Compromising Lagging-strand DNA Polymerase alpha Enhances Fly Midgut Stem Cell Regeneration In Response to Chemical Damage

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This project investigates the role of reduced DNA polymerase alpha (Polα) activity in Drosophila intestinal stem cells (ISCs). Heterozygous flies with compromised Polα (polα50+/-) show enhanced regenerative capabilities during aging and tissue regeneration in both germlines and the midgut ISC system. These flies survive longer when infected with lethal bacteria and maintain higher ISC percentages. Using Dextran Sulfate Sodium (DSS) to induce controlled gut damage, polα50+/- females exhibit improved survival at various DSS concentrations and temperatures. Future experiments will assess gut damage, track ISC activity, analyze cellular features, and perform genome-wide analyses to investigate differential gene expression post-DSS feeding. This research reveals a novel role for DNA replication components in enhancing regenerative capabilities in response to tissue damage and aging, providing insights into their non-traditional functions in development, homeostasis, and tissue regeneration.

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Arid1a controls key transcriptional regulators of lineage specification in mammary epithelial cells

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Chromatin accessibility is key for gene regulation during cell differentiation, but the epigenetic regulators of mammary cell fate remain unclear. We identify Arid1a as a critical regulator of mammary gland morphogenesis, with Arid1a knockout in mice reducing branching. Arid1a-deficient mammary organoids lose granularity and display a cystic phenotype with impaired differentiation. Using sc-multiomic sequencing, we show ARID1A maintains differentiation of cell states found in the mammary epithelium. Arid1a deletion restricts cellular identity, favoring undifferentiated mature luminal cells, with diminished estrogen receptor responsiveness, chromatin accessibility and SWI/SNF binding at lineage-defining TFs loci. CRISPR/Cas9-mediated LOF screening identifies Foxa1 and Meis1 as TFs whose loss recapitulates the Arid1a-deficient phenotype. Our findings highlight Arid1a's role in mammary epithelial cell identity and differentiation by collaborating with lineage-specific TFs.

Epigenetic Age Acceleration and Mortality among Persons who Inject Drugs with Poorly Controlled HIV

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HIV infection and substance use have been associated with accelerated epigenetic aging. However, limited data exist on the long-term implications associated with age acceleration and HIV among persons who inject drugs (PWID). We included participants from the AIDS Linked to the Intravenous Experience (ALIVE) study, a community-based cohort of PWID. DNA was isolated from buffy coat samples and treated with bisulfite before being analyzed using the Illumina MethylationEPIC BeadChip. Four measures of epigenetic age were generated: PhenoAge, Horvath age, Hannum age, and GrimAge. All participants were linked annually to the National Death Index-Plus to determine time and vital status since 1988. HIV infection and detectable viral load are associated with epigenetic age acceleration. Age acceleration and HIV infection independently and jointly increase mortality risk among persons who inject drugs.

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Intermolecular Profiling of Epigenetic Heterogeneity of Rare Biomarkers

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Trace amounts of tumor-derived, heterogeneously methylated DNA molecules can be found non-invasively in blood samples, or “liquid biopsies,” but at very low fractions compared to healthy molecules (<0.01%). This work presents a digital PCR and HRM microfluidic platform for the absolute quantification and comprehensive assessment of intermolecular heterogeneity. A microfluidic device digitizes the sample into 4096 1-nL well chambers for single-molecule detection and analysis. The digital HRM (dHRM) platform discriminates small sequence variations within the amplicons to assess sample heterogeneity at a target to background ratio as low as 0.0005%. The sensitivity of the platform marks a significant step towards rare biomarker detection in challenging samples such as liquid biopsies. This all-in-one molecular profiling and analysis platform provides simple fabrication and operation, robustness and flexibility to multiple assays, and the practicality necessary for routine use.

Promoter methylation gains in aging and cancer are independent of replication

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DNA methylation alterations occur early in cancer initiation, but their evolution remains poorly understood at the clonal level. We developed a method for profiling genome-wide DNA methylation from low DNA amounts and applied it to BRAFV600E oncogene-induced colon cancer organoids. Single organoid clones were profiled monthly for 5 months. Control organoids showed epigenetic drift leading to clonal convergence, while BRAFV600E-induced organoids exhibited step-wise divergence. Methylation changes in BRAFV600E organoids were attributed to age-in-culture, not oncogene induction. Affected CpG sites in control and BRAFV600E comparisons mapped to the same genomic elements, suggesting common drivers of methylation changes. Promoter CpG-island methylation gains were found to be independent of replication. Our studies provide insights into early epigenetic changes in BRAFV600E-driven carcinogenesis, challenging previous assumptions about oncogene-induced epigenetic changes.

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Prostruc: An Open-source Tool for 3D Structure Prediction using Homology Modeling

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Homology modeling is a widely used computational technique for predicting the three-dimensional (3D) structures. However, existing tools often require significant expertise and computational resources. Prostruc is a Python-based homology modeling tool designed to simplify protein structure prediction through an intuitive, automated pipeline. Integrating Biopython, BLAST for template identification, and ProMod3 for structure generation. Prostruc implements a validation process: it uses TM-align for structural comparison, assessing RMSD and TM scores against reference models and it evaluates model quality via QMEANDisCo to ensure high accuracy. The top five models are selected based on these metrics and provided to the user. It is accessible via a cloud-based web interface or as a Python package for local use, ensuring adaptability across research environments. Prostruc is a significant advancement, making protein structure prediction more accessible to the scientific community.

Ubiquitination pathway factors Effete and Cullin 4 affect nuclear organization of the gypsy chromatin insulator

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Chromatin insulators control higher-order genome organization. In Drosophila, Su(Hw), CP190, and Mod(mdg4)67.2 are core protein components of the gypsy insulator complex. Post-translational modifications of insulator proteins affect insulator body localization and are required for full insulator activity. We performed a high-throughput visual screen for Mod(mdg4)67.2-GFP localization using a ubiquitination-related RNAi library. We identified Effete (Eff) and Cullin 4 (Cul4) as novel regulators of gypsy insulator complex localization and function. Both Eff and Cul4 physically associate with gypsy insulator proteins and promote gypsy-dependent insulator barrier activity. Moreover, Cul4 extensively colocalizes with CP190 on chromatin and assists recruitment of CP190 to gypsy sites. Both Eff and Cul4 affect transcription near topologically associating domain borders, with Eff specifically altering the 3D nuclear positioning of gypsy insulator sites.

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H2A.Z facilitates Sox2-nucleosome interaction by promoting DNA and histone H3 tail mobility

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The conserved variant H2A.Z has been linked to pioneer factors Sox2 and Oct4 that open chromatin and initiate cell fate-specific expression. Using NMR spectroscopy, molecular dynamics simulations, and biochemistry, we examine the role of H2A.Z nucleosome dynamics in pioneer factor interaction. We find that H2A.Z facilitates Sox2 and Oct4 binding at distinct locations in 601 nucleosomes. We further link this to increased DNA accessibility and perturbed dynamics of the H3 N-terminal tail, which we show competes with Sox2 for DNA binding. Our simulations validate a coupling between H2A.Z-mediated DNA unwrapping and altered H3 N-tail conformations with fewer contacts to DNA and the H2A.Z C-terminal tail. This destabilizing effect is DNA sequence-dependent and enhanced with less stable nucleosomes. Together, our findings suggest that H2A.Z promotes pioneer factor binding by increasing access to DNA and reducing competition with H3 tails, thus regulating chromatin structure and function.

DROSOPHILA HNRNP M HOMOLOG RUMPELSTILTSKIN PROMOTES HOMIE INSULATOR BARRIER ACTIVITY AND REGULATES POLYCOMB-DEPENDENT 3D INTERACTIONS

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The Drosophila Homing insulator at eve (Homie) blocks Polycomb (PcG) repressive chromatin originating at even-skipped (eve) from spreading into the ubiquitous, essential TER94 gene. We found that Homie barrier activity relies on Rumpelstiltskin (Rump) and two insulator proteins, Centrosomal Protein 190kD (CP190) and CTCF. 3C analysis of the eve PcG domain showed increased cis-looping after Rump but not CP190 or CTCF depletion. ChIP-seq after Rump depletion revealed spreading of H3K27me3, increased binding of the PRC2 methyltransferase, decreased binding of two core PRC1 proteins, and differential binding of a PRE-binding protein at 60% of normally bound PREs. Finally, Oligopaint DNA FISH revealed distances between distal PcG domains increase after Rump depletion. Taken together, Rump depletion impacts association of PcG proteins with chromatin, influencing both cis and trans interactions.

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Delayed lagging strand synthesis drives asymmetric histone incorporation and promotes progenitor cell reprogramming in the Drosophila male germline

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In the Drosophila male germline lineage, stem cells display asymmetric histone inheritance. We report that an essential molecular mechanism underlying this is delayed lagging strand synthesis, which drives old histone incorporation into the leading strand during DNA replication. A candidate screen identified that proteins involved in lagging strand synthesis are expressed at reduced levels in stem cells compared to non-stem cells in the same germline lineage. Compromising DNA Polymerase α (Polα) genetically or pharmacologically is sufficient to induce the replication-coupled histone incorporation pattern in non-stem cells to be indistinguishable from that in stem cells. These altered chromatin features in progenitor cells allow them to act like stem cells under conditions in which bona fide stem cells are lost. Together, these results indicate that manipulating even a single DNA replication component can induce histone dynamics in non-stem cells resembling the dynamics in stem cells.

Targeting HPV-induced Vulnerabilities for tumor-specific sensitization

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Driven by high-risk HPV, cervical cancer progresses through the oncogenic effects of E6 and E7 proteins. We investigated whether the Aurora Kinase inhibitor(AZD1152) provides radio-sensitization specifically in HPV-positive cells as it is required for the mitotic function of the MIS12 complex that we uncovered to be critical for the survival of E6-expressing cells after radiation. Our findings show that 50nM of AZD1152 sensitizes SiHa HPV-positive cervical cancer cells to ionizing radiation. As a reciprocal approach, we generated MCF7 and C33A cell lines expressing eGFP and E6 via lentiviral transduction. We also performed MIS12 complex knockdowns, which sensitized SiHa WT but not SiHa E6 KO cells, suggesting E6-dependent radio-sensitization. These results indicate that disrupting the centromeric protein complex MSI12 causes radio-sensitization specifically in HPV-positive cells opening an opportunity for a cancer-specific radio-sensitization of HPV-positive tumors.

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Collaboration between two layers of gene repression establishes and maintains cell fate

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The C. elegans ASE neurons are a well-studied paradigm for transcriptional cell fate decisions because their left/right asymmetry is dictated by the expression of one gene, lsy-6, in the left ASE and not in the right. These cells are specified by the same transcription factor (TF) which should bind to lsy-6 in both neurons, but we know that lsy-6 in ASER is refractory to activation. However, the molecular players blocking transcription of lsy-6 in ASER are unknown. We hypothesize that chromatin is involved because HP1 deletion causes early, low-penetrance de-repression of lsy-6 in ASER. Intriguingly, heterochromatin appears insufficient for repression maintenance since deletion of a motif in the lsy-6 promoter results in later, robust de-repression. By RNAi, we found the NuRD complex is essential for continued repression of lsy-6. Therefore, we propose a model where repressive chromatin and a sequence-specific TF with co-repressors collaborate to specify and maintain cell identity.

Exploring novel strategies to improve BRAF inhibitor therapy in BRAFV600E-driven Colorectal Cancers

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BRAFV600E, a driver mutation, occurs in approximately 8–10% of all metastatic colorectal cancer (CRC). The limited efficacy of BRAF inhibitors (BRAFi) in BRAFV600E-driven CRC requires novel strategies to improve BRAFi therapy. Suppression of transcription factors (TFs) such as CDX2 showed a dependency for BRAFV600E on CRC initiation. We hypothesize that targeting pathways regulated by such TFs can enhance the anti-tumorigenic effects of BRAFi in BRAFV600E-driven CRC. PDX1, binding to CDX2 regulatory elements, is one over-expressed TF in BRAFV600E CRC and was chosen for a proof-of-concept study. Knockdown (KD) PDX1 significantly reduced cell proliferation rate and strongly upregulated CDX2 expression in Colo205. We then tested and found that PDX1-KD significantly enhanced the anti-tumor effects of BRAFi in BRAFV600E-mutant CRC cell lines. Targeting TFs, such as PDX1, provides a novel orthogonal strategy to enhance the efficacy of the potent BRAFi in BRAFV600E-driven CRC treatment.

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Nucleosome Remodeler Exclusion by Histone Deacetylation Enforces Heterochromatic Silencing and Epigenetic Inheritance

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Heterochromatin enforces transcriptional gene silencing and can be epigenetically inherited, but the underlying mechanisms remain unclear. Here we show that histone deacetylation, a conserved feature of heterochromatin domains, blocks SWI/SNF subfamily remodelers involved in chromatin unraveling, thereby stabilizing modified nucleosomes that preserve gene silencing. Histone hyperacetylation, resulting from either the loss of a histone deacetylase (HDAC) activity or the direct targeting of a histone acetyltransferase to heterochromatin, permits remodeler access, leading to silencing defects. The requirement for HDAC in heterochromatin silencing can be bypassed by impeding SWI/SNF activity. Merely targeting SWI/SNF to heterochromatin, increases nucleosome turnover, causing defective gene silencing and compromised epigenetic inheritance. Histone hypoacetylation in heterochromatic regions, therefore, ensures stable gene silencing and epigenetic inheritance by preventing SWI/SNF activity.

Epigenetic Instability Based Metrics for Multi-Cancer Early Detection

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Cancers present significant changes in DNA methylation, which has proven to be highly useful in cell-free DNA (cfDNA) based cancer detection. Cancer epigenomes are marked by a high degree of intra- and inter-tumor epigenetic variation, indicative of the high level of epigenetic instability. However, the nature of the regions with high instability and its utility as biomarker has not been explored. We developed a methodology to measure the degree of epigenetic perturbation, the Epigenetic Instability Index (EII), for cancer screening. Through machine learning, we have elucidated 269 CpG-island regions which sufficiently capture the epigenetic instability in cancers. We have built classifier models using EII metrics of these 269 regions and demonstrate that they can efficiently identify breast and lung cancer cases from cfDNA methylation data. The models can differentiate even Stage IA of NSCLC with ~75% sensitivity at 95% specificity and early-stage breast cancer at ~68% and 95%.

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Transcriptomic and Epigenetic Analyses Uncover Tumor-Specific Gene Expression Programs in Aged Colon Epithelium.

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The risk of cancer increases with age, highlighting the need to understand how aging drives carcinogenesis. Age-related epigenetic changes, such as DNA methylation, disrupt transcription factor (TF) networks that regulate vital processes like proliferation and differentiation. This study investigates how aging-associated epigenetic alterations influence cancer progression. Using EPCAM+ cells from young, late-adult, and geriatric mice, we analyzed DNA methylation changes and gene expression profiles. Results showed progressive DNA methylation changes during aging, impacting transcriptional regulators. Differential gene expression analysis identified regulators driving metabolic and inflammatory shifts. Downregulation of metabolic regulators and upregulation of growth-promoting genes mirrored cancer-associated signatures. These findings suggest aging primes the colon epithelium for cancer, warranting further functional studies in colon organoids to explore tumorigenic potential.

Suppression of transcription-associated homologous recombination by a G4 helicase

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Active transcription is emerging as an important driver of Homologous Recombination (HR). RNA supports HR in part by facilitating strand invasion via R-loop-initiated D-loop formation. Here, we identify the RNA/DNA G4 helicase DHX36 as a robust suppressor of HR. DHR induction upon DHX36 loss occurred in the absence of canonical, BRCA1- or RAD52-supported repair pathways. Instead, HR was entirely dependent on UAF1 and USP1, two proteins linked to R/D-loop-mediated strand invasion and consequently was epistatic with inactivation of the R-loop resolvase SETX. Supporting clinical relevance, low DHX36 expression correlated with poor survival in ovarian cancer patients treated with platinum, a drug less effective in HR-proficient tumors. our findings point to DHX36, and G4 resolution more broadly, as a novel paradigm in restraining potentially pathological, transcription-associated HR with implications for both ALT and BRCA1-mutant cancer genome maintenance.

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Multi-omic Mapping of the Human Habenula

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The habenula (Hb) is a bilateral midline structure in the brain containing a unique distribution of cell types implicated in reward processing. Due to its small size, few studies have investigated the molecular anatomy of the human Hb and little is known about the molecular signatures of spatially organized cell types in this region. Here we generated a spatiomolecular map of the human Hb using 10X Genomics Multi-ome and Visium platforms. We performed combined single nucleus RNA and ATAC sequencing on post-mortem human Hb from 10 adult neurotypical control donors to identify 13 transcriptomic signatures for medial and lateral Hb cell types and cis-regulatory elements associated with these spatially distinct cell types. Using Visium, we also identified data-driven spatial domains enriched in Hb cell types across the anterior-posterior axis of the human Hb. In summary, we present an integrated single cell multi-omic and spatial transcriptomic atlas of the human Hb.

HPV driven epigenetic reprogramming confers radiosensitivity to cervical cancer cells

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Radiotherapy is a standard of care for many types of cancer; however, the full potential of radiotherapy is limited by nonspecific targeting of normal cells. We hypothesized that HPV infection can rewire DNA damage signaling making HPV-positive tumor cells dependent on alternative pathways to survive radiation, which can be exploited for radio-sensitization of HPV-induced cancers. We performed a negative selection CRISPR screen to identify genes whose knockdown would radio-sensitize cells. We identified NSL1 as one of the potential targets for radio-sensitization. NSL1 KO cells have higher mitotic error rates, which contribute to higher sensitivity of these cells to radiation. E6 expression induces epigenetic alterations in host cells, which may be responsible for rewiring cellular response to radiation. Our findings reveal that E6 expression rewires cellular pathways to survive radiation, which can be attractive targets for radio-sensitization.

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DNA supercoiling and chromatin dynamics across the human genome

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National Institutes of Health National Cancer Institute

We characterized DNA supercoiling across the human genome using our newly developed ATMP-seq assay. In addition to identifying twin-supercoiled domains around genes, we observed negative supercoiling accumulation at TAD boundaries and discovered genome-wide supercoiling domains. We found that transcription influences supercoiling beyond the immediate vicinity of genes, affecting TAD boundaries and broader genomic regions. Specifically, RNAP-stimulated topoisomerase preferentially relax positive supercoiling, resulting in excessive negative supercoiling around genes. This excessive negative supercoiling is a major contributor to supercoiling observed at TAD boundaries and across the genome. Additionally, SMC movements generate supercoiling, that is differentially relaxed by topoisomerase across the genome. Together, multiple chromatin processes shape the distribution of genome-wide supercoiling, an understudied chromatin feature modulating biological processes including gene expression.

MicroRNAs provide negative feedback and stability in gene regulatory network models of cell-state transitions

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Gene regulatory networks (GRNs) connect transcription factors (TFs) and enhancers in nonlinear circuits capable of producing complex behavior such as bifurcations in cell state transition and differentiation. Our dynamic modeling of the Embryonic Stem Cell (ESC) to Definitive Endoderm (DE) transition requires an unknown negative feedback mechanism for epigenomic stability. We show that state-specific microRNAs (miRNAs) can provide this feedback by inactivating other lineage-determining TFs during the transition. Our model offers a mechanism to maintain stable cell states without requiring many cell-type-specific repressive TFs, of which few examples exist. Supporting this model, we use computational models of gene expression and chromatin accessibility from human cell lines to detect enhancers activating the miRNAs consistent with our network. Our analysis highlights the interplay between TFs and miRNAs during the ESC to DE transition and proposes a novel model for gene regulation.

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High-resolution analysis of human centromeric chromatin

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Centromeres are marked by the centromere-specific histone H3 variant CENP-A/CENH3. Throughout the cell cycle, the constitutive centromere-associated network is bound to CENP-A chromatin, but how this protein network modifies CENP-A nucleosome conformations in vivo is unknown. Here, we purified two distinct CENP-A populations with unique nucleosomal configurations; the taller variant was associated with the CENP-C complex. To investigate the role of the two CENP-A population in centromere biology, we found that CENP-A mutants partially corrected CENP-C overexpression–induced centromeric transcription and mitotic defects. In all, our data support a working model in which CENP-C is critical in regulating centromere homeostasis by supporting a unique higher order structure of centromeric chromatin and altering the accessibility of the centromeric chromatin fiber for transcriptional machinery.

Live-cell imaging uncovers dynamic coupling between enhancer transcription and gene activation

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Despite decades of research, how enhancers find and activate target genes remains poorly understood. Enhancer transcription has emerged as a robust marker of enhancer activity, yet its regulation and role in gene activation remain elusive. We investigated the transcriptional dynamics of the endogenous MYC gene and its upstream super-enhancer in non-transformed human cells using dual-color live-cell imaging. Both the MYC gene and enhancer are transcribed in bursts, but with distinct dynamics, suggesting different regulatory mechanisms. Although individual bursts are weakly coordinated, gene activity increases following enhancer transcription and decreases when the enhancer is not transcribed. Disruption of the enhancer or its RNA reduces MYC transcription by prolonging the “deep OFF” state. Together, these findings reveal dynamic coupling between enhancer and gene transcription, highlighting the role of enhancer transcription as a facilitator of gene activation.

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Direct observation of transcription factor binding at target genes in living cells

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Specificity in gene expression relies on discriminating the binding of specific transcriptional regulators from those of non-specific factors at a given gene. With approximately 1,600 genes encoding for transcription factors, a key question is how genes discriminate specific TF binding from the overwhelming presence of non-specific TFs in the nucleus. Here, we use dual-color, single-molecule tracking (SMT) to measure the kinetics of endogenous transcription factors binding at native genes in living human cells. We show that the ligand-inducible TF, glucocorticoid receptor (GR), displays markedly longer residence near GR-responsive gene ERRFI1 compared to a non-target locus (MYH9). In combination with the conceptual framework of kinetic proofreading, these data suggest promoters are ‘dwell time detectors’ rather than simply ‘occupancy detectors’, highlighting dissociation rate as a potential kinetic signature distinguishing specific from non-specific TF binding.

Arid1a directs lineage specification in mammary epithelial cells

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Epigenetic regulation is critical to many developmental processes, including mammary gland differentiation. However, the precise epigenetic regulators governing mammary cell fate remain poorly understood. Here, we identify Arid1a as a critical regulator of mammary gland morphogenesis. Arid1a knockout in mice disrupts key developmental structures such as ductal branching and terminal end buds. Mammary organoids derived from Arid1a-deficient mice exhibit abnormal granularity and adopt a cystic morphology, with impaired differentiation. Multiomic single-cell RNA and chromatin accessibility profiling reveals that Arid1a is critical for maintaining differentiation across all cell states found in the mammary epithelium, including basal, luminal progenitor, and alveolar cells. Depletion of Arid1a restricts cells to an undifferentiated luminal hormonal state, diminishes estrogen receptor responsiveness, and decreases chromatin accessibility and SWI/SNF targeting at key lineage-defining transcription factors (TF). CRISPR/Cas9-mediated loss-of-function screening identifies Foxa1 and Meis1 as key TFs, whose deletion recapitulates the Arid1a-deficient phenotype. These findings uncover a specific epigenetic mechanism governing cell-fate specification in the mammary epithelium. Our work highlights Arid1a and lineage-specific TFs as essential factors for mammary epithelial cell fate specification with important implications for both normal development and breast cancer.

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Folding the Epigenome: Reconstructing Chromatin with AlphaFold3

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Structural biology provides insights into how chromatin factors engage nucleosomes to regulate gene expression. AlphaFold3 (AF3) enables prediction of nucleosome-containing complexes, thus serving as a potential tool to study chromatin factors. While AF3 excels at predicting protein-protein interactions (PPIs), its predictions of nucleosome-containing complexes remain unassessed. We benchmarked AF3 on all nucleosome-containing structures after the training cutoff. We find AF3 excels at predicting histone interactions, such as complexes with readers and writers of post-translational modifications (PTMs). In contrast, AF3 struggles with intricate nucleosomal DNA and multi-nucleosomes. As a test of prediction accuracy, we found that AF3 can recapitulate experimental nucleosomal panel screens by identifying the highest-affinity PTM for chromatin readers. This assessment serves as a guide to leveraging AF3 for mechanistic chromatin studies and highlights its potential in screening for PPIs.

Is it Tumor or Virus? Methylation Analysis to Characterize EBV DNA in Bone Marrow Transplant Recipients

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Background: For allogeneic bone-marrow transplant (aBMT) patients, detection of EBV after transplantation is associated with the development of post-transplant lymphoproliferative disorder (PTLD). Many transplant centers use plasma EBV viral copy number measured by qPCR to screen and monitor for the development of EBV(+) PTLD. A major drawback of this assay is limited specificity. qPCR does not distinguish between EBV released from tumor cells and EBV virions not associated with tumor cells. High plasma copy-number is often detected in the absence of malignancy. Clinically, the features of PTLD overlap with other EBV-driven syndromes and end-organ dysfunction. A distinguishing feature of EBV DNA released from tumor cells is the presence of methylation, imparted by cellular DNA replication enzymes. In contrast, EBV DNA released from virions is never methylated.

Methods: Our group has developed a simple and inexpensive assay to detect methylated plasma EBV DNA. We are conducting an exploratory study to understand whether methylation analysis can be used to improve the specificity of EBV screening for PTLD. We obtained plasma samples from six bone marrow transplant recipients with detectable plasma EBV DNA measured by qPCR. One patient was confirmed to have EBV(+) PTLD. The remaining patients did not have clinical evidence of PTLD. We will assess all six samples for the presence of methylation.

Anticipated Results and Implications: We hypothesize that methylated DNA will only be detected in the specimen from the patient with confirmed PTLD. Such results would suggest that methylation can be used to distinguish EBV(+)PTLD from non-malignant EBV states. Future studies would incorporate a larger prospective patient sample cohort to evaluate the specificity of methylation analysis for EBV(+) PTLD.