

REVIEW ARTICLE

Programmable organoids and the emergence of circuit-inspired genetic and epigenetic control in human development

Moawiah M. Naffaa*

Independent Researcher, Mountain View, California, United States of America

*Corresponding author: Moawiah M. Naffaa (Moawiah.Naffaa@proton.me)

Citation: Naffaa MM. Programmable organoids and the emergence of circuit-inspired genetic and epigenetic control in human development. *Organoid Res.* 2026;2(1):025490038. doi: 10.36922/OR025490038

Received: December 6, 2025

Revised: February 26, 2026

Accepted: February 26, 2026

Published online: March 11, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, which provided that the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract

Programmable organoids refer to organoid systems in which developmental trajectories are experimentally influenced by engineered genetic, epigenetic, material, or computational interventions. At present, these systems are dominated by externally imposed and feedback-limited control strategies rather than internally implemented or autonomous developmental architectures. Traditional organoids rely on spontaneous self-organization, but this intrinsic variability limits reproducibility, causal inference, and translational relevance. Recent advances in Clustered Regularly Interspaced Short Palindromic Repeats-based transcriptional and epigenetic engineering, optogenetic and chemogenetic patterning technologies, reaction-diffusion design, and real-time biosensing now allow developmental trajectories to be biased, stabilized, and interrogated with increasing experimental precision, without enabling fully autonomous or self-correcting control. This review organizes these approaches into a tiered, evidence-based framework, spanning genetic circuit construction, epigenetic modulation, synthetic morphogenesis, multi-scale sensing, adaptive regulation, and artificial intelligence-guided design, explicitly distinguishing experimentally validated strategies from fragile, context-dependent implementations and conceptual architectures. Applications across human developmental biology, disease modeling, and regenerative medicine are highlighted, alongside the technical, biosafety, and ethical considerations associated with exploring increasingly structured, yet predominantly externally guided, approaches to developmental regulation. Collectively, programmable organoids are presented here not as autonomous developmental systems, but as experimentally steerable platforms whose capabilities and limitations are jointly shaped by biological variability, maturation constraints, and the need for external guidance.

Keywords: Programmable organoids; Synthetic gene circuits; Epigenetic engineering; Morphogenesis; Synthetic organizers; Cybergenetic and feedback-regulated developmental control; Cybergenetic systems; Developmental synthetic biology

1. Introduction

Organoids have transformed the study of human biology by providing self-organizing, three-dimensional (3D) systems that recapitulate key features of embryonic and tissue development. Built from pluripotent or adult stem

cells, these structures recreate the intrinsic patterning logic of morphogenesis, including symmetry breaking, lineage diversification, and the emergence of spatially ordered architectures.^{1,2} Their rise has made it possible to observe human developmental processes at a level of physiological relevance and ethical accessibility that traditional *in vivo*

models cannot offer. Yet despite these advances, organoid development remains largely governed by spontaneous self-organization.³ While powerful, this uncontrolled process often leads to heterogeneity, batch-to-batch variability, incomplete maturation, loss of axial fidelity, and limited reproducibility across laboratories.⁴ These constraints restrict the ability to interrogate causality in developmental pathways and limit the translational potential of organoid platforms.

An emerging direction involves shifting from passive observation to externally guided modulation of organoid development. Achieving this requires tools that can tune lineage decisions, sculpt spatial domains, regulate morphogen exposure, and shape emergent architectures with precision. The idea of programmable development captures this transition. Rather than allowing organoids to follow intrinsic yet unpredictable trajectories, researchers are beginning to apply externally guided interventions to genetic and epigenetic regulatory layers. Through the integration of synthetic biology, CRISPR-based logic systems, epigenomic engineering, optogenetic control, and inducible signaling modules, organoids are evolving into platforms where developmental outcomes can be experimentally biased or transiently stabilized under externally imposed interventions.^{5,6}

Central to this transformation is the convergence of several previously distinct technological streams. Synthetic gene circuits provide the computational logic needed to control cell behavior, enable conditional decision-making, feedback-limited regulation, and multicellular coordination. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) interference and activation expand this logic space by allowing programmable modulation of specific transcriptional programs. Epigenetic engineering introduces a heritable layer of control, enabling modulation and memory mechanisms that record signals, stabilize fates, or impose synthetic competence windows. Optogenetic and chemogenetic tools enable spatiotemporal precision, turning organoids into systems where morphogenetic cues can be triggered at defined times and locations.^{5,7-10} Together, these technologies form a growing toolkit for experimentally influencing developmental processes in ways that were previously inaccessible, although most current implementations remain proof-of-concept or technically constrained.

1.1. Definition and scope of “programmable organoids”

In this review, the term programmable organoids is used in a deliberately constrained and non-autonomous sense. It does not imply self-sustaining genetic circuitry, intrinsic decision-making, or fully closed-loop developmental control. Instead, programmable organoids refer to systems

in which developmental trajectories are experimentally steered, most often through externally imposed interventions such as inducible gene expression, optogenetic or chemogenetic modulation, controlled morphogen delivery, or computer-in-the-loop (cybergenetic) actuation.

Crucially, no current organoid system demonstrates fully reversible, causal epigenetic programming of development, nor does it implement intrinsically autonomous genetic or epigenetic modulation modules comparable to engineered control systems in unicellular organisms. Existing epigenetic engineering approaches in organoids primarily function by biasing developmental trajectories, stabilizing lineage decisions, recording prior signaling history, or correlating chromatin state with fate, rather than by implementing dynamic, programmable epigenetic controllers. Rather, they rely on externally scheduled or externally interpreted inputs, with limited internal feedback and no persistent, self-correcting regulatory autonomy. Throughout this manuscript, claims of programmability are therefore interpreted as degrees of experimental steerability, not as evidence of independent developmental computation or self-regulation.

Within this emerging landscape, programmable organoids should be understood as occupying a continuum of externally guided to partially internalized feedback control, rather than constituting a single technological class. Most current systems operate at the level of stimulus scheduling and conditional perturbation, with only limited demonstrations of internal feedback or memory, and no established examples of fully internally implemented developmental circuitry. Throughout this review, we classify these programmable approaches using a four-tier framework (Figure 1) that distinguishes externally imposed control from progressively more internally implemented regulatory architectures. Importantly, programmable organoids exist along a continuum of regulatory sophistication. In many current platforms, control is externally imposed through inducible transcription factors, optogenetic or chemogenetic modulation, or CRISPR-based perturbations that guide development without autonomous circuit behavior. Only a subset of emerging systems incorporate feedback, logic integration, memory, or state-dependent regulation that more closely resembles true genetic or epigenetic circuitry. At present, externally imposed control remains the dominant and most reliable mode of programmability, while internally implemented circuit-based regulation remains experimental, fragile, and largely conceptual in organoid systems.

Programmable organoids are depicted as an input-control-output system in which regulatory inputs—genetic, epigenetic, material, and cybergenetic cues—are processed by layered control architectures, including genetic circuit regulation, epigenetic memory systems,

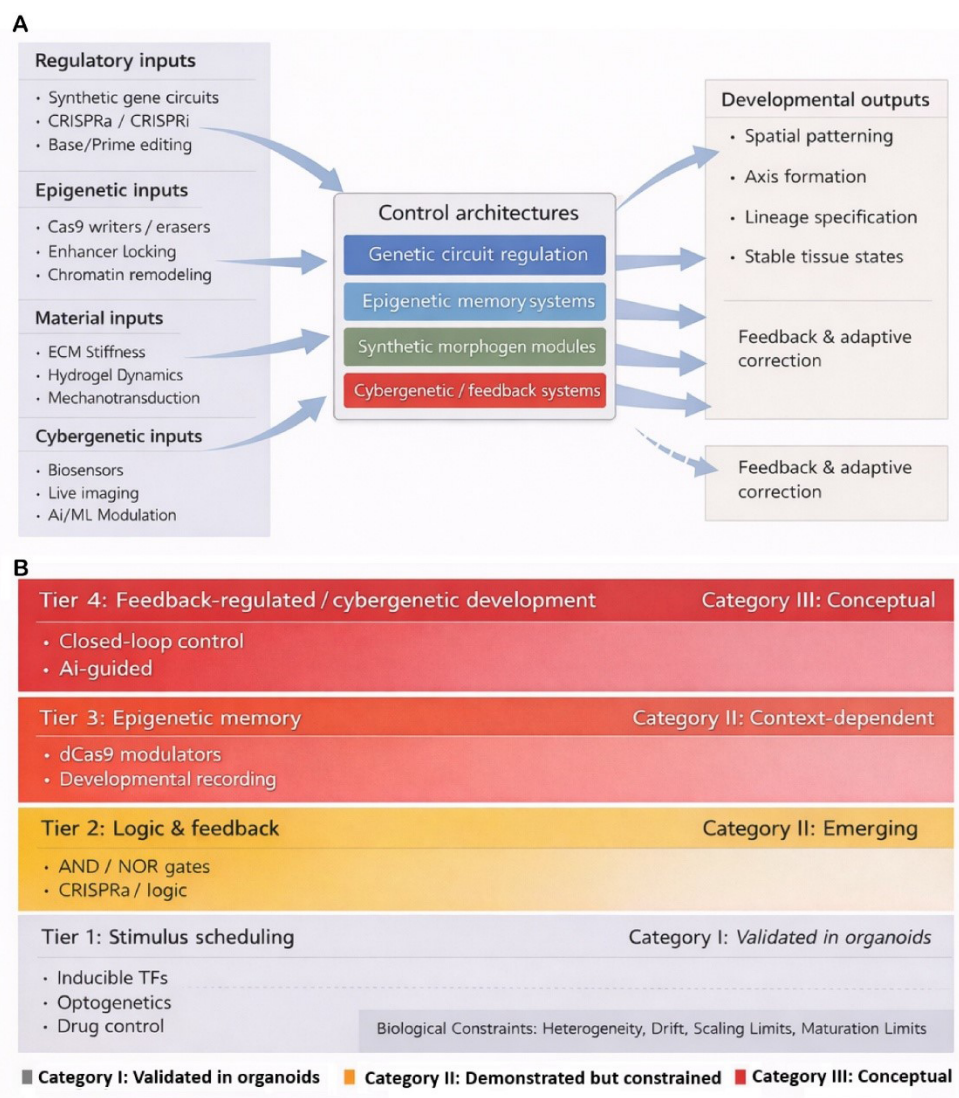


Figure 1. Programmable organoids: systems architecture and tiered regulatory framework for experimentally guided developmental control. (A) System architecture. (B) Tiered regulatory framework. Image created by the author using OpenAI's ChatGPT image-generation tool. The conceptual design, scientific content, and final edits were performed and verified by the author.

Abbreviations: AI: Artificial intelligence; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; ML: Machine learning; TF: Transcription factor.

synthetic morphogen modules, and cybergenetic or feedback systems. Material properties function as upstream regulatory inputs rather than as independent control-architecture blocks. These control layers influence developmental outputs such as spatial patterning, axis formation, lineage specification, and stabilization of tissue states under defined experimental conditions. Figure 1B presents a tiered regulatory framework that organizes programmable organoid systems according to increasing levels of regulatory sophistication: Tier 1 (stimulus scheduling), Tier 2 (logic and feedback), Tier 3 (epigenetic memory), and Tier 4 (feedback-regulated or cybergenetic developmental control). Each tier is mapped to an evidence

category: Tier 1 corresponds to Category I (validated in organoids), Tiers 2–3 correspond to Category II (demonstrated but constrained or context-dependent), and Tier 4 corresponds to Category III (conceptual or hybrid architectures not yet established as autonomous systems). Biological constraints—including heterogeneity, drift, scaling limits, and incomplete maturation—bound the feasibility of higher-tier architectures and currently prevent the implementation of fully internalized, self-maintaining developmental controllers in organoid systems. Feedback relationships between developmental outputs and control architectures illustrate how externally guided or hybrid cybergenetic systems may support adaptive, feedback-

limited modulation of developmental trajectories in defined experimental contexts.

1.2. Evidence classification and scope of claims

To ensure a strict and transparent separation between experimentally demonstrated capability and aspirational design, this review formally classifies all programmable organoid strategies into three evidence categories:

(a) Category I: Experimentally validated in organoids

Approaches that have been directly implemented and functionally demonstrated within 3D organoid systems, with reproducible effects on developmental trajectories, spatial patterning, lineage specification, or stability.

(b) Category II: Demonstrated in related systems but constrained in organoids

Strategies validated in mammalian cells, two-dimensional (2D) cultures, embryoid bodies, spheroids, or early-stage 3D systems, but which remain technically limited, short-lived, or incompletely demonstrated in mature organoid contexts.

(c) Category III: Conceptual or forward-looking architectures

Design frameworks, control architectures, or synthetic developmental strategies that are theoretically grounded or supported by partial experimental components, but have not yet been demonstrated as robust, autonomous, or long-term functional systems in organoids.

Throughout this manuscript, each section explicitly indicates the evidence category being discussed. When multiple categories are referenced within a section, they are clearly distinguished to prevent conflation of validated capability with conceptual or forward-looking proposals.

This distinction is critical: while all programmable organoids involve engineered intervention, not all represent internally, circuit-driven developmental systems. Rather, they span progressive levels of control, ranging from externally scheduled inputs to internally regulated feedback architectures. This concept shifts organoids from purely observational models toward experimentally steerable developmental systems while acknowledging the varying degrees of autonomy currently achievable. A detailed clarification of scope, limits, and interpretive framing is provided in [Appendix](#).

While programmable organoids are broadly defined as systems in which developmental trajectories are influenced by engineered genetic, epigenetic, material, or cybergenetic interventions, current platforms vary widely in their degree of regulatory sophistication. To clarify this landscape, we propose a tiered framework that organizes programmable developmental systems by increasing levels of control:

(a) Tier 1: Stimulus scheduling

Development is guided by externally timed inputs such as inducible promoters, optogenetic switches, or drug-controlled signaling. These systems impose temporal or spatial order but do not autonomously interpret tissue state.

(b) Tier 2: Logic and feedback

Synthetic circuits integrate multiple inputs using logic gates and feedback loops to stabilize boundaries, filter noise, and implement conditional fate decisions.

(c) Tier 3: Epigenetic memory (context-dependent and non-autonomous)

Transient signals are converted into heritable states using chromatin or genomic modification, enabling competence windows, lineage stabilization, and developmental history recording.

(d) Tier 4: Feedback-regulated and cybergenetic developmental control (conceptual or externally mediated)

Biosensors monitor tissue state, circuits or computers interpret this information, and corrective actions are initiated internally or through cybergenetic closed-loop control (computer-in-the-loop), enabling adaptive regulation via internally implemented or cybergenetic feedback mechanisms, often requiring external computation or intervention.

This tiered framework provides a structured lens for interpreting programmable organoids across varying levels of regulatory sophistication ([Figure 1](#)). Interpretive boundaries and scope clarifications are summarized in [Appendix](#).

To avoid misinterpretation of this tiered framework, it is important to emphasize that the tiers represent degrees of conceptual and technical maturity rather than a roadmap of imminent capability. Tier 1 systems (stimulus scheduling) are routine and widely implemented across organoid platforms. Tier 2 systems (logic and feedback) have been partially demonstrated and can stabilize specific developmental features, but remain sensitive to context, scale, and biological variability. Tier 3 systems (epigenetic memory) are fragile and highly context-dependent, with current implementations limited to lineage biasing, stabilization, or recording rather than robust, reversible control. Tier 4 systems (feedback-regulated and cybergenetic control) remain conceptual or require external computation and intervention, and should be interpreted as directional design frameworks rather than emerging autonomous capabilities.

Importantly, this review discusses programmable organoids as an emerging research framework rather than an established, reliable engineering practice. While the conceptual framework presented here organizes

existing tools into increasingly sophisticated tiers of control, many current implementations remain proof-of-concept demonstrations, technically constrained, or limited to simplified systems. Throughout the manuscript, programmable strategies are therefore interpreted in the context of these practical limitations, distinguishing what has been experimentally demonstrated from what remains aspirational.

Despite these advances, several fundamental biological constraints currently define the design space of programmable organoids and explain why autonomous developmental control remains infeasible. Variability between organoids, batch effects, incomplete maturation, lack of vascularization, and imperfect correspondence to *in vivo* developmental timing introduce sources of stochasticity and drift that no existing genetic or epigenetic architecture can reliably sense, interpret, and correct *in situ*.

These constraints are therefore not peripheral caveats but first-order design limits: they bound achievable control architectures, necessitate external guidance or cybergeneic intervention, and prevent the reliable deployment of fully internalized, self-maintaining regulatory systems. Throughout this review, Tier 3 and Tier 4 architectures are treated as constrained by these biological realities, with their aspirational status arising directly from unresolved variability, scaling, and maturation limits rather than from a lack of conceptual frameworks.

The programmable organoid framework shows how regulatory inputs are transformed by layered control architectures into defined developmental outcomes across increasing tiers of control (Figure 1).

Examples of Tier 1 systems include doxycycline-inducible lineage programs and optogenetic modulation of pathways such as wingless-related integration site (WNT), bone morphogenetic protein (BMP), or Sonic hedgehog (SHH).^{11,12} Tier 2 includes CRISPR activation/interference (CRISPRa/i)-based logic gates, synthetic Notch receptors, toggle switches, and feedback-stabilized patterning circuits.^{13,14} Tier 3 encompasses dCas9-based epigenetic writers, base-editing recorders, and enhancer-locking systems that impose heritable fate decisions or record developmental history.¹⁵ Tier 4 includes cybergeneic patterning platforms, cell-in-the-loop control systems, and machine-learning-guided optogenetic or chemogenetic morphogenesis.¹⁶

This review synthesizes and unifies these emerging directions, focusing on the integration of genetic circuits, epigenomic memory systems, and synthetic developmental control strategies to actively engineer morphogenesis. While prior reviews have examined genetic manipulation,

organoid patterning, or synthetic biology in isolation, this review integrates genetic, epigenetic, and synthetic patterning strategies into a single framework for programmable development.

1.3. Literature search strategy and inclusion criteria

To ensure comprehensive and reproducible coverage of the rapidly evolving field of programmable organoids and synthetic developmental engineering, we conducted a structured literature search across multiple databases, including PubMed, Web of Science, Scopus, and Google Scholar. Searches were performed between January 2010 and October 2025, reflecting the emergence of modern organoid technologies and synthetic biology tools relevant to developmental programming.

Search terms included combinations of: “organoids,” “programmable development,” “synthetic gene circuits,” “CRISPRa,” “CRISPRi,” “epigenetic engineering,” “synthetic organizers,” “morphogenesis,” “reaction–diffusion,” “optogenetics,” “chemogenetics,” “cybergeneic,” and “closed-loop control.” Boolean operators and iterative refinement were used to capture both foundational and emerging work.

Studies were included if they (i) involved 3D stem-cell-derived systems or organoid-like models, (ii) implemented genetic, epigenetic, or synthetic regulatory strategies to control developmental outcomes, or (iii) introduced theoretical or experimental frameworks for programmable morphogenesis. Both primary research articles and high-impact methodological or conceptual reviews were considered.

Articles were excluded if they focused solely on conventional organoid differentiation without engineered control, lacked relevance to developmental regulation, or were limited to 2D culture systems without translational relevance to 3D morphogenesis. Reference lists of key papers were manually screened to identify additional relevant studies.

2. Genetic control of cell fate in organoids: Validated strategies and emerging circuit-inspired architectures (primarily Categories I–II)

This section primarily covers experimentally demonstrated and emerging genetic control strategies (Categories I and II), with explicit indication of where approaches represent proof-of-concept demonstrations versus robust implementations in organoid systems.

2.1. Synthetic gene circuits and circuit-inspired regulatory architectures in stem cells and organoids (primarily Category II; selected Category I demonstrations)

Synthetic gene circuits and circuit-inspired regulatory architectures represent one class of tools within programmable organoids and correspond primarily to Tier 2 of the framework introduced above, where logic integration and feedback regulation begin to appear. As defined in Section 1, Tier 1 tools impose externally scheduled control, whereas Tier 2 architectures incorporate logic integration or feedback and therefore qualify as circuit-like regulatory systems in this framework. These architectures operate by linking regulatory DNA elements such as promoters, enhancers, and untranslated regions with engineered transcriptional regulators to create experimentally defined input–output relationships under controlled conditions^{17,18}. Similar to electronic components, circuit motifs such as logic gates, toggle switches, excitable loops, and feedback controllers allow cells to process, store, and respond to information in controlled ways (Figure 2).

In developmental contexts, logic gates have been demonstrated primarily in cell culture, embryoid bodies, and simplified early-stage differentiation systems to integrate combinations of morphogens or intracellular

signals that influence lineage outcomes when precise conditions are met. In organoids, these approaches should currently be interpreted as Category II proof-of-concept demonstrations rather than robust, long-term controllers of multi-lineage development. Toggle switches drive bistable transitions that stabilize cell fates against environmental fluctuations, while excitable or pulse-generating loops create transient activation patterns that reflect natural oscillatory behaviors such as segmentation rhythms.¹⁹ Feedback controllers, both positive and negative, can enhance stability, sharpen boundaries, and buffer stochastic noise, which is especially critical in 3D organoid environments characterized by variable cell density and heterogeneous microenvironments.^{20,21}

CRISPR-based platforms have rapidly expanded the sophistication and modularity of circuit-inspired regulatory systems. CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) (Category II in organoids) enable fine-grained transcriptional control of endogenous pathways, allowing developmental regulators to be modulated without exogenous overexpression.^{9,22,23} Catalytically inactive Cas9 (dCas9) can be fused to activators, repressors, or chromatin modifiers and programmed with multiple guide RNAs, permitting construction of scalable logic circuits capable of implementing AND, NOR, NAND, and

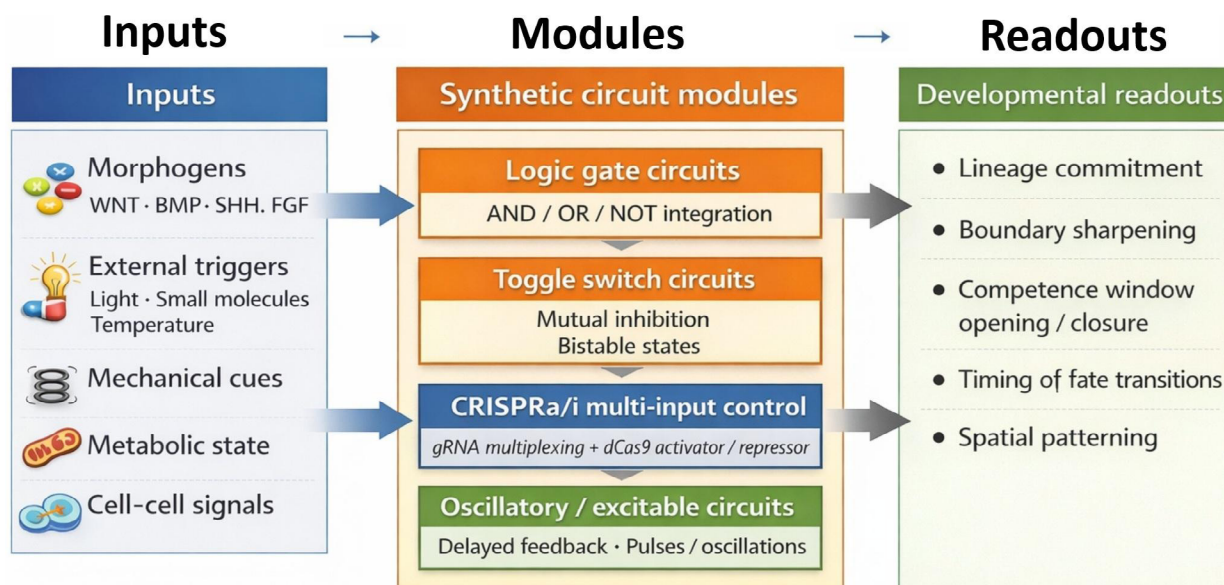


Figure 2. Inputs → Modules → Readouts: Circuit-inspired regulatory architectures for causal control of developmental fate. Synthetic gene circuits and circuit-inspired regulatory architectures in programmable organoids function as circuit-inspired regulatory modules that translate defined experimental inputs into context-dependent morphogenetic responses. Cells sense morphogens (WNT, BMP, SHH, FGF), external stimuli (light, small molecules, temperature), mechanical cues, metabolic state, and cell–cell interactions. These inputs are interpreted through distinct classes of synthetic circuit architectures, including logic gate circuits, toggle switch circuits, CRISPRa/i-based multi-input regulatory systems, and oscillatory or excitable feedback circuits. Together, these modules regulate lineage commitment, boundary sharpening, competence window opening and closure, timing of fate transitions, and spatial patterning during organoid development. Examples illustrated correspond primarily to Category II implementations in organoid or related 3D systems, with selected Category I demonstrations, as defined in Section 1.2. Image created by the author using OpenAI’s ChatGPT image-generation tool. The conceptual design, scientific content, and final edits were performed and verified by the author.

multi-input regulatory architectures.^{9,24,25} In parallel, base editors introduce irreversible single-nucleotide changes to create genomic “recording” modules, whereas prime editors enable precise rewriting of regulatory sequences to reshape enhancer logic or correct developmental regulators with minimal genomic disruption.^{26–28} These tools create a regulatory landscape in which gene networks can be experimentally rewired to influence cell fate, responsiveness, and competence windows. However, most demonstrations to date occur in 2D systems or early organoid stages and do not yet represent stable, long-term developmental programming across complex, multi-lineage organoid architectures.

A major advantage of synthetic circuits is their ability to integrate diverse multimodal inputs that are relevant to organoid development. Cells can be engineered to sense canonical morphogens such as WNT, BMP, SHH, and FGF, to interpret mechanical cues including stiffness, strain, or compression, and to respond to external stimuli such as light or small molecules.^{29–31} This combinatorial flexibility allows circuits to reproduce the complex information-processing tasks carried out by early embryos, where spatial gradients, mechanical constraints, and temporal cues converge to define identity. In 3D organoids, where morphogen diffusion, mechanical patterning, and stochastic interactions strongly influence developmental trajectories, multi-input circuits can improve reproducibility in constrained contexts and allow experimentally defined modulation of morphogenetic outcomes in 3D organoids.^{31,32}

However, despite strong mechanistic validation in mammalian cells and 2D differentiation systems, comparatively fewer CRISPRa/i and synthetic logic architectures have been demonstrated as stable, spatially reproducible controllers across long-duration 3D organoid development. In organoids, performance is often limited by (i) tissue heterogeneity (cell-state diversity, asynchronous differentiation, mosaic transduction/integration), which changes circuit input distributions over time; (ii) diffusion and access constraints (gradients of inducers, oxygen, nutrients) that create spatially varying thresholds; (iii) circuit burden (dCas9 expression load, multi-gRNA competition, promoter/enhancer load) that can slow growth or select for circuit-loss variants; and (iv) silencing and instability (epigenetic shutdown of transgenes, gRNA cassette instability, copy-number drift), which can cause progressive loss of control. Practical mitigation strategies reported across platforms include genomic safe-harbor integration, lower-burden or inducible dCas9 designs, redundant/insulated regulatory elements, and readout-linked selection or monitoring to detect failure early.^{33,34}

2.2. Inducible and spatiotemporal control: Experimentally validated but externally guided systems (Category I)

Temporal and spatial precision is essential for programming morphogenesis, and inducible systems provide a powerful set of tools to impose such control in developing organoids. These inducible systems correspond to Tier 1 (stimulus scheduling) in our framework. They impose externally timed or spatially patterned inputs but do not constitute internally implemented genetic circuitry, as they lack internal feedback, logic integration, or state-dependent regulation. Optogenetic systems (Category I) enable developmental pathways to be regulated with millisecond responsiveness and micron-level spatial resolution. By linking critical regulators of WNT, SHH, or BMP signaling to light-responsive proteins, investigators can generate synthetic morphogen gradients with tunable amplitude, geometry, and duration.³⁵ Spatially patterned illumination can guide symmetry breaking, define neural tube-like dorsal and ventral domains, or induce localized axis formation.^{12,36} Because optogenetic cues can be pulsed, stepped, or oscillated, these systems permit experimental reconstruction of dynamic developmental processes such as transient competence windows, morphogen decay profiles, or traveling waves of differentiation. In addition, combining optogenetics with live imaging enables real-time, closed-loop modulation of organoid behavior, which enables externally guided, feedback-limited modulation of developmental processes, without constituting internally autonomous regulatory control.^{12,35,37}

Chemogenetic and drug-inducible systems complement optical control by offering robust, scalable, and reversible mechanisms to modulate gene expression. Tet-On and Tet-Off systems allow transcriptional activation or repression through doxycycline, enabling researchers to titrate differentiation factor expression with fine temporal precision.^{38,39} CreERT2 recombination provides tamoxifen-dependent genetic switching for lineage tracing, fate restriction, or the induction of irreversible developmental transitions.⁴⁰ Rapalog-inducible modules can assemble or disassemble signaling complexes, activating developmental pathways only in the presence of defined synthetic ligands.^{41,42} Newer inducible platforms, including red light-responsive regulators, UV-sensitive repressors, and small-molecule-stabilized protein domains, further expand the range of regulatory mechanisms available for developmental programming.^{43,44} These systems are especially powerful for probing processes that depend on precise timing, such as stage-specific germ layer specification or tightly defined patterning intervals that are otherwise difficult to control in self-organizing tissues.

At a higher organizational level, inducible systems enable the creation of synthetic organizers, which are engineered

analogs of the signaling centers that orchestrate embryonic patterning. Engineered cells implanted within organoids can be programmed to secrete morphogens such as *WNT3A*, *SHH*, *BMP4*, or *FGF8* under defined inducible conditions. Alternatively, endogenous cells can be reprogrammed *in situ* using CRISPRa-driven secretion circuits to convert them into temporally controlled signaling hubs.^{12,31,45,46} These synthetic organizers recapitulate key developmental roles such as axial induction, competence specification, and boundary formation, while allowing their activity to be externally modulated or dynamically tuned. Incorporating feedback modules allows synthetic organizers to modulate morphogen output in response to measured tissue states; however, these implementations remain externally mediated and do not constitute fully self-correcting developmental control. As a result, synthetic organizers provide a means to reduce variability in defined experimental settings and to bias patterning toward geometrically interpretable outcomes, though reproducibility remains constrained by biological heterogeneity.

Throughout this section, inducible and perturbation-based systems are therefore discussed as stimulus-scheduled interventions and should not be interpreted as evidence of autonomous or internally self-regulating circuit architectures within organoids.

2.3. Synthetic morphogenetic programs: Demonstrated modules and forward-looking circuit-inspired designs (Categories I–II; selected Category III conceptual designs)

Synthetic morphogenetic programs arise from the combination of Tier 1 stimulus scheduling tools and Tier 2 logic-based circuits, which together allow externally guided patterning to incorporate limited feedback stabilization in constrained contexts, without achieving fully autonomous developmental control. A foundational application is guided symmetry breaking, where synthetic circuits introduce controlled anisotropy into initially isotropic stem-cell aggregates. Inducible activation of *WNT/β-catenin*, *NODAL*, or *LEFTY* pathways can steer germ-layer formation toward precise spatial domains, while optogenetic gradients introduce directional cues that orient early axes.^{12,47,48} These approaches allow researchers to systematically vary spatial and temporal parameters of symmetry breaking, revealing how early asymmetries propagate and shape downstream patterning. Most reported implementations of these strategies occur in small aggregates, early-stage organoids, or simplified patterning contexts, and should be interpreted as mechanistic demonstrations rather than fully reproducible morphogenetic control in mature, multi-

lineage organoids. More advanced circuit-inspired designs incorporate symmetry sensors, enabling cells to detect uniformity in gene expression, tension, or morphogen levels and trigger asymmetry only when appropriate.^{31,47,49} These programs provide a mechanistic handle on one of the most fundamental yet elusive events in embryogenesis.

In early-stage organoids and controlled small-aggregate systems, synthetic circuits have enabled experimental engineering of axis induction and spatial pattern formation, recreating key organizational principles of multicellular development. Engineered signaling centers that produce region-defining morphogens such as *SHH* for ventral identities, *WNT* for posteriorization, and *FGF* for axial elongation can induce spatially biased anterior–posterior or dorsal–ventral domains in early-stage organoids under controlled conditions.^{50,51} Optogenetically sculpted gradients can create graded or sharply bordered domains, while chemogenetic control allows temporal gating of axis induction to mimic developmental progression.^{12,52} Incorporating feedback circuits that reinforce or sharpen boundary formation can improve stability of synthetic axes even as organoids grow and undergo morphogenetic remodeling.^{53,54} These systems provide a unique framework to dissect and reconstruct the logic of positional information and to explore in a systematic way how tissue level patterns emerge from molecular cues.

Finally, directed lineage specification uses synthetic circuits to guide cells through multi step differentiation cascades. Bistable *SOX2* and *OCT4* toggle switches can drive neural induction, while inducible expression of mesodermal regulators such as *T*, *EOMES*, or *MIXL1* initiates orderly mesoderm formation.^{55,56} CRISPRa-based activation cascades guide sequential induction of endodermal programs without dependence on exogenous morphogens, improving reproducibility and reducing protocol variability.^{57,58} When combined with spatial cues, such as patterned illumination or engineered organizers, these circuits enable the simultaneous formation of multiple germ layers or region-specific subdomains within a single organoid. This capability positions organoids as experimentally steerable developmental platforms under externally scheduled interventions where lineage interactions and patterning hierarchies can be systematically interrogated and partially reconstructed in defined experimental contexts.

To facilitate comparison across the major technological strategies for programmable organoids, we summarize their core advantages, limitations, and optimal use cases in Table 1.

Table 1. Comparison of technologies for programmable organoids: Strengths and limitations

Technology class	Core strategy	Advantages	Key limitations	Best use cases	Evidence maturity (Category I/II/III)
Inducible/optogenetic systems	External temporal or spatial control of signaling	Simple, reversible, precise timing	No intrinsic autonomy, diffusion/light limits	Short-term patterning, perturbation studies	Category I
CRISPRa/i & logic circuits	Genetic logic and feedback	Experimentally programmable in constrained contexts	Burden, silencing, heterogeneity	Lineage control, boundary stabilization	Category II
Epigenetic memory systems	Heritable chromatin changes	Long-term stability	Drift, irreversibility, off-target	Lineage locking, history recording	Category II
Reaction–diffusion/organizers	Self-patterning morphogen systems	Emergent spatial structure (validated primarily in small or early-stage systems)	Parameter sensitivity, scaling issues	Axis formation, symmetry breaking	Category I–II (early-stage organoid validation)
Cybergenetic control	Cell–computer feedback	Enables adaptive intervention in defined experimental systems	Instrumentation, latency	Long-term pattern maintenance	Category II (hybrid implementations)
Artificial intelligence-guided control (hybrid/proof-of-concept)	Machine learning-driven interventions	Handles complexity	Data-hungry, opaque	Optimization and exploratory adaptive morphogenesis in hybrid or proof-of-concept systems	Category III (conceptual/proof-of-concept)

3. Epigenetic modulation and circuit-inspired memory in organoids: Constraints, capabilities, and design space (primarily Category II; emerging Category III elements)

Epigenetic modulation and circuit-inspired memory add a stabilizing and state-constraining dimension to organoid engineering by stabilizing cell states, biasing lineage trajectories, and recording developmental history. Importantly, current implementations function as stabilizing or memory-encoding layers rather than fully reversible epigenetic controllers. Whereas genetic circuits operate through transcription factor logic and promoter architecture, epigenetic systems act through modifications that persist across cell divisions, integrate environmental cues over time, and define the competence of individual cells and tissues.^{59–61} These properties make epigenetic engineering well-suited for developmental systems that depend on timing, history, and spatial context. Recent advances in dCas9-based epigenetic tools, enhancer engineering, and chromatin-state modulation position organoids as systems capable of stabilizing, biasing, and recording memory-guided developmental programs (Figure 3).^{8,62}

3.1. Epigenome engineering as a programmable memory layer: Demonstrations and limitations in organoids (Category II demonstrations; limited Category I in mature organoids)

A central strategy for engineering epigenetic modulation in organoids relies on dCas9 fused to “writer” or “eraser” enzymes that deposit or remove chromatin marks at precise loci. Targeted DNA methylation using DNMT3A or locus-specific demethylation via TET1 can durably shift the regulatory state of key developmental genes, while histone-directed fusions such as KRAB (H3K9me3 deposition), p300 (H3K27 acetylation), and LSD1 (removal of H3K4me1/2) provide highly tunable regulation of enhancers and promoters.^{8,9,63} These tools allow precise reconfiguration of regulatory landscapes that dictate lineage competence, germ-layer commitment, and spatial identity within organoids.

It is important to note that, while these tools provide powerful locus-specific chromatin manipulation, most current applications in organoids represent Category II demonstrations, rather than Category I epigenetic control systems as defined in Tier 3 of our framework. In many studies, chromatin profiling correlates with developmental outcomes without causal circuit-level feedback. Only a subset of recent work begins to approach causal epigenetic modulation in 3D organoid contexts, and no current system

demonstrates fully reversible, circuit-level epigenetic programming.

An equally important design axis is the distinction between persistent and reversible epigenetic states. Transient chromatin opening can generate competence windows during which cells are sensitive to morphogen cues, recapitulating temporally constrained phenomena such as gastrulation, neural induction, or axial patterning.^{64,65} In contrast, stable marks create epigenetic memory that endures through multiple divisions, converting past morphogen exposure or mechanical signals into long-term lineage commitments.^{59,60} Balancing reversibility and persistence is essential for engineering orderly developmental sequences.

Epigenetic modulation and memory systems can also operate as biological recorders, encoding the duration, intensity, or temporal ordering of morphogen signals. For example, “write-once” methylation marks can permanently record transient WNT or SHH signals,

enabling reconstruction of developmental trajectories long after the original cues have dissipated.^{8,66} Multistep dCas9-based systems can encode graded or cumulative exposure, offering a means to understand how early signaling history constrains later morphogenesis. These recorders are particularly valuable in organoids, where spatial heterogeneity makes it challenging to interpret developmental events from endpoint phenotypes alone.^{67,68}

Finally, epigenetic memory can be intentionally integrated into synthetic circuits as a design principle. Memory modules can serve as developmental checkpoints that restrict progression unless specific chromatin states are met; they can prevent premature lineage switching; and they can enforce sequential transitions by locking cells into intermediate states until circuit-defined conditions are satisfied.^{69,70} In this way, epigenome engineering creates programmable, heritable rules that guide how organoids progress through complex developmental landscapes.

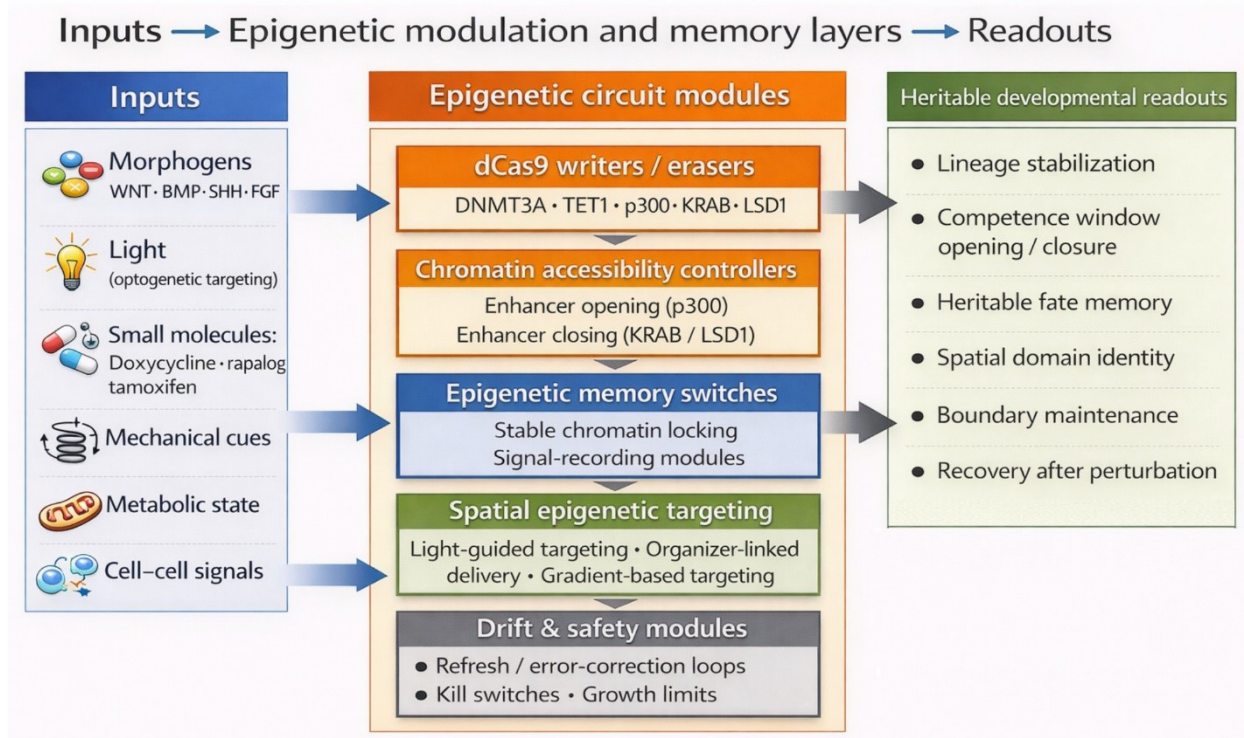


Figure 3. Inputs → Epigenetic modulation and memory layers → Readouts: Epigenetic modulation and memory mechanisms for stable lineage and patterning bias. Epigenetic engineering in programmable organoids functions as a chromatin-level information-processing layer that converts developmental inputs into stable and heritable cellular states. Cells sense morphogens (WNT, BMP, SHH, FGF), optogenetic signals, small molecules (doxycycline, rapalog, tamoxifen), mechanical cues, metabolic state, and cell–cell signals. These inputs are interpreted through distinct classes of epigenetic modulation modules, including locus-specific dCas9 writers and erasers (DNMT3A, TET1, p300, KRAB, LSD1), chromatin accessibility controllers at enhancers, epigenetic memory switches that lock or record prior signals, spatial epigenetic targeting strategies, and drift- and safety-control modules that maintain chromatin fidelity over time. Together, these modules enable lineage stabilization, regulation of competence window opening and closure, heritable fate memory, spatial domain identity, boundary maintenance, and recovery after perturbation during organoid development. Epigenetic modulation and memory mechanisms illustrated here correspond primarily to Category II demonstrations, with limited Category I validation in mature organoids. Image created by the author using OpenAI’s ChatGPT image-generation tool. The conceptual design, scientific content, and final edits were performed and verified by the author.

3.1.1. Evidence strength and failure modes of epigenetic memory modules in organoids

Epigenetic memory modules (e.g., dCas9-linked writers/erasers and recording architectures) provide a powerful route to convert transient developmental inputs into heritable states, but in organoids their reliability depends on chromatin context, division history, and spatial microenvironments. Key 3D failure modes include (i) mosaic memory formation due to variable editor delivery or expression across depth; (ii) state erosion or drift as organoids expand and undergo stress, remodeling, or selection; (iii) context-dependent silencing of engineered loci or transgene cassettes; and (iv) off-target or collateral chromatin effects that can subtly bias lineage trajectories. A practical design implication is that memory modules should be paired with longitudinal state readouts (reporters, targeted profiling) and, where possible, refresh or error-correcting architectures to reassert intended chromatin states when drift is detected.^{15,71}

3.1.2. Timescales, reversibility, drift, and safety in programmable epigenetic systems

Programmable developmental systems operate across multiple biological timescales. Transcriptional circuits typically act over minutes to hours, enabling rapid responses to morphogens or external inputs. Epigenetic programs, in contrast, function over days to weeks, stabilizing lineage identity across many cell divisions. Closed-loop and cybergenetic systems span both regimes, combining fast sensing and actuation with slow, heritable state control. Designing effective programmable organoids therefore requires explicit alignment of control strategies with the relevant developmental timescales.

Reversibility is a central design dimension. Some applications require transient control—such as opening short competence windows or inducing temporary symmetry breaking—while others require irreversible transitions, including lineage commitment or long-term regional stabilization. Reversible systems, such as inducible transcriptional regulators or optogenetic switches, enable flexible experimentation but are vulnerable to drift once control is withdrawn. Irreversible systems, including base editing or stable epigenetic writing, provide durability but reduce the ability to correct design errors or respond to unexpected developmental outcomes.

Drift emerges as a fundamental long-term challenge. Genetic drift arises from mutation, silencing, or selection against burdensome circuits. Epigenetic drift, in particular, affects systems that rely on heritable chromatin states: engineered marks can erode, spread, or become mosaic during prolonged growth, mechanical stress, or metabolic perturbation. Such epigenetic drift and memory erosion

threaten lineage fidelity, reproducibility, and long-term stability of programmable organoids.

Safety considerations are tightly coupled to timescale and reversibility. Short-lived, reversible control modules carry lower long-term risk but may fail to stabilize developmental trajectories. Persistent or irreversible systems increase control but elevate risks related to off-target effects, uncontrolled growth, or the emergence of aberrant cell states. For this reason, long-timescale systems should incorporate layered safeguards, including kill switches, growth-restriction modules, and error-correcting or refreshable epigenetic modulation modules.

Taken together, timescale alignment, reversibility design, drift management, and safety engineering must be treated as a unified design problem. Programmable organoids aimed at long-term developmental fidelity will require not only precise initial programming, but continuous monitoring, drift detection, and corrective capacity to remain both functional and safe during extended culture or translational use.⁷²⁻⁷⁴

3.2. Chromatin state control for fate stabilization

Precise chromatin organization is essential for establishing and maintaining stable lineage identities, and synthetic biology now provides tools for controlling chromatin architecture with high specificity. Synthetic nucleosome-positioning frameworks allow researchers to tune accessibility at promoters and enhancers, thereby regulating transcriptional responsiveness without altering the underlying DNA sequence. Targeted chromatin remodelers recruited through dCas9 or other DNA-binding platforms can shift nucleosome arrays to prime developmental genes, silence alternative fates, or modify transcription factor access during critical differentiation events.^{75,76}

Assay for Transposase-Accessible Chromatin-seq guided engineering expands this level of control by linking synthetic circuits to the endogenous chromatin landscape. Enhancers that become accessible during neural, mesodermal, or endodermal differentiation can be selectively activated using p300-based systems, while competing enhancer programs can be silenced using KRAB or LSD1. These approaches enable rational engineering of lineage-specific enhancer hierarchies.⁶³ In organoids, where spatial domains often correspond to differential enhancer activation, chromatin-guided engineering ensures that regional identities emerge consistently and remain resistant to disruption caused by local variations in microenvironmental signals.^{77,78}

A key application of chromatin editing is the generation of synthetic competence windows. Temporarily opening lineage-specific enhancers (e.g., SOX17 for endoderm, NEUROG2 for neural induction) before reclosing them

at later stages allows organoids to recapitulate the narrow developmental intervals during which cells respond to specific cues.^{79,80} This ensures that differentiation proceeds in a controlled, stage-aligned manner, improving reproducibility and reducing lineage ambiguity. Synthetic competence windows mirror natural mechanisms governing early embryonic transitions, making them powerful tools for engineering high-fidelity developmental programs.

Beyond competence, synthetic epigenetic modulation modules can lock in lineage trajectories, reinforcing chromatin states compatible with chosen fates while suppressing alternative programs. Stable deposition of activating marks at lineage-defining enhancers, coupled with inhibitory marks on competing networks, prevents lineage drift and ensures that regional identities persist as organoids expand, fold, and mature.^{15,62} This stabilization is crucial for multi-domain organoids such as patterned brain or gut models, where early specification must be maintained over extended periods of morphological remodeling.

3.3. Multicellular epigenetic patterning

Epigenetic modulation does not operate only at the level of individual cells; it also organizes multicellular assemblies by creating spatially distinct chromatin states that encode positional identity. Region-specific chromatin landscapes, engineered using spatially restricted delivery of dCas9 modifiers through optogenetics, ligand gradients, or synthetic organizers, allow organoids to develop compartments that mirror embryonic domains long before transcriptional divergence is detectable.^{81,82} These epigenetically encoded territories form a scaffold upon which tissue-level patterning unfolds, enabling early establishment of neural, mesodermal, or endodermal zones.

A critical feature of multicellular epigenetic structure is the formation of epigenetic boundaries, which are sharp transitions in chromatin state that prevent inappropriate mixing of developmental programs. Synthetic circuits can generate such boundaries by activating one chromatin state in a defined region, for example rostral HOX genes, while enforcing a mutually exclusive state in another region, for example caudal HOX repression through KRAB.⁸³ This engineered opposition stabilizes anterior to posterior or dorsal to ventral patterning and shields developing domains from the effects of morphogen diffusion or growth related distortion.

Epigenetic modulation and memory mechanisms also contribute to emergent pattern formation through feedback loops that propagate or restrict chromatin states across cell populations. Positive feedback can reinforce domain

identity, allowing spatial domains to expand or consolidate, while negative feedback sharpens borders and prevents lineage intermixing.⁸⁴ These emergent dynamics resemble natural mechanisms such as lateral inhibition, mutual repression, and community effects, all of which generate robust developmental patterns in embryos. In organoids, such synthetic feedback loops can bias pattern formation under defined experimental conditions, contributing to partially self-organizing structures shaped by engineered epigenetic constraints.^{85,86}

As spatially resolved epigenome editing becomes more precise, organoid systems are approaching the construction of multicellular epigenetic architectures, which function as blueprints that encode positional cues, lineage boundaries, and long-term identity in chromatin rather than relying solely on signaling gradients. These architectures provide a strategy for engineering tissues with more stable developmental trajectories and more interpretable compartmentalization under defined conditions. Integration of epigenetic, genetic, and signaling-based networks may contribute to the development of organoid systems in which developmental logic is encoded across multiple regulatory layers, potentially supporting more stable and experimentally steerable morphogenesis under defined *in vitro* conditions.

3.4. Integrating epigenetic logic with genetic and morphogenetic programs

Across organoid development, epigenetic modulation and memory mechanisms interact continuously with genetic circuits, signaling pathways, and mechanical forces. As synthetic organoid engineering advances, a central challenge is designing systems where these layers operate cooperatively rather than independently. Genetic circuits provide rapid, signal-responsive regulation; signaling pathways set spatial gradients; mechanical cues shape tissue geometry; and epigenetic programs provide stability, memory, and heritable identity.^{54,87} A coherent synthetic framework integrates these modalities so that transient genetic inputs produce long-term chromatin commitments, morphogen gradients modify epigenetic signatures, and mechanical forces are translated into chromatin remodeling. Such multi-layered integration may support the development of experimentally steerable systems in which developmental outcomes are increasingly influenced by engineered regulatory constraints and heritable chromatin states.^{88,89} The emergence of this integrative logic suggests a conceptual transition toward more highly structured and experimentally steerable developmental systems in which epigenetic architecture acts as a durable, context-aware foundation for engineered morphogenesis.

3.5. Clinical translation: Epigenetic biomarkers and therapeutic control of cell fate

Unlike genetic circuit control, which remains largely proof-of-concept in organoid systems, epigenetic modulation has long-standing clinical precedent, making it a useful bridge between programmable organoids and established therapeutic practice. However, organoid-to-organoid variability, batch effects, and incomplete maturation currently limit the reliability of organoid platforms as predictive translational systems, even when epigenetic modulation is technically feasible. In medicine, chromatin state is not only a correlate of disease but a measurable and therapeutically manipulable driver of cell identity. Patterns of DNA methylation, histone acetylation, chromatin accessibility, and enhancer usage are widely employed as diagnostic and prognostic biomarkers across oncology, developmental disorders, and regenerative medicine. These measurements reveal that stable cell states are frequently maintained by epigenetic configurations rather than by irreversible genetic mutations.⁹⁰

Notably, even in clinical settings, epigenetic therapies operate by shifting chromatin states and lineage competence, not by executing precise, reversible epigenetic programs, reinforcing the view that epigenetic mechanisms function as biasing and stabilizing layers rather than programmable controllers.

In cancer, for example, global hypomethylation combined with locus-specific hypermethylation, enhancer rewiring, and histone modification changes define malignant cell identity and predict therapeutic response. Similarly, imprinting disorders, enhanceropathies, and chromatin-boundary defects arise from misregulated epigenetic architecture rather than alterations in coding sequences. These observations establish that chromatin state can *causally determine* lineage competence and pathological identity.⁹¹

This clinical reality provides important context for programmable epigenetic systems in organoids. While fully autonomous epigenetic modulation and memory systems have not yet been demonstrated in organoid platforms and remain biologically premature, the principle that controlled chromatin modification can redirect cell fate is already firmly established in therapeutic practice. Histone deacetylase inhibitors, histone acetyltransferase modulators, DNA methyltransferase inhibitors, BET bromodomain inhibitors, and LSD1 or EZH2 inhibitors are used clinically or are in advanced trials to induce differentiation, suppress malignancy, or restore developmental competence.⁹²

These drugs act as coarse-grained epigenetic regulators, globally shifting chromatin accessibility and transcriptional potential. In contrast, dCas9-based epigenetic editing, enhancer locking, and chromatin-state programming in

organoids represent fine-grained, locus-specific analogs of the same biological principle. Programmable organoids therefore offer a platform to study, with spatial and temporal precision, how targeted chromatin modifications influence developmental trajectories in ways that parallel therapeutic epigenetic modulation in patients.

A particularly important connection lies in differentiation therapy. Several epigenetic drugs do not kill cells directly but instead push malignant or dysfunctional cells toward terminal differentiation by altering chromatin states. Similar principles are increasingly explored in regenerative medicine, where restoring lineage competence or stabilizing therapeutic identity depends on epigenetic reinforcement rather than transient transcriptional activation.⁹³

Epigenetic biomarkers also carry strong prognostic value. DNA methylation patterns, histone marks, and enhancer activity are used to predict tumor aggressiveness, treatment response, and disease recurrence. By integrating epigenetic memory modules with reporters, organoids can be engineered to record how transient signals or drug exposures become stabilized as heritable chromatin states. This capability enables direct modeling of how early epigenetic events translate into long-term tissue behavior, something difficult to infer from static patient samples.⁹⁴

Finally, this clinical perspective clarifies the role of epigenetic modulation mechanisms in programmable organoids. Rather than being viewed as speculative control layers inferred from chromatin profiling, epigenetic modules in organoids can be understood as experimentally tractable, spatially resolved implementations of mechanisms that are already exploited therapeutically in humans. In this way, programmable organoids bridge developmental biology and clinical epigenetics, allowing causal interrogation of chromatin-driven cell fate decisions within controlled 3D developmental systems.

4. Programmable morphogenesis in organoids: Experimentally demonstrated patterning and scaling constraints (primarily Categories I–II)

This section primarily reflects Category I and Category II evidence, emphasizing experimentally demonstrated patterning and morphogenetic control in organoids while explicitly acknowledging scaling, maturation, and long-term stability limitations.

Unless otherwise specified, examples discussed in this section reflect early-stage, small-scale, or proof-of-concept implementations, rather than fully mature, long-term multi-lineage organoid control systems.

Organoid morphogenesis arises from a dynamic interplay between intrinsic self-organizing principles and extrinsic molecular or physical cues. While this spontaneous behavior reveals fundamental aspects of human development, it also introduces variability, incomplete patterning, and divergence from canonical embryonic trajectories.^{78,95} Programmable morphogenesis aims to reduce, rather than eliminate, the inherent unpredictability of organoid development through engineered, externally guided regulatory interventions. However, variability between organoids, batch effects, incomplete maturation, lack of vascularization, and imperfect alignment with *in vivo* developmental timing remain major constraints that limit how fully such control can be realized in practice. These strategies rely on synthetic gene and epigenetic modulation modules that modulate responsiveness to environmental signals, optogenetic and chemogenetic systems that precisely regulate temporal and spatial patterning, and engineered signaling hubs that recapitulate organizer-like functions.^{81,85} Collectively, these strategies enable more

controlled and reproducible morphogenesis in defined experimental contexts; however, organoid heterogeneity, batch effects, and incomplete maturation currently constrain scalability, long-term stability, and translational robustness (Figure 4). Taken together, they coalesce into two overarching conceptual domains that frame the discussion that follows, encompassing the deliberate engineering of self-organizing behaviors and the systematic reconstruction of canonical human developmental events.

Despite these advances, the feasibility of fully autonomous morphogenetic control remains fundamentally constrained by biological variability, incomplete maturation, and the absence of vascular and metabolic integration in current organoid systems. These constraints do not merely reduce performance; they actively shape which morphogenetic control architectures are viable, favoring externally guided, feedback-limited designs over autonomous patterning systems. As a result, assessments of feasibility in this review should be interpreted relative to these biological constraints, which presently necessitate externally guided

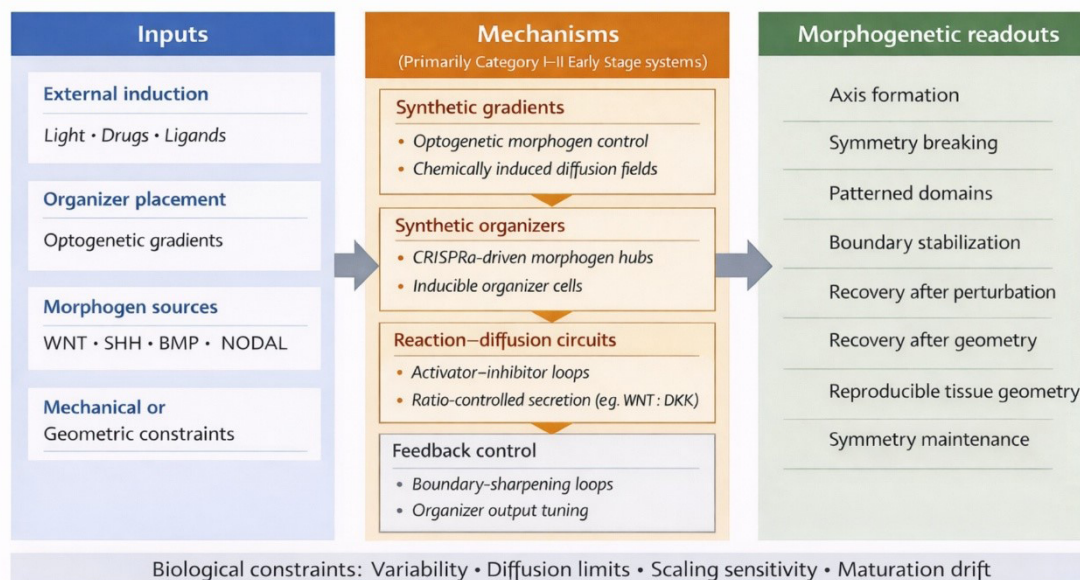


Figure 4. Inputs → Mechanisms → Morphogenetic Readouts: Experimentally Guided Spatial and Temporal Patterning in Organoids. Programmable morphogenesis is depicted as a progression from defined experimental inputs to synthetic patterning mechanisms and resulting morphogenetic readouts. External induction (light, drugs, ligands), organizer placement, optogenetic gradients, morphogen sources (WNT, SHH, BMP, NODAL), and mechanical or geometric constraints are applied as experimentally imposed inputs. These inputs are interpreted through synthetic mechanisms that bias pattern formation, including optogenetically or chemically generated morphogen gradients, inducible synthetic organizers, reaction–diffusion circuits based on activator–inhibitor dynamics and ratio-controlled secretion (e.g., WNT:DKK), and feedback control loops that modulate boundary sharpening or organizer output under defined conditions. In early-stage aggregates, constrained organoid geometries, or proof-of-concept 3D systems, these engineered mechanisms have been used to experimentally induce or bias symmetry breaking, axis formation, patterned domains, boundary stabilization, partial recovery after perturbation, and more reproducible tissue geometries relative to spontaneous self-organization. However, such implementations remain sensitive to parameter choice, diffusion limits, tissue scaling, and biological variability. Examples shown correspond primarily to Categories I–II early-stage or context-dependent implementations rather than stable, long-term morphogenetic control across mature, multi-lineage organoid systems. Biological constraints—including variability, diffusion limits, scaling sensitivity, and maturation drift—limit the extent to which autonomous or fully self-maintaining morphogenetic architectures can presently be achieved. Image created by the author using OpenAI’s ChatGPT image-generation tool. The conceptual design, scientific content, and final edits were performed and verified by the author.

or hybrid control strategies for reproducible outcomes.

4.1. Engineering self-organization and pattern formation

Intrinsic self-organization in organoids reflects the natural capacity of stem cells to interpret local morphogen signals, mechanical forces, and cell-to-cell interactions to generate symmetry breaking, axial specification, and spatial patterning. However, these processes are often stochastic, sensitive to small fluctuations, and difficult to replicate across experiments.^{96,97} Programmable morphogenesis introduces engineered regulatory systems that override or redirect these endogenous programs, enabling more experimentally constrained developmental outcomes under defined induction conditions. In constrained organoid geometries and early developmental windows, synthetic gene circuits can modulate a cell's sensitivity to key morphogens such as WNT, BMP, SHH, and FGF, or impose synthetic thresholds that restrict developmental transitions to defined conditions. By dampening or amplifying endogenous feedback loops, these circuits reduce noise and stabilize desired outcomes.^{98,99} This level of control allows researchers to bias organoid development toward more interpretable trajectories rather than relying solely on the probabilistic unfolding of intrinsic signaling networks.

A major conceptual framework underlying pattern formation is rooted in reaction–diffusion mechanisms described by Turing, in which interacting activators and inhibitors generate spatial patterns such as stripes, spots, and periodic domains. Synthetic biology provides tools for constructing experimental versions of these systems by engineering cells to produce activators, inhibitors, or both in precisely controlled ratios. For instance, cells can be programmed to secrete WNT agonists in conjunction with DKK inhibitors, creating synthetic landscapes that can generate periodic patterning under defined parameter regimes or spatial differentiation within organoids.^{48,100} Manipulating production rates, diffusion dynamics, and degradation parameters allows partial reconstruction of selected aspects of natural patterning events under constrained experimental conditions. In proof-of-concept organoid and spheroid systems, these engineered reaction–diffusion networks serve not only as patterning tools but also as experimental platforms for testing mathematical models of morphogenesis in a biologically realistic context.

Beyond reaction–diffusion systems, programmable morphogenesis enables the construction of synthetic spatial gradients that define positional information with high precision. Optogenetic tools can generate sharply defined, customizable illumination patterns that activate or repress signaling pathways with spatial specificity. By adjusting wavelength, intensity, or exposure duration,

synthetic SHH, BMP, or WNT gradients can be sculpted with nearly arbitrary geometry.^{12,101} Chemogenetic systems complement this approach by producing morphogen gradients through controlled diffusion of ligands or small-molecule inducers.¹⁰² These synthetic gradients provide a foundational scaffold for reproducible regionalization, enabling organoids to acquire stable polarity, directional identity, and multi-domain patterning without reliance on endogenous variability.

Finally, programmable morphogenesis incorporates engineered signaling hubs, also known as synthetic organizers, that replicate the role of embryonic organizers in orchestrating large scale tissue patterning.^{85,99,103} These signaling centers can be built from engineered cells implanted into organoids or generated *in situ* through inducible expression systems. Synthetic organizers impose spatially restricted secretion of morphogens, directing symmetry breaking, axis initiation, and boundary formation. Their activity can be regulated by light, chemical inducers, or genetic circuits, allowing highly precise temporal control. Synthetic organizers can bias organoid patterning toward experimentally defined developmental sequences in controlled settings, enabling researchers to define the architecture of the developing tissue through strategic placement and timing of organizer activation.

4.1.1. Parameter sensitivity, model fitting, and reproducibility in reaction–diffusion and organizer-based systems

Reaction–diffusion and organizer-based patterning systems are highly sensitive to quantitative parameter choices, including morphogen production rates, diffusion coefficients, degradation kinetics, receptor abundance, and feedback strengths. Small changes in these parameters can shift systems between qualitatively distinct regimes, such as uniform expression, unstable oscillation, or periodic pattern formation. In organoids, additional sensitivity arises from geometry, tissue size, and growth rate, which directly alter diffusion distances and effective reaction times. As a result, parameter sets that generate patterned domains under constrained experimental conditions in small aggregates may fail or collapse when scaled to larger 3D tissues.

Model-guided design has become essential for engineering these systems. Reaction–diffusion architectures are typically first explored using partial differential equations or agent-based models that predict spatial patterning as a function of kinetic and transport parameters. Experimental data—such as spatial reporter intensity, domain width, or pattern periodicity—are then used to fit model parameters using regression, Bayesian inference, or optimization-based approaches. Iterative cycles of modeling and experimentation allow refinement

of production, degradation, and feedback strengths until predicted and observed patterns converge.^{104,105}

Scaling to 3D organoids introduces major reproducibility challenges. Diffusion gradients become depth-dependent, leading to spatially heterogeneous effective parameters. Cell density, extracellular matrix composition, and tissue compaction further alter transport and signaling kinetics. In addition, stochastic variation in initial aggregate size, organizer placement, or circuit expression leads to divergence of patterning outcomes across replicates. These effects explain why reaction–diffusion or organizer designs that are robust in 2D or small spheroids often display increased variability, distorted pattern geometry, or complete pattern failure in large organoids.

Improving reproducibility therefore requires strategies that reduce parameter fragility, including feedback-stabilized organizer circuits, self-scaling gradient designs, size-normalized induction protocols, and dynamic closed-loop adjustment of morphogen output. Combining quantitative modeling with real-time imaging and feedback control is increasingly necessary to maintain intended patterning trajectories as tissues grow and remodel in three dimensions.¹⁰⁶ These scaling sensitivities and parameter fragilities illustrate why reproducibility and translational generalization remain challenging despite the mechanistic elegance of reaction–diffusion and organizer-based designs.

4.1.2. Material regulation as a control layer in programmable organoids

Material environments are not passive scaffolds for organoid growth but active regulatory layers that shape cell fate, patterning, and morphogenesis. Extracellular matrix (ECM) composition, stiffness, viscoelasticity, degradability, and ligand presentation influence how cells interpret genetic and biochemical programs. As programmable organoids increasingly rely on engineered gene circuits, epigenetic memory, and closed-loop control, material regulation should be treated as a parallel control layer that can act as an input, constraint, and feedback element within developmental systems.^{107,108}

Extracellular matrix mechanics strongly influence lineage decisions and tissue organization. Variations in stiffness and viscoelastic relaxation alter cytoskeletal tension, nuclear mechanics, and mechanotransduction pathways such as YAP/TAZ, integrins, and focal adhesion signaling. In organoids, these effects translate into shifts in proliferation, differentiation timing, and regional identity. For example, softer matrices tend to favor neural and epithelial fates, whereas stiffer environments promote mesenchymal or contractile phenotypes. Thus, mechanical properties can function as programmable parameters that

bias developmental trajectories, analogous to morphogen concentration or gene circuit state.^{109,110}

Hydrogels and synthetic matrices provide a particularly powerful platform for material programming. Modern biomaterials allow independent tuning of stiffness, degradability, ligand density, and dynamic remodeling. Light-, enzyme-, or chemically responsive hydrogels enable temporal control of material properties, allowing environments to soften, stiffen, or release ligands during development. Such dynamic materials can be aligned with developmental timing, for example, by softening during early patterning and stiffening during maturation, thereby acting as a temporal “scheduler” of morphogenesis.¹¹¹

Mechanical cues can also be integrated as explicit inputs to genetic and epigenetic modulation and memory mechanisms. Mechanosensitive promoters, tension-responsive transcription factors, and signaling pathways that respond to substrate stiffness can be wired into synthetic logic or feedback systems. In this way, material state becomes part of the sensing layer in closed-loop developmental control.³⁰ For instance, deviations in tissue compaction or stiffness could be detected by mechanosensitive reporters and used to trigger corrective genetic or optogenetic responses, linking material and genetic regulation into a unified control architecture.¹¹²

Materials can further participate in feedback and closed-loop control. In cybergenetic or hybrid systems, imaging of tissue geometry or mechanical state can guide external actuation of material properties, such as light-triggered hydrogel remodeling or controlled release of matrix-bound factors.¹¹³ Conversely, genetically programmed cells can secrete matrix-modifying enzymes, crosslinkers, or ECM components, enabling tissues to reshape aspects of their material environment within engineered constraints. This creates bidirectional coupling between genetic programs and material state, analogous to biochemical feedback but operating through physical properties.¹¹⁴

Integration of material regulation with synthetic circuits and cybergenetic systems highlights the interdisciplinary nature of programmable organoids. Genetic logic, epigenetic memory, biochemical signaling, and physical environment together define developmental state. Treating materials as a formal control layer—rather than as background support—allows design of systems in which inputs (morphogens, light, mechanics), modules (gene circuits, epigenetic writers, material properties), and readouts (lineage choice, pattern geometry, stability) are jointly optimized. As programmable organoids incorporate increasingly layered regulatory architectures, coupling genetic and computational control with programmable materials will be essential for achieving robust, scalable, and physiologically relevant morphogenesis.

4.2. Reconstructing canonical human developmental events

Programmable morphogenesis enables organoids to reenact developmental events that are otherwise inaccessible in human embryos, particularly early processes such as gastrulation, germ layer specification, and axial formation. It is important to emphasize that most current demonstrations of these capabilities occur in early-stage organoids, small aggregates, or simplified patterning contexts. While these studies provide compelling mechanistic insight, they do not yet represent a fully reproducible reconstruction of human developmental events across mature, multi-lineage organoid systems. Through engineered control of WNT, BMP, and NODAL pathways, researchers can induce spatially asymmetric signaling that results in organized mesendoderm formation, an essential hallmark of gastrulation-like transitions. In early-stage organoid and aggregate models, synthetically guided gastrulation-like patterning can produce more anatomically interpretable structures than spontaneous systems.^{54,85,87} This creates a powerful model for studying early human development and for investigating congenital abnormalities that originate during gastrulation but cannot be ethically examined *in vivo*.

The ability to induce and orient body axes with precision is a central goal of developmental engineering. Synthetic organizers, together with optogenetic and chemogenetic tools, enable experimentally guided induction of anterior–posterior, dorsal–ventral, and medial–lateral axes in constrained contexts within organoids. For example, localized activation of SHH pathways can generate stable ventral domains, while spatially confined WNT stimulation can posteriorize tissues or induce primitive streak-like structures.^{54,115,116} Synthetic manipulation of activator–inhibitor dynamics further refines these axes by sharpening boundaries and maintaining stable domain identities. These engineered processes recapitulate selected and simplified aspects of the logic of human axis formation in organoid models, providing an experimentally tractable system for testing hypotheses about symmetry breaking, positional information, and the integration of signaling cues across developing tissues.

Reconstructing neural tube patterning is another achievement made possible through programmable morphogenesis. The neural tube emerges from gradients of SHH, BMP, and WNT signaling, which establish dorsal–ventral neural progenitor domains. In neural organoid models and early-stage patterned aggregates to respond to light-controlled SHH induction or CRISPRa-mediated BMP repression, researchers can create controlled dorsal or ventral regions that recapitulate key features of neural tube development.^{85,117} Manipulating the steepness or duration of these gradients reveals how progenitor populations

interpret positional cues and provides an experimental platform for understanding congenital disorders of neural tube closure or patterning.

At a higher organizational level, programmable morphogenesis enables precise regionalization of the forebrain, midbrain, and hindbrain within cerebral organoids. Controlled activation of FGF8, SHH, WNT, and retinoic acid signaling pathways allows researchers to impose distinct anterior–posterior identities.^{118–120} Epigenetic boundary engineering can further stabilize the segregation of these regions, ensuring that forebrain, midbrain, and hindbrain compartments develop in proper proportion and orientation. These strategies permit the creation of patterned brain organoids with region-specific architectures, enabling the study of inter-regional communication, developmental timing, and mechanisms underlying region-specific neurodevelopmental disorders.

5. Feedback, sensing, and closed-loop control in organoids: Emerging and conceptual architectures (primarily categories II–III)

This section focuses primarily on emerging and conceptual frameworks (Categories II and III) that outline how biosensing, cybergenetics, and AI-guided systems (Category III conceptual architectures) may expand the scope of closed-loop developmental control beyond what is currently routine in organoid platforms.

As organoid engineering advances toward increasingly structured and experimentally interpretable developmental systems, the field is beginning to integrate the concepts of biosensing, adaptive regulation, and closed-loop control into living tissues. Traditional organoid protocols rely on exogenous interventions delivered according to fixed schedules, with cells responding passively to environmental cues.^{97,121,122} Yet organoid development itself is dynamic and nonlinear, shaped by stochastic molecular fluctuations, variable mechanical environments, and emergent multicellular interactions.^{96,123} Improving the reliability of morphogenesis therefore motivates exploration of a paradigm in which organoids not only receive developmental instructions but may, in emerging hybrid systems, be coupled to sensing and feedback mechanisms that detect deviations and support externally mediated corrective responses through hybrid or cybergenetic feedback architectures, often requiring external computation. At present, most implementations of such adaptive frameworks remain limited to hybrid or proof-of-concept systems rather than long-term, fully internalized organoid controllers. In particular, stochastic heterogeneity, spatial diffusion limits, and maturation drift reduce the reliability of internally implemented controllers

and complicate long-term translational deployment without external monitoring.

This emerging framework integrates several layers of control, including biosensors that monitor molecular, metabolic, or mechanical states, internal genetic circuits that interpret this information and implement regulatory actions, and external computational systems powered by machine learning that analyze complex developmental data and recommend optimal interventions. Together, these capabilities form the foundations of closed-loop developmental control, including genetic, cybergenetic, and AI-guided implementations, a field that merges synthetic biology, developmental biomechanics, systems control, and artificial intelligence. Throughout this review, we use “closed-loop developmental control” as the general term for systems that sense developmental state and adjust regulatory outputs in response. We use “genetic closed-loop control” for fully biological implementations, “cybergenetic closed-loop control” when a computer is in the loop, and “AI-guided closed-loop control” when machine-learning algorithms drive intervention. In current organoid platforms, such closed-loop architectures remain largely externally mediated or proof-of-concept implementations, rather than fully internalized or autonomously operating developmental controllers. The interpretive limits of higher-tier and closed-loop architectures are further clarified in [Appendix](#).

5.1. Biosensors and reporting circuits for emerging closed-loop developmental control

Biosensors constitute the primary interface through which programmable organoids become aware of their internal physiological state. Classical reporter systems, including fluorescent, luminescent, and transcriptionally encoded indicators, provide real-time visibility into the activation of key signaling pathways such as WNT, BMP, SHH, Notch, Ras-ERK, or Hippo. Fluorescent protein-based reporters reveal spatial domains of pathway activity, allowing visualization of symmetry-breaking events or lineage specification boundaries.^{124,125} Fluorescent timers further enrich these systems by encoding the temporal history of gene expression through predictable shifts in emission spectra, enabling reconstruction of lineage dynamics with temporal resolution.

Luminescent reporters extend these capabilities by offering long-term, low background measurements suitable for monitoring slow developmental processes or large 3D structures where phototoxicity may compromise viability.^{126,127} When coupled to enhancer-specific barcodes or immediate early regulatory elements, these systems allow researchers to map the dynamics of developmental enhancers and track the emergence of spatial heterogeneity across complex tissues.

Beyond protein-based reporters, the field is now rapidly adopting probe-free genetic sensors that operate entirely within endogenous molecular networks. RNA-based regulatory circuits, including toehold switches, riboregulators, and synthetic RNA logic gates, can detect the presence of specific transcripts or small molecules and convert these signals into transcriptional or translational outputs. Aptamer-based sensors recognize intracellular ligands such as ATP, NAD⁺, acetyl CoA, or cAMP, providing real-time readouts of metabolic states or signaling flux.¹²⁸⁻¹³¹ Light up RNA aptamers, such as Broccoli, Mango, and Pepper, enable fluorescent reporting without protein intermediates, reducing metabolic burden and allowing deep integration into endogenous RNA dynamics.¹³²⁻¹³⁴

Together, these systems increasingly position organoids as self-reporting developmental platforms that can reveal their internal states with spatial and temporal precision and lay the groundwork for more advanced and increasingly internally integrated control architectures ([Table 2](#)).

5.1.1. Classes of closed-loop developmental control systems: From hybrid implementations to conceptual architectures

Closed-loop developmental control systems can be classified according to how sensing, computation, and actuation are distributed between biological and external computational layers. Each class exhibits characteristic experimental implementations, technical constraints, and domains in which it is most effective.

Fully genetic closed-loop controllers implement sensing, decision-making, and actuation entirely within engineered cells. Typical examples include transcription-factor-based or CRISPRa/i-based feedback circuits, genetic integral controllers, toggle-switch regulators, and feedback-regulated morphogen secretion systems operating under internally implemented feedback control that modulate output in response to internal reporters. These systems aim to reduce reliance on external scheduling and, in constrained settings, may modestly improve scalability but are constrained by biological response times, limited computational complexity, genetic burden, and vulnerability to mutation or silencing during long-term culture. Once deployed, parameter tuning is difficult, and adaptation to unforeseen system states is limited. As a result, fully genetic controllers are best suited for stabilizing lineage identity, buffering noise in morphogen signaling, enforcing competence windows, and supporting more stable developmental states under defined conditions in relatively stable environments.^{135,136} Their long-term stability in organoids is further constrained by heterogeneity, silencing, and developmental state drift, which limit reliable scaling beyond controlled experimental contexts.

Table 2. Biosensors and feedback control modules for programmable development

Sensor/Control module	Detection modality	Response speed	Integration with synthetic circuits	Developmental role	Evidence maturity (Category I/II/III)
Fluorescent pathway reporters (e.g., WNT, SHH, BMP, ERK reporters)	Fluorescent protein expression downstream of pathway-responsive elements	Minutes to hours	Provides real-time readout of morphogen activity; can feed signals into downstream logic gates	Tracks lineage decisions, monitors signaling gradients, validates circuit performance	Category I
Luminescent reporters	Luciferase expression controlled by signaling-responsive promoters	Minutes to hours (low noise)	Compatible with long-term monitoring and closed-loop algorithms	Non-invasive tracking of slow developmental processes, stability assessments	Category I
RNA-based toehold switches/ riboregulators	RNA hybridization, endogenous transcript detection	Seconds to minutes	Direct coupling to CRISPRa/i or translational control modules	Context-dependent activation of lineage programs, sensing endogenous developmental states	Category II
Metabolic aptamer sensors (e.g., ATP, SAM, NAD ⁺ , acetyl-CoA)	Metabolite binding to RNA aptamers	Seconds	Link metabolic state to gene circuit activation; modulate fate transitions	Synchronizes metabolic state with developmental progression, models metabolic disorders	Category II
Mechanosensitive gene reporters (e.g., YAP/TAZ activity sensors)	Nuclear localization or transcription driven by mechanical forces	Seconds to minutes	Feeds mechanical information into gene-expression circuits	Regulates morphogenesis, symmetry breaking, and niche sensing	Categories I–II (limited 3D validation)
Synthetic feedback controllers (negative or positive feedback)	Internal sensing of circuit output or key developmental genes	Fast to intermediate	Maintains gene expression within defined ranges; buffers noise	Stabilizes lineage identity, shapes gradients, enforces temporal order	Category II
Integral feedback controllers	Accumulates deviations over time; adjusts circuit output	Slow to intermediate	Ensures robust homeostasis even under perturbation	Maintains stable morphogen thresholds and timing of transitions	Category II (limited organoid-scale validation)
Optogenetic feedback loops	Light-controlled sensing and actuation	Milliseconds to seconds	Enables precision tuning of pathway activity using ML-guided stimulation	Fine control of spatial patterning, gradient sharpening, and axis formation	Categories I–II
Chemogenetic control modules (e.g., Tet-On, CreERT2, rapalog switches)	Small-molecule activation	Minutes to hours	Couples external control inputs to developmental regulators	Inducible lineage switching, timed activation of transient competence windows	Category I
Hybrid cybergenetic systems (cell–computer feedback)	Live sensing + computational prediction	Variable; usually fast	Reinforcement learning or ML algorithms adjust circuit inputs	Predictive control of morphogenesis, pattern stabilization, correction of deviations	Category II (hybrid implementations)

Optogenetic and chemogenetic feedback systems introduce an external control layer. In these platforms, cells express light- or drug-responsive regulators that control morphogen production, lineage factors, or circuit activity, while reporters are monitored through live imaging and stimulation patterns are adjusted manually or semi-

automatically. These systems enable precise spatiotemporal perturbation but require continuous instrumentation and human or algorithmic intervention. Performance is limited by phototoxicity, light penetration, drug diffusion kinetics, and the difficulty of scaling to large numbers of organoids. Consequently, such systems are most appropriate for

mechanistic studies of pattern formation, gradient shaping, axis induction, and reversible perturbation experiments rather than long-term internally self-maintaining development.^{137,138}

Hybrid cybergenetic systems couple biological sensors to external computational controllers. Fluorescent, luminescent, RNA-based, or metabolic reporters provide real-time state information, which is processed by algorithms that determine corrective actions delivered via optogenetics, chemogenetics, or inducible gene circuits. Representative examples include cell-in-the-loop patterning systems and computer-guided morphogen modulation platforms. These systems can support adaptive correction of developmental drift and stabilization of spatial patterning under defined experimental conditions, but they depend on continuous imaging, data processing, and hardware integration. Latency between sensing and actuation limits fast dynamics, and system complexity increases experimental overhead. Hybrid cybergenetic control is therefore best suited for stabilizing tissue-scale patterning, maintaining gradients, and studying adaptive morphogenesis under variable conditions.^{16,139}

Artificial intelligence-guided cybergenetic systems extend this framework by incorporating machine learning models that predict developmental trajectories and recommend interventions. Real-time imaging and multi-omic sensing feed into predictive or reinforcement-learning algorithms that guide optogenetic, chemogenetic, or genetic actuation. These systems may be useful for optimizing complex morphogenetic programs and compensating for biological variability, but they require large training datasets, risk overfitting to specific experimental conditions, and often lack interpretability. Additional regulatory and data-governance challenges arise for translational applications. AI-guided platforms are therefore most appropriate for exploratory design of complex developmental architectures and for systems in which adaptability outweighs interpretability.^{140,141}

Finally, conceptual multi-layer developmental controllers aim to integrate genetic feedback, epigenetic memory, biosensing, and embedded logic within cybergenetic architectures to approximate more internally coordinated regulation, while still requiring external oversight and constraint. These architectures may include internal error-correction and safety modules. While they offer the greatest degree of internalized regulation and potential long-term stability, they are also the most difficult to engineer, debug, and regulate, and they carry heightened biosafety considerations due to the potential for emergent behavior. Such systems are conceptually positioned as potential candidates for long-term tissue engineering, regenerative medicine constructs, and developmental models that require stable identity and resilience to

perturbation with minimal external control.^{5,142}

Taken together, no single cybergenetic class is universally optimal. Control architectures should be selected based on developmental timescale, required autonomy, acceptable risk, and experimental complexity. Early-stage mechanistic studies favor externally guided or hybrid systems, whereas long-term developmental and translational platforms may benefit from layered control architectures with increasing integration of internal feedback that integrates genetic, epigenetic, material, and cybergenetic regulation.

Under the evidence taxonomy used here, hybrid cybergenetic platforms currently approach Category II, whereas largely self-contained multi-layer controllers remain Category III conceptual architectures.

Importantly, the primary barrier to fully internalized closed-loop developmental control is not the absence of sensing, logic, or computational frameworks, but the biological variability inherent to organoid systems. Batch effects, asynchronous differentiation, metabolic heterogeneity, incomplete maturation, and lack of vascular integration prevent reliable state estimation and correction at the tissue scale. As a result, Tier 3 and Tier 4 architectures remain aspirational, not because they are theoretically underspecified, but because current organoid platforms lack the stability and observability required for autonomous control. Accordingly, Tier 3 and Tier 4 should be read throughout this review as biologically premature but conceptually informative, serving as design targets rather than indicators of near-term feasibility.

5.2. Closed-loop developmental control

Once biosensors provide insight into organoid state, adaptive regulatory systems translate this information into dynamic modulation of developmental processes. Natural tissues maintain functional integrity through networks of regulatory feedback loops that stabilize signaling activity and coordinate growth. Synthetic biology now offers tools that may allow engineering of analogous regulatory architectures within organoids, potentially supporting correction of deviations during morphogenesis under feedback regulation.^{31,143}

Adaptive circuits modulate gene expression or signaling output in response to fluctuations detected by biosensors. For example, engineered pathways can increase morphogen production in regions where signaling becomes insufficient, preventing collapse of axis formation, or reduce proliferative activity when tissue density surpasses a desirable threshold.^{10,144,145} Other designs stabilize differentiation trajectories by buffering against stochastic pulses of transcription factor activity that would otherwise push cells into inappropriate lineages.^{100,146,147} Such circuits can help stabilize selected developmental trajectories under

defined perturbation conditions, whether arising from intrinsic noise or external influences.

5.2.1. Evaluation metrics for closed-loop developmental control

To enable systematic comparison of closed-loop and AI-guided developmental systems, standardized evaluation metrics are required. Four core performance dimensions are particularly relevant for programmable organoids:

(a) **Stability:** the ability of a system to maintain a desired developmental state or pattern over time without oscillation, collapse, or drift. Stability can be quantified by variance of reporter signals, persistence of spatial domains, or maintenance of lineage proportions across extended culture.

(b) **Response time:** the delay between detection of a deviation and corrective action. This can be measured as the time required for reporters or phenotypes to return within a predefined tolerance after perturbation.

(c) **Robustness:** the capacity to preserve developmental trajectories across biological noise, batch variation, and parameter uncertainty. Robustness is assessed by reproducibility of outcomes across replicates, genetic backgrounds, and environmental conditions.

(d) **Disturbance rejection:** the ability to recover from imposed perturbations such as transient morphogen depletion, mechanical disruption, or circuit inhibition. Performance can be measured by recovery speed, overshoot, and final-state accuracy after disturbance.

Together, these metrics provide a control-theoretic framework for evaluating whether cybergenetic systems merely function or function reliably under realistic developmental variability.^{148,149}

5.2.2. Standard experimental workflow for closed-loop developmental systems

A standard experimental workflow is needed to evaluate cybergenetic closed-loop systems in a reproducible manner. A general pipeline consists of:

(a) **Define target trajectory:** specify desired lineage proportions, spatial domains, or signaling dynamics as quantitative reference states.

(b) **Embed sensing layer:** introduce reporters or biosensors that continuously monitor relevant molecular, metabolic, or mechanical states.

(c) **Implement controller:** connect sensors to genetic, optogenetic, chemogenetic, or computer-guided actuators using defined logic or learning algorithms.

(d) **Baseline characterization:** measure stability, response time, robustness, and disturbance rejection under

unperturbed conditions.

(e) **Perturbation testing:** introduce controlled disturbances (signal withdrawal, mechanical stress, circuit inhibition) to quantify recovery behavior.

(f) **Long-term drift analysis:** track performance across extended culture to assess stability, memory erosion, or controller fatigue.

(g) **Iterative refinement:** adjust sensor placement, controller logic, or actuation parameters based on quantitative performance metrics.

This workflow provides a common experimental language for comparing different cybergenetic and AI-guided platforms and for distinguishing proof-of-concept demonstrations from robust developmental control systems.^{149,150}

Recent advances have demonstrated the feasibility of constructing complex cybergenetic closed-loop systems in mammalian cells, where molecular sensing is directly linked to signal processing and corrective actuation. These systems can preserve stable gene-expression states under sustained perturbations and maintain morphogen gradients with remarkable robustness.^{99,151} When adapted to organoid contexts, these systems have so far been demonstrated primarily in simplified or early-stage 3D tissues and should be interpreted as proof-of-concept implementations rather than mature controllers of multi-lineage development. In such settings, they illustrate how closed-loop strategies may improve stability of spatial patterning, reduce lineage drift, and prolong maintenance of desired tissue organization, but significant challenges related to heterogeneity, scaling, and long-term robustness remain.^{152,153}

The integration of machine learning guided intervention further enhances these capabilities. High-resolution imaging, multimodal reporters, and real-time transcriptomic or epigenomic profiling generate rich datasets that capture the evolving state of an organoid. Machine learning models, including predictive neural networks and reinforcement learning agents, can interpret these data streams to forecast developmental trajectories, detect early divergence from intended outcomes, and recommend targeted interventions.¹⁵⁴⁻¹⁵⁷ These interventions may include optogenetic stimulation, timed induction of signaling pathways, or modulation of synthetic circuit activity.

The result is a hybrid closed-loop (cybergenetic) framework in which biological systems perform local, autonomous regulation while computational systems provide global oversight and predictive decision-making. Such integration paves the way for organoids that can maintain stable developmental programs even under complex dynamic conditions.

5.3. Merging artificial intelligence with programmable organoids

Artificial intelligence plays a pivotal role in elevating programmable organoids from engineered tissues to predictive and designable developmental systems. AI-driven modeling platforms enable researchers to simulate how synthetic gene circuits, signaling networks, or mechanical perturbations influence morphogenesis across scales.^{158,159} By incorporating single-cell transcriptomics, chromatin landscapes, proteomic profiles, and live-imaging data, these models achieve increasingly accurate representations of cellular decision-making and tissue morphodynamics.

Generative AI models further expand the design space by proposing novel circuit architectures or morphogenetic strategies that align with specified developmental outcomes. Deep generative networks, including diffusion models, graph neural networks, and transformer-based sequence design algorithms, analyze high-dimensional datasets from both natural embryos and synthetic organoid systems to infer robust and generalizable design rules.¹⁶⁰⁻¹⁶² These systems can recommend unexpected combinations of signaling cues, feedback loops, or circuit topologies that are capable of producing complex patterning behaviors that would otherwise be difficult to engineer manually.

Artificial intelligence also optimizes circuit performance through evolutionary search and reinforcement-learning frameworks.¹⁶³⁻¹⁶⁵ These algorithms iteratively refine promoter strengths, enhancer configurations, transcription factor ratios, and optogenetic stimulation patterns, converging on parameter sets that produce stable and reproducible outcomes. When coupled with real-time biosensing, such systems can conduct closed-loop optimization directly on living organoids, adjusting circuit activity or environmental conditions as tissues develop.

Collectively, the integration of AI with programmable organoids establishes a new class of cybergenetic developmental systems that function as systems that integrate biological sensing with computational control to guide development, executing synthetic genetic logic, and evolving under computational guidance. This convergence of synthetic biology and artificial intelligence positions programmable organoids as powerful platforms for modeling human development, dissecting disease mechanisms, and designing entirely new morphogenetic processes with unprecedented precision.

6. Applications of programmable organoids: Current capabilities and forward-looking opportunities

This section integrates Category I and Category II applications, with Category III implications discussed

explicitly as forward-looking translational opportunities rather than established practice.

Programmable organoids enable more precise experimental control of human developmental processes than conventional organoid systems. By integrating genetic circuits, epigenetic modulation, synthetic organizers, and feedback-regulated or cybergenetic regulatory architectures, organoids can reconstruct developmental processes, model disease mechanisms, and engineer therapeutic tissues in a manner that is both mechanistically rigorous and experimentally controllable, a direction supported by recent analyses emphasizing the expanding role of organoid systems in neurodevelopmental disease research.^{5,32,166,167} These advances position programmable organoids as a cornerstone technology for developmental biology, mechanistic disease modeling, and regenerative medicine, with broad implications for understanding human-specific biology, dissecting complex disorders, and building next-generation therapeutic systems (Figure 5).

These applications should be interpreted in the context of emerging capabilities rather than standardized practice. Many examples discussed below represent experimental or proof-of-concept studies performed in controlled settings, and significant variability, scaling challenges, and biological constraints currently limit routine implementation across complex organoid systems.

6.1. Deep mechanistic interrogation of human development

Programmable organoids create opportunities to explore aspects of human embryogenesis that cannot be ethically or technically accessed *in vivo*. Classical animal models diverge markedly from humans in critical developmental parameters, including enhancer logic, chromatin remodeling tempo, morphogen sensitivity thresholds, and neurodevelopmental patterning, which limits their ability to capture human-specific regulatory grammars.^{168,169} Organoids derived from human pluripotent stem cells retain this species-restricted architecture, enabling the study of primate-specific developmental programs, human germ layer patterning dynamics, and region-specific enhancer promoter interactions.^{170,171}

Synthetic control systems allow causal replay of developmental hypotheses. Engineered organizers can initiate gastrulation-like events with precise spatial localization; optogenetic gradients can reproduce morphogenetic waves with tunable strength and duration; and synthetic epigenetic modulation modules can impose competence windows that mirror temporally restricted embryonic transitions.^{36,172} These capabilities enable rigorous testing of long-standing questions regarding the sequence of axial specification, the necessity of symmetry-

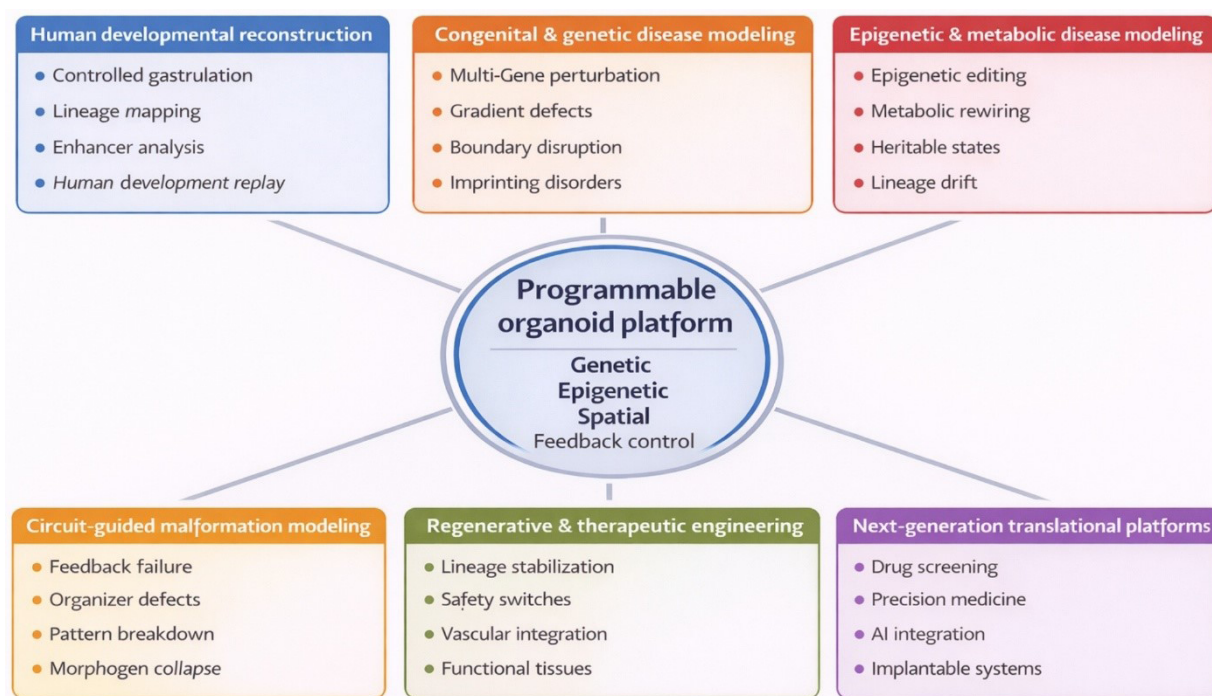


Figure 5. Applications and forward-looking opportunities of programmable organoids across development, disease modeling, and regenerative engineering. Programmable organoid systems provide a framework for experimentally guided investigation of developmental biology, disease mechanisms, and regenerative engineering by integrating genetic, epigenetic, spatial, and feedback-based regulatory strategies. (i) Human developmental reconstruction supports partial and experimentally guided modeling of early developmental processes, including gastrulation-like transitions, axis specification, and lineage diversification in constrained organoid contexts. These systems enable mapping of lineage hierarchies, enhancer dynamics, and chromatin-state changes to test causal developmental hypotheses not readily accessible *in vivo*. (ii) Modeling congenital and genetic disease networks enables controlled multi-gene perturbation, manipulation of morphogen gradients, and interrogation of spatial patterning defects or imprinting-associated dysregulation within defined three-dimensional systems, allowing longitudinal analysis of disease-relevant phenotypes under experimentally constrained conditions. (iii) Modeling epigenetic and metabolic disease states employs targeted epigenetic editors and metabolic modulation (e.g., acetyl-CoA, NAD⁺ perturbation) to induce defined chromatin and metabolic alterations associated with disease, enabling investigation of how these perturbations bias lineage specification and morphogenesis in controlled contexts. (iv) Circuit-guided modeling of developmental malformations uses synthetic organizers, feedback modules, and reaction-diffusion-inspired systems to experimentally perturb boundary formation, symmetry breaking, and tissue specification, providing mechanistic insight into neural tube, cardiac, and forebrain patterning defects in early-stage organoid models. (v) Regenerative and therapeutic engineering applications explore strategies for lineage stabilization, incorporation of safety switches, and partial vascular or stromal integration within organoid systems. While translation remains experimental, these approaches provide design principles for improving stability and maturation of engineered tissues under defined *in vitro* conditions. (vi) Next-generation translational platforms position programmable organoids as human-specific systems for drug screening, mechanistic testing, and exploratory precision-medicine modeling. In combination with computational and artificial intelligence (AI)-guided optimization strategies, these platforms represent a conceptual foundation for future engineered tissue constructs, though implantable applications remain a long-term goal rather than established practice. Image created by the author using OpenAI's ChatGPT image-generation tool. The conceptual design, scientific content, and final edits were performed and verified by the author.

breaking signals, or the thresholds that govern lineage bifurcation.

Programmable organoids further permit systematic perturbation across molecular, cellular, and tissue scales, revealing how gene regulatory networks, chromatin states, mechanical cues, and spatial signaling domains converge to construct human form. Emerging directions include incorporation of immune-developmental interfaces, such as fetal macrophage signaling or early thymic epithelial interactions, which provide insights into how immune elements participate in shaping organ architecture during early human life.^{173,174}

6.2. Disease modeling with programmable perturbations

Programmable organoids provide a transformative framework for modeling congenital, genetic, and acquired diseases by enabling precise perturbation of developmental pathways in a controlled and human-specific context. Traditional organoid models often rely on passive observation or isolated gene disruptions, whereas programmable systems allow explicit reconstruction of multi-gene network dysfunctions, temporal mispatterning, and disrupted intercellular communication, which are features central to many developmental disorders.^{78,175}

Synthetic epigenetic editors enable faithful modeling of epigenetic disease states, such as imprinting disorders, enhanceropathies, and chromatin-boundary defects.^{15,176,177} Locus-specific methylation, histone modification, or chromatin-loop engineering can recreate pathogenic epigenomic landscapes and allow longitudinal tracking of their consequences for lineage specification and morphogenesis.

Programmable organoids also permit circuit-guided reconstruction of maldevelopment, such as neural tube closure defects, cardiac outflow tract malformations, or regionalization errors in the developing forebrain.^{178,179} By modulating organizer function, adjusting reaction–diffusion dynamics, or altering cell–cell feedback loops, researchers can simulate pathological contexts with unprecedented mechanistic precision.^{180,181}

A rapidly advancing frontier involves metabolic disease modeling, where engineered circuits control intracellular levels of acetyl-CoA, NAD⁺, or ROS to mimic metabolic congenital disorders. Similarly, synthetic immune–epithelial interfaces allow modeling of autoimmune and inflammatory developmental diseases by programming immune-like signals within organoids.^{182–184}

Together, these approaches enable programmable organoids to dissect disease mechanisms in a manner that unites molecular precision with tissue-level phenotypic relevance.

6.3. Regenerative medicine and cell therapy engineering

Programmable organoids offer powerful opportunities for regenerative medicine by enabling the construction of tissues that recapitulate developmental logic rather than relying solely on terminal differentiation cues. Regeneration often requires niche environments rich in spatially patterned signals, mechanical constraints, and lineage-supportive microstructures. Synthetic organizers and engineered gradients allow reconstruction of developmental niches *in vitro*, promoting accurate cell fate specification and functional maturation.^{47,185–187}

Programmable epigenetic and signaling circuits allow long-term stabilization of therapeutic cell fates, addressing a major challenge in cell therapy where transplanted cells frequently lose identity or respond aberrantly to inflammatory cues.^{10,15} Lineage-locking circuits, enhancer reinforcement modules, and self-stabilizing feedback programs help maintain differentiation states and prevent lineage drift after transplantation.^{188,189}

Advances in programmable morphogenesis also enable the assembly of multi-tissue constructs incorporating vasculature, stromal components, and innervation. Synthetic pro-angiogenic circuits coordinate vascular

network formation; axon-guiding signaling architectures facilitate organized neuronal wiring; and programmed stromal cells support matrix remodeling and tissue integration.^{190–192} These developments move beyond isolated organoids toward integrated organ assemblies capable of supporting physiological function, modeling organ–organ interactions, or serving as pre-transplantation therapeutic units.

At the translational interface, layered safety circuits, including inducible kill switches, growth restriction modules, and immune evasive epigenetic programs, provide essential control for clinical applications. These synthetic safeguards help ensure compatibility, prevent uncontrolled proliferation, and enable external regulation of therapeutic constructs *in vivo*.^{193,194}

Collectively, programmable organoids establish a new paradigm for regenerative medicine: therapeutic tissues built not only from differentiated cell types but from engineered developmental programs that ensure structural integrity, functional stability, and long-term physiological performance.

7. Challenges and ethical considerations

Despite rapid progress, it is equally important to state clearly what programmable organoids are not yet capable of achieving. Current systems cannot reliably enforce deterministic development across large, heterogeneous, multi-lineage organoids; cannot fully overcome variability arising from diffusion limits, growth dynamics, and epigenetic drift; and cannot yet sustain long-term, autonomous control of complex morphogenesis without substantial external intervention. Most programmable strategies remain sensitive to parameter tuning, tissue size, and circuit stability, and many demonstrations occur in early-stage or simplified 3D systems. Recognizing these limitations is essential for realistically assessing the current state of the field and for guiding future engineering efforts.

As programmable organoids move toward increasingly sophisticated developmental capabilities, their scientific promise is accompanied by technical challenges, biosafety risks, and important ethical considerations. Engineering tissues that contain synthetic gene circuits, epigenetic programs, and self-organizing developmental logic requires careful evaluation of stability, predictability, and regulatory oversight.^{195–197} This section outlines the major scientific and ethical questions that must be addressed to ensure the responsible advancement of programmable organoid systems (Table 3).

7.1. Safety of engineered genetic and epigenetic modulation strategies

The introduction of synthetic regulatory modules into

Table 3. Technical and ethical challenges in programmable organoid systems

Challenge	Description	Risk	Mitigation Strategy
Off-target genetic or epigenetic activity	Unintended CRISPRa/i activation, base-editing changes, or ectopic chromatin modification	Aberrant lineage specification, mispatterning, unpredictable morphogenesis	High-fidelity Cas variants, genome-wide off-target profiling, orthogonal control systems
Genetic instability of synthetic circuits	Mutations, silencing, or loss of constructs during long-term culture or high transcriptional load	Circuit collapse, emergence of escape mutants, divergence across replicates	Safe-harbor integration, low-burden design, redundancy in key modules
Epigenetic drift and memory erosion	Loss or blurring of engineered chromatin states during division or stress	Loss of lineage fidelity, mosaic chromatin patterns, reduced reproducibility	Error-correcting epigenetic modulation modules, re-establishment modules, longitudinal chromatin profiling
Horizontal transfer of circuit components	DNA/RNA elements spreading via extracellular vesicles, viral vectors, or cell fusion	Mislocalized circuit function, loss of spatial specificity, unintended lineage rewiring	Genetic insulation motifs, compartment-specific promoters, containment sequences
Unintended emergent self-organization	Nonlinear interactions between endogenous and synthetic signals produce unplanned patterning	Aberrant domains, spontaneous symmetry breaking, uncontrolled morphogenetic events	Real-time imaging, predictive modeling, negative-feedback designs
Evolution or drift of embedded circuits	Adaptive mutation or silencing that alters thresholds or logic	Loss of developmental control, variability between replicates	Evolutionarily stable design principles, periodic reseeded, multi-layer redundancy
Lineage rewiring in human-derived tissues	Engineering neural, germline-like, or embryo-like domains raises conceptual concerns	Ambiguity regarding biological status or moral boundaries	Defined lineage limits, ethical oversight, transparent reporting
Embryo-like developmental features	Synthetic organizers and patterned epigenetic systems approach early embryonic structure	Regulatory uncertainty, potential reclassification as embryo models	Adherence to ISSCR guidelines, developmental boundary definitions
Governance gaps for autonomous/artificial intelligence-coupled systems	Lack of regulatory frameworks for self-regulated or ML-guided organoids	Biosafety concerns, ungoverned autonomy, reduced public trust	Algorithmic transparency, data-provenance safeguards, interdisciplinary governance
Clinical translation risks	Uncertain long-term stability of transplanted engineered tissues	Uncontrolled proliferation, mispatterning, immune activation	Safety switches, growth-restriction modules, staged preclinical testing

Abbreviation: ISSCR: International Society for Stem Cell Research.

organoids brings inherent biosafety concerns, particularly regarding off-target effects, genetic stability, epigenetic integrity, and interactions between engineered components and surrounding cellular populations.¹⁹⁸⁻²⁰⁰

Off-target activity remains a central challenge, especially for CRISPR-based transcriptional regulators, base editors, and epigenetic modifiers. Even modest levels of unintended promoter activation, enhancer modification, or chromatin remodeling can alter lineage trajectories or generate aberrant cell states. As organoids grow in 3D environments, these off-target effects may propagate through cell–cell interactions, amplifying subtle errors into large-scale patterning defects.^{28,201,202}

Genetic instability represents an additional risk. Long-term culture, repeated circuit activation, and high transcriptional load may promote DNA damage, structural variants, or loss of synthetic constructs. Some complex circuits exert metabolic or proteostatic burden, selecting for

escape mutants that disable circuit components or rewire regulatory modules.^{198,203} Ensuring genetic robustness will require the design of circuits that minimize fitness penalties and incorporate fail-safe mechanisms.

Epigenetic drift poses unique concerns in systems that rely on heritable chromatin states. Synthetic epigenetic programs, including methylation-based memories, enhancer locking modules, and histone modification circuits, may lose fidelity over time as cells divide, differentiate, or experience mechanical stress.^{15,204} Drift can destabilize lineage commitment, impair long-term reproducibility, or generate mosaic epigenetic states within a tissue.

Horizontal transfer of circuits in co-culture environments or multi-lineage systems represents an emerging issue. Circuit-bearing DNA or RNA elements may theoretically spread between cells through extracellular vesicles, viral vectors, or fusion events, potentially altering intended

compartmentalization.^{205,206} As programmable organoids are increasingly used in human–animal chimeric models to study integration, vascularization, or *in vivo* maturation, preventing unintended circuit dissemination across species boundaries becomes particularly critical.

7.2. Predictability, controllability, and emergent behavior

Despite careful engineering, organoids remain inherently complex adaptive systems with emergent properties that cannot always be fully predicted from the behavior of their individual components.¹⁶⁸ This poses biological and design challenges for programmable morphogenesis.

One major concern is the risk of unintended self-organization. Synthetic organizers, reaction–diffusion networks, or feedback-modulated gradients may generate patterns that deviate from intended designs, especially when interacting with endogenous signaling pathways.²⁰⁷ Complex 3D geometries can amplify small stochastic differences in initial conditions, producing divergent morphogenetic outcomes across replicates.²⁰⁸

Synthetic gene circuits embedded within organoids may evolve over time, especially during prolonged growth or differentiation. Mutations, epigenetic silencing, and circuit–host interactions can reshape circuit behavior, altering response thresholds, introducing instability, or reversing intended logic.²⁰⁹ Evolutionary drift is particularly problematic in circuits governing growth, survival, or metabolic regulation, where selective pressures may favor aberrant states.

Moreover, engineered circuits may interact nonlinearly with mechanical forces, metabolic gradients, and spatial constraints, giving rise to emergent behaviors that are difficult to anticipate computationally. Morphogenetic feedback loops, once inserted into a 3D system, may spontaneously generate new spatial domains, unexpected pattern boundaries, or novel modes of symmetry breaking.^{54,210}

As closed-loop regulatory systems incorporate machine learning, predictive algorithms, and autonomous control architectures, additional considerations arise related to algorithmic transparency, data provenance, and the governance of AI-driven decision frameworks. Ensuring predictability in programmable organoids will therefore require advances in modeling, high-resolution monitoring, and circuit designs that prioritize robustness, modularity, and containment of emergent dynamics.

7.3. Ethical dimensions

Increasing sophistication in programmable organoid systems raises important ethical questions, particularly as engineered developmental programs enable

reconstructions of events previously restricted to intact human embryos.^{196,211} These concerns span lineage identity, developmental potential, cross-species integration, and governance of synthetic biological systems.

Lineage rewiring in human tissues raises questions about the moral status of constructs engineered to acquire complex or unique developmental identities.^{196,212,213} Programming neural, germline-like, or early embryonic structures requires clear ethical boundaries on what types of lineage transformations are permissible, especially when circuits enable higher-order patterning or quasi-embryonic features.

The generation of synthetic developmental events that approach embryo-like organization, including gastrulation, axial formation, or early neural tube patterning, pushes existing regulatory frameworks to their limits. Although current organoids lack the full integrated architecture or totipotent potential of embryos, the convergence of multiple engineered features may require a reevaluation of conceptual and legal definitions, particularly regarding embryo models, integrated human models, and permissible limits on developmental fidelity.^{214,215}

The use of programmable organoids in chimeric transplantation studies, where human-engineered tissues integrate into animal hosts^{216,217}, poses additional ethical challenges regarding species boundaries, functional integration, and potential neurodevelopmental implications.

Finally, the regulation of engineered organoid systems lags behind their technical capabilities. There is limited consensus on oversight for constructs containing synthetic gene circuits, epigenetic memory systems, or autonomous closed-loop regulatory modules.^{213,215,218} As organoid systems move closer to therapeutic applications, new considerations arise regarding clinical-grade manufacturing, long-term patient safety, and the responsible deployment of tissues with engineered developmental logic.

Collectively, these issues emphasize the need for ethical foresight, transparent governance structures, and interdisciplinary dialogue to ensure that programmable organoids advance in a manner aligned with societal values and biomedical responsibility.

7.4. Triggers for enhanced oversight, data/artificial intelligence governance, and reporting standards

As programmable organoids acquire increasing autonomy, complexity, and developmental realism, ethical and regulatory oversight should scale with functional capability rather than remain uniform across all platforms. Oversight frameworks should therefore be adaptive, responding to what systems can do rather than what they are nominally called. To operationalize this principle, we propose three

integrated components: defined triggers for enhanced oversight, governance expectations for data- and AI-driven systems, and standardized reporting criteria.

Enhanced ethical and regulatory review should be initiated when specific functional thresholds are crossed. These include the emergence of embryo-like organization, such as coordinated axis formation, germ-layer-like segregation, or organizer-like signaling centers; long-term autonomous developmental progression beyond short-term patterning or lineage induction; use of irreversible genetic or epigenetic modifications that alter lineage potential or developmental trajectory; deployment of closed-loop or AI-guided systems capable of autonomous developmental decision-making; transplantation or functional integration into animal hosts; and generation of structures plausibly associated with sentience-related substrates, including organized neural activity or sensory-like integration. These conditions do not imply prohibition, but rather signal the need for heightened ethical scrutiny, greater transparency, and potentially specialized oversight mechanisms.²¹⁹

Cybergenetic and AI-guided organoid systems also introduce new responsibilities related to data use, algorithmic control, and accountability. Governance expectations should therefore include transparency of algorithmic design, including description of model architecture, training data sources, and decision logic; traceability, enabling reconstruction of how specific developmental interventions were generated; assessment of bias and generalization across batches, cell lines, and laboratories; clear policies for storage, sharing, and reuse of high-dimensional biological data; and human-in-the-loop safeguards that allow manual override during unsafe or unexpected developmental trajectories.^{141,220}

To improve reproducibility, ethical clarity, and regulatory readiness, programmable organoid studies should also adopt standardized reporting practices. At minimum, reports should specify the developmental capabilities targeted, the control architecture employed (genetic, epigenetic, cybergenetic, or AI-based), the degree of system autonomy, the reversibility of developmental changes, whether predefined oversight triggers were reached, the safety measures implemented (such as kill switches, growth limits, or containment strategies), the approach to data and AI governance, and the ethical review pathway used. Together, these measures move ethical discussion from abstract concern to operational governance, enabling programmable organoids to advance responsibly as increasingly powerful developmental systems.^{221,222}

8. Future directions and conclusion toward fully programmable human developmental systems

As emphasized throughout this review, the concept of programmable organoids currently represents an emerging and rapidly developing capability rather than a fully mature technological reality. Many of the strategies discussed remain proof-of-concept demonstrations, early organoid implementations, or technically constrained approaches that illustrate mechanistic control without yet achieving reliable, long-term developmental programmability in complex, multi-lineage systems. The significance of this field lies not in claiming present-day completeness, but in defining a coherent framework that connects existing tools to the future possibility of experimentally steerable human developmental systems.

The integration of synthetic biology, stem cell engineering, computational modeling, and organoid technology is steering the field toward an ambitious new horizon in which fully programmable human developmental systems become feasible. These systems will not simply emulate embryogenesis but will execute engineered developmental programs with precision, tunability, and robustness. Achieving this vision requires coordinated advances in genetic circuitry, epigenetic memory frameworks, spatial patterning technologies, autonomous regulatory systems, and artificial intelligence-guided developmental design. Together, these innovations will redefine the capabilities of organoid platforms, enabling the construction of biological systems that can be directed, shaped, and stabilized according to engineered developmental logic (Figure 6).

A central direction for future research involves multi-circuit developmental programming in which several synthetic regulatory networks operate in parallel to control lineage specification, morphogen signaling, mechanical feedback, metabolic states, and temporal progression. Current organoid systems largely depend on single-input perturbations, but next-generation architectures will employ hierarchical and layered control reminiscent of natural developmental hierarchies. These multi-circuit frameworks may enable sequential activation of developmental modules, context-dependent fate decisions guided by local microenvironments, division-of-labor regulatory designs distributed across specific lineages, and coordinated tissue-level governance across complex 3D assemblies. Such integrated programming may allow organoids to progress through intricate developmental sequences with engineered reliability and reduced stochastic variability.

In parallel, substantial advances are anticipated in the development of synthetic organizers with higher-order spatial and temporal control. Early organizers functioned primarily as engineered morphogen sources, but future constructs will incorporate sensing, computation, and adaptive responses. These organizers may transition

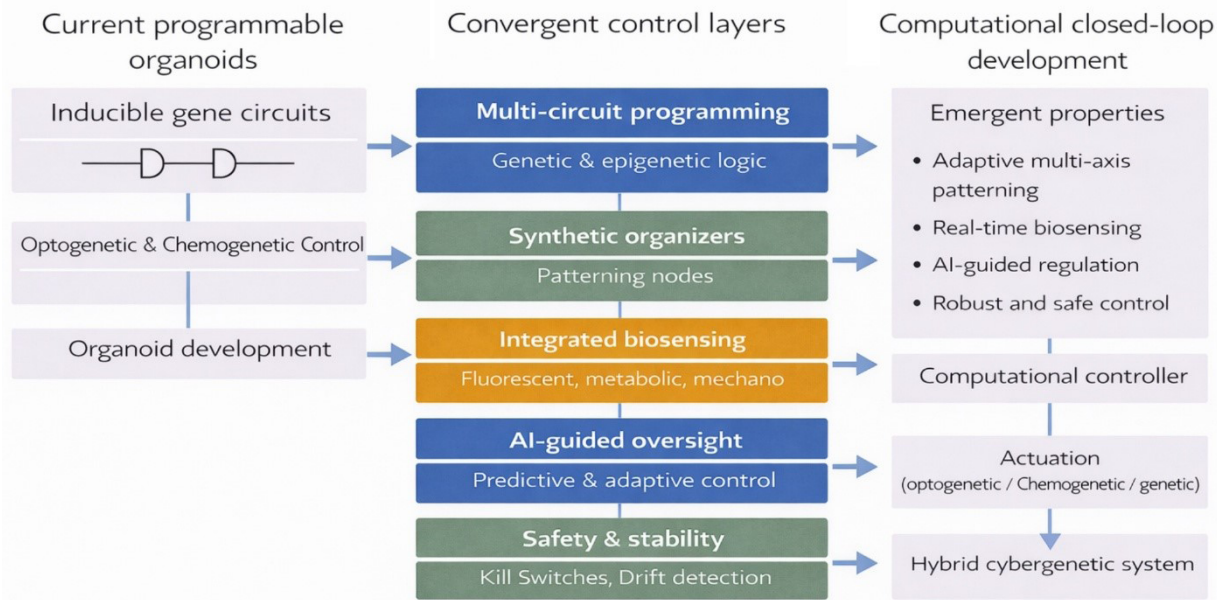


Figure 6. Conceptual roadmap toward integrated programmable organoids and hybrid computational feedback control. The figure outlines a conceptual integration roadmap illustrating how current programmable organoid approaches may evolve toward more coordinated, feedback-informed developmental control architectures. Present implementations rely primarily on inducible gene circuits and optogenetic or chemogenetic perturbations applied to developing organoids under externally guided conditions. The central column highlights convergent control layers that are being explored in early-stage or context-dependent systems, including multi-circuit programming (genetic and epigenetic logic modules), synthetic organizers functioning as patterning nodes, integrated biosensing platforms (e.g., fluorescent, metabolic, or mechanosensory readouts), artificial intelligence (AI)-assisted analytical oversight, and safety or stability modules such as kill switches or drift-detection strategies. These components are depicted as layered integration targets rather than as uniformly mature capabilities. The right panel illustrates a hybrid cybergnetic framework in which biosensing data may be transmitted to an external computational controller that determines subsequent actuation (optogenetic, chemogenetic, or genetic intervention). Such configurations represent computer-in-the-loop or hybrid feedback systems rather than fully autonomous developmental controllers. The listed emergent properties—adaptive multi-axis patterning, real-time biosensing, AI-assisted regulation, and improved robustness—are presented as forward-looking integration goals consistent with Categories II–III evidence levels and remain constrained by biological variability, scaling sensitivity, diffusion limits, and incomplete maturation in current organoid platforms. Accordingly, this roadmap should be interpreted as a structured synthesis of ongoing experimental directions and conceptual design trajectories rather than as a depiction of fully realized, self-regulating developmental systems. Image created by the author using OpenAI’s ChatGPT image-generation tool. The conceptual design, scientific content, and final edits were performed and verified by the author.

between anteriorizing and posteriorizing influences, impose orthogonal patterning axes within a single structure, reposition themselves according to dynamic tissue gradients, or function collectively as distributed networks that sculpt multi-domain architectures. Through these mechanisms, it will become possible to generate controlled axes, compartment boundaries, regionalized neural territories, and complex tissue interfaces that closely mirror early human organogenesis.

Equally transformative are emerging epigenetic memory systems designed to encode, stabilize, and propagate developmental instructions across extended temporal windows. Because long-term pattern fidelity depends on durable and heritable regulatory states, synthetic epigenetic architectures offer essential tools for controlling developmental history. Future designs will include multi-state chromatin switches capable of storing graded information, synthetic epigenetic timers that record

the duration or order of signaling events, spatially encoded enhancer landscapes that define and maintain positional identity, and feedback mechanisms that correct drifted chromatin states. These systems will reinforce lineage trajectories, preserve spatial domains, and markedly improve reproducibility of long-duration morphogenesis.

As these technologies evolve, organoids will increasingly adopt features of autonomous and self-regulating within cybergnetic architectures, developmental entities. Through integration of real-time biosensing with embedded computation and feedback-adjusted gene expression, future organoids will be capable of identifying deviations from intended developmental pathways and initiating corrective responses without external intervention. Embedded robustness modules will buffer against fluctuations in morphogen levels, metabolic stresses, or mechanical perturbations, and safety systems will prevent uncontrolled proliferation, aberrant differentiation, or pathological

patterning. Over time, these capabilities will produce engineered tissues that maintain stable organization across diverse conditions, bringing organoid systems closer to controlled developmental machines.

Collectively, these advances point toward the emergence of developmental synthetic biology, an interdisciplinary field dedicated to engineering the principles that govern multicellular organization in human-specific contexts. This discipline will draw together synthetic biology, developmental and stem cell biology, systems engineering, control theory, machine learning, computational morphodynamics, and epigenetic and evolutionary science. Its overarching aim is to shift developmental biology from a descriptive discipline to a programmable engineering framework capable of designing new developmental trajectories, constructing synthetic body plans, and generating multicellular systems with emergent structural and functional complexity.

Programmable organoids therefore occupy a pivotal position at the intersection of developmental research, disease modeling, and regenerative medicine. Through the integration of synthetic circuits, engineered epigenetic memory, synthetic organizers, and multi-scale feedback systems, these platforms now provide causally interpretable and developmentally informed models that far exceed the capabilities of conventional organoids. This progress enables precise reconstruction of early human developmental pathways, mechanistic dissection of congenital and epigenetic disorders, and engineering of therapeutic tissues with stable identity and long-term function. However, the increasing sophistication of programmable organoids also introduces significant responsibilities, including the need for rigorous biosafety evaluation, management of emergent behaviors, careful consideration of ethical boundaries, and development of adaptive regulatory frameworks capable of evolving alongside technological capability.

Looking ahead, the convergence of synthetic biology, artificial intelligence, and programmable developmental systems suggests a future in which these technologies enable increasingly controlled experimental manipulation of developmental processes, with important implications for biology and medicine. The coming decade will determine how this unprecedented capability is used, regulated, and integrated into both scientific and clinical practice. Programmable organoids thus stand at the threshold of a fundamentally new era in which the processes that shape human form become programmable substrates for discovery, therapeutic innovation, and regenerative medicine.

Acknowledgments

None.

Funding

None.

Conflict of interest

The author declares no conflict of interest.

Author contributions

This is a single-authored article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

Further disclosure

The paper has been uploaded to and deposited on the preprint server preprints.org (DOI: 10.20944/preprints202512.1646.v1).

References

1. Lancaster MA, Knoblich JA. Generation of cerebral organoids from human pluripotent stem cells. *Nat Protoc.* 2014;9(10):2329-2340.
doi: 10.1038/nprot.2014.158
2. Sasai Y. Next-generation regenerative medicine: Organogenesis from stem cells in 3D culture. *Cell Stem Cell.* 2013;12(5):520-530.
doi: 10.1016/j.stem.2013.04.009
3. Brassard JA, Lutolf MP. Engineering Stem Cell Self-organization to Build Better Organoids. *Cell Stem Cell.* 2019;24(6):860-876.
doi: 10.1016/j.stem.2019.05.005
4. Zhao Z, Chen X, Dowbaj AM, et al. Organoids. *Nat Rev Methods Primers.* 2022;2(1).
doi: 10.1038/s43586-022-00174-y
5. Trentesaux C, Yamada T, Klein OD, Lim WA. Harnessing synthetic biology to engineer organoids and tissues. *Cell Stem Cell.* 2023;30(1):10-19.
doi: 10.1016/j.stem.2022.12.013
6. McNamara HM, Ramm B, Toettcher JE. Synthetic developmental biology: New tools to deconstruct and rebuild developmental systems. *Semin Cell Dev Biol.* 2023;141:33-42.
doi: 10.1016/j.semcdb.2022.04.013
7. Santorelli M, Lam C, Morsut L. Synthetic development: Building mammalian multicellular structures with artificial

- genetic programs. *Curr Opin Biotechnol.* 2019;59:130-140.
doi: 10.1016/j.copbio.2019.03.016
8. Liu XS, Wu H, Ji X, *et al.* Editing DNA Methylation in the Mammalian Genome. *Cell.* 2016;167(1):233-247.e17.
doi: 10.1016/j.cell.2016.08.056
9. Gilbert LA, Larson MH, Morsut L, *et al.* CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell.* 2013;154(2):442-451.
doi: 10.1016/j.cell.2013.06.044
10. Morsut L, Roybal KT, Xiong X, *et al.* Engineering Customized Cell Sensing and Response Behaviors Using Synthetic Notch Receptors. *Cell.* 2016;164(4):780-791.
doi: 10.1016/j.cell.2016.01.012
11. Lu C, Garipler G, Dai C, *et al.* Essential transcription factors for induced neuron differentiation. *Nat Commun.* 2023;14(1):8362.
doi: 10.1038/s41467-023-43602-7
12. Repina NA, Johnson HJ, Bao X, *et al.* Optogenetic control of Wnt signaling models cell-intrinsic embryogenic patterning using 2D human pluripotent stem cell culture. *Development.* 2023;150(14).
doi: 10.1242/dev.201386
13. Malaguti M, Portero Migueles R, Annoh J, Sadurska D, Blin G, Lowell S. SynPL: Synthetic Notch pluripotent cell lines to monitor and manipulate cell interactions in vitro and in vivo. *Development.* 2022;149(12).
doi: 10.1242/dev.200226
14. Yin J, Wan H, Kong D, *et al.* A digital CRISPR-dCas9-based gene remodeling biocomputer programmed by dietary compounds in mammals. *Cell Syst.* 2024;15(10):941-955.e5.
doi: 10.1016/j.cels.2024.09.002
15. Nunez JK, Chen J, Pommier GC, *et al.* Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing. *Cell.* 2021;184(9):2503-2519.e17.
doi: 10.1016/j.cell.2021.03.025
16. Kumar S, Beyer HM, Chen M, Zurbriggen MD, Khammash M. Image-guided optogenetic spatiotemporal tissue patterning using muPatternScope. *Nat Commun.* 2024;15(1):10469.
doi: 10.1038/s41467-024-54351-6
17. Nissim L, Perli SD, Fridkin A, Perez-Pinera P, Lu TK. Multiplexed and programmable regulation of gene networks with an integrated RNA and CRISPR/Cas toolkit in human cells. *Mol Cell.* 2014;54(4):698-710.
doi: 10.1016/j.molcel.2014.04.022
18. Xie M, Fussenegger M. Designing cell function: Assembly of synthetic gene circuits for cell biology applications. *Nat Rev Mol Cell Biol.* 2018;19(8):507-525.
doi: 10.1038/s41580-018-0024-z
19. Sonnen KF, Lauschke VM, Uraji J, *et al.* Modulation of Phase Shift between Wnt and Notch Signaling Oscillations Controls Mesoderm Segmentation. *Cell.* 2018;172(5):1079-1090.e12.
doi: 10.1016/j.cell.2018.01.026
20. Gao Y, Wang L, Wang B. Customizing cellular signal processing by synthetic multi-level regulatory circuits. *Nat Commun.* 2023;14(1):8415.
doi: 10.1038/s41467-023-44256-1
21. De Carluccio G, Fusco V, di Bernardo D. Engineering a synthetic gene circuit for high-performance inducible expression in mammalian systems. *Nat Commun.* 2024;15(1):3311.
doi: 10.1038/s41467-024-47592-y
22. Chavez A, Scheiman J, Vora S, *et al.* Highly efficient Cas9-mediated transcriptional programming. *Nat Methods.* 2015;12(4):326-328.
doi: 10.1038/nmeth.3312
23. Kampmann M. CRISPRi and CRISPRa Screens in Mammalian Cells for Precision Biology and Medicine. *ACS Chem Biol.* 2018;13(2):406-416.
doi: 10.1021/acscchembio.7b00657
24. Zalatan JG, Lee ME, Almeida R, *et al.* Engineering complex synthetic transcriptional programs with CRISPR RNA scaffolds. *Cell.* 2015;160(1-2):339-350.
doi: 10.1016/j.cell.2014.11.052
25. Gao Y, Xiong X, Wong S, Charles EJ, Lim WA, Qi LS. Complex transcriptional modulation with orthogonal and inducible dCas9 regulators. *Nat Methods.* 2016;13(12):1043-1049.
doi: 10.1038/nmeth.4042
26. Chen PJ, Liu DR. Prime editing for precise and highly versatile genome manipulation. *Nat Rev Genet.* 2023;24(3):161-177.
doi: 10.1038/s41576-022-00541-1
27. Anzalone AV, Randolph PB, Davis JR, *et al.* Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature.* 2019;576(7785):149-157.
doi: 10.1038/s41586-019-1711-4
28. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature.* 2016;533(7603):420-424.
doi: 10.1038/nature17946
29. Teng F, Cui T, Zhou L, Gao Q, Zhou Q, Li W. Programmable synthetic receptors: The next-generation of cell and gene therapies. *Signal Transduct Target Ther.* 2024;9(1):7.
doi: 10.1038/s41392-023-01680-5
30. Nims RJ, Pferdehirt L, Ho NB, *et al.* A synthetic mechanogenetic gene circuit for autonomous drug delivery in engineered tissues. *Sci Adv.* 2021;7(5).

doi: 10.1126/sciadv.abd9858

31. Toda S, McKeithan WL, Hakkinen TJ, Lopez P, Klein OD, Lim WA. Engineering synthetic morphogen systems that can program multicellular patterning. *Science*. 2020;370(6514):327-331.
doi: 10.1126/science.abc0033
32. Velazquez JJ, Su E, Cahan P, Ebrahimkhani MR. Programming Morphogenesis through Systems and Synthetic Biology. *Trends Biotechnol*. 2018;36(4):415-429.
doi: 10.1016/j.tibtech.2017.11.003
33. Lo YH, Horn HT, Huang MF, *et al*. Large-scale CRISPR screening in primary human 3D gastric organoids enables comprehensive dissection of gene-drug interactions. *Nat Commun*. 2025;16(1):7566.
doi: 10.1038/s41467-025-62818-3
34. Ahmed A, Di Molfetta D, Iaconisi GN, *et al*. Human Genome Safe Harbor Sites: A Comprehensive Review of Criteria, Discovery, Features, and Applications. *Cells*. 2026;15(1).
doi: 10.3390/cells15010081
35. Zhang K, Cui B. Optogenetic control of intracellular signaling pathways. *Trends Biotechnol*. 2015;33(2):92-100.
doi: 10.1016/j.tibtech.2014.11.007
36. Beyer HM, Kumar S, Nieke M, *et al*. Genetically-stable engineered optogenetic gene switches modulate spatial cell morphogenesis in two- and three-dimensional tissue cultures. *Nat Commun*. 2024;15(1):10470.
doi: 10.1038/s41467-024-54350-7
37. Komatsu N, Terai K, Imanishi A, *et al*. A platform of BRET-FRET hybrid biosensors for optogenetics, chemical screening, and in vivo imaging. *Sci Rep*. 2018;8(1):8984.
doi: 10.1038/s41598-018-27174-x
38. Sim X, Cardenas-Diaz FL, French DL, Gadue P. A Doxycycline-Inducible System for Genetic Correction of iPSC Disease Models. *Methods Mol Biol*. 2016;1353:13-23.
doi: 10.1007/7651_2014_179
39. Gossen M, Bujard H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc Natl Acad Sci USA*. 1992;89(12):5547-5551.
doi: 10.1073/pnas.89.12.5547
40. Kretschmar K, Watt FM. Lineage tracing. *Cell*. 2012;148(1-2):33-45.
doi: 10.1016/j.cell.2012.01.002
41. Kim S, Park J, Jeon BW, *et al*. Chemical control of receptor kinase signaling by rapamycin-induced dimerization. *Mol Plant*. 2021;14(8):1379-1390.
doi: 10.1016/j.molp.2021.05.006
42. Liu L, Chen L, Chung J, Huang S. Rapamycin inhibits F-actin reorganization and phosphorylation of focal adhesion proteins. *Oncogene*. 2008;27(37):4998-5010.
doi: 10.1038/onc.2008.137
43. Fan LZ, Lin MZ. Optical control of biological processes by light-switchable proteins. *Wiley Interdiscip Rev Dev Biol*. 2015;4(5):545-554.
doi: 10.1002/wdev.188
44. Shamala LF, Zhou HC, Han ZX, Wei S. UV-B Induces Distinct Transcriptional Re-programing in UVR8-Signal Transduction, Flavonoid, and Terpenoids Pathways in *Camellia sinensis*. *Front Plant Sci*. 2020;11:234.
doi: 10.3389/fpls.2020.00234
45. Li L, Klim JR, Derda R, Courtney AH, Kiessling LL. Spatial control of cell fate using synthetic surfaces to potentiate TGF-beta signaling. *Proc Natl Acad Sci USA*. 2011;108(29):11745-11750.
doi: 10.1073/pnas.1101454108
46. Pandelakis M, Delgado E, Ebrahimkhani MR. CRISPR-Based Synthetic Transcription Factors In Vivo: The Future of Therapeutic Cellular Programming. *Cell Syst*. 2020;10(1):1-14.
doi: 10.1016/j.cels.2019.10.003
47. Cheng D, Clark CT, Smith Q. Advances in engineered models of peri-gastrulation. *iScience*. 2025;28(6):112659.
doi: 10.1016/j.isci.2025.112659
48. Chhabra S, Liu L, Goh R, Kong X, Warmflash A. Dissecting the dynamics of signaling events in the BMP, WNT, and NODAL cascade during self-organized fate patterning in human gastruloids. *PLoS Biol*. 2019;17(10):e3000498.
doi: 10.1371/journal.pbio.3000498
49. Heisenberg CP, Bellaiche Y. Forces in tissue morphogenesis and patterning. *Cell*. 2013;153(5):948-962.
doi: 10.1016/j.cell.2013.05.008
50. Feuerstein M, Chleilat E, Khakipoor S, Michailidis K, Ophoven C, Roussa E. Expression patterns of key Sonic Hedgehog signaling pathway components in the developing and adult mouse midbrain and in the MN9D cell line. *Cell Tissue Res*. 2017;370(2):211-225.
doi: 10.1007/s00441-017-2664-2
51. Scuderi S, Khouri-Farah N, Rauthan R, *et al*. Engineering human neuronal diversity: Morphogens and stem cell technologies for neurodevelopmental biology. *Stem Cell Rep*. 2025;20(9):102615.
doi: 10.1016/j.stemcr.2025.102615
52. Repina NA, McClave T, Johnson HJ, Bao X, Kane RS, Schaffer DV. Engineered Illumination Devices for Optogenetic Control of Cellular Signaling Dynamics. *Cell Rep*. 2020;31(10):107737.
doi: 10.1016/j.celrep.2020.107737
53. Sagner A, Briscoe J. Morphogen interpretation:

- Concentration, time, competence, and signaling dynamics. *Wiley Interdiscip Rev Dev Biol.* 2017;6(4).
doi: 10.1002/wdev.271
54. Warmflash A, Sorre B, Etoc F, Siggia ED, Brivanlou AH. A method to recapitulate early embryonic spatial patterning in human embryonic stem cells. *Nat Methods.* 2014;11(8):847-854.
doi: 10.1038/nmeth.3016
 55. Streibinger D, Deluz C, Friman ET, Govindan S, Alber AB, Suter DM. Endogenous fluctuations of OCT4 and SOX2 bias pluripotent cell fate decisions. *Mol Syst Biol.* 2019;15(9):e9002.
doi: 10.15252/msb.20199002
 56. Schroder CM, Zissel L, Mersowsky SL, *et al.* EOMES establishes mesoderm and endoderm differentiation potential through SWI/SNF-mediated global enhancer remodeling. *Dev Cell.* 2025;60(5):735-748.e5.
doi: 10.1016/j.devcel.2024.11.014
 57. Weltner J, Balboa D, Katayama S, *et al.* Human pluripotent reprogramming with CRISPR activators. *Nat Commun.* 2018;9(1):2643.
doi: 10.1038/s41467-018-05067-x
 58. Liu Y, Yu C, Daley TP, *et al.* CRISPR Activation Screens Systematically Identify Factors that Drive Neuronal Fate and Reprogramming. *Cell Stem Cell.* 2018;23(5):758-771.e8.
doi: 10.1016/j.stem.2018.09.003
 59. Bragdon MDJ, Patel N, Chuang J, Levien E, Bashor CJ, Khalil AS. Cooperative assembly confers regulatory specificity and long-term genetic circuit stability. *Cell.* 2023;186(18):3810-3825.e18.
doi: 10.1016/j.cell.2023.07.012
 60. Schuettengruber B, Bourbon HM, Di Croce L, Cavalli G. Genome Regulation by Polycomb and Trithorax: 70 Years and Counting. *Cell.* 2017;171(1):34-57.
doi: 10.1016/j.cell.2017.08.002
 61. Nashun B, Hill PW, Hajkova P. Reprogramming of cell fate: Epigenetic memory and the erasure of memories past. *EMBO J.* 2015;34(10):1296-1308.
doi: 10.15252/embj.201490649
 62. Black JB, Adler AF, Wang HG, *et al.* Targeted Epigenetic Remodeling of Endogenous Loci by CRISPR/Cas9-Based Transcriptional Activators Directly Converts Fibroblasts to Neuronal Cells. *Cell Stem Cell.* 2016;19(3):406-414.
doi: 10.1016/j.stem.2016.07.001
 63. Hilton IB, D'Ippolito AM, Vockley CM, *et al.* Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. *Nat Biotechnol.* 2015;33(5):510-517.
doi: 10.1038/nbt.3199
 64. Zaret KS, Mango SE. Pioneer transcription factors, chromatin dynamics, and cell fate control. *Curr Opin Genet Dev.* 2016;37:76-81.
doi: 10.1016/j.gde.2015.12.003
 65. Du Z, Zhang K, Xie W. Epigenetic Reprogramming in Early Animal Development. *Cold Spring Harb Perspect Biol.* 2022;14(6).
doi: 10.1101/cshperspect.a039677
 66. Cambuli F, Murray A, Dean W, *et al.* Epigenetic memory of the first cell fate decision prevents complete ES cell reprogramming into trophoblast. *Nat Commun.* 2014;5:5538.
doi: 10.1038/ncomms6538
 67. Shipman SL, Nivala J, Macklis JD, Church GM. CRISPR-Cas encoding of a digital movie into the genomes of a population of living bacteria. *Nature.* 2017;547(7663):345-349.
doi: 10.1038/nature23017
 68. Chen W, Choi J. Molecular circuits for genomic recording of cellular events. *Trends Genet.* 2025;41(8):647-659.
doi: 10.1016/j.tig.2025.04.004
 69. Tomljanovic M, Muflihah CH, Rajkovski D, Mikulski P. The epigenetic circle: Feedback loops in the maintenance of cellular memory. *Epigenetics Chromatin.* 2025;18(1):56.
doi: 10.1186/s13072-025-00621-6
 70. Bell CC, Faulkner GJ, Gilan O. Chromatin-based memory as a self-stabilizing influence on cell identity. *Genome Biol.* 2024;25(1):320.
doi: 10.1186/s13059-024-03461-x
 71. Lin K, Zou C, Hubbard A, *et al.* Multiplexed epigenetic memory editing using CRISPRoff sensitizes glioblastoma to chemotherapy. *Neuro Oncol.* 2025;27(6):1443-1457.
doi: 10.1093/neuonc/noaf055
 72. Campos OA, Migliara A, Toda S, Lopez P, Lim WA, Almeida R. Engineering Inducible Cell Fate Transitions by Harnessing Epigenetic Silencing. *bioRxiv.* Preprint online 2025.
doi: 10.1101/2025.09.29.679324
 73. O'Laughlin R, Cheng F, Song H, Ming GL. Bioengineering tools for next-generation neural organoids. *Curr Opin Neurobiol.* 2025;92:103011.
doi: 10.1016/j.conb.2025.103011
 74. Santorelli M, Bhamidipati PS, Courte J, *et al.* Control of spatio-temporal patterning via cell growth in a multicellular synthetic gene circuit. *Nat Commun.* 2024;15(1):9867.
doi: 10.1038/s41467-024-53078-8
 75. Wang H, Xu X, Nguyen CM, *et al.* CRISPR-Mediated Programmable 3D Genome Positioning and Nuclear Organization. *Cell.* 2018;175(5):1405-1417.e14.
doi: 10.1016/j.cell.2018.09.013
 76. Wei Y, Sun J, Zhu R. CRISPR-epigenetic crosstalk: From

- bidirectional regulation to therapeutic potential. *Comput Struct Biotechnol J*. 2025;27:4496-4504.
doi: 10.1016/j.csbj.2025.10.031
77. Pollen AA, Bhaduri A, Andrews MG, *et al*. Establishing Cerebral Organoids as Models of Human-Specific Brain Evolution. *Cell*. 2019;176(4):743-756.e17.
doi: 10.1016/j.cell.2019.01.017
 78. Velasco S, Kedaigle AJ, Simmons SK, *et al*. Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. *Nature*. 2019;570(7762):523-527.
doi: 10.1038/s41586-019-1289-x
 79. Hawkins RD, Hon GC, Yang C, *et al*. Dynamic chromatin states in human ES cells reveal potential regulatory sequences and genes involved in pluripotency. *Cell Res*. 2011;21(10):1393-1409.
doi: 10.1038/cr.2011.146
 80. Tsankov AM, Gu H, Akopian V, *et al*. Transcription factor binding dynamics during human ES cell differentiation. *Nature*. 2015;518(7539):344-349.
doi: 10.1038/nature14233
 81. Polstein LR, Gersbach CA. A light-inducible CRISPR-Cas9 system for control of endogenous gene activation. *Nat Chem Biol*. 2015;11(3):198-200.
doi: 10.1038/nchembio.1753
 82. Deng Y, Bartosovic M, Ma S, *et al*. Spatial profiling of chromatin accessibility in mouse and human tissues. *Nature*. 2022;609(7926):375-383.
doi: 10.1038/s41586-022-05094-1
 83. Noordermeer D, Leleu M, Schorderet P, Joye E, Chabaud F, Duboule D. Temporal dynamics and developmental memory of 3D chromatin architecture at Hox gene loci. *eLife*. 2014;3:e02557.
doi: 10.7554/eLife.02557
 84. Amberg N, Laukoter S, Hippenmeyer S. Epigenetic cues modulating the generation of cell-type diversity in the cerebral cortex. *J Neurochem*. 2019;149(1):12-26.
doi: 10.1111/jnc.14601
 85. Manfrin A, Tabata Y, Paquet ER, *et al*. Engineered signaling centers for the spatially controlled patterning of human pluripotent stem cells. *Nat Methods*. 2019;16(7):640-648.
doi: 10.1038/s41592-019-0455-2
 86. Moris N, Anlas K, van den Brink SC, *et al*. An in vitro model of early anteroposterior organization during human development. *Nature*. 2020;582(7812):410-415.
doi: 10.1038/s41586-020-2383-9
 87. Etoc F, Metzger J, Ruzo A, *et al*. A Balance between Secreted Inhibitors and Edge Sensing Controls Gastruloid Self-Organization. *Dev Cell*. 2016;39(3):302-315.
doi: 10.1016/j.devcel.2016.09.016
 88. Mammoto T, Mammoto A, Ingber DE. Mechanobiology and developmental control. *Annu Rev Cell Dev Biol*. 2013;29:27-61.
doi: 10.1146/annurev-cellbio-101512-122340
 89. Treutlein B, Brownfield DG, Wu AR, *et al*. Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-seq. *Nature*. 2014;509(7500):371-375.
doi: 10.1038/nature13173
 90. Feinberg AP. The Key Role of Epigenetics in Human Disease Prevention and Mitigation. *N Engl J Med*. 2018;378(14):1323-1334.
doi: 10.1056/NEJMra1402513
 91. Sur I, Taipale J. The role of enhancers in cancer. *Nat Rev Cancer*. 2016;16(8):483-493.
doi: 10.1038/nrc.2016.62
 92. Jones PA, Issa JB, Baylin S. Targeting the cancer epigenome for therapy. *Nat Rev Genet*. 2016;17(10):630-641.
doi: 10.1038/nrg.2016.93
 93. de The H. Differentiation therapy revisited. *Nat Rev Cancer*. 2018;18(2):117-127.
doi: 10.1038/nrc.2017.103
 94. Koch A, Joosten SC, Feng Z, *et al*. Analysis of DNA methylation in cancer: Location revisited. *Nat Rev Clin Oncol*. 2018;15(7):459-466.
doi: 10.1038/s41571-018-0004-4
 95. Kadoshima T, Sakaguchi H, Nakano T, *et al*. Self-organization of axial polarity, inside-out layer pattern, and species-specific progenitor dynamics in human ES cell-derived neocortex. *Proc Natl Acad Sci USA*. 2013;110(50):20284-20289.
doi: 10.1073/pnas.1315710110
 96. Sasai Y. Cytosystems dynamics in self-organization of tissue architecture. *Nature*. 2013;493(7432):318-326.
doi: 10.1038/nature11859
 97. Lancaster MA, Renner M, Martin CA, *et al*. Cerebral organoids model human brain development and microcephaly. *Nature*. 2013;501(7467):373-379.
doi: 10.1038/nature12517
 98. Anand AA, Khan M, V M, Kar D. The Molecular Basis of Wnt/beta-Catenin Signaling Pathways in Neurodegenerative Diseases. *Int J Cell Biol*. 2023;2023:9296092.
doi: 10.1155/2023/9296092
 99. Toda S, Blaich LR, Tang SKY, Morsut L, Lim WA. Programming self-organizing multicellular structures with synthetic cell-cell signaling. *Science*. 2018;361(6398):156-162.
doi: 10.1126/science.aat0271

100. Sekine R, Shibata T, Ebisuya M. Synthetic mammalian pattern formation driven by differential diffusivity of Nodal and Lefty. *Nat Commun.* 2018;9(1):5456.
doi: 10.1038/s41467-018-07847-x
101. Bugaj LJ, Choksi AT, Mesuda CK, Kane RS, Schaffer DV. Optogenetic protein clustering and signaling activation in mammalian cells. *Nat Methods.* 2013;10(3):249-252.
doi: 10.1038/nmeth.2360
102. Lende-Dorn BA, Atkinson JC, Bae Y, Galloway KE. Chemogenetic tuning reveals optimal MAPK signaling for cell-fate programming. *Cell Rep.* 2025;44(9):116226.
doi: 10.1016/j.celrep.2025.116226
103. Liu TL, Upadhyayula S, Milkie DE, *et al.* Observing the cell in its native state: Imaging subcellular dynamics in multicellular organisms. *Science.* 2018;360(6386).
doi: 10.1126/science.aaq1392
104. Ramirez Sierra MA, Sokolowski TR. AI-powered simulation-based inference of a genuinely spatial-stochastic gene regulation model of early mouse embryogenesis. *PLoS Comput Biol.* 2024;20(11):e1012473.
doi: 10.1371/journal.pcbi.1012473
105. Matas-Gil A, Endres RG. Unraveling biochemical spatial patterns: Machine learning approaches to the inverse problem of stationary Turing patterns. *iScience.* 2024;27(6):109822.
doi: 10.1016/j.isci.2024.109822
106. Scuderi S, Kang TY, Jourdon A, *et al.* Specification of human brain regions with orthogonal gradients of WNT and SHH in organoids reveals patterning variations across cell lines. *Cell Stem Cell.* 2025;32(6):970-989.e11.
doi: 10.1016/j.stem.2025.04.006
107. Garibyan M, Hoffman T, Makaske T, *et al.* Engineering programmable material-to-cell pathways via synthetic notch receptors to spatially control differentiation in multicellular constructs. *Nat Commun.* 2024;15(1):5891.
doi: 10.1038/s41467-024-50126-1
108. Chen X, Liu C, McDaniel G, *et al.* Viscoelasticity of Hyaluronic Acid Hydrogels Regulates Human Pluripotent Stem Cell-derived Spinal Cord Organoid Patterning and Vascularization. *Adv Healthc Mater.* 2024;13(32):e2402199.
doi: 10.1002/adhm.202402199
109. Roth JG, Huang MS, Navarro RS, Akram JT, LeSavage BL, Heilshorn SC. Tunable hydrogel viscoelasticity modulates human neural maturation. *Sci Adv.* 2023;9(42):eadh8313.
doi: 10.1126/sciadv.adh8313
110. Elosegui-Artola A, Gupta A, Najibi AJ, *et al.* Matrix viscoelasticity controls spatiotemporal tissue organization. *Nat Mater.* 2023;22(1):117-127.
doi: 10.1038/s41563-022-01400-4
111. Kopyeva I, Goldner EC, Hoye JW, *et al.* Stepwise Stiffening/Softening of and Cell Recovery from Reversibly Formulated Hydrogel Interpenetrating Networks. *Adv Mater.* 2024;36(44):e2404880.
doi: 10.1002/adma.202404880
112. Cosgrove BD, Bounds LR, Taylor CK, *et al.* Mechanosensitive genomic enhancers potentiate the cellular response to matrix stiffness. *Science.* 2025;390(6778):eadl1988.
doi: 10.1126/science.adl1988
113. Li X, Wang H, Dong X, *et al.* Accurate modulation of photoprinting under stiffness imaging feedback for engineering ECMs with high-fidelity mechanical properties. *Microsyst Nanoeng.* 2022;8:60.
doi: 10.1038/s41378-022-00394-y
114. Narasimhan BN, Fraley SI. Matrix degradation enhances stress relaxation, regulating cell adhesion and spreading. *Proc Natl Acad Sci USA.* 2025;122(13):e2416771122.
doi: 10.1073/pnas.2416771122
115. Martyn I, Brivanlou AH, Siggia ED. A wave of WNT signaling balanced by secreted inhibitors controls primitive streak formation in micropattern colonies of human embryonic stem cells. *Development.* 2019;146(6).
doi: 10.1242/dev.172791
116. Meinhardt A, Eberle D, Tazaki A, *et al.* 3D reconstitution of the patterned neural tube from embryonic stem cells. *Stem Cell Rep.* 2014;3(6):987-999.
doi: 10.1016/j.stemcr.2014.09.020
117. Cao J, Spielmann M, Qiu X, *et al.* The single-cell transcriptional landscape of mammalian organogenesis. *Nature.* 2019;566(7745):496-502.
doi: 10.1038/s41586-019-0969-x
118. Muguruma K, Nishiyama A, Kawakami H, Hashimoto K, Sasai Y. Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells. *Cell Rep.* 2015;10(4):537-550.
doi: 10.1016/j.celrep.2014.12.051
119. Cederquist GY, Asciolla JJ, Tchieu J, *et al.* Specification of positional identity in forebrain organoids. *Nat Biotechnol.* 2019;37(4):436-444.
doi: 10.1038/s41587-019-0085-3
120. Qian X, Nguyen HN, Song MM, *et al.* Brain-Region-Specific Organoids Using Mini-bioreactors for Modeling ZIKV Exposure. *Cell.* 2016;165(5):1238-1254.
doi: 10.1016/j.cell.2016.04.032
121. Huch M, Knoblich JA, Lutolf MP, Martinez-Arias A. The hope and the hype of organoid research. *Development.* 2017;144(6):938-941.
doi: 10.1242/dev.150201
122. Rossi G, Manfrin A, Lutolf MP. Progress and potential in organoid research. *Nat Rev Genet.* 2018;19(11):671-687.

doi: 10.1038/s41576-018-0051-9

123. Gao Q, Yang Y, Yang H, Jiang H. Beyond biochemical patterning: How mechanical bistability governs robust organoid morphogenesis. *Mechanobiol Med.* 2025;3(2):100134.
doi: 10.1016/j.mbm.2025.100134
124. Depry C, Mehta S, Zhang J. Multiplexed visualization of dynamic signaling networks using genetically encoded fluorescent protein-based biosensors. *Pflugers Arch.* 2013;465(3):373-381.
doi: 10.1007/s00424-012-1175-y
125. Zou F, Bai L. Using time-lapse fluorescence microscopy to study gene regulation. *Methods.* 2019;159-160:138-145.
doi: 10.1016/j.jymeth.2018.12.010
126. Hall MP, Unch J, Binkowski BF, *et al.* Engineered luciferase reporter from a deep sea shrimp utilizing a novel imidazopyrazinone substrate. *ACS Chem Biol.* 2012;7(11):1848-1857.
doi: 10.1021/cb3002478
127. Taylor A, Sharkey J, Plagge A, Wilm B, Murray P. Multicolour In Vivo Bioluminescence Imaging Using a NanoLuc-Based BRET Reporter in Combination with Firefly Luciferase. *Contrast Media Mol Imaging.* 2018;2018:2514796.
doi: 10.1155/2018/2514796
128. Wang T, Simmel FC. Riboswitch-inspired toehold riboregulators for gene regulation in *Escherichia coli*. *Nucleic Acids Res.* 2022;50(8):4784-4798.
doi: 10.1093/nar/gkac275
129. Takahashi K, Galloway KE. RNA-based controllers for engineering gene and cell therapies. *Curr Opin Biotechnol.* 2024;85:103026.
doi: 10.1016/j.copbio.2023.103026
130. Paige JS, Wu KY, Jaffrey SR. RNA mimics of green fluorescent protein. *Science.* 2011;333(6042):642-646.
doi: 10.1126/science.1207339
131. Chappell J, Takahashi MK, Lucks JB. Creating small transcription activating RNAs. *Nat Chem Biol.* 2015;11(3):214-220.
doi: 10.1038/nchembio.1737
132. Chen Z, Chen W, Reheman Z, Jiang H, Wu J, Li X. Genetically encoded RNA-based sensors with Pepper fluorogenic aptamer. *Nucleic Acids Res.* 2023;51(16):8322-8336.
doi: 10.1093/nar/gkad620
133. Dolgosheina EV, Jeng SC, Panchapakesan SS, *et al.* RNA mango aptamer-fluorophore: A bright, high-affinity complex for RNA labeling and tracking. *ACS Chem Biol.* 2014;9(10):2412-2420.
doi: 10.1021/cb500499x
134. Filonov GS, Moon JD, Svensen N, Jaffrey SR. Broccoli: Rapid selection of an RNA mimic of green fluorescent protein by fluorescence-based selection and directed evolution. *J Am Chem Soc.* 2014;136(46):16299-16308.
doi: 10.1021/ja508478x
135. Zhang Y, Zhang S. CRISPR perfect adaptation for robust control of cellular immune and apoptotic responses. *Nucleic Acids Res.* 2024;52(16):10005-10016.
doi: 10.1093/nar/gkac665
136. Frei T, Chang CH, Filo M, Arampatzis A, Khammash M. A genetic mammalian proportional-integral feedback control circuit for robust and precise gene regulation. *Proc Natl Acad Sci USA.* 2022;119(24):e2122132119.
doi: 10.1073/pnas.2122132119
137. Benzinger D, Briscoe J. Investigating morphogen and patterning dynamics with optogenetic control of morphogen production. *Dev Cell.* 2025;60(24):3421-3430 e6.
doi: 10.1016/j.devcel.2025.07.019
138. Legnini I, Emmenegger L, Zappulo A, *et al.* Spatiotemporal, optogenetic control of gene expression in organoids. *Nat Methods.* 2023;20(10):1544-1552.
doi: 10.1038/s41592-023-01986-w
139. Perkins ML, Benzinger D, Arcak M, Khammash M. Cell-in-the-loop pattern formation with optogenetically emulated cell-to-cell signaling. *Nat Commun.* 2020;11(1):1355.
doi: 10.1038/s41467-020-15166-3
140. Lugagne JB, Blassick CM, Dunlop MJ. Deep model predictive control of gene expression in thousands of single cells. *Nat Commun.* 2024;15(1):2148.
doi: 10.1038/s41467-024-46361-1
141. Passmore JB, Rates A, Schroder J, *et al.* Closed-loop optogenetic control of cell biology enables outcome-driven microscopy. *Nat Commun.* 2025.
doi: 10.1038/s41467-025-67848-5
142. Jerez-Longres C, Gomez-Matos M, Becker J, *et al.* Engineering a material-genetic interface as safety switch for embedded therapeutic cells. *Biomater Adv.* 2023;150:213422.
doi: 10.1016/j.bioadv.2023.213422
143. Lewis A, Keshara R, Kim YH, Grapin-Botton A. Self-organization of organoids from endoderm-derived cells. *J Mol Med.* 2021;99(4):449-462.
doi: 10.1007/s00109-020-02010-w
144. Del Vecchio D, Abdallah H, Qian Y, Collins JJ. A Blueprint for a Synthetic Genetic Feedback Controller to Reprogram Cell Fate. *Cell Syst.* 2017;4(1):109-120.e11.
doi: 10.1016/j.cels.2016.12.001
145. Ma Y, Budde MW, Mayalu MN, *et al.* Synthetic mammalian signaling circuits for robust cell population control. *Cell.* 2022;185(6):967-979.e12.

- doi: 10.1016/j.cell.2022.01.026
146. Kotula JW, Kerns SJ, Shaket LA, *et al.* Programmable bacteria detect and record an environmental signal in the mammalian gut. *Proc Natl Acad Sci USA*. 2014;111(13):4838-4843.
doi: 10.1073/pnas.1321321111
 147. Holmes WR, Reyes de Mochel NS, Wang Q, *et al.* Gene Expression Noise Enhances Robust Organization of the Early Mammalian Blastocyst. *PLoS Comput Biol*. 2017;13(1):e1005320.
doi: 10.1371/journal.pcbi.1005320
 148. Gutierrez Mena J, Kumar S, Khammash M. Dynamic cybergenetic control of bacterial co-culture composition via optogenetic feedback. *Nat Commun*. 2022;13(1):4808.
doi: 10.1038/s41467-022-32392-z
 149. Kumar S, Rullan M, Khammash M. Rapid prototyping and design of cybergenetic single-cell controllers. *Nat Commun*. 2021;12(1):5651.
doi: 10.1038/s41467-021-25754-6
 150. Caringella G, Bandiera L, Menolascina F. Recent advances, opportunities and challenges in cybergenetic identification and control of biomolecular networks. *Curr Opin Biotechnol*. 2023;80:102893.
doi: 10.1016/j.copbio.2023.102893
 151. Miliadis-Argeitis A, Rullan M, Aoki SK, Buchmann P, Khammash M. Automated optogenetic feedback control for precise and robust regulation of gene expression and cell growth. *Nat Commun*. 2016;7:12546.
doi: 10.1038/ncomms12546
 152. Amin ND, Kelley KW, Kaganovsky K, *et al.* Generating human neural diversity with a multiplexed morphogen screen in organoids. *Cell Stem Cell*. 2024;31(12):1831-1846.e9.
doi: 10.1016/j.stem.2024.10.016
 153. Johnson MB, March AR, Morsut L. Engineering multicellular systems: Using synthetic biology to control tissue self-organization. *Curr Opin Biomed Eng*. 2017;4:163-173.
doi: 10.1016/j.cobme.2017.10.008
 154. Monzel AS, Hemmer K, Kaoma T, *et al.* Machine learning-assisted neurotoxicity prediction in human midbrain organoids. *Park Relat Disord*. 2020;75:105-109.
doi: 10.1016/j.parkreldis.2020.05.011
 155. Bai L, Wu Y, Li G, Zhang W, Zhang H, Su J. AI-enabled organoids: Construction, analysis, and application. *Bioact Mater*. 2024;31:525-548.
doi: 10.1016/j.bioactmat.2023.09.005
 156. Huang K, Li M, Li Q, Chen Z, Zhang Y, Gu Z. Image-based profiling and deep learning reveal morphological heterogeneity of colorectal cancer organoids. *Comput Biol Med*. 2024;173:108322.
doi: 10.1016/j.compbimed.2024.108322
 157. Fu Z, Chen C, Wang S, Wang J, Chen S. scRL: Utilizing Reinforcement Learning to Evaluate Fate Decisions in Single-Cell Data. *Biology*. 2025;14(6).
doi: 10.3390/biology14060679
 158. Etcheverry M, Moulin-Frier C, Oudeyer PY, Levin M. AI-driven automated discovery tools reveal diverse behavioral competencies of biological networks. *eLife*. 2025;13.
doi: 10.7554/eLife.92683
 159. Zhu D, Jerby L. Gradient-aware modeling advances AI-driven prediction of genetic perturbation effects. *bioRxiv*. Preprint online 2025.
doi: 10.1101/2025.10.03.680360
 160. Ali M, Richter S, Erturk A, Fischer DS, Theis FJ. Graph neural networks learn emergent tissue properties from spatial molecular profiles. *Nat Commun*. 2025;16(1):8419.
doi: 10.1038/s41467-025-63758-8
 161. Chen K, Qin KR, Na J, Gao G, Yang C, Fu J. Deep manifold learning reveals hidden developmental dynamics of a human embryo model. *Sci Adv*. 2025;11(32):eadr8901.
doi: 10.1126/sciadv.adr8901
 162. Stillman NR, Mayor R. Generative models of morphogenesis in developmental biology. *Semin Cell Dev Biol*. 2023;147:83-90.
doi: 10.1016/j.semcdb.2023.02.001
 163. Li Z, Zhang Y, Peng B, *et al.* A novel interpretable deep learning-based computational framework designed synthetic enhancers with broad cross-species activity. *Nucleic Acids Res*. 2024;52(21):13447-13468.
doi: 10.1093/nar/gkae912
 164. Peleke FF, Zumkeller SM, Gultas M, Schmitt A, Szymanski J. Deep learning the cis-regulatory code for gene expression in selected model plants. *Nat Commun*. 2024;15(1):3488.
doi: 10.1038/s41467-024-47744-0
 165. Moeckel C, Mouratidis I, Chantzi N, Uzun Y, Georgakopoulos-Soares I. Advances in computational and experimental approaches for deciphering transcriptional regulatory networks: Understanding the roles of cis-regulatory elements is essential, and recent research utilizing MPRA, STARR-seq, CRISPR-Cas9, and machine learning has yielded valuable insights. *Bioessays*. 2024;46(7):e2300210.
doi: 10.1002/bies.202300210
 166. Ho C, Morsut L. Novel synthetic biology approaches for developmental systems. *Stem Cell Rep*. 2021;16(5):1051-1064.
doi: 10.1016/j.stemcr.2021.04.007
 167. Naffaa MM. Bridging molecular mechanisms and therapeutic innovations: The role of brain organoids in

- neurodevelopmental disorder research. *Organoid Res.* 2025;1(3):025100010.
doi: 10.36922/OR025100010
168. Kanton S, Boyle MJ, He Z, *et al.* Organoid single-cell genomic atlas uncovers human-specific features of brain development. *Nature.* 2019;574(7778):418-422.
doi: 10.1038/s41586-019-1654-9
 169. Won H, Huang J, Opland CK, Hartl CL, Geschwind DH. Human evolved regulatory elements modulate genes involved in cortical expansion and neurodevelopmental disease susceptibility. *Nat Commun.* 2019;10(1):2396.
doi: 10.1038/s41467-019-10248-3
 170. Sullivan AE, Santos SD. The ever-growing world of gastruloids: Autogenous models of mammalian embryogenesis. *Curr Opin Genet Dev.* 2023;82:102102.
doi: 10.1016/j.gde.2023.102102
 171. Klein JC, Keith A, Agarwal V, Durham T, Shendure J. Functional characterization of enhancer evolution in the primate lineage. *Genome Biol.* 2018;19(1):99.
doi: 10.1186/s13059-018-1473-6
 172. De Santis R, Rice E, Croft G, Yang M, Rosado-Olivieri EA, Brivanlou AH. The emergence of human gastrulation upon in vitro attachment. *Stem Cell Rep.* 2024;19(1):41-53.
doi: 10.1016/j.stemcr.2023.11.005
 173. Mezu-Ndubuisi OJ, Maheshwari A. Role of macrophages in fetal development and perinatal disorders. *Pediatr Res.* 2021;90(3):513-523.
doi: 10.1038/s41390-020-01209-4
 174. Park JE, Botting RA, Dominguez Conde C, *et al.* A cell atlas of human thymic development defines T cell repertoire formation. *Science.* 2020;367(6480).
doi: 10.1126/science.aay3224
 175. Sun Y, Pan W. Brain organoids: A new paradigm for studying human neuropsychiatric disorders. *Front Neurosci.* 2025;19:1699814.
doi: 10.3389/fnins.2025.1699814
 176. Li K, Liu Y, Cao H, *et al.* Interrogation of enhancer function by enhancer-targeting CRISPR epigenetic editing. *Nat Commun.* 2020;11(1):485.
doi: 10.1038/s41467-020-14362-5
 177. Wang K, Escobar M, Li J, *et al.* Systematic comparison of CRISPR-based transcriptional activators uncovers gene-regulatory features of enhancer-promoter interactions. *Nucleic Acids Res.* 2022;50(14):7842-7855.
doi: 10.1093/nar/gkac582
 178. Karzbrun E, Khankhel AH, Megale HC, *et al.* Human neural tube morphogenesis in vitro by geometric constraints. *Nature.* 2021;599(7884):268-272.
doi: 10.1038/s41586-021-04026-9
 179. Rao KS, Kameswaran V, Bruneau BG. Modeling congenital heart disease: Lessons from mice, hPSC-based models, and organoids. *Genes Dev.* 2022;36(11-12):652-663.
doi: 10.1101/gad.349678.122
 180. Abdel Fattah AR, Daza B, Rustandi G, *et al.* Actuation enhances patterning in human neural tube organoids. *Nat Commun.* 2021;12(1):3192.
doi: 10.1038/s41467-021-22952-0
 181. Lee JH, Shin H, Shaker MR, *et al.* Production of human spinal-cord organoids recapitulating neural-tube morphogenesis. *Nat Biomed Eng.* 2022;6(4):435-448.
doi: 10.1038/s41551-022-00868-4
 182. Suhito IR, Sunil C, Tay A. Engineering human immune organoids for translational immunology. *Bioact Mater.* 2025;44:164-183.
doi: 10.1016/j.bioactmat.2024.10.010
 183. Harter MF, Recaladin T, Gjorevski N. Organoids as models of immune-organ interaction. *Cell Rep.* 2025;44(9):116214.
doi: 10.1016/j.celrep.2025.116214
 184. Gunther C, Winner B, Neurath MF, Stappenbeck TS. Organoids in gastrointestinal diseases: From experimental models to clinical translation. *Gut.* 2022;71(9):1892-1908.
doi: 10.1136/gutjnl-2021-326560
 185. Nikolaev M, Mitrofanova O, Broguiere N, *et al.* Homeostatic mini-intestines through scaffold-guided organoid morphogenesis. *Nature.* 2020;585(7826):574-578.
doi: 10.1038/s41586-020-2724-8
 186. Lewis-Israeli YR, Wasserman AH, Gabalski MA, *et al.* Self-assembling human heart organoids for the modeling of cardiac development and congenital heart disease. *Nat Commun.* 2021;12(1):5142.
doi: 10.1038/s41467-021-25329-5
 187. Thomson M. Signaling Boundary Conditions Drive Self-Organization of Human "Gastruloids". *Dev Cell.* 2016;39(3):279-280.
doi: 10.1016/j.devcel.2016.10.016
 188. Del Vecchio D. Epigenetic memory: The role of the crosstalk between histone modifications and DNA methylation. *Comput Struct Biotechnol J.* 2025;27:4019-4025.
doi: 10.1016/j.csbj.2025.08.034
 189. Maier JAH, Mohrle R, Jeltsch A. Design of synthetic epigenetic circuits featuring memory effects and reversible switching based on DNA methylation. *Nat Commun.* 2017;8:15336.
doi: 10.1038/ncomms15336
 190. Cakir B, Xiang Y, Tanaka Y, *et al.* Engineering of human brain organoids with a functional vascular-like system. *Nat*

- Methods*. 2019;16(11):1169-1175.
doi: 10.1038/s41592-019-0586-5
191. Andersen J, Revah O, Miura Y, *et al*. Generation of Functional Human 3D Cortico-Motor Assembloids. *Cell*. 2020;183(7):1913-1929.e26.
doi: 10.1016/j.cell.2020.11.017
 192. Takebe T, Sekine K, Enomura M, *et al*. Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature*. 2013;499(7459):481-484.
doi: 10.1038/nature12271
 193. Di Stasi A, Tey SK, Dotti G, *et al*. Inducible apoptosis as a safety switch for adoptive cell therapy. *N Engl J Med*. 2011;365(18):1673-1683.
doi: 10.1056/NEJMoa1106152
 194. Deuse T, Hu X, Gravina A, *et al*. Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. *Nat Biotechnol*. 2019;37(3):252-258.
doi: 10.1038/s41587-019-0016-3
 195. Simsek E, Yao Y, Lee D, You L. Toward predictive engineering of gene circuits. *Trends Biotechnol*. 2023;41(6):760-768.
doi: 10.1016/j.tibtech.2022.11.001
 196. Hyun I, Scharf-Deering JC, Lunshof JE. Ethical issues related to brain organoid research. *Brain Res*. 2020;1732:146653.
doi: 10.1016/j.brainres.2020.146653
 197. Sandoval SO, Cappuccio G, Kruth K, *et al*. Rigor and reproducibility in human brain organoid research: Where we are and where we need to go. *Stem Cell Rep*. 2024;19(6):796-816.
doi: 10.1016/j.stemcr.2024.04.008
 198. Lamm N, Ben-David U, Golan-Lev T, Storchova Z, Benvenisty N, Kerem B. Genomic Instability in Human Pluripotent Stem Cells Arises from Replicative Stress and Chromosome Condensation Defects. *Cell Stem Cell*. 2016;18(2):253-261.
doi: 10.1016/j.stem.2015.11.003
 199. Lackner M, Helmbrecht N, Paabo S, Riesenberger S. Detection of unintended on-target effects in CRISPR genome editing by DNA donors carrying diagnostic substitutions. *Nucleic Acids Res*. 2023;51(5):e26.
doi: 10.1093/nar/gkac1254
 200. Su Z, Dong H, Fang X, Zhang W, Duan H. Frontier progress and translational challenges of pluripotent differentiation of stem cells. *Front Genet*. 2025;16:1583391.
doi: 10.3389/fgene.2025.1583391
 201. Hanna RE, Doench JG. Design and analysis of CRISPR-Cas experiments. *Nat Biotechnol*. 2020;38(7):813-823.
doi: 10.1038/s41587-020-0490-7
 202. Li A, Mitsunobu H, Yoshioka S, Suzuki T, Kondo A, Nishida K. Cytosine base editing systems with minimized off-target effect and molecular size. *Nat Commun*. 2022;13(1):4531.
doi: 10.1038/s41467-022-32157-8
 203. Henry MP, Hawkins JR, Boyle J, Bridger JM. The Genomic Health of Human Pluripotent Stem Cells: Genomic Instability and the Consequences on Nuclear Organization. *Front Genet*. 2018;9:623.
doi: 10.3389/fgene.2018.00623
 204. Andrews PW, Barbaric I, Benvenisty N, *et al*. The consequences of recurrent genetic and epigenetic variants in human pluripotent stem cells. *Cell Stem Cell*. 2022;29(12):1624-1636.
doi: 10.1016/j.stem.2022.11.006
 205. Ghosh S, Brown AM, Jenkins C, Campbell K. Viral Vector Systems for Gene Therapy: A Comprehensive Literature Review of Progress and Biosafety Challenges. *Appl Biosaf*. 2020;25(1):7-18.
doi: 10.1177/1535676019899502
 206. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol*. 2007;9(6):654-659.
doi: 10.1038/ncb1596
 207. Arendt D, Musser JM, Baker CVH, *et al*. The origin and evolution of cell types. *Nat Rev Genet*. 2016;17(12):744-757.
doi: 10.1038/nrg.2016.127
 208. Lancaster MA, Corsini NS, Wolfinger S, *et al*. Guided self-organization and cortical plate formation in human brain organoids. *Nat Biotechnol*. 2017;35(7):659-666.
doi: 10.1038/nbt.3906
 209. Helenek C, Krzyszton R, Petreczky J, *et al*. Synthetic gene circuit evolution: Insights and opportunities at the mid-scale. *Cell Chem Biol*. 2024;31(8):1447-1459.
doi: 10.1016/j.chembiol.2024.05.018
 210. Gjorevski N, Sachs N, Manfrin A, *et al*. Designer matrices for intestinal stem cell and organoid culture. *Nature*. 2016;539(7630):560-564.
doi: 10.1038/nature20168
 211. Lavazza A, Massimini M. Cerebral organoids: Ethical issues and consciousness assessment. *J Med Ethics*. 2018;44(9):606-610.
doi: 10.1136/medethics-2017-104555
 212. Nicolas P, Etoc F, Brivanlou AH. The ethics of human-embryoids model: A call for consistency. *J Mol Med*. 2021;99(4):569-579.
doi: 10.1007/s00109-021-02053-7
 213. Rivron NC, Martinez Arias A, Pera MF, Moris N, M'Hamdi H I. An ethical framework for human embryology with embryo models. *Cell*. 2023;186(17):3548-3557.

- doi: 10.1016/j.cell.2023.07.028
214. De Miguel Beriain I, Rueda J, Villalba A. Re-defining the human embryo : A legal perspective on the creation of embryos in research. *EMBO Rep.* 2024;25(2):467-470.
doi: 10.1038/s44319-023-00034-0
215. Writing Group of the EEC, Pennings G, Dondorp W, Popovic M, Chuva de Sousa Lopes S, Mertes H. Ethical considerations on the moral status of the embryo and embryo-like structuresdagger. *Hum Reprod.* 2024;39(11):2387-2391.
doi: 10.1093/humrep/deae228
216. Koplin JJ. Response to the ISSCR guidelines on human-animal chimera research. *Bioethics.* 2023;37(2):192-198.
doi: 10.1111/bioe.13104.
217. Real R, Peter M, Trabalza A, *et al.* In vivo modeling of human neuron dynamics and Down syndrome. *Science.* 2018;362(6416).
doi: 10.1126/science.aau1810
218. Ou Y, Guo S. Safety risks and ethical governance of biomedical applications of synthetic biology. *Front Bioeng Biotechnol.* 2023;11:1292029.
doi: 10.3389/fbioe.2023.1292029
219. Hartung T, Morales Pantoja IE, Smirnova L. Brain organoids and organoid intelligence from ethical, legal, and social points of view. *Front Artif Intell.* 2023;6:1307613.
doi: 10.3389/frai.2023.1307613
220. Gao S, Fang A, Huang Y, *et al.* Empowering biomedical discovery with AI agents. *Cell.* 2024;187(22):6125-6151.
doi: 10.1016/j.cell.2024.09.022
221. Ahn SJ, Lee S, Kwon D, *et al.* Essential Guidelines for Manufacturing and Application of Organoids. *Int J Stem Cells.* ay 30 2024;17(2):102-112.
doi: 10.15283/ijsc24047
222. Clark AT, Cook-Andersen H, Franklin S, *et al.* Stem cell-based embryo models: The 2021 ISSCR stem cell guidelines revisited. *Stem Cell Rep.* 2025;20(6):102514.
doi: 10.1016/j.stemcr.2025.102514

Appendix

Scope, limits, and interpretation of this review.

I. What this review is

This review provides a structured, evidence-based framework for understanding how genetic, epigenetic, material, and cybergenetic interventions are currently used to experimentally steer developmental trajectories in organoid systems. The emphasis is on organizing heterogeneous tools and demonstrations into a tiered conceptual architecture that distinguishes validated capabilities from emerging, fragile, or conceptual approaches.

II. What this review is not

This review does not claim that current organoid platforms achieve autonomous, self-regulating, or fully closed-loop developmental control. It does not present programmable organoids as mature engineering systems, nor does it imply that genetic or epigenetic circuits currently function as robust, reversible controllers of human development. Higher-tier architectures are not intended to represent near-term feasibility.

III. How claims should be interpreted

Throughout this manuscript, claims of programmability should be interpreted as degrees of experimental steerability rather than intrinsic autonomy. Genetic circuits are discussed as circuit-inspired or feedback-limited architectures unless explicitly demonstrated as stable and internally regulated in organoids. Epigenetic mechanisms are treated as state-modifying layers that bias, stabilize, or record developmental trajectories, not as programmable controllers capable of fully reversible, causal regulation.

IV. Role of biological constraints

Biological variability, batch effects, incomplete maturation, lack of vascularization, and limited observability are treated as first-order design constraints rather than peripheral caveats. These constraints define the feasible control architectures that can currently be implemented and explain why higher-tier systems remain fragile, context-dependent, or conceptual despite strong theoretical grounding.

V. Purpose of higher-tier frameworks

Tier 3 and Tier 4 architectures are included as directional design frameworks that articulate how future programmable organoid systems might be structured if current biological and technical constraints are overcome. They are presented to guide hypothesis generation, system design, and experimental prioritization; not as indicators of imminent autonomous developmental control.