Review

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Glucocorticoid receptor biology through the omics lens: context matters

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Abstract: Over the past two decades, advances in omics technologies, combined with classical genetics, have fundamentally reshaped our understanding of glucocorticoid (GC) biology and informed strategies to optimise GC therapy. Traditional models focused on uniform, ligand-dependent gene regulation via the glucocorticoid receptor (GR). In contrast, genome-wide transcriptomic, epigenomic, and proteomic approaches have revealed a far more complex landscape in which GC responses are highly cell-type-specific and context-dependent. High-resolution transcriptomics and single-cell RNA sequencing have uncovered divergent GC-regulated gene networks across immune, metabolic, and structural cell lineages, providing mechanistic insight into both therapeutic efficacy and tissue-specific adverse effects. Epigenomic profiling, including ChIP-seq and ATAC-seq, has shown that chromatin accessibility, enhancer architecture, and GR cistromes vary across cell types and states, thereby shaping transcriptional outcomes. Complementary proteomic studies have defined GR-interacting protein networks that modulate receptor activity. Together, these multi-layered datasets support a systems-level model of GR signalling that integrates chromatin context, transcriptional regulation, and cellular state and begins to explain GC sensitivity, resistance, and context-specific side effects. Emerging multi-omics and single-cell multimodal approaches now enable high-resolution dissection of GC action in complex tissues and disease contexts, advancing precision therapeutic strategies.

Keywords: glucocorticoid receptor; single-cell; RNA-seq; ChIP-seq; ATAC-seq; context-specific

1 Introduction

Glucocorticoids (GCs), commonly referred to as corticosteroids in clinical practice, are steroid hormones widely used for their anti-inflammatory and immunomodulatory effects. They remain cornerstone therapies for inflammatory and autoimmune diseases, oncology, and replacement treatment in adrenal insufficiency (Ayroldi et al. 2022; Caetano and Malchoff 2022; Kim et al. 2023). Despite this broad utility, glucocorticoid action is not uniformly suppressive: under specific conditions, GCs can also potentiate inflammatory responses and opportunistic infections (Chastain et al. 2024; Horowitz et al. 2020). This functional duality reflects the context-dependent activity of the glucocorticoid receptor (GR; encoded by *NR3C1*), a ligand-activated transcription factor that integrates hormonal signals with cellular state; its cDNA was cloned from rat and human cells independently in the Yamamoto and Evans labs (Hollenberg et al. 1985; Miesfeld et al. 1984). Upon GC binding, the GR dissociates from cytoplasmic chaperone complexes, undergoes conformational rearrangements, and translocates to the nucleus (Beato 1989; Cairns et al. 1991). In the nucleus, GR recognises GR binding sites and modulates gene expression by engaging chromatin and recruiting coregulators, defined here as chromatin-associated proteins or protein complexes that influence transcription without sequence-specific DNA binding. Depending on genomic context and interacting partners, GR can both activate and repress transcription (Gouilleux 1999; Rousseau 1984) (Figure 1). Clinically, this complexity manifests in heterogeneous therapeutic responses, adverse effects upon long-term use, and the emergence of glucocorticoid resistance, for example in airway inflammatory diseases (Desmet and De Bosscher 2017; Xia et al. 2017).

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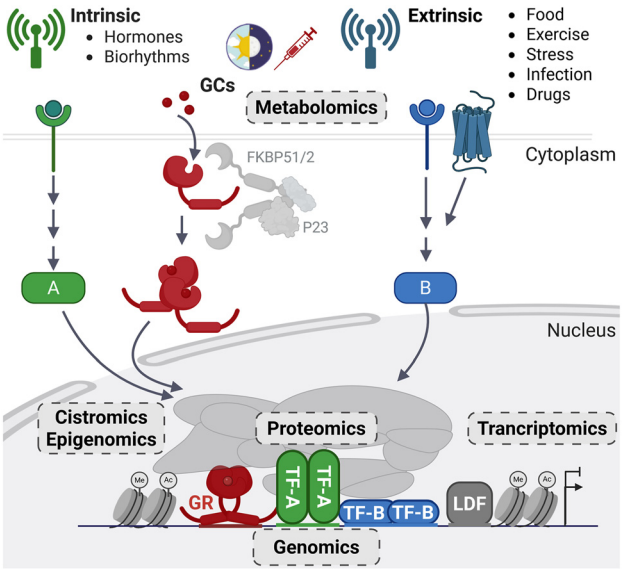

Figure 1: Multi-omics view of glucocorticoid receptor (GR) signalling. Intrinsic cues and systemic or synthetic glucocorticoids (GCs) shape ligand availability (metabolomics) and activate the cytoplasmic GR (red), which dissociates from chaperones and translocates to the nucleus. In a context-dependent manner (A (green), B (blue)), GR engages accessible regulatory elements defined by the epigenome and cistrome dependent on DNA sequence (genomics). GR cooperates with lineage- and signal-dependent transcription factors (TF-A, TF-B), and recruits protein complexes (proteomics) to modulate chromatin state and transcriptional output (transcriptomics) context-specifically. LDF, lineage-determining factor.

At the mechanistic level, understanding how GR produces such diverse outcomes requires genome-wide approaches that capture regulatory processes across multiple layers of gene control. Recent advances in multi-omics technologies have enabled a more integrated view of GR signalling by interrogating transcriptional outputs, chromatin state and protein interactions in parallel. These approaches provide a framework to dissect how GR engages gene regulatory networks in a context-dependent manner, including its interplay with other transcription factors and coregulators, and how these interactions contribute to therapeutic efficacy or resistance. Emerging single-cell and spatial profiling technologies further extend this perspective by resolving GC responses within complex tissues, revealing cellular heterogeneity that is masked in bulk analyses. Together, these developments are reshaping our understanding of GR biology from a largely linear signalling paradigm to a multilayered, context-dependent regulatory system, with direct implications for the design of more precise and effective glucocorticoid-based therapies.

2 Genome-wide approaches changed how we think about GR

Pre-genomic models assumed that ligand binding by GR would produce uniform transcriptional responses. Genome-wide studies have replaced this with a context-dependent model. Bulk RNA-seq demonstrated that glucocorticoids can induce, repress, or have no effect on gene expression depending on cell type and condition (Beato 1989; Greulich et al. 2021b; John et al. 2011; Reddy et al. 2009; Uhlenhaut et al. 2013). ChIP-based and accessibility assays revealed that GR binding, enhancer usage, and chromatin accessibility vary widely across tissues and cell types (Greulich et al. 2021a; John et al. 2011). These findings shifted the field from a receptor-centric perspective to one where tissue-specific chromatin landscapes and transcription factor networks influence transcriptional outcomes.

3 The conundrum of one receptor and many tissue-specific functions: insights from RNA-seq

Bulk transcriptomic approaches have been pivotal in resolving a long-standing paradox in GC biology: how a single, ubiquitously expressed receptor, the GR, can orchestrate highly divergent physiological programs across tissues. The transition from microarray-based profiling to high-throughput RNA sequencing (RNA-seq) marked a decisive inflexion point. Early comparative studies established that RNA-seq provides superior dynamic range, improved detection of low-abundance transcripts, and more accurate quantification relative to microarrays (Marioni et al. 2008), while enabling unbiased transcript discovery, including alternative splice isoforms and previously unannotated RNAs (Wang et al. 2009; Zhao et al. 2014). These technical advances shifted the conceptual framework of GC signalling from a handful of canonical targets to a systems-level perspective in which GR regulates extensive, context-dependent transcriptional networks.

Subsequent methodological refinements have further expanded the analytical resolution of RNA-seq and the biological layers accessible for studying GR function. For example, 3′-end sequencing strategies enable cost-efficient, high-throughput gene-level quantification through the use of unique molecular identifiers, whereas long-read sequencing technologies now provide full-length transcript information, allowing direct resolution of transcript isoforms, alternative

splicing, and transcript end usage (Stark et al. 2019; Su et al. 2024). Library preparation strategies further shape biological interpretation: Poly(A) selection enriches for mature mRNAs, excludes non-polyadenylated transcripts and introduces a characteristic 3′ coverage bias (Viscardi and Arribere 2022). In contrast, rRNA depletion captures a broader spectrum of coding and non-coding RNAs, thereby substantially expanding the detectable regulatory landscape. These advances have revealed that a considerable fraction of the GC response is mediated through non-coding RNAs (ncRNAs), including long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and enhancer RNAs (eRNAs). A recent comprehensive atlas identified more than 2,000 glucocorticoid-responsive ncRNAs across multiple human primary cell types, demonstrating pronounced cell-type specificity and widespread induction of previously unannotated, inducible ncRNAs (Tran et al. 2025). Earlier studies had already established that GR signalling modulates miRNA networks, for example through repression of the oncogenic miR-17~92 cluster, thereby promoting apoptosis via derepression of targets such as *BCL2L11* (BIM) (Molitoris et al. 2011). In parallel, nascent transcription profiling has uncovered tissue-specific GR-regulated eRNAs at active enhancers, which closely track the transcriptional activity of nearby genes and can contribute to both gene activation and repression (Greulich et al. 2021a).

In addition, strand-specific RNA-seq protocols improve the annotation of overlapping sense and antisense transcription and complex genomic loci, which is particularly relevant for resolving alternative promoter usage and polyadenylation site selection in GR target genes (Russcher et al. 2006). Complementary approaches, including sequencing of GR-associated RNAs, further indicate that glucocorticoids regulate not only transcriptional initiation but also RNA processing, stability, and decay, highlighting an additional post-transcriptional layer of GR-mediated gene regulation (Boo et al. 2024; Cho et al. 2015).

3.1 Tissue-specific glucocorticoid responses

A central insight emerging from bulk transcriptomics is the pronounced tissue specificity of GC responses. Cross-tissue analyses consistently reveal a limited set of conserved GR target genes such as *FKBP5*, *TSC22D3* (GILZ), *DUSP1*, and *PER1*, superimposed on a much larger repertoire of tissue-restricted targets (Figure 2). In metabolic organs, particularly the liver, GCs robustly induce genes involved in gluconeogenesis and energy metabolism, including *PCK1*, *G6PC*, and *ANGPTL4*, thereby supporting glucose homeostasis during stress (Murani et al. 2019; Quagliarini et al. 2019). In adipose tissue, short-term GC exposure stimulates lipolysis and causes insulin resistance, whereas in skeletal muscle proteolysis releases metabolic intermediates to fuel glucose homeostasis and the energy demands of the body, especially after fasting periods (Kuo et al. 2013; Makris et al. 2025; Ramshanker et al. 2019). In contrast, in immune cells, GR predominantly modulates inflammatory and differentiation pathways, repressing cytokine networks and inducing anti-inflammatory mediators as well as apoptosis (Chinenov et al. 2014; Franco et al. 2019; Greulich et al. 2021a; Sacta et al. 2018). In other tissues, GR-dependent transcriptional programs drive contractile function and maturation in cardiomyocytes (Rog-Zielinska et al. 2015), stress responses and adaptation in the brain (Itoi et al. 2019), epithelial maturation and surfactant production in the lung (Bridges et al. 2020; Pew et al. 2016), osteoclastogenesis in bone (Buetti-Dinh et al. 2026), and regulate barrier function of skin and intestine (Aranda et al. 2019; Sevilla et al. 2020) underscoring the role of GCs as systemic regulators of homeostasis.

Beyond defining tissue specificity, bulk RNA-seq has enabled the identification of previously unrecognised mediators of GC action relevant in therapeutic contexts. A representative example is provided by Himes and colleagues, who used RNA-seq to profile dexamethasone-treated airway smooth muscle cells. They identified *CRISPLD2* as a highly GC-inducible gene. Functional analyses revealed that CRISPLD2 modulates cytokine production and inflammatory signalling, positioning it as a potential mediator of GC responsiveness in asthma (Himes et al. 2014).

3.2 Glucocorticoids mediate gene activation and repression

Importantly, bulk RNA-seq has made clear that ligand-activated GR is a bidirectional regulator of gene activation and repression. In A549 epithelial cells, genome-wide dexamethasone time-course RNA-seq identified 2,184 regulated protein-coding genes with approximately equivalent numbers of induced and repressed transcripts (Reddy et al. 2009); comparable bidirectional outputs were subsequently reported in porcine liver, mouse skin, murine liver, primary bone marrow-derived macrophages, human bone marrow mesenchymal stromal cells and human trabecular meshwork cells (Buetti-Dinh et al. 2026; Greulich et al. 2021b; Kathirvel et al. 2022; Murani et al. 2019; Quagliarini et al. 2019; Sevilla et al. 2020).

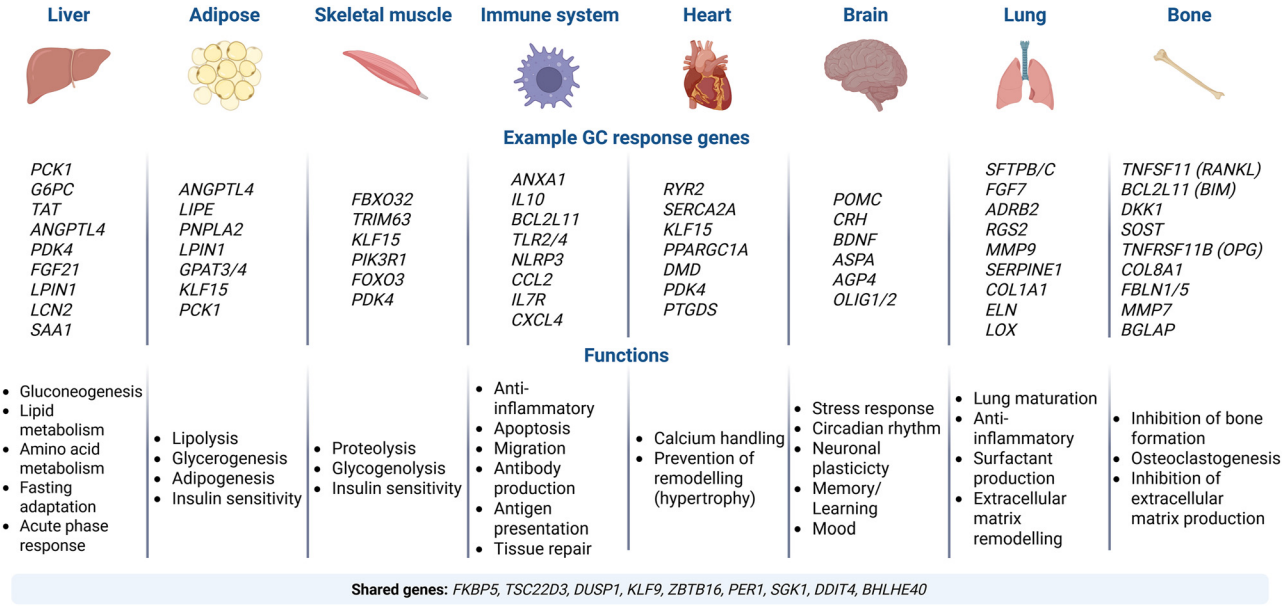

Figure 2: Tissue-specific glucocorticoid receptor (GR) actions. Summary of glucocorticoid (GC) responses across selected tissues, illustrating representative GR target genes alongside the dominant physiological processes regulated by GR in each tissue.

3.3 The role of cellular state, time and dose for glucocorticoid responses

A further layer of complexity revealed by bulk transcriptomics is the strong dependency of GC responses on cellular state. Comparative RNA-seq analyses of primary human monocytes and *in vitro* differentiated macrophages showed that differentiation status profoundly alters the transcriptional response to dexamethasone. Monocytes exhibited a broader and more dynamic transcriptional response, whereas macrophages displayed a more restricted program, with substantial overlap between glucocorticoid-regulated genes and differentiation-associated transcriptional signatures (Wang et al. 2019). These findings suggest that GCs integrate into ongoing transcriptional programs rather than act as independent signals. Similarly, in muscle satellite cells, conditional GR deletion revealed loss of quiescence-associated gene expression and a concomitant increase in proliferation and activation signatures, indicating that endogenous GR signalling contributes to satellite cell quiescence and muscle homeostasis (Rajgara et al. 2023).

Time-resolved transcriptomics has further refined the understanding of GR-mediated gene regulation by revealing a hierarchical and temporally structured response. In a landmark genome-wide study, Reddy et al. quantified transcriptional responses to dexamethasone in A549 epithelial cells across multiple time points and concentrations. They found that induced genes respond more rapidly than repressed genes, with median times to half-maximal response of approximately 40.5 min for activation and 52.5 min for repression. Notably, a subset of genes showed detectable induction as early as 18 min after ligand exposure, indicating that GR can drive rapid, gene-specific transcriptional changes (Reddy et al. 2009). Importantly, early-response genes are enriched for direct GR targets, including transcription factors and signalling molecules, whereas later responses reflect secondary transcriptional waves and indirect regulatory effects. Additionally, Reddy et al. reported a global EC50 of approximately 3 nM dexamethasone for transcriptional responses in A549 cells, yet individual genes displayed markedly different sensitivities. For example, the circadian regulator *PER1* responded at concentrations as low as ~0.5 nM, highlighting that distinct GR target genes occupy different positions along the ligand-response curve. This gene-specific sensitivity implies that GR signalling encodes information not only through the presence or absence of ligand but also through its concentration (Reddy et al. 2009). Extending these observations to metabolic tissues, Murani and colleagues performed RNA-seq analysis in porcine liver following dexamethasone administration at different doses. They demonstrated that hepatic transcriptional responses depend on both ligand concentration and receptor sensitivity, with canonical GR targets such as *FKBP5*, *TSC22D3*, *DUSP1*, and *PCK1* showing robust regulation even at low ligand concentration (Murani et al. 2019). Importantly, their data indicate that dose variation can shift the balance between different functional gene programs.

Because GR's function as a transcription factor is directly measurable by bulk transcriptomics, this approach

provides a framework for understanding the consequences of genetic variation in receptor sensitivity or signalling context, linking genotype to pharmacologic response. Although this remains an emerging area, transcriptome-based phenotyping could ultimately help stratify patients by GC sensitivity, risk of side effects, or likelihood of resistance. Finally, by identifying pathway crosstalk between GR and inflammatory, developmental, or metabolic signalling modules, bulk RNA-seq informed combination therapy approaches. Combining JAK inhibitors with GC therapy in rheumatoid arthritis, for example, reduces long-term GC dose and adverse effects (Conigliaro et al. 2024).

At the same time, the interpretive scope of bulk transcriptomics must be considered carefully. By measuring averaged signals across heterogeneous cell populations, bulk methods cannot resolve cell-type-specific contributions to observed transcriptional changes. This limitation is particularly relevant in GC biology, where treatment can alter cell composition through apoptosis, cell trafficking, or differentiation. Moreover, conventional short-read RNA-seq provides limited resolution of transcript isoforms, alternative promoter usage, and post-transcriptional regulation. Changes in transcript abundance do not necessarily translate directly into corresponding changes in protein levels or cellular function; nevertheless, because GR acts primarily as a transcriptional regulator, RNA-seq provides one of the most direct genome-wide readouts of its regulatory activity.

4 Understanding GR function in complex tissues at single-cell resolution

The physiological, molecular, and bulk omics eras of glucocorticoid research have largely relied on measurements averaged across heterogeneous cell populations. Critically, these approaches did not allow interrogation of GC responses at cellular resolution within intact tissues. Single-cell technologies have fundamentally transformed this landscape. Building on earlier cytometric and imaging-based approaches, modern high-throughput single-cell genomics enables direct quantification of cellular heterogeneity in GR signalling. Conceptually, this shift was anticipated by early live-cell imaging studies. McNally and colleagues demonstrated that GR-chromatin interactions are highly dynamic (McNally et al. 2000), while subsequent studies by Voss et al. revealed a non-linear relationship between GR binding and transcriptional output, even in clonal populations (Voss et al. 2006). These findings established that GR occupancy alone is insufficient to predict transcriptional responses and pointed to stochastic, post-binding regulatory processes. Although not genome-wide, these studies foreshadowed a central principle of modern single-cell biology: GR-mediated transcription is probabilistic rather than deterministic.

A major technological advancement was reached with the advent of single-cell RNA sequencing (scRNA-seq) (Tang et al. 2009), followed by the development of high-throughput platforms capable of profiling thousands to millions of individual cells.

4.1 Heterogeneous glucocorticoid responses

Collectively, the expanding single-cell literature establishes heterogeneity as a core organising principle of GR biology. This heterogeneity manifests at multiple levels: (i) intra-population variability, where individual cells activate distinct subsets of GR target genes; (ii) lineage and cell-state specificity, reflecting differences between developmental stages, differentiation states, and activation contexts; (iii) spatial heterogeneity, arising from tissue compartmentalisation and local micro-environments; (iv) dynamic heterogeneity, as cells traverse transcriptional trajectories asynchronously; and (v) inter-individual variation, driven by genetic differences affecting receptor sensitivity and downstream signalling (Figure 3).

At the intra-population level, single-cell transcriptomics has revealed that GC responses are broadly distributed rather than uniform within homogeneous cell populations (Figure 3A). In a breast cancer model, scRNA-seq across a dexamethasone time course showed that nearly all cells respond to GC exposure, yet each cell activates only a subset (approximately 30 %) of the detectable GR target gene repertoire (Hoffman et al. 2020). Notably, more than 100 GR-regulated genes were identified that were not detectable in bulk RNA-seq, demonstrating that single-cell approaches uncover both variability and previously hidden regulatory outputs. Complementary studies in primary immune cells further showed that GCs modulate not only mean expression levels but also transcriptional variability. By introducing a response pseudo-time framework, Resztak and colleagues demonstrated that genetic variants influence not only response magnitude but also response dynamics and heterogeneity (Resztak et al. 2023). A central mechanistic insight emerging from these studies is that the conversion of GR activation into mRNA production occurs only in a subset of cells, reflecting probabilistic transcriptional activation.

Single-cell approaches are particularly powerful in resolving GC biology within complex tissues. During prenatal lung development, scRNA-seq revealed that GR signalling

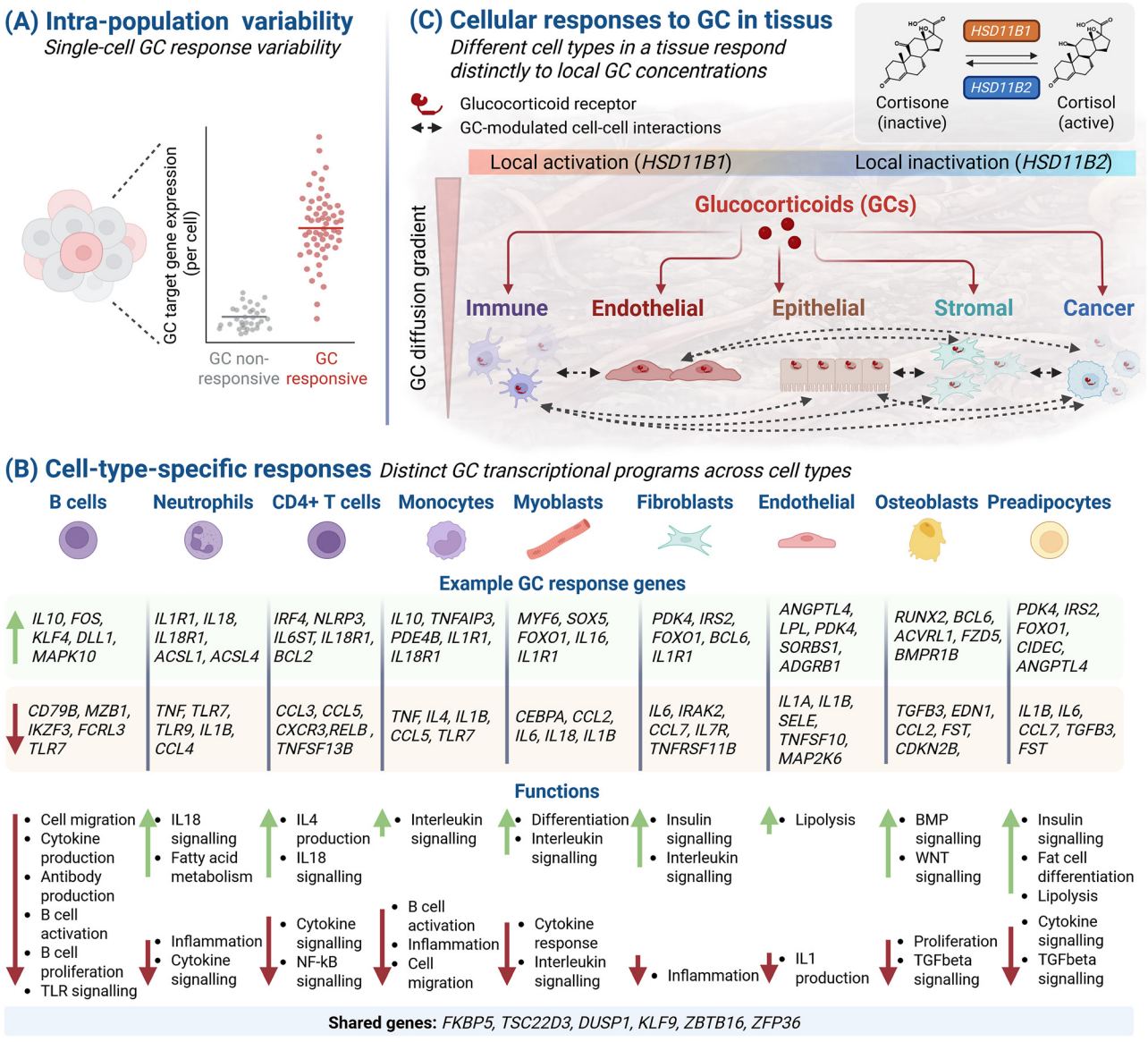

Figure 3: Insights into glucocorticoid (GC) biology from single-cell RNA sequencing. (A) Single-cell analyses reveal marked intra-population variability, with distinct GC-responsive and non-responsive cells within the same population. (B) A comparative study in human primary single cells revealed cell-type-specific GC responses, alongside a conserved core set of shared GR target genes (Franco et al. 2019). Green, activated by GCs; red, repressed by GCs (C) in tissues, GCs activate the glucocorticoid receptor (GR) across multiple cell types, thereby reshaping cell-intrinsic programs and intercellular communication within the tissue micro-environment. The enzymes 11β-hydroxysteroid dehydrogenase type 1 (*HSD11B1*) and type 2 (*HSD11B2*) regulate GC activity by interconverting inactive cortisone and active cortisol. The spatially restricted expression of these enzymes generates tissue-specific GC micro-environments that operate independently of systemic hormone levels. Abbreviations: BMP, bone morphogenetic protein; CD4+, cluster of differentiation 4-positive; IL1, interleukin-1; IL4, interleukin-4; IL18, interleukin-18; TGFbeta, transforming growth factor beta; TLR, Toll-like receptor; WNT, Wnt signalling pathway (Wingless/integrated).

in mesenchymal progenitors directs their differentiation into matrix fibroblasts and indirectly regulates epithelial maturation through pathways such as VEGF, JAK-STAT, and WNT (Bridges et al. 2020). In the central nervous system, single-cell analyses of the adult hippocampus demonstrated cell-type-specific expression of GR and mineralocorticoid receptor (MR), as well as divergent downstream transcriptional programs across neuronal and glial populations (Viho et al. 2022). Genetic perturbation studies further support this view: GR- and MR-deficient mouse models exhibit distinct stress-responsive signatures across glutamatergic neurons, oligodendrocytes, astrocytes, and endothelial cells (De Donno et al. 2026). Similarly, studies in human cortical organoids indicate that GCs act on highly

specific progenitor states rather than uniformly across neural lineages (Krontira et al. 2024). A systematic analysis of nine primary human cell types further mapped this diversity, revealing that cell-type specificity extends beyond target gene selection to include both the magnitude and direction of gene regulation (Franco et al. 2019) (Figure 3B).

Additionally, single-cell approaches enable the reconstruction of local glucocorticoid niches, where GR signalling is influenced not only by circulating hormone levels but also by local cellular ecosystems shaped by steroid metabolism and intercellular communication (Figure 3C). In the synovium, for instance, a homeostatic fibroblast state is maintained by adipocyte-derived cortisol and high expression of *hydroxysteroid 11-beta dehydrogenase 1* (*HSD11B1*), which converts inactive cortisone to active cortisol. These observations illustrate that local steroid activation supports tissue homeostasis and confers protection against inflammation (Faust et al. 2024). Similarly, locally synthesised GCs in the thymus act in a paracrine manner on specific thymocyte populations, highlighting highly selective intra-tissue signalling (Taves et al. 2019). In COVID-19, compartment-specific responses to dexamethasone have been characterised at cellular resolution (Neyton et al. 2024; Sinha et al. 2022), while studies in vascular disease have identified GC-associated transcriptional networks in endothelial cells alongside altered intercellular communication pathways, suggesting potential therapeutic entry points (Wang et al. 2025). In summary, single-cell approaches have redefined GC biology by establishing heterogeneity as a central organising principle.

Despite its transformative impact, scRNA-seq has important limitations. First, it requires tissue dissociation, thereby disrupting native spatial organisation and cell-cell interactions. This is particularly problematic in GC biology, where tissue context and local micro-environments strongly influence GR signalling. Computational approaches that infer spatial organisation from ligand-receptor interactions and transcriptional co-variation offer partial solutions but remain inferential and require experimental validation (Pong et al. 2024). Secondly, single-cell transcriptomics is limited in sensitivity and depth. Compared to bulk RNA-seq, fewer transcripts are detected per cell, and measurements are affected by technical noise, including dropout events and inflated zero counts. Additionally, long-read integration and non-coding RNA measurements are just emerging technologies. This constrains the analysis of lowly expressed genes, alternative isoforms, and regulatory effects, all of which are critical for understanding GR-mediated transcriptional mechanisms. Improving capture efficiency, quantification accuracy, and multimodal integration remains a key priority.

5 Glucocorticoid signalling in the spatial context

Single-cell analyses have established that GC responses are profoundly dependent on the local tissue environment, yet tissue dissociation disrupts the spatial organisation that underlies heterogeneity. Spatial methodologies overcome this limitation by preserving tissue architecture, thereby suggesting that GC biology is intrinsically spatial, an aspect that cannot be reliably inferred from bulk or dissociated single-cell datasets.

High-resolution mass spectrometry imaging (MSI) of the human adrenal gland, for example, has delineated distinct molecular zones of steroidogenesis, including a structured corticomedullary interface. Complementary spatial transcriptomic atlases have localised zona glomerulosa-, fasciculata-, and reticularis-like transcriptional programs *in situ*, while also identifying intermediate cortical states bridging the glomerulosa and fasciculata (Altieri et al. 2024; Sun et al. 2018). Furthermore, GC concentrations are not uniformly distributed across tissues. In cortisol-producing adrenal adenomas, MSI has linked the magnitude of cortisol excess to spatially distinct metabolic states, indicating intra-lesional heterogeneity in endocrine activity (Murakami et al. 2023). Beyond adrenal pathology, renal MSI demonstrates that different corticosteroids preferentially accumulate in specific kidney subregions (Stasinopoulos et al. 2026). In lower gastrointestinal acute graft-versus-host disease, spatial transcriptomics revealed that steroid-sensitive lesions are characterised by classical cell-mediated immune responses, differentiated epithelial compartments, and inflamed vasculature, whereas steroid-resistant lesions exhibit neutrophil-dominated inflammation, regenerative epithelial states, and angiogenic endothelial programs (Patel et al. 2023). Similarly, in giant cell arteritis, favourable GC responsiveness correlates with plasma cell-rich adventitial infiltrates, whereas resistance is associated with extracellular matrix remodelling and T-cell activation (Ansalone et al. 2026). These studies emphasise the importance of resolving therapeutic responses to GCs spatially.

Despite these advances, spatial approaches remain technically constrained. A central unresolved challenge is integrating ligand metabolism and distribution with GR transcriptional output at matched spatial coordinates. This is

particularly relevant for the enzymes 11β-hydroxysteroid dehydrogenase type 1 and type 2 (*HSD11B1* and *2*), which regulate local GC activation and inactivation but are rarely co-mapped with active cortisol concentrations and GR-dependent transcriptional readouts in intact tissue. More broadly, spatial transcriptomics is still limited by trade-offs between transcriptome coverage, cellular resolution, and sensitivity, and most datasets provide static snapshots rather than dynamic, time-resolved information. While MSI directly addresses ligand distribution, steroid imaging remains technically demanding due to the low abundance, poor ionisation efficiency, and structural similarity of steroid molecules (Cobice et al. 2013). Emerging spatial epigenomic approaches, including spatial assay for transposase-accessible chromatin (ATAC)-seq and spatial cleavage under targets and tagmentation (CUT&Tag), now offer the potential to connect local steroid exposure with chromatin state; however, these methods have not yet been widely applied in GC biology (Deng et al. 2022a, 2022b).

Taken together, these findings argue for a conceptual shift: GC signalling should not be viewed as a spatially uniform, circadian endocrine input, but rather as a tissue-organised axis in which local steroid availability, micro-anatomical context, and cell-state-specific competence jointly determine GR activity and GC responses, both physiologically and therapeutically.

6 Genome-wide chromatin profiling reframed GR biology

The preceding chapter established the breadth, heterogeneity, and context dependency of GC-responsive transcription. However, chromatin profiling hints at why those outputs are so variable. In the GR field, the decisive conceptual shift was the transition from inferring targets from expression changes to defining direct action by GR occupancy on chromatin and then interpreting that occupancy jointly with transcriptional output. Methodologically, this shift began with crosslinking-based chromatin immunoprecipitation followed by locus-specific PCR, promoter arrays, and then sequencing. Chromatin immunoprecipitation (ChIP)-seq removed the prior assumption of where relevant regulatory elements ought to lie, while cleavage under targets and release using nuclease (CUT&RUN) and cleavage under targets and tagmentation (CUT&Tag) later extended high-resolution mapping to far lower cell numbers and improved signal-to-noise, making primary tissues and clinically limited material more accessible to chromatin profiling (Kaya-Okur et al. 2019; Skene and Henikoff 2017).

Early studies established the logic that underpins GR cistromes. In A549 cells, ChIP-scanning across candidate regulatory neighbourhoods identified GR-binding regions for 8 of 11 GC-regulated genes, including both induced and repressed targets, thereby providing one of the earliest systematic demonstrations that direct GR action could be mapped on chromatin (Wang et al. 2004), while integrative analyses in mouse liver combined expression profiling with mapping GR binding events *in vivo* to identify more than 1,300 GC-responsive transcripts, more than 300 GR-bound promoters, and an intersecting set of 53 direct hepatic GR targets (Phuc Le et al. 2005). These studies were limited in genomic scope by later standards, but they introduced the key principle that neither binding nor expression alone is sufficient to define direct GR action.

6.1 Accessibility, lineage factors, and combinatorial control of glucocorticoid responses

Genome-wide accessibility profiling revealed that GR binding is strongly constrained by the pre-existing chromatin landscape (Figure 4). John and colleagues showed that up to 95 % of inducible GR occupancy occurs at sites that are already accessible before GC exposure (John et al. 2011), and time-resolved analyses showed that dexamethasone treatment drives GR rapidly onto nearly all pre-established enhancers while sparing many accessible regions that lack enhancer features (McDowell et al. 2018).

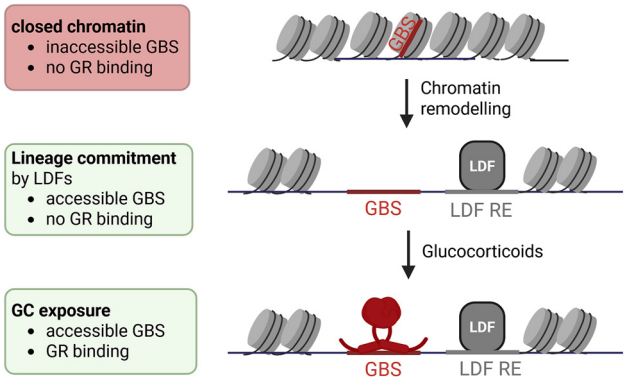

Figure 4: Chromatin accessibility and lineage define glucocorticoid receptor (GR) binding competence. In closed chromatin, glucocorticoid receptor binding sites (GBS) are embedded within nucleosome-dense regions, preventing GR access and binding. Upon lineage commitment, lineage-determining factors (LDF) engage their cognate motifs and locally remodel chromatin, resulting in nucleosome repositioning and increased accessibility. This process exposes previously occluded GBSs within accessible DNA. Subsequently, ligand-activated GR binds these accessible GBSs in nucleosome-depleted regions.

In the liver, the role of lineage-determining factors, which bookmark cell-type-specific enhancers, is particularly clear. Grøntved and colleagues showed that the CCAAT/enhancer-binding protein beta (C/EBPβ) maintains chromatin accessibility and facilitates GR recruitment to steroid-responsive elements *in vivo* (Grøntved et al. 2013). More recently, Hunter and colleagues demonstrated that hepatocyte nuclear factor 4 alpha (HNF4A) helps encode the tissue specificity of GC responses in the liver. Loss of HNF4A causes loss of GR binding at HNF4A-marked sites enriched for weak GR binding sites while allowing gain of GR occupancy at strong GR binding sites that are typical for non-liver tissues (Hunter et al. 2022). A related principle has emerged in prostate cancer: Sahu and colleagues first showed that forkhead box A1 (FOXA1) specifies a distinct subset of GR binding events (Sahu et al. 2013), and Helminen and colleagues extended that observation by showing that in enzalutamide-exposed prostate cancer cells, GR replaces androgen receptor predominantly at pre-accessible FOXA1-occupied chromatin. Counter-intuitively, FOXA1 can also restrain GR action by repressing its expression, illustrating that pioneer factors can both enable and limit GR function depending on the regulatory layer being examined (Helminen et al. 2024). Acute lymphoblastic leukaemia provides one of the clearest examples in which target selection can fail upstream of receptor binding itself. Jing and colleagues identified lymphocyte-specific open chromatin domains that pre-determine GC sensitivity, including enhancers close to the pro-apoptotic BIM gene where GR cooperates with CCCTC-binding factor (CTCF) in long-range chromatin looping. In GC-resistant leukaemic cells and in non-lymphoid contexts, these regions are hypermethylated and inaccessible (Jing et al. 2018). Follow-up work further implicated the Spi-1 proto-oncogene PU.1/SPI1 as a priming factor at lymphocyte-specific GC-response domains and super-enhancers, with PU.1/SPI1-mediated nucleosome eviction facilitating GR loading and apoptotic gene induction (Beck et al. 2024). In this framework, GC resistance can arise from failure to generate or preserve the permissive enhancer landscape on which GR depends.

In summary, this means that GR is recruited into a regulatory space that has already been delimited by lineage history and basal enhancer architecture.

6.2 A distal and context-specific GR cistrome

ChIP-chip provided two main conclusions that continue to frame the GR field. So and colleagues surveyed roughly 100-kb windows around 548 steroid-responsive candidate genes in A549 cells and identified 73 GR-binding regions. Of these 73 regions, 64 were associated with genes regulated by GR in A549 cells, and only a very small fraction mapped near genes known to be GC responsive in other cellular contexts. The regulatory geometry was predominantly distal: 63 % of the mapped GR binding sites lay more than 10 kb from the transcription start site, and the elements were distributed both upstream and downstream of target genes (So et al. 2007). Therefore, the enhancer-centred architecture of GR cistromes was identified before genome-wide sequencing became routine.

The transition to ChIP-seq marked a decisive conceptual shift by enabling unbiased, genome-wide discovery of GR-binding sites. In the study by Reddy and colleagues, 1 h of dexamethasone exposure yielded 4,392 GR-occupied genomic positions and 234 significantly responsive genes in A549 cells when binding was integrated with RNA-seq. The central insight was the distinct topological logic of activation and repression: 70 % of dexamethasone-responsive genes had no GR binding within 10 kb of the transcription start site. The nearest GR peak lay a median of 11 kb from induced genes but 146 kb from repressed genes, strongly implying that repression often cannot be explained by promoter-proximal receptor occupancy and must instead be interpreted in terms of distal regulation, indirect circuitry, or broader chromatin context (Reddy et al. 2009) (Figure 5A). A second correction followed quickly: the size of the GR cistrome is not a fixed property of the receptor but a context-dependent property of the target cell. In neuronal PC12 cells, Polman and colleagues identified 1,183 GR-binding sites, whereas A549 studies reported more than 10,000 occupied loci and lipopolysaccharide-activated macrophages harbour >10,000 GR binding events (Greulich et al. 2021b; Polman et al. 2012; Uhlenhaut et al. 2013; Vockley et al. 2016). This shifted the key question from where GR binds to under which circumstances it binds a given regulatory element. Just as importantly, binding proved to be permissive rather than deterministic. Vockley and colleagues functionally assayed nearly all GR-bound sites recovered from A549 cells and found that only 13 % displayed GC-induced reporter activity. In other words, receptor occupancy is necessary to define regulatory potential, but it is a poor proxy for transcriptional output when removed from its native chromatin, spatial, and coregulator environment (Vockley et al. 2016).

6.3 Sequence grammar and binding plasticity shape glucocorticoid receptor function

Genome-wide chromatin profiling has fundamentally reframed GR biology by revealing that its cistrome is not

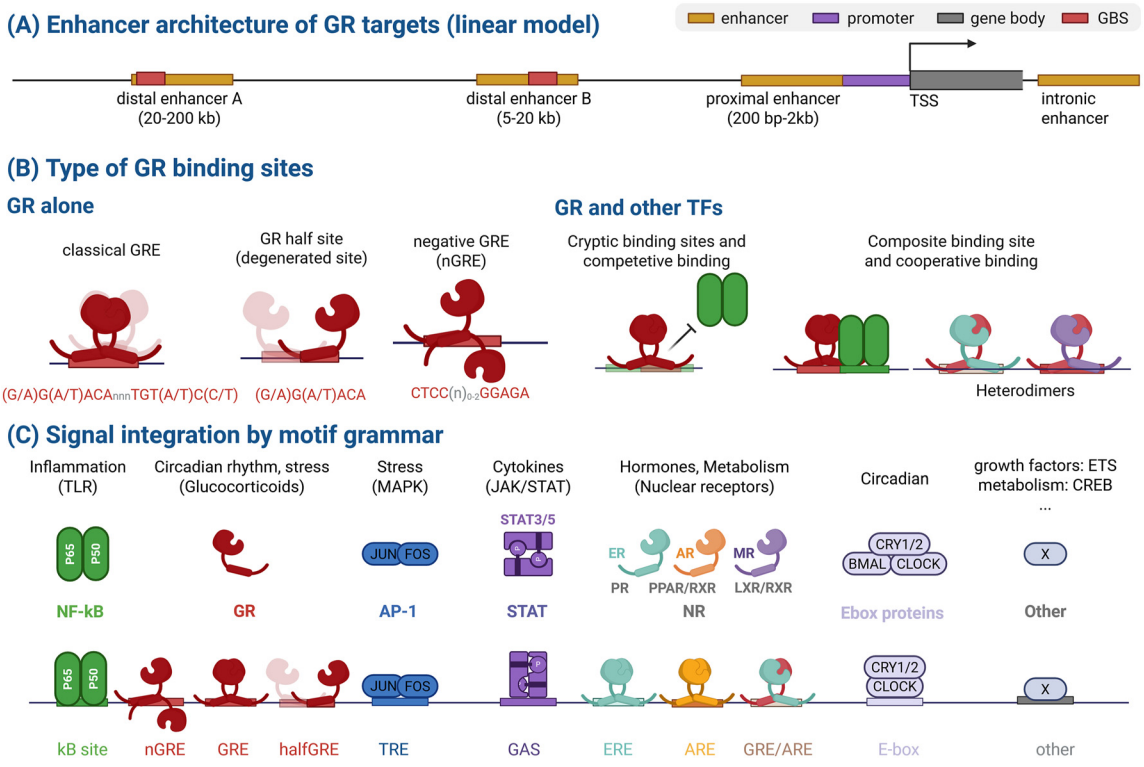

Figure 5: Enhancer grammar and motif architecture of glucocorticoid receptor (GR) binding. (A) Schematic linear model of a representative GR target locus illustrating enhancer architecture. (B) Classes of GR binding sites. GR can bind DNA directly via canonical glucocorticoid response elements (GREs), half-sites, or negative GREs (nGREs), or indirectly through interactions with other transcription factors (TFs) at cryptic or composite binding sites. (C) Signal integration by motif grammar. GR function is modulated through combinatorial interactions with signal-dependent TFs at adjacent or overlapping motifs, collectively shaping context-dependent transcriptional outputs. Abbreviations: AP-1, activator protein 1; AR, androgen receptor; ARE, androgen response element; BMAL1, brain and muscle ARNT-like 1; CLOCK, circadian locomotor output cycles kaput; CRY1/2, cryptochrome 1/2; E-box, enhancer box; ER, estrogen receptor; ERE, estrogen response element; GAS, gamma-activated sequence; GBS, GR binding site; GRE, glucocorticoid response element; LXR/RXR, liver X receptor/retinoid X receptor; MR, mineralocorticoid receptor; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; nGRE, negative GRE; NR, nuclear receptor; PPAR/RXR, peroxisome proliferator-activated receptor/retinoid X receptor; PR, progesterone receptor; STAT, signal transducer and activator of transcription; TRE, TPA response element.

dictated by a single consensus motif, but instead emerges from binding plasticity governed by a heterogeneous enhancer grammar. Early ChIP-seq studies already challenged the classical view of GR as a factor targeting a fixed set of glucocorticoid response elements (GREs). For example, in neuronal PC12 cells, only ~58 % of GR binding sites contained a recognisable GRE (Polman et al. 2012), indicating that canonical motifs account for a substantial but incomplete fraction of GR occupancy. This observation established a central question that subsequent high-resolution and integrative approaches have addressed: how can a single transcription factor engage such a diverse genomic landscape while maintaining regulatory specificity?

At the core of GR-DNA recognition lies the canonical GRE, composed of two inverted hexameric half-sites separated by a three-base pair spacer [(A/G)G(A/T)ACA nnn TGT(A/T)C(C/T); Figure 5B]. The term “glucocorticoid response element” (GRE) was first coined in 1983, when the Yamamoto lab identified a transcriptionally active GC-responsive DNA element in the mammary tumour virus promoter that lacked a transcriptional initiation sequence (Chandler et al. 1983). These sequences are recognised by GR’s DNA-binding domain (DBD), which contains two zinc fingers that mediate both sequence-specific DNA contacts and receptor dimerisation. Binding to palindromic GREs stabilises a head-to-head GR homodimer, the classical configuration associated with transcriptional activation (Dvorak and Pavek 2010; Luisi et al. 1991). This interaction is intrinsically cooperative: palindromic GREs exhibit higher affinity and more stable occupancy than isolated half-sites due to a defined DBD dimerisation interface (Lundbaeck et al. 1993, 1994). Beyond this minimal dimeric unit, accumulating evidence from live-cell imaging and biophysical analyses indicates that GR can form higher-order oligomers on chromatin, including tetramers, which further stabilise chromatin engagement and may enhance transcriptional output (Paakinaho et al. 2019; Presman et al. 2016).

A key conceptual advance has been the recognition that GREs are not merely passive docking sites but actively encode regulatory information. Subtle variations in half-site composition, spacer sequence, and local DNA shape can allosterically influence GR conformation, dimer geometry, and coregulator recruitment, thereby modulating transcriptional output (Meijsing et al. 2009). The principle of sequence-dependent allostery provides a mechanistic basis for how seemingly similar binding events can yield divergent regulatory outcomes. However, high-resolution chromatin profiling by ChIP-exo demonstrated that GR occupancy extends far beyond canonical GREs. At base-pair resolution, GR footprints can be reconciled with a broad spectrum of sequence configurations, including degenerate GRE variants, isolated half-sites, and composite elements in which GR recognition sequences are embedded within or adjacent to motifs for other transcription factors such as ETS and TEAD factors. In addition, a subset of binding events is best explained by indirect recruitment mechanisms, in which GR is stabilised through cooperative interactions with other DNA-bound factors such as FOX or STAT family members (Lim et al. 2015; Starick et al. 2015).

Importantly, binding to isolated half-sites generally exhibits reduced affinity and limited stability compared to canonical GREs and is unlikely to drive robust transcriptional regulation in isolation (Lundbaeck et al. 1993, 1994). Genome-wide analyses of monomeric GR variants have demonstrated that GR monomers fail to stably interact with DNA. These observations challenge earlier models that attributed specific regulatory functions, particularly anti-inflammatory repression, to GR monomers alone (Johnson et al. 2021).

The repertoire of functional GR DNA elements has been further expanded by the identification of negative GREs (nGREs), composite motifs, and cryptic binding sequences. Negative GREs, defined by inverted repeat sequences (CTCC(n)0-2GGAGA, Figure 5B), mediate direct transcriptional repression by agonist-bound GR (Surjit et al. 2011). Structural analyses revealed that GR binds these elements in a head-to-tail configuration on opposite DNA strands, thereby disrupting the canonical dimer interface and promoting negatively cooperative and asymmetric binding (Hudson et al. 2013). This provides a direct DNA-encoded mechanism for transcriptional repression that is mechanistically distinct from canonical GRE-driven activation. In parallel, several studies have demonstrated that GR can directly engage composite or overlapping regulatory elements (Figure 5B). For instance, GR can bind Activator Protein 1 (AP-1) response elements through a half-site embedded within the TPA (12-O-tetradecanoylphorbol-13-acetate) response elements (TRE) and regulate transcription in a DNA-binding-dependent manner (Weikum et al. 2017). A similar logic applies to Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) response elements, where GR can recognise cryptic sequences within κB motifs (Hudson et al. 2013). These findings undermine the traditional view that repression at such loci is mediated exclusively through protein-protein interactions and instead indicate that direct DNA binding contributes to many regulatory events previously classified as "tethering." Indeed, the concept of tethering has undergone substantial revision in light of genomic and biochemical data. While GR can modulate transcription through protein-protein interactions with other transcription factors, many loci previously interpreted as tethering sites show evidence of direct GR engagement on chromatin. Moreover, DNA-binding-deficient GR mutants exhibit markedly impaired transcriptional regulation and coregulator recruitment (Escoter-Torres et al. 2020), underscoring the central role of DNA binding even in contexts of apparent indirect regulation.

Importantly, motif grammar adds a layer of combinatorial control by signal-dependent transcription factors that contributes to context-specific GC responses (Figure 5C). AP-1 is the archetypal example: Biddie and colleagues showed that AP-1 maintains local baseline accessibility and is required for productive GR recruitment at co-occupied sites (Biddie et al. 2011). However, AP-1 can also oppose GR function. At certain locations, AP-1 occupancy has been found to be associated with reduced glucocorticoid-mediated repression or with competition for enhancer activity (De Bosscher et al. 2003; Jonat et al. 1990). Consequently, the relationship between AP-1 and GR is not uniformly antagonistic or cooperative, and might depend on dimer composition and motif spacing. For composite GREs with close spacing (14–18 base pairs) between the GRE and AP-1 site, GR can synergise with c-Jun alone to activate transcription but repress when c-Fos is present. Conversely, greater spacing (>26 base pairs) permits cooperative activation (Diamond et al. 1990; Pearce et al. 1998). NF-κB crosstalk is similarly more complex than simple mutual exclusion on DNA. Rao and colleagues showed that coactivation of GR and the NF-κB subunit P65 creates novel, mutually dependent binding sites and reshapes the regulated gene set (Rao et al. 2011), whereas Kadiyala and colleagues demonstrated that, in airway epithelium, GR can recruit P65 to dimeric GR-binding sites and cooperatively induce anti-inflammatory and injury-response genes such as SERPINA1 and FOXP4 (Kadiyala et al. 2016). However, recent work adds a caution: redistribution of the GR cistrome toward inflammatory loci under NF-κB control can correlate with reduced GC-mediated repression, emphasising that inflammatory recruitment of GR is not synonymous with productive

transrepression (Mostafa et al. 2025). However, these studies do not establish whether P65 directly drives altered GR function or whether the observed effects arise indirectly. Notably, inflammatory stimuli induce profound changes in cellular state, encompassing signalling network rewiring, metabolic shifts, and post-translational modification of GR (Pace et al. 2007; Selvarajoo 2013). Signal-dependent transcription factors implicated in context-specific GC responses extend beyond the canonical examples to include STAT5 in the liver (Quagliarini et al. 2019) and STAT3 in the pituitary tumour cell line AtT-20 (Langlais et al. 2012). Additional contributors comprise E-box-binding proteins and core components of the circadian clock, including BMAL1 (Cheon et al. 2013) and CLOCK (Nader et al. 2009), as well as E47 (Hemmer et al. 2019) and the cAMP-responsive element-binding protein (CREB), a key cofactor of GR activity during the hepatic fasting response (Goldstein et al. 2017). Many additional factors likely remain to be characterised, as suggested by the diversity of DNA motifs enriched at GR binding sites across cell types and tissues.

Chromatin profiling has likewise clarified GR cross-talk with other steroid receptors. In estrogen receptor (ER)-positive breast cancer models, GR reorganises chromatin accessibility and changes which genomic loci ER can use (Miranda et al. 2013), supporting combination therapies with glucocorticoids, which have been shown to mimic the beneficial effects of fasting on tumour growth in ER-positive breast cancer xenografts (Padrão et al. 2026). In prostate cancer, GR and the androgen receptor (AR) exploit partly overlapping but non-identical cistromes (Sahu et al. 2013), helping to explain how GR can bypass AR blockade in antiandrogen-resistant disease (Arora et al. 2013). The mineralocorticoid receptor (MR) is probably the clearest example of a true corticosteroid receptor partner for GR. MR and GR can form heterodimeric complexes with DNA-binding and transactivation properties distinct from either homodimer (Trapp et al. 1994). That model was strengthened by Ou et al., who showed MR-GR heterodimerisation at a negative GRE of the 5-hydroxytryptamine receptor 1A gene in SN-48 neuronal cells, directly linking receptor partnership to serotonergic gene repression (Ou et al. 2001). More recent *in vivo* work showed that acute stress enhances MR-GR heterodimerisation and DNA binding in the rat hippocampus, linking combinatorial MR/GR action to hippocampal health (Oakley et al. 2021). In PR+/GR+ breast cancer cells, Ogara et al. showed that the progesterone receptor (PR) and GR can be part of the same complex, based on co-immunoprecipitation and sequential ChIP, and that they co-occupy multiple genomic regions (Ogara et al. 2019). On the metabolic side, GR directly interacts with the Peroxisome Proliferator-Activated Receptor alpha (PPARα) in pull-down assays in the context of fasting (Ratman et al. 2016). The GR-PPARα interaction extends beyond the liver as PPARα and GR co-occupy chromatin in erythroid progenitors and synergise to promote progenitor self-renewal (Lee et al. 2015). Furthermore, the liver X receptor beta (LXRβ) is required for dexamethasone-induced GR recruitment to the PEPCK promoter in liver and primary mouse hepatocytes, and *Lxrβ-null* mice are protected from dexamethasone-induced hyperglycemia, hyperinsulinemia, and hepatic steatosis while remaining sensitive to glucocorticoid immunosuppression (Patel et al. 2011). Nader et al. then showed that activated LXRs can gene-specifically attenuate GR transcriptional activity, in part because endogenous LXRα can bind GRE-containing regions and interfere with GR binding (Nader et al. 2009).

Collectively, GR motifs, lineage-determining and signal-dependent factors constitute an enhancer grammar that integrates context-dependent GR binding and shapes downstream transcriptional outcomes (Figure 5C).

6.4 Enhancer state, coregulators, and chromosome topology

GR binding typically occurs within nucleosome-depleted regions of accessible chromatin; however, adjacent nucleosomes carry characteristic post-translational histone modifications that encode regulatory potential. Enhancers are distinguished from promoters by low or absent histone H3 lysine 4 trimethylation (H3K4me3) and enrichment of H3K4 monomethylation (H3K4me1), deposited by the histone lysine methyltransferases KMT2C and KMT2D. Active enhancers are further marked by H3K27 acetylation (H3K27ac), catalysed by the acetyltransferases P300 and CBP, whereas poised enhancers retain H3K4me1 but exhibit low H3K27ac (Allis and Jenuwein 2016). GR occupies both active and poised enhancers, as well as genomic regions lacking these canonical marks (Greulich et al. 2021b; McDowell et al. 2018; Quagliarini et al. 2019; Stavreva et al. 2026).

Proteomic approaches, particularly proximity labelling and chromatin-immunoprecipitation coupled to mass spectrometry, have identified an extensive network of GR-associated proteins, including more than 30 ligand-dependent interactors encompassing coactivators, corepressors, Mediator components, chromatin remodellers, and RNA-processing factors (Dendoncker et al. 2019; Greulich et al. 2021b; Paakinaho et al. 2019; Quagliarini et al. 2019; Van Moortel et al. 2024). These observations reinforce that GR functions within a highly interconnected regulatory environment, where transcriptional outcomes are modulated by the combinatorial assembly of coregulators with scaffolding

and enzymatic functions. Systematic chromatin-level mapping of all these interactions remains experimentally challenging, and functional dissection is complicated by their pleiotropic roles across transcriptional processes. Accordingly, Figure 6A presents a conceptual model situating GR-associated coregulators within the chromatin context. Importantly, the traditional binary classification of coregulators into coactivators and corepressors is increasingly insufficient. Functional roles are often context-dependent and influenced by chromatin state and post-translational modifications. For example, the histone deacetylase HDAC1 can mediate both transcriptional activation and repression by targeting non-histone substrates, including other coregulators and transcription factors (Peserico and Simone 2011; Qiu et al. 2006). Similarly, the canonical coactivator NCOA2 (GRIP1) can switch between activating and repressive functions depending on CDK9-dependent phosphorylation (Rogatsky et al. 2002; Rollins et al. 2017).

At active enhancers, GR cooperates with coactivators and chromatin modifiers that promote transcriptional competence. Histone lysine acetyltransferases such as P300/CBP deposit H3K27ac, while KAT2A/KAT2B catalyse histone H3 lysine 9 and 14 (H3K9/14) acetylation and TIP60 targets histone H4 and histone H2A. Histone methyltransferases, including KMT2C/D and SETD1A, maintain H3K4 mono- and dimethylation at enhancers and H3K4 trimethylation at promoters (Wang and Helin 2025; Wapenaar and Dekker 2016). Coactivator complexes such as NCOA1–3 and PGC-1α stabilise GR-dependent transcription, whereas chromatin readers and transcriptional mediators, including BRD4, BRD9, and RNA polymerase II, facilitate transcription initiation and elongation. Collectively, these factors sustain an open chromatin configuration characterised by high histone acetylation and H3K4 methylation, enabling productive transcription. Conversely, GR can recruit corepressor complexes that establish transcriptionally repressive chromatin environments. Histone deacetylases (HDAC1/2/3) remove acetyl groups, while histone lysine methyltransferases such as EZH2 (catalysing H3K27me3) and EHMT2/G9a (H3K9me) promote repressive chromatin states. These modifications can be further stabilised by DNA methyltransferases (DNMT1, DNMT3A, DNMT3B), contributing to long-term silencing. Corepressor scaffolds, including NCOR1/2, GPS2, TBL1/TBLR1, and LCOR, organise these enzymatic activities,

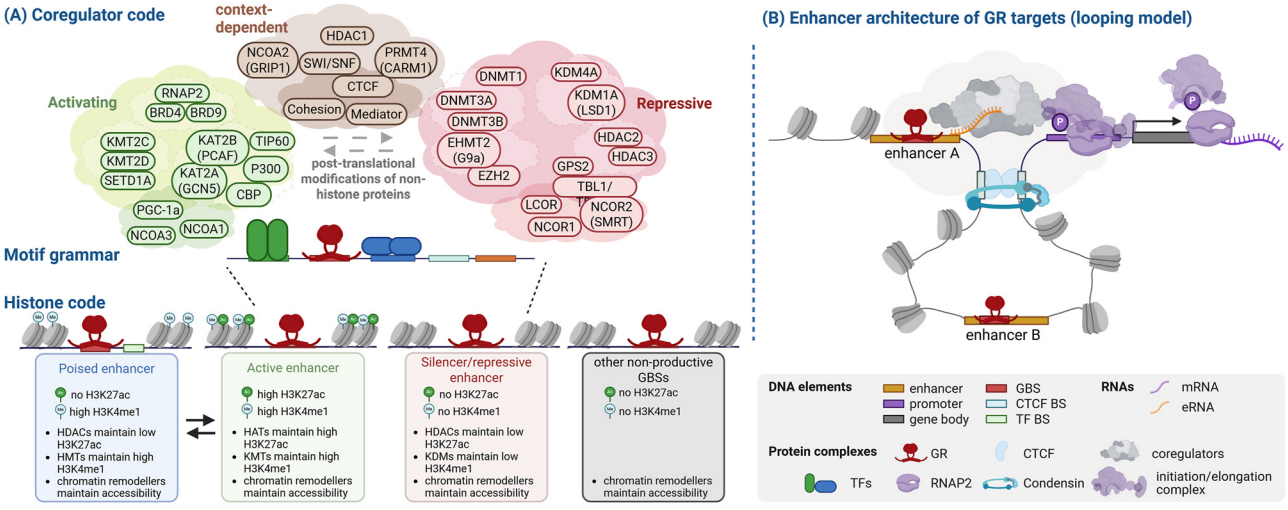

Figure 6: Coregulator code and enhancer architecture underlying glucocorticoid receptor (GR) function. (A) GR-dependent transcription is shaped by a context-dependent coregulator code that integrates activating, repressive, and architectural factors while interpreting local DNA sequence features (motif grammar) and chromatin state (histone code). Activating coregulators promote chromatin accessibility and enhancer activity, whereas repressive complexes drive deacetylation, repressive methylation states (H3K27, H3K9) or removal of activating methylation marks (H3K4), and transcriptional silencing. Context-dependent regulators further modulate chromatin topology and protein function, including post-translational modification of non-histone proteins. These combinatorial inputs define distinct regulatory states: poised enhancers (high H3K4me1, low H3K27ac), active enhancers (high H3K4me1 and H3K27ac), repressive enhancers/silencers (low H3K4me1 and H3K27ac), and non-productive GR binding sites lacking canonical enhancer features. (B) GR-bound enhancers regulate gene expression through three-dimensional chromatin looping. Distal glucocorticoid binding sites (GBSs) are brought into proximity with target promoters via CTCF- and Cohesin-mediated interactions, enabling assembly of transcriptional complexes, including mediator and RNA polymerase II (RNAP2). Active enhancers produce enhancer RNAs (eRNAs), which may contribute to stabilisation of enhancer–promoter contacts, while promoters generate messenger RNAs (mRNAs). Together, coregulator composition and higher-order genome organisation determine context-specific GR transcriptional outputs. Abbreviations: CTCF, CCCTC-binding factor; H3K4me1, histone H3 lysine 4 monomethylation; H3K27ac, histone H3 lysine 27 acetylation, H3K9, histone H3 lysine 9; H3K27, histone H3 lysine 27; HAT, histone acetyl transferase; HDAC, histone deacetylase; KDM, histone demethylase; KMT, histone methyltransferase; TF, transcription factor.

resulting in chromatin environments with low acetylation and reduced H3K4 methylation, characteristic of silencers or repressed enhancers. A third class of factors integrates chromatin architecture and signalling inputs. CTCF and Cohesin shape higher-order genome organisation and facilitate enhancer-promoter communication, while Mediator serves as a central hub integrating transcription factor inputs with the transcriptional machinery. SWI/SNF chromatin remodelling complexes maintain accessibility, and enzymes such as CARM1/PRMT4 and HDACs modify non-histone proteins, including GR itself (Oakley and Cidlowski 2013). These regulators modulate GR activity in a context-dependent manner defined by motif composition, chromatin state, and signalling environment (Figure 6A).

Functional studies underscore the importance of this dynamic coregulatory landscape. Inflammatory signalling, for instance, can impair GR-dependent transcription without affecting receptor nuclear translocation, dimerisation, or DNA binding, instead reshaping the nuclear cofactor repertoire, most notably by reducing GR-P300 interactions (Dendoncker et al. 2019). In primary macrophages, GR recruits SETD1A to specific enhancer subsets, leading to locus-specific changes in H3K4 dimethylation and transcriptional output (Greulich et al. 2021b). Ligand-specific proteomics further demonstrated that selective GR modulators and antagonists diminish interactions with P300/CBP and Mediator compared to dexamethasone (Van Moortel et al. 2024), while post-translational modification of GR, such as SUMOylation, alters its chromatin-associated interactome and enhancer accessibility (Paakinaho et al. 2019).

In conclusion, productive gene regulation by GR depends on which effector complexes the receptor can assemble at each gene locus (Figure 6A).

Chromosome conformation capture assays added a spatial dimension to this model. D'Ippolito and colleagues showed that GR binding generally acts through chromatin interactions that already exist before hormone exposure rather than creating target-gene loops *de novo*, and that both induced and repressed genes increase interactions with distal GR-binding sites (D'Ippolito et al. 2018). In a complementary study, most activated GR joins pre-existing enhancer hubs, although a subset of loci acquires an active enhancer state and additional long-range interactions after ligand exposure (Kuznetsova et al. 2015). More recent work adds chromatin content to the older "squelching" ideas. Portuguez and colleagues showed that P300 can be spatially sequestered away from non-GR loci (Portuguez et al. 2022), whereas Tav and colleagues demonstrated regionalised gain or loss of MED1 and BRD4 within topologically associating domains (Tav et al. 2023), creating transcriptionally favourable or unfavourable nuclear neighbourhoods. However, studies are still technically limited by a lack of measurements of locus-specific protein abundance. Finally, studies on nuclear compartmentalisation suggest that DNA element class itself can influence coregulator partitioning into GR hubs or condensates (Frank et al. 2021), with more recent imaging technologies linking subsets of GR condensates to both initiating and elongating RNA polymerase II compartments (Benítez et al. 2025). The unified implication is that GR output is specified locally by motif grammar and histone code dictating downstream coregulator recruitment, but also by whether a bound enhancer resides in a coregulator-rich or -poor three-dimensional micro-environment (Figure 6B).

6.5 Translational extension and methodological limits

Updated ChIP-seq and CUT&RUN protocols achieve usable GR binding profiles from very small inputs, and CUT&Tag similarly enables efficient high-resolution mapping from small samples and even single cells. This has made it feasible to apply GR-centered chromatin profiling to primary tissues, patient-derived material, and inter-individual comparisons. In human disease contexts, chromatin accessibility studies in mouse models of diabetic kidney disease have implicated reduced accessibility of GR-binding sites in the proximal tubule (Wilson et al. 2022), while CUT&RUN in human lung tumours has shown that environmental stress exposure can be associated with altered GR recruitment (Heath et al. 2024). Individual-specific functional epigenomics in stem-cell-derived adipocytes and hepatocytes further suggests that donor-specific chromatin architecture can shape GR-dependent metabolic responses, pointing toward a genuinely precision-epigenomic view of glucocorticoid action (Hu et al. 2021).

At the same time, ChIP-seq, CUT&RUN, CUT&Tag, accessibility maps, and chromosome conformation capture assays are still fundamentally snapshot measurements. They do not by themselves reveal binding order, residence time, kinetic coupling to transcription, or whether a given chromatin change is causal or consequential. GR biology repeatedly exposes these limitations: many robust peaks are non-productive, repression often lacks nearby GR motifs, and even strong occupancy can be uncoupled from transcription if enhancer state, coregulator availability, or 3D proximity are unfavourable. For that reason, GR cistromes are most informative when integrated with RNA-seq, nascent transcription, histone modifications, or proteomics rather than treated as self-sufficient mechanistic evidence. The major contribution of chromatin profiling to GR biology is therefore not a static list of peaks. It is the demonstration

that GC responses are encoded in a layered regulatory architecture in which lineage factors define accessible space, motif grammar and partner factors shape receptor engagement, coregulators execute local biochemical programs, and chromosome topology constrains which bound elements can productively communicate with genes.

Taken together, GR is a transcriptional integrator of environmental signals that translates enhancer grammar, together with the local chromatin environment and architecture, into transcriptional outputs.

Based on distinct GR binding modalities, pharmacological efforts have been made to develop selective glucocorticoid receptor modulators to improve beneficial anti-inflammatory effects and avoid adverse effects associated with long-term glucocorticoid therapy. However, only a few of those have entered clinical trials, potentially due to the complex GR biology. For example, a study from the Reddy lab compares 10 selective GR ligands (agonists, partial agonists, and antagonists) with dexamethasone using bulk mRNA-seq followed by self-transcribing active regulatory region sequencing (STARR-seq), in A549 cells. They showed that transcriptional responses were highly correlated with dexamethasone, arguing that many "selective" ligands primarily attenuate response magnitude rather than generate orthogonal gene programs. Gene expression responses correlated well with gene regulatory activity, with agonists activating regulatory elements and antagonists showing differential activity at fewer regulatory elements (Giroux et al. 2025). Even as this study investigates these compounds outside chromatin and signalling contexts, it suggests that current strategies for GR modulators should focus on GR's context-dependent interactome (e.g., cotranscription factors or coregulators) to preferentially elicit one cellular output over another, in addition to cell-type-specific targeting.

7 Where does the future go?

7.1 Context-matched multi-omics

The next phase of GR research should move beyond cataloguing GR occupancy and toward defining causal, context-specific rules that explain why the same receptor activates, represses, or leaves nearby genes unchanged in different settings. Achieving that goal will require matched multi-omics designs in which one variable at a time is perturbed systematically (e.g. cell type, ligand, dose, inflammatory input, metabolic state, or time). Therefore, future studies should prioritise internally matched datasets over retrospective integration of heterogeneous public data.

In practice, the most informative experimental workflow will align receptor occupancy maps with motif analysis of GREs, local sequence-flanking effects and DNA shape, chromatin accessibility, enhancer-state measurements, followed by both steady-state and nascent transcription readouts. For the transcript and contact layers, RNA-seq should be complemented by global run-on sequencing (GRO-seq), precision run-on sequencing (PRO-seq), or capped small RNA sequencing (csRNA-seq), together with 3D chromatin assays such as high-throughput chromosome conformation capture (Hi-C), proximity ligation-assisted chromatin immunoprecipitation sequencing (PLAC-seq), or Capture-C. Functional prioritisation should then proceed to massively parallel reporter assays (MPRAs), STARR-seq and targeted synthetic enhancer libraries, as these are the assays most capable of converting descriptive chromatin maps into testable rules of enhancer grammar.

7.2 From prediction to endogenous causality

Predicted grammar rules should be tested experimentally in two stages. First, MPRAs or STARR-seq can compare wild-type and motif-edited sequences while varying GRE copy number, spacing, orientation, flanking sequence, and composite motif content. Second, the strongest candidates should be re-tested at endogenous loci using clustered regularly interspaced short palindromic repeats interference (CRISPRi), CRISPR activation (CRISPRa), focused deletions, or precise base and prime editing to establish both enhancer necessity and the causal nucleotides that tune GC responsiveness. This endogenous step is essential because perturbation databases and recent screens make clear that many genes are regulated by multiple enhancers, with each candidate enhancer having small, conditional, or no detectable effects when challenged in their native genomic environment (Zhou et al. 2024).

7.3 Biological layers that remain unresolved

Even with richer datasets, several methodological limitations remain. Occupancy maps identify where proteins are found, not whether those binding events are sufficient for activation or repression; accessibility assays identify candidate regulatory DNA, not activity; motif enrichment and genomic foot-printing remain probabilistic and can fail for rapidly exchanging transcription factors; and physical chromatin contacts do not, on their own, establish causal enhancer-promoter communication. Steady-state transcriptomics adds another layer of ambiguity because it cannot readily separate direct from indirect transcriptional

effects unless early time points or nascent RNA measurements support it. For GR, this means that future studies should explicitly distinguish mapping assays from causal assays rather than treating them as interchangeable.

A second unresolved layer concerns receptor heterogeneity itself. Multiple GR isoforms arise through alternative splicing and alternative translation initiation (Lu and Cidlowski 2005), and their functions are further diversified by phosphorylation, acetylation, ubiquitylation, and SUMOylation, with documented consequences for transcriptional specificity and GC sensitivity or resistance (Mao et al. 2023; Oakley et al. 2021). Yet most genomic studies still treat GR as a single molecular species. A major opportunity, therefore, is to generate isoform- and proteoform-resolved maps of chromatin occupancy, coregulator recruitment, and transcriptional output across physiological and disease contexts, because this is one of the clearest ways to connect molecular diversity at the receptor level to phenotypic diversity in target-gene control.

Single-cell and spatial approaches should also become routine in GR studies. scRNA-seq has already revealed substantial cell-to-cell heterogeneity in GC responses, and single-cell and spatial omics are rapidly expanding across transcriptomic and chromatin modalities. In parallel, chromatin proteomics and emerging single-cell or spatial metabolomics can begin to resolve how local chromatin composition, coregulator assembly, and metabolite availability shape GR action.

7.4 Time as a regulatory variable

Finally, time should be treated as an organising variable rather than a parameter. Endogenous GCs are secreted in circadian and ultradian pulses, and GR-chromatin interactions are highly dynamic, with residence times ranging from seconds to minutes (McNally et al. 2000); in pulsatile stimulation settings, GR occupancy itself can track hormone pulses. Future experimental designs should therefore combine rapid time courses, pulse-chase or pulsatile hormone delivery, live-cell imaging, and matched multi-omics readouts to determine how signal amplitude, duration, and frequency influence enhancer engagement, transcriptional bursting, and gene-network stabilisation. In that framework, time is likely to emerge as an additional layer of regulatory encoding, spanning seconds-scale receptor exchange, minutes-scale signal integration, and hours-to-days-scale physiological synchronisation.

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List of abbreviations

3D	3-dimension
ANGPTL4	angiopoietin-like protein 4
AP-1	activator protein 1
AR	androgen receptor
ARE	androgen response element
ATAC-seq	assay for transposase-accessible chromatin sequencing
BCL2L11	Bcl-2-like protein 11
BIM	Bcl-2 interacting mediator of cell death
BMAL1	brain and muscle ARNT-like 1
BMP	bone morphogenetic protein
BRD	bromodomain-containing protein
CBP	CREB-binding protein
CD	cluster of differentiation
C/EBPβ	CCAAT/enhancer-binding protein beta
ChIP-exo	chromatin immunoprecipitation coupled with exonuclease digestion
ChIP-seq	chromatin immunoprecipitation sequencing
CLOCK	circadian locomotor output cycles kaput
CRISPLD2	cysteine-rich secretory protein LCCL domain-containing 2
CRISPR	clustered regularly interspaced short palindromic repeats
CRISPRa	CRISPR activation
CRISPRi	CRISPR interference
CRY1/2	cryptochrome 1/2
CTCF	CCCTC-binding factor
CUT & RUN	cleavage under targets and release using nuclease
CUT & Tag	Cleavage under targets and tagmentation
csRNA-seq	capped small RNA sequencing
DBD	DNA-binding domain
DNA	deoxyribonucleic acid
DNMT	DNA methyltransferase
DUSP1	dual specificity protein phosphatase 1
EC50	half maximal effective concentration
EHMT	euchromatic histone lysine methyltransferase
eRNA	enhancer RNA
ER	estrogen receptor
ERE	estrogen response element
ETS	E26 transformation-specific
EZH	enhancer of zeste homolog

IL	interleukin
FKBP5	FK506 binding protein 5
FOX	forkhead box
FOXA1	forkhead box A1
G6PC	glucose-6-phosphatase, catalytic subunit
GAS	gamma-interferon activated sequence
GC	glucocorticoid
GBS	glucocorticoid receptor binding site
GILZ	glucocorticoid-induced leucine zipper
GPS2	G protein pathway suppressor 2
GRE	glucocorticoid response element
GR	glucocorticoid receptor
GRIP1	glucocorticoid receptor-interacting protein 1
GRO-seq	global run-on sequencing
H3K27ac	histone H3 lysine 27 acetylation
H3K27me3	histone H3 lysine 27 trimethylation
H3K4me1	histone H3 lysine 4 monomethylation
H3K4me3	histone H3 lysine 4 trimethylation
H3K9	histone H3 lysine 9
HAT	histone acetyltransferase
HDAC	histone deacetylase
Hi-C	high-throughput chromosome conformation capture
HMT	histone methyltransferase
HNF4A	hepatocyte nuclear factor 4 alpha
HSD11B1/2	11β-hydroxysteroid dehydrogenase type 1 and type 2
JAK	Janus-activated kinase
LDF	lineage-determining factor
lncRNA	long non-coding RNA
KAT	histone acetyltransferase
KDM	histone lysine demethylase
KMT	histone lysine methyltransferase
LCOR	ligand-dependent nuclear receptor corepressor
LXR	liver X receptor
mediator	mediator complex
miRNA	microRNA
MPRA	massively parallel reporter assay
mRNA	messenger RNA
MR	mineralocorticoid receptor
MSI	mass spectrometry imaging
ncRNA	non-coding RNA
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
nGRE	negative glucocorticoid response element
NCOA	nuclear receptor coactivator
NCOR	nuclear receptor corepressor
NR	nuclear receptor
Omics	high-throughput molecular profiling technologies
P300	histone acetyltransferase p300
PCK1	phosphoenolpyruvate carboxykinase 1
PER1	period 1
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1 alpha
PLAC-seq	proximity ligation-assisted ChIP sequencing
PPAR	peroxisome proliferator-activated receptor
PR	progesterone receptor
PRO-seq	precision run-on sequencing
RNAP2	RNA polymerase II
RNA-seq	ribonucleic acid sequencing
PRMT	protein arginine methyltransferase
RXR	retinoid X receptor
scRNA-seq	single-cell RNA sequencing
SETD1A	SET domain containing 1A
SMRT	silencing mediator of retinoid and thyroid receptors
SPI1	Spi-1 proto-oncogene
STARR-seq	self-transcribing active regulatory region sequencing
STAT	signal transducer and activator of transcription
SUMO	small ubiquitin-like MOdifier
SWI/SNF	switch/sucrose non-fermentable chromatin remodelling complex
TBL/TBLR	transducin beta-like/transducin beta-like related
TEAD	transcriptional enhanced associate domain
TF	transcription factor
TGF	transforming growth factor
TIP60	tat-interactive protein, 60
TLR	toll-like receptor
TPA	12-O-tetradecanoylphorbol-13-acetate
TRE	TPA response element
TSC22D3	TSC22 domain family protein 3
VEGF	vascular endothelial growth factor
WNT	Wingless and Int

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