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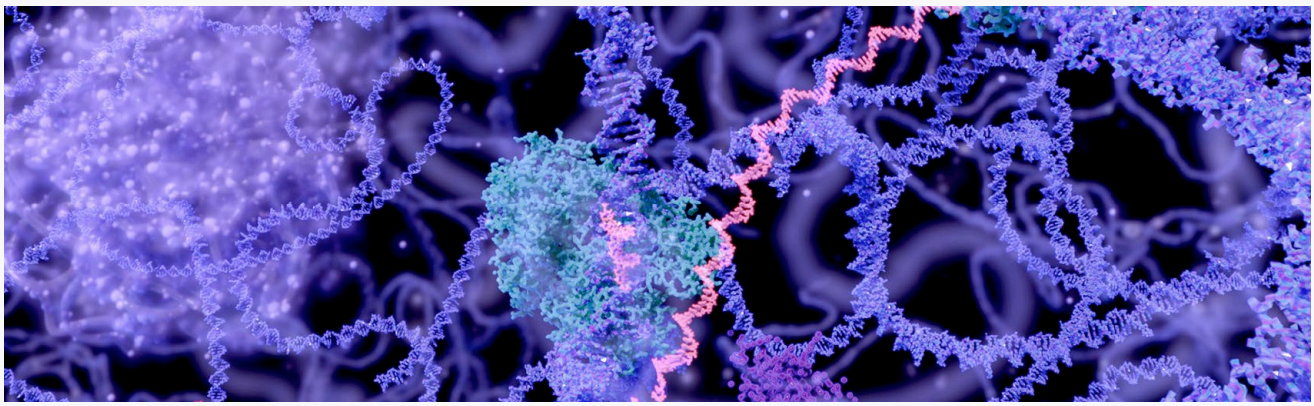

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TARGETS & MECHANISMS

# EXPLORING 3-D GENOMIC WHITE SPACE

By Karen Tkach Tuzman, Associate Editor

Gene regulation is offering a new class of targets that operate on a higher level than DNA sequences and epigenetic markers: 3-D genomic structures. While strategies to drug the 3-D genome are still in their infancy, the growing accessibility of mapping technologies is paving the way for translation in cancer and developmental disorders.

DNA has long been known to wrap around histone protein complexes in stretches of about 150 bases, forming chromatin that is made more or less accessible by epigenetic modifications, such as lysine and methyl tags. However, less is understood about the higher order chromatin loops and clusters, known as topologically associating domains (TADs), which involve DNA stretches on the order of tens of kilobases to several megabases.

In the last five years, advances in sequencing- and imaging-based methods to characterize these structures have catalyzed research studies dissecting their role in controlling gene expression.

The question is whether better understanding of the 3-D genome will yield therapies that correct dysfunctional gene expression in disease.

3-D genome targeting is farther away from translation than targeting enhancer and promoters or developing epigenetic modulators. But the payoff, compared with those approaches, could be greater potency, particularly in diseases such as cancer or fragile X syndrome, where gene expression is dysregulated on a large scale.

"The factors that govern chromatin topology are likely to represent powerful targets for drug discovery," said Cigall Kadoch, co-founder of Foghorn Therapeutics Inc."We now know from a wide range of studies by our lab and others that perturbation to chromatin regulating machines, particularly remodeling complexes, can massively affect global DNA accessibility."

Kadoch is assistant professor of pediatric oncology at Dana-Farber Cancer Institute, and an institute member and Epigenomics Program co-director at the Broad Institute of Harvard and MIT.

Foghorn, which launched last year with a $50 million series A round from Flagship Pioneering, is developing small molecules that disrupt the pathogenic activity of mutated chromatin remodeling complexes, to restore normal 3-D genomic structure and function. Former Bristol-Myers Squibb Co. SVP and head of discovery Carl Decicco joined as CSO in December.

In addition, Jennifer Phillips-Cremins, assistant professor of bioengineering at the University of Pennsylvania, published a study last year suggesting many DNA repeat-driven diseases are characterized by dysfunctional genomic structures, meaning a single drug platform could theoretically be adapted to address the whole class (see "Repeat Offenders").

Hers is one of at least four academic groups taking a different tack: physically forcing chromatin into desired conformations using catalytically inactive gene editing machinery, specifically zinc finger and Cas proteins.

 

## STRUCTURED TRANSLATION

Foghorn's focus on genomic structure stems from the frequency of mutations in chromatin remodeling complexes, which are estimated to be present in around one quarter of all cancers.

"It just so happens that probably the most exciting targets right now, because of the cancer genetics, are these remodeling machines, because of the very central role they play in orchestrating the three-dimensional structure of chromatin," said Steven Bellon, VP of drug discovery.

> "The factors that govern chromatin topology are likely to represent powerful targets for drug discovery."
>
> Cigall Kadoch, Dana-Farber Cancer Institute

Work from Kadoch's lab has shown mutations in SWI/SNF complex subunits, or in the transcription factors that recruit them, change where the complexes bind to the genome, dysregulating chromatin structure and gene expression. Because these complexes are present at tens of thousands of genomic sites, their misplacement has a big impact on chromatin architecture, she said.

The company's platform enables it to isolate remodeling complexes from human cancer cells and perform biophysical, biochemical and cellular assays to identify targets and screen compounds.

Its lead programs, slated to enter the clinic in 2020, are dual and selective inhibitors of the SWI/SNF complex subunits SMARCA2 and SMARCA4. Foghorn is assessing the range of indications these programs can address, and is considering clinical trials in several indications in which the SWI/SNF complex is mutated.

Selvita S.A. also has a dual SMARCA2 and SMARCA4 inhibitor in preclinical development for cancer.

Decicco told BioCentury Foghorn has more than 10 cancer programs underway. The company plans to expand beyond cancer into immunology and neurology, including intelligence-deficit diseases associated with mutations in the SWI/SNF complex, and will likely seek a partner for its neurology programs.

Bellon said that while the mechanistic effects of SWI/SNF subunit mutations vary across different disease contexts, "the general theme that unites many of these mutations is this concept of relocalizing the complex to sites on chromatin where it doesn't belong, changing the 3-D structure of that chromatin, which then results in an aberrant gene expression program."

Fulcrum Therapeutics Inc. has a platform that could also yield 3-D genome-targeted therapies. The company uses patient-derived cells to screen for compounds that change the expression of genes dysregulated in monogenic disorders such as fragile X syndrome.

Fulcrum launched in 2016 with a $55 million series A round from Third Rock Ventures; GV topped off the round with an undisclosed amount in May 2017. Fulcrum raised an $80 million series B round last September led by Foresite Capital.

Fulcrum CSO Owen Wallace said the company is not specifically looking for compounds that interact with 3-D chromatin, but its assay approach would pick up compounds with direct or indirect effects on genomic structures.

## DETECTING DIMENSIONS

Diagnostic and tool companies are developing technologies that could precipitate a surge in translational activity in 3-D genomics.

For example, Arima Genomics Inc. sells kits for detection of 3-D genomic structures. Diagnostics company Oxford Biodynamics plc's EpiSwitch platform detects chromosomal conformation signatures in cancers, autoimmune disease and neurological disorders. And 3D Signatures Inc. is developing cancer tests based on telomere structures.

Arima is commercializing Hi-C technology, a workhorse of the 3-D genome field.

"The goal of the company was to really democratize the Hi-C technology," said Jay Clark, a strategic account manager at Arima. "Historically, only a few labs in the world could use it."

The method cross-links DNA, ligates and tags nearby loci, and then shears the genome into fragments, which are sequenced. Researchers can quantify the frequency with which different loci interact with each other, and use that information to infer 3-D chromatin structures, revealing distal interactions caused by looping of the linear sequence.

Arima director of products Joe Spidle told BioCentury the company has worked to make the technology more amenable to translation by reducing the amount of sequencing and number of input cells needed to get high-resolution maps. These make it more feasible to use the technology on tissue samples from large patient cohorts.

Clark said since Arima established proof of concept with academic clients the company has seen interest from biotechs and pharmas.

Deals from biomarker and diagnostics company Oxford Biodynamics also hint that drug developers are paying attention to the 3-D genome space.

> "We certainly think it is possible to engineer the 3-D genome on demand."
>
> Jennifer Phillips-Cremins, University of Pennsylvania

In the last two years, the company has signed three deals with undisclosed pharmas involving EpiSwitch: one to develop predictive biomarkers for immuno-oncology therapies, another to identify non-small cell lung cancer (NSCLC) biomarkers and a third to find new targets and biomarkers for fibrosis and immuno-oncology.

Oxford Biodynamics did not return requests for comment.

More routine use of tests characterizing the 3-D genome could reveal whether other approaches in gene regulation induce substantial changes in chromatin structure.

In this scenario, the tests could become biomarkers of response, for example for epigenetic modulators and compounds targeting enhancers and promoters.

That includes therapies targeting epigenetic modulators that add, remove or read epigenetic tags — such as HDACs and BET bromodomain proteins — which have sorely lacked biomarkers that capture the state of chromatin regulation in patients (see "On Your Marks").

Likewise, the search for compounds that interact with linear DNA sequences involved in gene regulation, such as enhancers and promoters, is benefitting from screening methods that take 3-D structure into account (see "Ripe for Enhancement").

## FORCING THE ISSUE

While no companies have yet disclosed programs that directly manipulate 3-D chromatin structure, engineering approaches are gaining traction in academic labs.

"We certainly think it is possible to engineer the 3-D genome on demand," said Phillips-Cremins.

Work from the lab of Gerd Blobel has shown forced chromatin looping can re-activate expression of fetal hemoglobin in mature cells, which could help treat sickle cell disease or β-thalassemia.

Blobel is a researcher in pediatric hematology at The Children's Hospital of Philadelphia and professor of pediatrics at the UPenn School of Medicine. He did not respond to requests for comment.

In a 2014 *Cell* study in collaboration with researchers at Sangamo Therapeutics Inc., Blobel's team showed an artificial zinc finger protein that tethered the transcriptional regulator LDB1 to the fetal globin gene turned on fetal hemoglobin expression in mouse and human erythroblasts. The tether created a chromatin loop that forced promoter-enhancer contacts.

A 2016 paper in *Blood* showed zinc finger-based chromosome looping activated fetal hemoglobin expression and reduced sickling in erythroid cells from adult sickle cell patients. The 2016 study authors did not include Sangamo researchers.

Sangamo spokesperson Aron Feingold told BioCentury the chromatin looping approach is "not a focus of our therapeutic development."

In 2017, two independent labs published studies in *Nature Communications* describing programmable Cas9-based gene editing systems to force chromatin looping and turn on gene expression.

A group from Stanford University described a ligand-inducible forced looping system in mammalian cells, while a team from University of Adelaide showed its system could be multiplexed to form multiple chromatin loops in bacteria.

Last June, Phillips-Cremins' team at UPenn published a manuscript in *biorxiv* describing a light-activated Cas9-based forced looping system to control endogenous gene expression. *Biorxiv* is a preprint platform for manuscripts that have not yet been peer reviewed.

Phillips-Cremins said her team hopes to commercialize the technology, which could enable spatial cell type-specific targeting, for example, of specific neuron populations in the brain.

Kadoch thinks forced looping strategies could be challenging to translate into therapies given the hurdles of delivering gene editing machinery, but said there's room for academics to push the approach forward and use it as a tool to better understand 3-D chromatin biology.

## COMPANIES AND INSTITUTIONS MENTIONED

**3D Signatures Inc.** (TSX-V: DXD), Toronto, Ontario
**Arima Genomics Inc.**, San Diego, Calif.
**Bristol-Myers Squibb Co.** (NYSE:BMY), New York, N.Y.
**Broad Institute of MIT and Harvard**, Cambridge, Mass.
**Dana-Farber Cancer Institute**, Boston, Mass.
**Foghorn Therapeutics Inc.**, Cambridge, Mass.
**Fulcrum Therapeutics**, Cambridge, Mass.
**Oxford BioDynamics plc** (LSE:OBD), Oxford, U.K.
**Sangamo Therapeutics Inc.** (NASDAQ:SGMO), Richmond, Calif.
**Selvita S.A.** (Warsaw:SLV), Krakow, Poland
**Stanford University**, Stanford, Calif.
**The Children's Hospital of Philadelphia**, Philadelphia, Pa.
**University of Adelaide**, Adelaide, Australia
**University of Pennsylvania**, Philadelphia, Pa.

## TARGETS

**HDAC** - Histone deacetylase
**LDB1** - LIM domain binding 1
**SMARCA2** (BRM) - SWI/SNF related matrix associated actin dependent regulator of chromatin subfamily a member 2
**SMARCA4** (BRG1) - SWI/SNF related matrix associated actin dependent regulator of chromatin subfamily a member 4

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