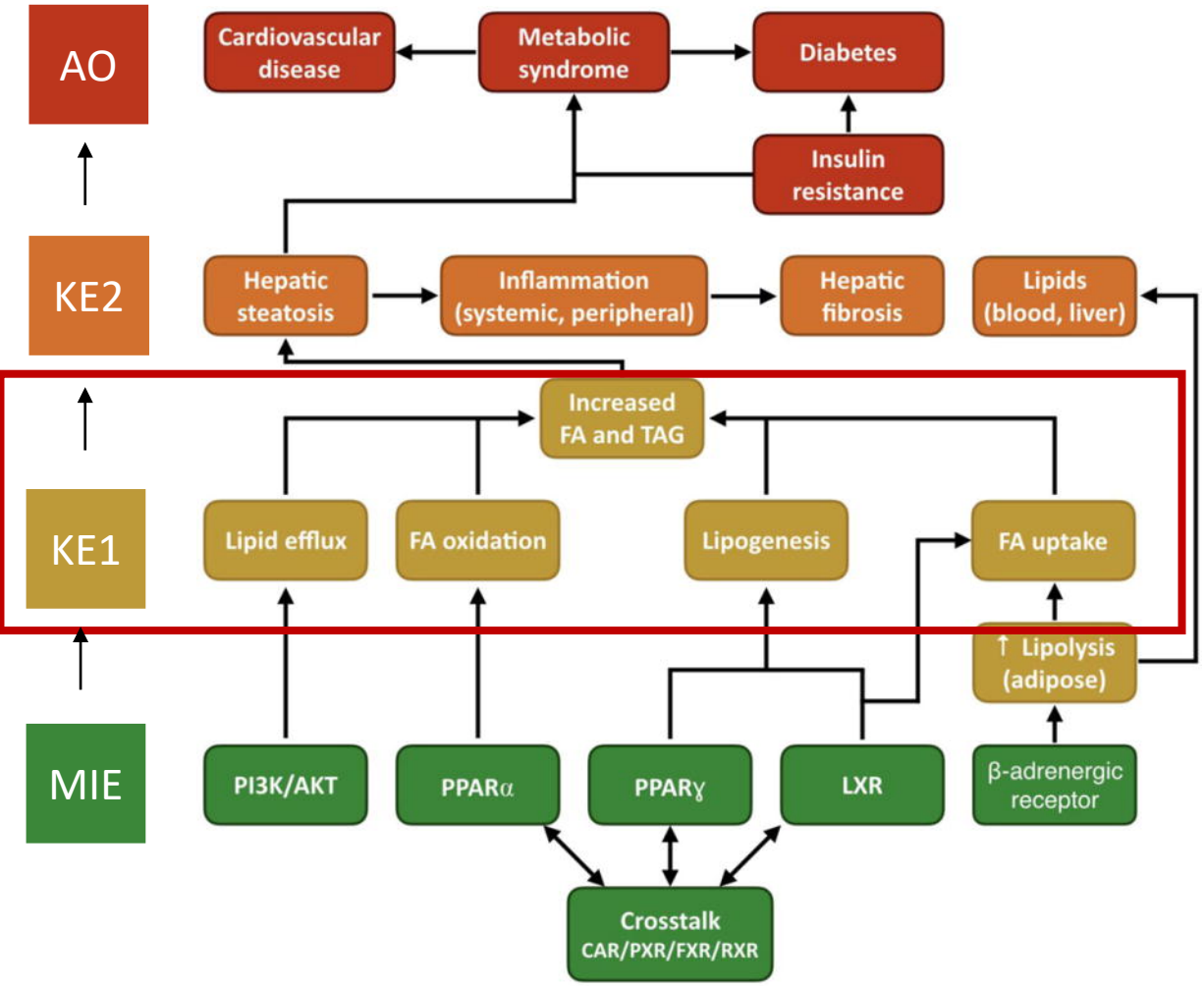
New. Example 3. Gene editing can advance AOP networks



Adapted from Dries *et al.* 2018, *ET&C*

- Multiple receptors involved
- Converge at FA and TAG
- Identify genes involved
- Edit gene function
- Replicate and confirm chemical response
- Define most critical key event(s)
- Assist in NAM development

New. Example 3. Gene editing to confirm key event linkages in fatty liver



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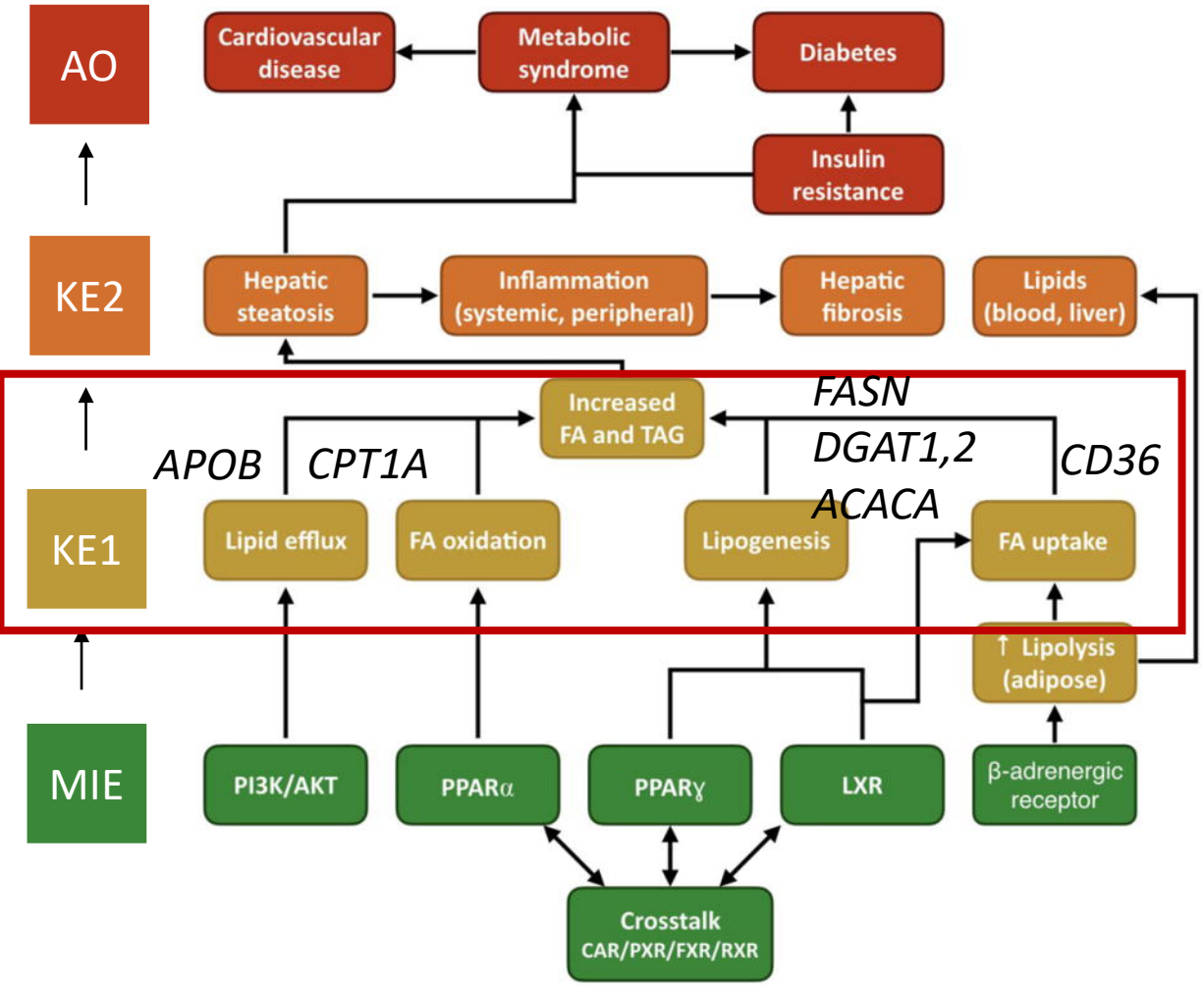

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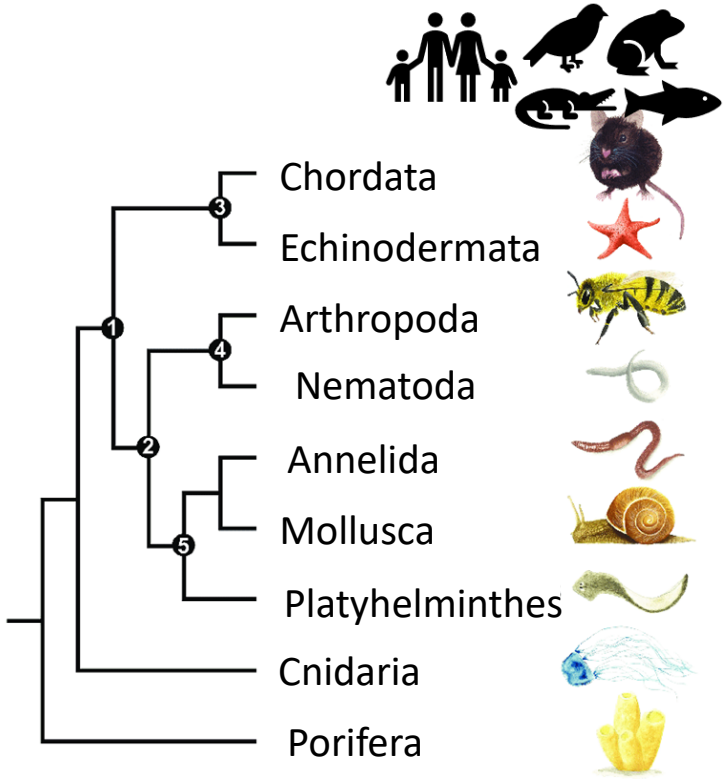
- Replicate and confirm chemical response
- Define most critical key event(s)
- Assist in NAM development

New. Example 4. Comparative functional toxicogenomics can aid AOPs and NAMs

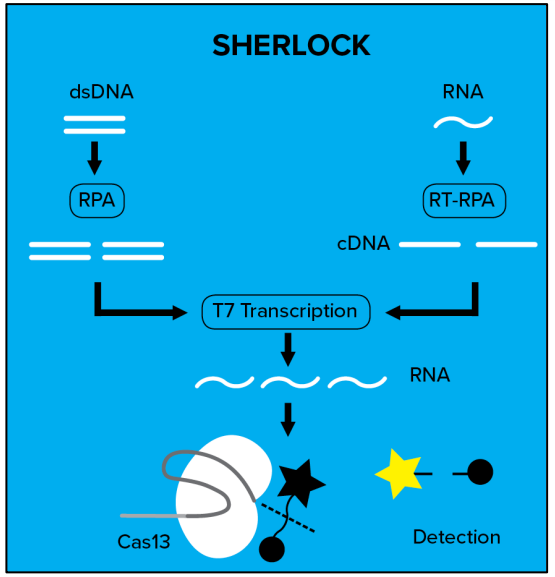
- Confirm conserved genes
- ID key signaling pathways for AOP networks
- Translate toxic effects across species
- Evaluate/refine existing tools
- Assist in NAM development

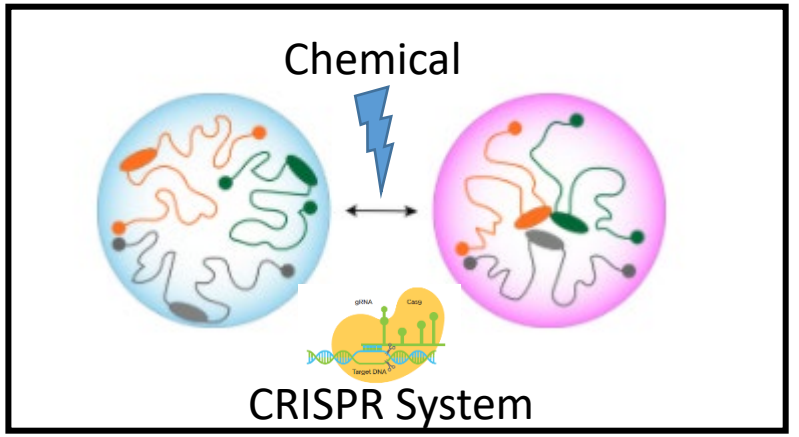


Adapted from Telford *et al.* 2015, *Current Biol*

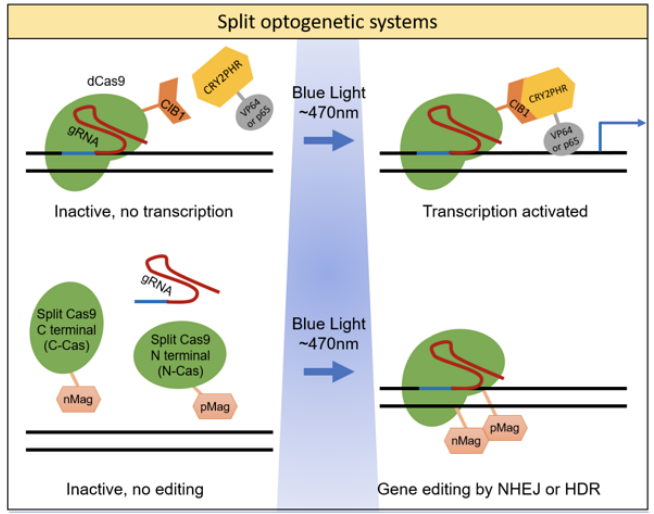
New. Example 5. Gene editing for biomarker detection and real time toxicodynamics

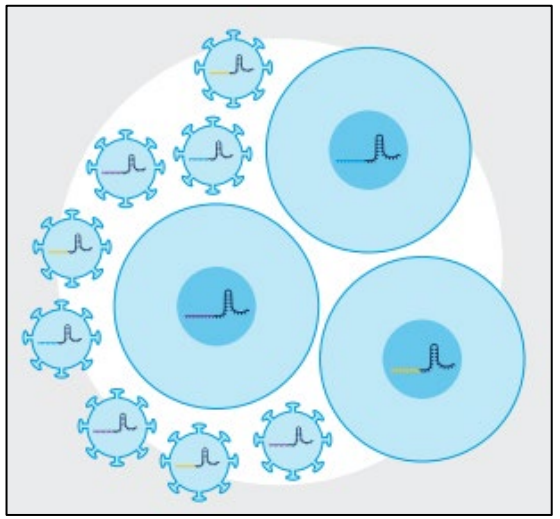
- Biomarker detection
- Chemical effects on 4D Nucleome
- CRISPR optogenetics
- CRISPR single cell RNA-seq





https://blog.addgene.org/
https://commonfund.nih.gov/4Dnucleome





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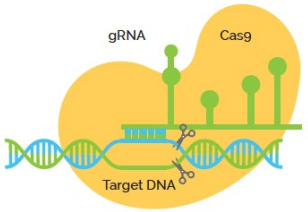

CRISPR System

Molecular Initiating Event → Key Events → Adverse Outcome

Acknowledgments

- Society of Toxicology
- Scientific Program Committee
- Molecular and Systems Biology Specialty Section
- Mechanisms Specialty Section
- Symposia presenters
- US EPA Chemical Safety for Sustainability Adverse Outcome Pathway Task