BioE 134 Final Project Notes

Next steps

- Use OpenTronics Python API for wetlab automation of constructs, following previous design but with more concrete protocol and ability for variable setup for the initial oligo plate
    - Create a PlateDesigner class to intelligently design 96 well pcr plates with combos and variations
- Add more constructs
    - Casilio-ME, better activation than Tet-1 ?? https://www.addgene.org/browse/article/28207030/
- Add default-off promoters right behind the dCas9 CDS, put all plasmids in 1 organism, but with all different promoters, so you can easily test different combinations of genes in a big plate
- Make GPT able to talk to it, ie. finetune model on some papers and let it browse GEO

Notes

1. Synthetic epigenetics—towards intelligent control of epigenetic states and cell identity (2015)
2. CRISPR 101: Epigenetics and Editing the Epigenome (2020)
3. A CRISPR-based approach for targeted DNA demethylation (2016)

Synthetic epigenetics — towards intelligent control of epigenetic states and cell identity (2015) (Link)

Summary of Relevant Info

- DNA binding domain targets a loci
- Effector domain attaches an epigenetic mark
- Gene expression stays changed even after the construct goes away, so it's safer.
- Changing methylation of promoters can induce loss or gain of function (silence/activate)
- Changes are generally stable for many days
    - Need to target a large area with spreading mechanism or multiple targets
    - Stability also depends on
        - pre-existence of other epigenetic marks
        - location of the locus in euchromatic or heterochromatic region
        - extent of modification and nature of the mark
- CRISPR/Cas9 is probably best
- Delivery method is important

Full Notes

- ENCODE , Roadmap Epigenomics Mapping consortium and Blueprint: repositories of epigenetic states of various tissues
- Currently we can:
    - Understand epigenetic signaling pathways
    - Characterize epigenetic marks (DNA/histone)
    - Read/write/remove marks with enzymatic machinery.
- Questions that remain:
    - Are epigenetic signals the cause or consequence of cell transcriptional profile?
    - What is the sequential order of epigenetic transitions between repressed and activated states?
    - Are epimutations drivers or by-products of a diseased state?
    - What is the contribution of epigenetics to disease development?
- Synthetic epigenetics is "the design and construction of novel specific artificial epigenetic pathways or redesign of existing natural biological systems to intentionally change epigenetic information of the cell at desired loci"
    - Targeted genome editing by programmable DNA binding domains fused to epigenetic modifiers (epigenetic editing)
- Epigenetic editing – change epigenome by targeting effector domain to locus.
    - DNA binding domains (DBDs) target an epigenetic modifier to desired loci and deposit the corresponding epigenetic mark at nearby chromatin.

        - Better than RNAi, gene knockout, recombinant protein expression, programmable activators/silencers, because those require either constitutive expression or irreversible changes to the genome.
        - Epigenetic signal and change in gene expression are heritably maintained even after the initial editing construct is cleared from cells (Transient introduction)
            - Lets you change gene expression without genomic damage
            - Safer and better for therapeutic use.
        - Changing methylation uses:
            - to silence overexpressed oncogenes
            - reactivate tumor suppressor genes
            - Imprinting defects can be reverted
            - Chronic diseases (ie psoriasis) are often correlated with abnormal epigenetic changes
- Bottom-up synthetic epigenetics AKA epigenetic editing
    - Artificial DNA binding domain which binds to a unique sequence in desired locus, with effector domain that edits the epigenetic state of that locus.
    - Genome targeting domains and epigenetic modifiers for activating or repression marks to desired loci
        - 1. Epigenetic Editing: targeted rewriting of epigenetic marks to modulate expression of selected target genes - PMC
        - 2. Silencing of gene expression by targeted DNA methylation: concepts and approaches
        - 3. Broad Specificity Profiling of TALENs Results in Engineered Nucleases With Improved DNA Cleavage Specificity - PMC,
        - 4. Application of DNA methyltransferases in targeted DNA methylation
    - Using CRISPR/Cas9 for genome targeting
        - orthologous Cas9 systems fused to different epigenetic modifiers could be simultaneously used in a single experiment to target various epigenetic modifications to selected loci (can be same or different loci)
        - Catalytically inactive Cas9 variant has to be used, which still can recognize and bind the target sequence, but cannot cleave it
            - 1. Orthogonal Cas9 proteins for RNA-guided gene regulation and editing
            - 2. Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression - PMC
            - 3. CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering
        - Downsides: potentially high immunogenicity, worse target specificity
        - Upsides: multiplexing (2+ sites), independence of DNA modifications
    - Selecting effector domains / Epigenetic modification domains
        - DNA methyltransferases
            - bacterial M.SssI targeted in vitro to synthetic DNA [98]

            - M.HpaII targeted to reporter plasmids and integrated viral DNA [99,100] and eukaryotic Dnmt3a catalytic domain and full-length proteins
            - Dnmt3a-Dnmt3L single-chain constructs targeted to endogenous loci [38,101]
        - ten-eleven translocation DNA demethylases
            - targeted to endogenous loci [102,103]
        - thymine DNA glycosylase
            - targeted to endogenous locus [104]
        - histone methyltransferases
            - G9a—targeted to integrated Gal4 binding site [105]
            - G9a [106] and Suv39H1 [106,107] targeted to endogenous locus
            - Ezh2 targeted to reporter construct [108]) and histone demethylase
                - Lsd1 targeted to endogenous locus [109]
        - histone deacetylases
            - targeted to reporter plasmid [110]
    - Most focused on changing DNA methylation state of promoters to repress oncogenes or activate tumor suppressor genes.
        - we don't understand maintenance of histone modifications during mitotic division [112], but we do know about setting up and inheritance of DNA methylation
            - Loss of function: Targeted DNA methylation can heritably switch off gene expression [37-39]
            - Gain of function: Targeted DNA demethylation [102-104] to activate gene expression from native locus
        - Straightforward clinical application: demethylate promoters, thereby activate expression of tumor suppressor genes, which are commonly silenced in cancer cells [117-120].
        - New functions to differentiated cells could be conveyed by activating genes which are normally not expressed in that cell type.
        - No matter which effector domain is selected for targeting, evaluate the extent and stability of the introduced modification and its biological effect
- Stability of epigenetic modifications
    - Cell and global epigenetic states are heritable and stable over multiple mitotic divisions (vs transient overexpression of transcription factors)
        - [100] – DNA methylation and repressive effect observed 17 days post transfection, when expression of ZF-HpaII F35H construct was no longer detectable
        - [34] – After clearance of the construct, stable DNA methylation (up to 50 days post transfection) and gene repression were maintained in ZF-Dnmt3a CD, but lost in ZF-KRAB transcription repression domain
    - Histone marks also seem to be stably maintained in the cells

        - [108] – H3K27 methylation (target Ezh2 next to the Gal4 binding site) was maintained 4 days post clearance of the targeting construct
        - [122]. – Spreading and long-term stability (over multiple cell divisions) of H3K9me3 triggered by recruitment of HP1α to Oct4 promoter
    - Might not be stable in all genomic contexts, depends on:
        - pre-existence of other epigenetic marks
        - location of the locus in euchromatic or heterochromatic region
        - extent of modification and nature of the mark
    - Small and local changes might not be efficiently maintained [114,115]
        - So, modify as big a part of the region of interest as possible
            - Spreading mechanism or targeting fusion construct to multiple places in the locus. [102]
        - Simultaneous targeting of multiple epigenetic modifier to the same locus, which reinforce the same effect, might strengthen the stability
- Spreading of the mark across the genome
    - length of the region which can be directly modified is limited
        - epigenetic modifier is tethered to the DNA binding domain, which binds tightly to its recognition sequence
    - Extent of the linker between targeting device and epigenetic modifier is the main determinant of the possible reach
    - Most efficient introduction of modification was in the nearest vicinity of the binding site of the targeting domain (10–40 bp [98,102,123])
        - is in line with the typical distance that the linker region between the DBD and effector domain can provide
    - Unlikely that a single and even very stable binding event would lead to a widespread modification at larger distances (mechanical restraints)
        - Histone modification [106] and DNA methylation spreading was observed beyond the expected distance that could be reached when considering the provided domain linker
        - Observed deposition of DNA methylation marks up to 300 bp and more from the targeted site when using the catalytic domain of Dnmt3a (or the Dnmt3a-Dnmt3L single-chain construct) to human EpCAM and VEGFA promoters [37,38]
            - Whether the broad modification of these regions is due to DNA looping and nucleosome wrapping or the proposed spreading mechanism needs to be further investigated.
                - Can be explained by extensive looping of the DNA in this region, which would allow tethered DNA methyltransferase to reach further
                - OR, Dnmt3a cooperatively polymerases along the DNA molecule [124-126] and that its methylation activity is stimulated by the filament formation [127].
                    - So Dnmt3a would recruit additional molecules of the enzyme (maybe even the endogenous protein)

to the modified region, leading to an efficient methylation of a larger genomic region adjacent to the targeted sequence (nucleation point).

- Delivery of programmable epigenetic editors for epigenetic therapy
    - Efficiency mostly depends on vehicle used for delivery of constructs
    - So far, only cultured cells and not whole organisms were used
    - problem with viral delivery systems is limited insert size capacity, which becomes restricting
        - the epigenome editing constructs tend to be very large (esp. Cas9 fusions) and targeting multiple loci at once can be difficult.
    - Approach: hydrodynamic injection method, plasmids encoding targeting constructs are injected into the bloodstream. cells internalize the DNA and express the protein, which can exert the desired effect [131]
    - Approach: deliver purified proteins to cells by attaching cell-penetrating peptides to the purified protein constructs or RNA:protein complexes (in the case of CRISPR/Cas9), therefore allowing spontaneous uptake by the cells [132-134]
    - Lipid nanoparticles?
    - Tethered TALE-VP64 proteins with negatively supercharged domains (containing large amounts of acidic residues) deliver proteins using poly-cationic transfection reagents.
        - Cas9 when complexed with guide RNA could be delivered without this additional domain [136].
- The most exciting progress is expected from the CRISPR/Cas9 system, as it allows the biggest flexibility and ease of design of new targets and the possibility to construct target libraries [137,138], which will allow unprecedented control of the epigenetic states at desired loci.

CRISPR 101: Epigenetics and Editing the Epigenome (2020)

Summary of Relevant Info

- For activation, probably use Tet1 demethylase with Casilio-ME system
- For repression, probably use DNMT3A

Full Notes

- With gRNAs, fusions between Cas proteins and the epigenetic modifier could be targeted to specific DNA sequences
- Non-editing CRISPR is used, with catalytically inactive dCas9 fused to epigenetic modifiers to specific loci (no double strand breaks)
- DNMT3A-induced methylation persisted throughout a 100 day experimental period
- Transcriptional activation
    - p300 acetyltransferase
        - increases levels of H3K27ac histone modification at specified loci
    - Tet1 demethylase
        - targeted cytosine demethylation in mammalian cells
        - available in lentiviral vector
        - Tet1 initiates cytosine demethylation of DNA.
            - Several proteins restore the DNA after cytosine removal.
            - Casilio-ME system for targeted delivery of Tet1 alone, or coupled with DNA oxidation and repair factors for increased gene activation at the target site compared to other Tet1 delivery systems
- Transcriptional repression
    - DNA Methyltransferase 3 Alpha (DNMT3A)
        - targeted cytosine methylation in mammalian cells
        - EGFP and PuroR enable sorting and selection of transduced cells
        - constitutive and Tet-dependent constructs available
        - lentiviral expression available
    - DNA Methyltransferase MQ1
        - fusion of dCas9 to a small DNA methyltransferase
        - Q147L mutation improves methylation kinetics so cytosine methylation is within 24 hours rather than several days
    - Lysine-specific Demethylase 1 (LSD1)
        - targeted removal of H3K4me1/2 and H3K9me2 histone modifications
        - inactivates targeted enhancers
        - functions in mammalian expression systems
        - fused LSD1 to x-Cas9(3.7)
            - increased PAM flexibility, and allows for LSD1 targeting to a wider set of genomic targets

A CRISPR-based approach for targeted DNA demethylation (2016) (pdCas9-Tet1-CD and pcDNA3.1-MS2-Tet1-CD)

Summary

- Describes pdCas9-Tet1-CD and pcDNA3.1-MS2-Tet1-CD
    - https://www.addgene.org/83340/ and https://www.addgene.org/83341/

Full Notes

- Abstract
    - sgRNAs tether Tet1 catalytic domain (Tet-CD) with dCas9 / MS2 to gene loci.
        - Efficiently demethylated target genes with low off-target effects.
- Introduction
    - DNA methylation usually happens in CpG (C and G next to each other)
        - Methylating CpG islands (places w/ lots of CpG) stably silences the gene
            - 70% of promoters in humans have CpG islands
        - Hypermethylation in tumor suppressors can silence, causing cancer
        - Hypermethylation is common in neuromuscular diseases
    - Tet oxidation of the 5-methylcytosine promotes DNA methylation
        - Tet-CD is the smallest functional module
        - Generally annoying to design and assemble
    - Tet is made better with CRISPR
        - dCas9 is the deactivated version of Cas9
        - sgRNA2.0 has added RNA elements that get recognized by RNA-specific binding protein effectors, which can increase efficiency of the dCas9.
    - Strategy for targeted demethylation:
        - Tether Tet1-CD to dCas9 w/ MS2 RNA sgRNA2.0 and MS2 coat protein
- Results
    - dCas9 fused to Tet1-CD to create dCas9-Tet1-CD.
    - sgRNA2.0 w/ two MS2 RNA elements in the same vector
    - MS2 coat protein fused to Tet1-CD to form MS2-Tet1-CD
    - With sgRNA2.0 targeting, both dCas9-Tet1-CD and MS2-Tet1-CD can be used at the same time in the target gene locus.
- What you gotta do:
    - Design sgRNAs to target the promoter region of a gene.
        - In RANKL, that's 800bp upstream of transcription start.
        - They made 8 sgRNAs, and 3 of them increased transcription a lot
    - Molar ratio of dCas9-Tet1-CD to MS2-Tet1-CD should be 1:2.6 for high efficiency.
    - To minimize off target effects of the dCas9:
        - Scoring algorithm for the sgRNAs
            - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3969858/
    - Carefully titrated amounts/ratios of system components

The Role of Epigenetic Factors in Psoriasis

- Possible targets:
    - Promoter 2 of SHP-1 hypomethylation
    - ID4 hypermethylation
    - p15 hypomethylation
    - p21 hypomethylation
    - p16 hypomethylation

https://sci-hub.se/https://www.nature.com/articles/nbt.3437

To read

In Vitro Disease Models for Understanding Psoriasis and Atopic Dermatitis

Genetic and Epigenetic Mechanisms of Psoriasis.(2023)

The Role of Epigenetic Factors in Psoriasis - PMC

Epigenetics of Psoriasis

Toward the Development of Epigenome Editing-Based Therapeutics: Potentials and Challenges

CRISPR technologies for precise epigenome editing | Nature Cell Biology (2021)

Epigenome engineering: new technologies for precision medicine (2020)

Engineering Epigenetic Regulation Using Synthetic Read-Write Modules (2019)

Designing Epigenome Editors: Considerations of Biochemical and Locus Specificities (2018)

Epigenetics knocks on synthetic biology's door (2016)

Editing the Epigenome: Technologies for Programmable Transcriptional Modulation and Epigenetic Regulation (2016)

The epigenome: the next substrate for engineering (2016)

Integrative computational epigenomics to build data-driven gene regulation hypotheses | GigaScience | Oxford Academic (2020)