Epigenetics Communications

MEETING REPORT

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Bridging bioengineering and epigenetics: from technical innovations to clinical applications

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Abstract

The 7th International Conference on Epigenetics & Bioengineering held in Amsterdam, The Netherlands was a successful event covering cutting-edge research utilizing innovative technologies from multidisciplinary international scientists in the fields of epigenetics and bioengineering, with an emphasis on development and disease. This conference report highlights the outstanding research presented and the engaging discussions that took place. Throughout the sessions, leading experts demonstrated novel technologies to explore epigenetic mechanisms, including advanced data analysis pipelines and bioengineered systems. Several speakers uncovered emerging fundamental principles and demonstrated how these insights are being applied to address (bio)medical challenges, underscoring that the field is progressing toward (pre)clinical applications of targeted epigenetic therapies. The conference featured stimulating discussions on the causal relationship between epigenetic marks and transcription, emphasizing the importance of standardizing epigenetic editing methodologies.

Keywords Epigenetics, Bioengineering, Emerging technologies, Chromatin organization, Gene regulation, Biomedical applications

Introduction

The 7th international conference *Epigenetics and Bioengineering* [1] held October 3–5, 2024 in Amsterdam (EpiBio-24), chaired by Dr. Karmella Haynes (Emory Univ, USA), Dr. Nate Hathaway, (Univ. of North Carolina at Chapel Hill, USA) and Prof. Dr. Pernette Verschure (Univ. of Amsterdam, the Netherlands and Amsterdam University Medical Centers), was a successful event featuring outstanding research presentations, dynamic discussions, and an inclusive atmosphere.

The EpiBio-24 conference covered cutting-edge research utilizing innovative technologies and served as a platform for multidisciplinary international scientists to explore the field of epigenetics and bioengineering with an emphasis on development and disease. The program included high-profile speakers, such as a special EMBO (European Molecular Biology Organization) Keynote

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lecture by professor Wendy Bickmore, Director of the MRC Human Genetics Unit at the University of Edinburgh, along with a keynote lecture from Lei Stanley Qi, associate professor of Bioengineering at Stanford University and pioneer in CRISPR technology, and a keynote by Angelo Lombardo, professor of tissue biology and regenerative medicine at Vita-Saluta San Raffaele University (Italy) and co-founder of Chroma Medicine Inc. (Boston, USA), developing new gene therapy modalities based on targeted epigenetic editing. Additional highlights included a poster networking session and a workshop on responsible innovation led by Dr. Michelle Habets from the Rathenau Institute, The Hague, The Netherlands. Several key areas for future development were discussed, aimed at facilitating biomedical applications and the translation of this research into practical technology transfer. This meeting report provides a concise summary of the highlights from the event.

From pioneering research to technological innovation and biomedical application

On the first day of the conference, EMBO keynote speaker Wendy Bickmore, professor of human genetics and pioneer in functional 3D genome organization in health and disease, gave an inspiring lecture on how the non-coding genome regulates gene activity, including how distant enhancers communicate with their target gene promoters. Wendy Bickmore illustrated that although direct contact is not always necessary for enhancers to activate gene expression, it is essential that they are located close to a promoter (200-300nm). She showed that cohesin-mediated loop-extrusion is essential for long-range enhancement, but she also challenged the loop-extrusion model as the only model by discussing results from her lab pointing towards 'leaky' insulations across TAD boundaries causing bystander activation. In addition to exploring non-coding genome regulation, she presented her findings on a novel histone post-translational modification, H3K115 acetylation, which is located within the histone core rather than on the tail. Intriguingly, this mark is enriched exactly at the transcription start site (TSS) in the 'nucleosome-depleted region', rather than in the nucleosomes flanking the TSS. This modification is suggested to mark a fragile nucleosome, strongly associated with active transcription [2, 3].

On the second day, keynote speaker Lei Stanley Qi, a pioneer in CRISPR technology, delivered an insightful lecture on how to best create and utilize epigenetic bioengineering tools to study epigenome and transcription functioning in human cells [4–6]. Lei Stanley Qi illustrated that TAD boundaries are just snapshots of chromatin interactions, while a cell functions as a living, dynamic system. He made a beautiful comparison with Leonardo da Vinci's *Vitruvian Man*, which captures optionable configurations of the human body much like many epigenetic figures capture the state of many cells across time and space. Qi discussed the functionality of enhancer interactions through an epistasis mapping approach, explaining how redundant, independent or synergistic enhancer interactions can respectively provide compensatory, fine-tuning or robust regulatory effects. He noted that histone acetyltransferase BRD4-mediated condensation can facilitate such enhancer interactions [7]. Interestingly, Qi confirmed Wendy Bickmore's findings that enhancers need to be spatially close to a promoter to exert their function effectively.

The final day of the conference opened with an intriguing keynote lecture from Angelo Lombardo, presenting his lab's pioneering work on strategies for diseases where gene silencing is a valid therapeutic option and also presenting novel cell engineering approaches for cancer immunotherapy. The Lombardo group discovered that stable gene repression requires simultaneous methylation of DNA and repressive histone marks [8]. They observed that epigenetic regulators such as DNMT3A/3L and KRAB domains, which catalyse DNA and histone methylation, play a crucial role in silencing retroviral elements in embryonic stem cells [9, 10]. Inspired by this natural system, Lombardo's group fused DNMT3A/3L and the KRAB domain of ZNF10 to zinc finger domains targeting specific genomic regions, to achieve stable repression of genes across multiple cell lines. They recently demonstrated long-term repression of *PCSK9*, a gene expressed in hepatocytes involved in cholesterol homeostasis [10]. This repression, observed in mice for almost one year, reduced low-density lipoprotein (LDL) receptor presence on the plasma membrane of hepatocytes in the liver. Notably, even after partial liver resection, the induced DNA methylation state and repressed gene expression were retained in regrown tissue, showcasing how stable these modifications are inherited. Lombardo also highlighted the potential of epigenetic editing to stably silence Hepatitis B virus via induced DNA methylation. His lecture boosted confidence in epigenetic editing as a viable therapeutic approach for clinical applications.

Progress in epigenetic bioengineering tools

During the conference, a host of different methods for improving epigenetic enzymes were discussed. Using Deep Enzymology, Albert Jeltsch (University of Stuttgart, Germany) has focussed on DNA methyltransferases (DNMTs) and systematically investigated their preferred flanking DNA sequences. Intriguingly, his findings indicate that different DNA substrates influence the efficacy of DNMT1 so drastically that it is possible to perform allele-specific DNA methylation [11–17]. The group of

Saulius Klimašauskas (Vilnius University, Lithuania), also working on DNMTs, has developed a technique using click-chemistry to perform biorthogonal labelling at sites where individual DNMTs catalyse DNA methylation in live cells. This method enables them to trace high-resolution chemical "tracks" of epigenetic writers throughout the cell cycle or cell state transitions [18–21]. Another way to improve functionality is to reduce off-target effects. Jacob Goell (Rice University, USA) successfully reduced P300 cytotoxicity while preserving its enzymatic activity by introducing a single point-mutation in the P300 core. Furthermore, his findings indicate that P300 primes genes for activation and enhances prime editing efficiency, independent of its enzymatic activity [22].

A recent trend in research shows many labs focusing on epigenetic reader domains, which have evolved over millions of years, to recognize specific post-translational modifications (PTMs). These reader proteins offer a distinct advantage, particularly when certain moieties are challenging targets for developing high-quality antibodies. Tuncay Baubec (Utrecht University, The Netherlands) developed a systematic approach, ChromID, to understand how chromatin modifications regulate protein recruitment. Chromatin readers are employed as recruitment vehicles to target specific proteins and enzymes to defined chromatin modifications. The tool contains a biotin ligase fused to a reader domain that biotinylates proteins in close proximity. After pull-down and mass-spectrometry, the epigenetic proteome at the targeted PTM is mapped, giving insights in gene regulation pathways [23]. Another use of reader domains is highlighted by Matthew Meiners (EpiCypher, USA). They developed chimeric tandem reader domains, as well as synthetic fully chemically defined heterotypically and homotypically modified nucleosomes that can be used as spike-ins when performing CUT&RUN/CUT&Tag. Together these products can be used to map reader-PTM interactions and also to detect histone PTM co-occurrences [24].

In the context of pioneering work on novel technologies that meet the need for tools to examine epigenetic states in living cells, Anja Köhler (University of Stuttgart, Germany) showed a notable technology they developed to examine epigenetic states in living cells. The Bimolecular Anchor Detector (BiAD) technology uses live-cell fluorescence imaging to visualize epigenetic marks with locus resolution. It combines a sgRNA/dCas9 complex as a programmable DNA binding module with reader domains that specifically bind epigenetic modifications as detector modules. Both modules are fused to complementary parts of a split fluorophore allowing the reconstitution of a full fluorophore upon binding of both modules in close proximity, offering a versatile toolbox with broad application [25, 26].

While gene regulation is a critical first step in producing functional proteins, many post-transcriptional factors are at play that influence final protein production. Until recently, it was challenging to visualize ribosome kinetics in real-time. Marvin Tanenbaum (Hubrecht Institute, The Netherlands) created a technique that uses Stopless-ORF circular mRNAs (socRNA) encoding SunTag epitopes that, upon translation, enable direct fluorescence labelling of the nascent polypeptides. This technique can be used to study ribosome kinetics via live-cell single-molecule imaging. Using this approach, Tanenbaum discovered that ribosome collisions facilitate translation through difficult-to-translate sequences by resolving ribosome stalls [27, 28].

Epigenetic screening technologies for unbiased approaches

With the development of new functionally improved tools for epigenetic editing, the need for large-scale, high-throughput screening methods also increases. Several speakers demonstrated their incredible work comparing numerous different targets and effectors.

Targeted recruitment of a protein to a reporter gene allows identification of the effect of the recruited protein on the reporter expression, thereby providing information about the protein's potential function. Thus, the question emerged: are we using the best transcriptional activator and repressor domains? Many of the annotated protein families have not been linked to a function. Lacramioara Bintu (Stanford, USA) and her lab developed dCas9-mediated high-throughput recruitment (HT-recruit) to test the function of protein domains and their ability to silence or activate gene expression [29]. Bintu tested more than 5000 nuclear protein Pfam domains from human and viral origin across multiple contexts and also mapped context dependencies from unannotated protein regions using a library containing 114,288 sequences tiling both transcription factors (TFs) and chromatin regulators. She illustrated that activators are often 'greasy acidic noodles with a little salt, pepper and queso', i.e. rich in both acidic residues and particular hydrophobic residues like leucine and aromatics, interspersed with serine, proline and/or glutamine. Repressors have more 'flavours', being not so strictly tied to a specific subset of residues. KRAB domains are the best repressors across context, with the KRAB domain from ZNF705F being a notable find, as it has higher efficacy than the more commonly used KRAB from ZNF10. Interestingly, a strong transcriptionally activating KRAB domain was also found, highlighting how informative such large unbiased screenings can be [30–32].

While the group of Bintu focused mostly on smaller domains, Michael Herschl (UC Berkeley, USA) utilized

catalytic domains of up to 6.3 kilobases to screen over 50,000 pairs of epigenetic editors. During his EpiBio presentation, he reported on the COMBINE (COMbinatorial INteraction Exploration) high-throughput and inducible screening platform. Using this platform, they identified combinations of editors capable of imparting long-term epigenetic changes in target gene expression. Furthermore, a novel bidirectional CRISPR perturbation system capable of both activating and repressing target genes concurrently was highlighted. One of the main takeaways of his talk was that domains that are in the same or similar pathways have good perturbation synergy, mimicking the natural collaboration of these domains [33].

In search for regenerative therapies to restore neuronal loss by direct reprogramming of astrocytes into neurons, Samuel Reisman (Duke University, USA) used CRISPR activation (CRISPRa)-based strategies to conduct high throughput screens of over 1600 human TFs, followed by single-cell RNA-seq with sgRNA capture (Perturb-seq). Using these methods, they could map the fidelity and subtype-specificity of each TF, widely expanding the list of potential therapeutic targets (unpublished data).

Data analysis and experimentally applicable computational tools for epigenetic bioengineering

With the growing influence of bioinformatics on data analysis and also on experimental engineering, know-how on how to utilize both dry- and wet-lab techniques is becoming more essential. Kim Kira Witetzek (Academia Sinica, Taiwan) demonstrated the novel pipeline called ATAC-Mass. This technology capitalizes on isotopic labelling to detect accessible parts of the genome by ion beam imaging with 100nm resolution, combined with mass cytometry, enabling multi-parameter three-dimensional imaging of nuclear features. This results in a tool integrating epigenomics, proteomics and high-resolution imaging on a single-cell level, enhancing the understanding of molecular mechanisms involved in gene regulation (unpublished data).

Jennifer Spangle (Emory University School of Medicine, USA) demonstrated another novel technique, which does not only work on a single-cell level, but also in vivo. Her lab makes use of an L-Methionine analogue ProSeMet which is converted into the S-Adenosyl methionine (SAM) analogue ProSeAm, thereby tagging proteins with a biorthogonal alkyne. This alkyne can then directly be detected via LC–MS/MS. Even without enrichment, this technique can identify mono-, di-, and trimethylation, histidine methylation and arginine methylation with site-specific resolution, while with enrichment 221 proteins with novel methylation sites were identified [34]. As the technique can also be used in vivo and even across the blood–brain barrier, this promises a bright future for understanding the methyl proteome across multiple contexts.

Seeing how enzymes behave and interact over time can provide valuable insights and suggest possible optimizations. Philipp Schnee (University of Stuttgart, Germany) showed the beauty of innovation through 3D molecular dynamic simulation and developed a model to simulate how atoms move over time. This makes it possible to infer changes that would for instance make enzymes less or more efficient, but also predict their preferred substrate. Using this model, they designed a Super-Substrate for a protein lysine methyltransferase (PKMT). This Super-Substrate outcompetes the natural substrate due to its substantially increased specificity, significantly reducing off-targets when using it as a small peptide inhibitor, even for PKMTs with the same substrate. Models like this aid in both the engineering of enzymes with increased or decreased activity, and the engineering of small peptide inhibitors with an incredible amount of specificity, providing a deeper understanding of the catalytic mechanism in question at nearly the smallest possible spatial resolution [35–37].

With increasingly larger experimental setups providing complicated multi-omics data, building and streamlining the data analysis pipelines and necessary tools is of utmost importance. Kimberley Glass (Brigham and Women's Hospital Boston, USA) presented the computational modeling tool SPIDER (Seeding PANDA Interactions to Derive Epigenetic Regulation) [38]. SPIDER uses DNase-seq, ATAC-seq, or DNA methylation data to estimate gene regulatory networks, including cell-line-specific regulatory interactions. The initial model is created using TF-TF, TF-gene and gene–gene interactions, before being pruned of false-positives using chromatin states. What makes SPIDER especially attractive for use is that it not only ameliorates the noise that is often present in multi-omics data, but also that it reduces false negatives and increases true positives in its output, thereby accurately predicting ChIP-seq TF binding events that do not have a corresponding sequence motif. This is a clear improvement that older tools and pipelines struggle with. The SPIDER tool can give an accurate feel for the general direction of regulatory pathways and discover missing links within those pathways.

Emerging principles in epigenetic gene regulation

In the field of epigenetics, a large effort is made to better understand the causal relationship between histone PTMs and gene expression. Jamie Hackett (European Molecular Biology Laboratory Rome, Italy) has utilized CRISPR-dCas9 epigenetic perturbation screens to systematically dissect context-dependent interactions and define causal regulatory roles. A foremost finding is

that blocking histone tail acetylation prevents transcription activation after H3K4me3 deposition. Furthermore, CTCF binding sites act as repressors when located within promoters where H3K36me3 is present. When looking across cell types, it was found that gene permissiveness to epigenetic reprogramming is dependent on cell type, which (co)transcription factors are expressed, and the underlying DNA sequence [39–41].

On the topic of causality in gene expression, Domitilla del Vecchio (Massachusetts Institute of Technology, USA) proposed that H3K9me3 causally follows DNA methylation. The argument that is brought forward is that targeting KRAB alone to a locus does not provide long-term epigenetic memory, while DNMT3A is capable of doing so [42]. Furthermore, experiments point to DNA methylation in the promoter and upstream binding site having a direct inverse log-linear relationship with gene expression. When the methylation level in cells remains stable over time, so does the repressed state of gene expression. Alexander Gimelbrant (Altius Institute for Biomedical Sciences, USA) investigated DNA methylation between alleles and discovered that DNA methylation in most cases drives monoallelic autosomal expression [43]. However, in a subset of genes, allelic imbalance remained unchanged upon DNA demethylation, suggesting the involvement of additional mechanisms in the maintenance of allele-specific expression in these loci.

In the field of epigenetic reprogramming there is currently a large focus on perturbing gene expression by targeting promoters with epigenetic effector domains. However, it is also interesting to look at the effect that other genomic features have on transcription and whether targeting those would increase editing efficacy. Bas van Steensel (Netherlands Cancer Institute, The Netherlands) made clever use of transposon systems to translocate genomic elements such as promoters, enhancers, CTCF binding sites and LoxP recombination sites to hundreds of positions within a 2Mb window and detected functional consequences on gene expression. This led to the discovery that enhancers not only talk to promoters but also to gene bodies to influence gene expression [44–46].

During the conference it became clear that the field is moving towards (pre)clinical application of targeted epigenetic therapies with many examples of successful and long-lasting reprogramming. Possibly, as important as on-target effects are potential off-target effects of epigenetic editing. Though, different labs tend to have different thresholds of acceptance for unwarranted editing. Therefore, Henriette O'Geen (UC Davis, USA) proposed to have a discussion within the field to establish a gold standard for reporting off-target effects. Her research demonstrates that hundreds of CpGs retain off-target methylation even after 24 days of culture following transient epigenetic editing with dCas9-DNMT3A/3L and KRAB [47]. Furthermore, she reports that bivalent genes, containing coinciding H3K27me3 and H3K4me3 marks. These genes are 'poised' for transcription and play a role in oncogenesis. These data indicate that we should pay attention to off-target methylation.

Majid Pahlevan Kakhki (Maja Jagodic's lab, Karolinska Institutet, Sweden) also highlighted the issue of widespread off-target methylation. They have characterized several existing and novel CRISPR-dCas9 constructs for their efficiency, stability and specificity of targeting and they uncovered a strong pattern of unintended on-target and off-target DNA methylation by almost all the tools including CRISPRoff (DNMT3A/3L, KRAB) [48]. This effect, particularly pronounced in genomic regions with low to moderate DNA methylation, appears to be specific to both the sgRNA and effector domains used (unpublished data).

Applications of epigenetic bioengineering in biomedical research

With more and more epigenetic mechanisms becoming established in literature, the field advances to applying this knowledge in a biomedical setting. Pernette Verschure (University of Amsterdam, The Netherlands) has focused on improving treatment of hormone-sensitive breast cancer. Her lab investigates how the relationship between transcription dynamics and epigenetic regulation drives cell diversification, potentially leading to resistance against hormonal treatment in breast cancer. Through studies involving epigenetic drugs combined with CRISPR/dCas9-based epigenetic editing, they found that the transcription burst size serves as a predictive parameter of gene responsiveness (unpublished data). Overall, her research highlights significant variability in readouts across different target gene combinations, underscoring the importance of single-cell analysis [49–52]. As part of the Epi-Guide-Edit consortium that she coordinates, her group is preparing a CRISPR/dCas9 perturbation screen using multiple epigenetic editor combinations and single-cell analysis to identify genes that are permissive to sustained epigenetic reprogramming based on their epigenetic context, following hit-and-run targeting.

Gabriella Ficz (Barts Cancer Institute, UK) also shared an interesting example of epigenetic editing in an applied setting and showed that it is possible to perform *ex-vivo* epigenetic editing of *CDKN2B* (p15) in hematopoietic stem cells from the umbilical cord with dCas9-DNMT3A/3L. This produced long-lasting DNA methylation and gene repression, which was maintained

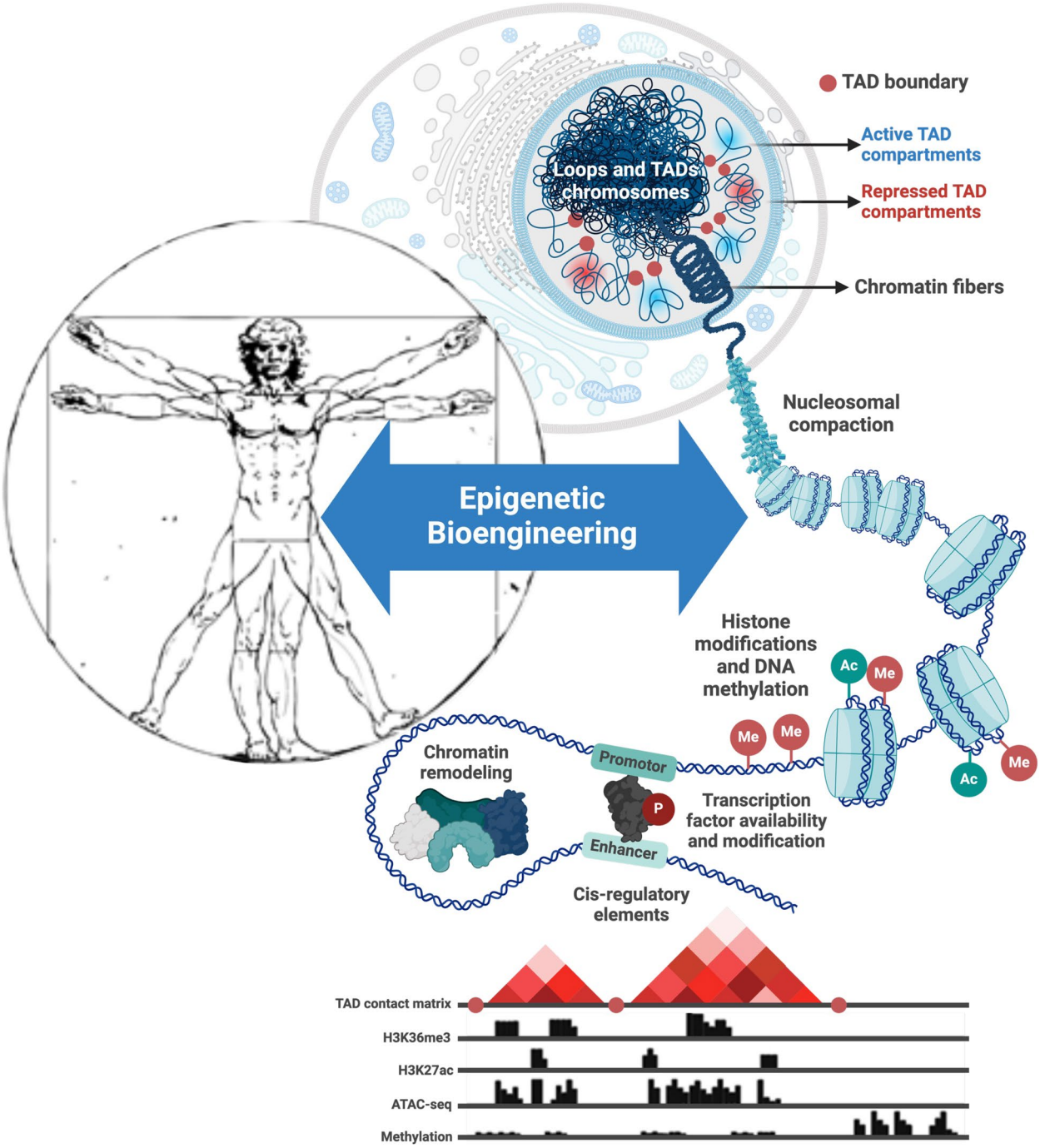

Fig. 1 Bridging bioengineering and epigenetics: From technical innovations to fundamental understanding. The figure illustrates how epigenetic bioengineering has advanced our understanding of mechanisms underlying epigenetic gene regulation in human cells. By integrating high-throughput sequencing with high-resolution microscopy, multiple regulatory levels can now be analyzed, including DNA methylation, chromatin remodeling, transcription factor modifications and their availability, cis-regulatory elements, chromatin loops, topologically associated domains, histone modifications, nucleosomal compaction, 3D chromatin structures and nuclear organization. Despite these advancements, the dynamics and functional interactions between these levels remain only partially understood. Additionally, findings at one technological level may not always align with findings at another, highlighting the need for open-minded exploration and improved conceptual frameworks. The reference to Leonardo da Vinci's *Vitruvian Man*, showing a figure with extended arms and legs, symbolizes this transition from conceptual understanding to the dynamic interplay of functional processes.

even after engraftment and differentiation in mice. The reprogrammed cells home to the bone marrow and after differentiation, epigenetic changes are conserved and inherited in both the myeloid and lymphoid lineage. Moreover, monocytes derived from reprogrammed cells appear to be activated and show increased inflammatory transcriptional programs, which indicates promising application in clinical settings [53]. Abnormal changes in DNA methylation are associated with aging and cancer. Being able to sustainably change methylation patterns in primary cells could thus prove a useful tool for the clinic.

Epigenetic regulation is thought to underlie various immunogenic responses and cell-to-cell variation. Therefore, research into epigenetic pathways is crucial. The lab of Ivana Parker (Herbert Wertheim College of Engineering, University of Florida, USA) studies the immunomodulatory effects of the tuberculosis vaccine bacillus Calmette-Guérin (BCG), which has profound effects on memory-like responses in macrophages. Using multilevel proteomics to examine DNA and histone modifications, she discovered epigenetic writers and erasers that modulate the observed effect and identified the pathways leading to potential BCG-induced activation [54]. Her research demonstrates a different approach to understanding epigenetic pathways without using targeted epigenetic editing, but rather by investigating the cause and consequence of natural cellular epigenetic responses to external stimuli such as vaccines.

Concluding remarks

A wide variety of topics was discussed at the 7th International Conference on Epigenetics and Bioengineering. Pioneers in the field highlighted novel technologies that can be used to investigate epigenetic mechanisms, ranging from data analysis pipelines to bioengineered systems. Furthermore, we saw presenters who, using clever tools, uncovered emerging fundamental principles and others who were able to apply fundamental principles to solve (bio)medical questions. This conference was accompanied by many interesting discussions regarding the causal relation of epigenetic marks and transcription. Finally, researchers reported on off-target effects of epigenetic editing, proposing to prepare a white paper to discuss and standardize epigenetic editing methodologies.

Within only one decade after coining the term [55], the epigenetic editing field has made impressive progress with currently over 10 companies developing epigenetic editing therapies and a clinical trial ongoing [56]. This meeting thus underscored the importance of researchers coming together to discuss the direction of the field. It also provides a valuable platform to share insights on enhancing the safety of epigenetic editing by minimizing off-target effects.

Conferences like these inspire and energize researchers from both academia and industry, fostering collaboration and bridging the gap between (bio)medical sciences to informatics and physics. They provide a platform to discuss ongoing work, address challenges, and explore potential improvements, all in the context of rapidly advancing technologies in the epigenetics field. Together, such gatherings create an optimistic outlook for the future of epigenetics and bioengineering (Figure 1).

Abbreviations

ATACseq	Assay for Transposase-Accessible Chromatin using sequencing
BCG	Bacillus Calmette–Guérin Vaccine
BRD4	Bromodomain-containing protein 4
CDKN2B	Cyclin-Dependent Kinase 4 inhibitor B
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CRISPRa	CRISPR activation
CRISPRi	CRISPR inactivation
CTCF	CCCTC-Binding factor
DNA	Deoxyribonucleic acid
DNMT	DNA Methyltransferase
EMBO	European Molecular Biology Organization
HT	High Throughput
Kb	Kilobase
KRAB	Krüppel associated box
LC–MS	Liquid Chromatography–Mass Spectrometry
LDL	Low-Density Lipoprotein
Mb	Megabase
PCSK9	Proprotein Convertase Subtilisin/Kexin type 9
PKMT	Protein Lysine Methyltransferase
PTM	Post-Translational Modification
SAM	S-Adenosyl Methionine
sgRNA	Single guide ribonucleic acid
socRNA	Stopless-ORF circular mRNA
TAD	Topology Associated Domain
TF	Transcription Factor
TSS	Transcription Start Site
UK	United Kingdom of Great Britain and Northern Ireland
USA	United States of America
ZNF	Zinc finger protein

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Authors' contributions

JJ, QCvL, ACHvdBvS, FS drafted the manuscript. PJV supervised the writing of the manuscript. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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