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Current Opinion in Plant Biology

Synthetic biology unlocks programmable control of complex plant glycan metabolism

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Advances in synthetic biology now offer spatiotemporal, conditional, and precise control of plant metabolism, enabling engineering of pathways that have resisted constitutive approaches. Plant cell wall biosynthesis and metabolite glycosylation are industrially valuable pathways that are also acutely sensitive to constitutive perturbation. We use these pathways as a lens to examine the potential impact of emerging programmable tools, including synthetic, tissue-specific, and inducible promoter design; RNA-, protein-, and pathway-level regulatory switches; and CRISPR-based systems. We then examine how these tools are overcoming barriers in cell wall engineering, production of glycosylated therapeutics, and emerging applications in signalling and herbicide tolerance. Together, these advances move plant metabolic engineering from static modification toward self-regulating, precise metabolic control, with complex glycosylation emerging as both a significant test and a key beneficiary of these advances.

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Introduction

Through the design, construction, and testing of heterologous and novel biological functions, synthetic biology (SynBio) iteratively expands the possibilities for engineering plant metabolism [1]. Traditionally, metabolic engineering has relied on static modifications such as constitutive overexpression or gene knockout, which impose significant limitations [2,3]. In contrast, SynBio advances now enable spatial, temporal, and conditional control of metabolic processes [4–7]. By introducing these additional dimensions, pathways that were previously inaccessible due to essentiality or developmental constraints can now be reprogrammed in a controlled and predictable manner. This minimises pleiotropic effects while expanding the range of achievable outcomes. We distinguish throughout between these two approaches: static engineering, defined as constitutive modifications of plant metabolism, and programmable control, defined as dynamic regulation in which gene expression is conditionally activated, repressed, or tuned according to spatial, temporal, or environmental context.

Among plant metabolic pathways, those governing cell wall and glycan metabolism present some of the most stringent constraints on static engineering. These pathways coordinate the biosynthesis and modification of structural polysaccharides, glycoproteins, glycolipids, and other metabolites, which commonly resist static engineering due to their essentiality [8,9]. For example, constitutive perturbations to the cell wall can lead to wall failure (e.g. pollen tube bursting) or can trigger cell wall integrity (CWI) sensing mechanisms which in turn activate defence responses, leading to deleterious trade-offs [10]. Many glycosylation outputs are of direct interest for industrial applications, including bioenergy feedstocks, forage, biomaterials and platform chemicals and therapeutics. Therefore, these pathways provide an ideal test case to demonstrate the potential of shifting from static engineering to programmable control.

In this review, we examine the current state of SynBio technical advances that expand what can be controlled.

We also highlight critical limitations revealed by current efforts to engineer plant cell wall and glycan metabolism constitutively and illustrate how programmable control offers strategies to overcome these barriers. Overall, we propose that programmable control marks a new era in plant SynBio, moving beyond static modifications to precise engineering, enabling the full exploitation of cell wall engineering and other complex glycosylation.

Programmable control of plant metabolism

Plant metabolism can be engineered at multiple layers, defined here as distinct aspects of gene expression and protein function that are amenable to targeted intervention. The simplest layer is transcriptional control, targeting core transcription factors (TFs) and rate-limiting enzymes, interventions that can reshape entire pathways and plant responses [11]. The next layer is post-transcriptional control, which enables the modulation of messenger RNA (mRNA) abundance and translation [12]. After translation, protein activity can be modulated at the post-translational layer through direct modification or inhibition [13]. Beyond individual proteins, plant metabolism can also be regulated through signalling cascades that coordinate the activity of multiple proteins. Unlike post-translational regulation of individual proteins, controlling signalling involves harnessing existing signalling pathways by inducing or repressing key upstream inputs [14,15]. Finally, the development of gene editing tools based on the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)−CRISPR associated protein (Cas) system introduces an additional layer of control, by mutating or rewriting genomic DNA, or regulating transcription [16]. In this section we explore existing tools to control these different metabolic layers and discuss their current implementation in plants and associated limitations.

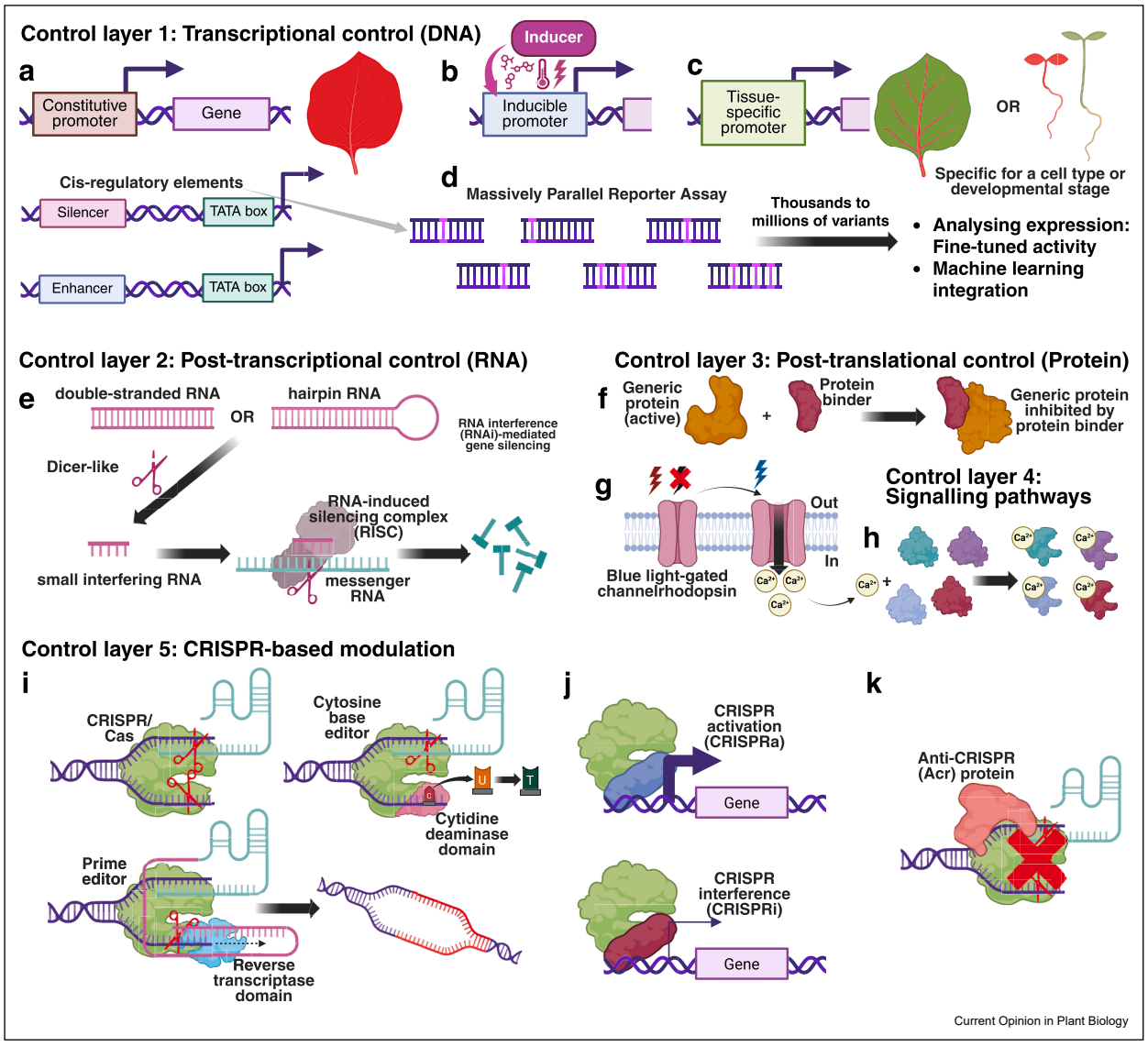
Promoter elements, which drive transcription, constitute the first layer of control [17]. Constitutive promoters, such as the widely used *cauliflower mosaic virus* (CaMV) 35S promoter, offer high expression amounts [18]. Expression from these promoters can be fine-tuned, as demonstrated for the CaMV 35S promoter by mutating conserved DNA motifs such as the TATA box [19], or through the positioning of enhancers, silencers, and insulators, which are DNA motifs that recruit transcriptional regulators [20,21] (Figure 1a). Inducible promoters provide conditional control, activated by externally applied stimuli such as temperature [4] or small molecules [5] (Figure 1b). The main limitation is that inducible promoters can show “leakage”, resulting in low-level basal expression even in the absence of an inducer [22]. To prevent leakage, transgenic constructs need to be carefully designed and validated, since neighbouring elements such as introns or enhancers from adjacent transgene cassettes can elevate basal expression [23]. Furthermore, tissue-specific promoters target expression to certain tissues or cell types [6], while promoters of developmentally regulated genes can be harnessed for transgene expression at specific growth stages and tissues [7] (Figure 1c). Advances in single-cell RNA sequencing have expanded the repertoire of identified tissue-specific promoters and improved detection of off-target expression in other tissues [24]. Combined, inducible and tissue-specific promoters enable finely tuned spatiotemporal regulation of gene expression [6].

Promoter diversity is theoretically powerful, but practically constrained due to the limited number of characterised promoters that are effective in plant systems. As a result, complex metabolic engineering strategies often reuse the same promoter elements, increasing the risk of targeted silencing [18]. Repeated use of the same promoter sequences can trigger epigenetic silencing via RNA-directed DNA methylation (RdDM) or repeat-induced gene silencing (RIGS) [25,26], both of which represent a real constraint in multigene engineering strategies [27]. These effects can be mitigated through promoter diversification, minimisation of homologous regulatory sequence repetition, and incorporation of chromatin boundary elements such as insulators or matrix attachment regions (MARs) to reduce epigenetic interference between transgene cassettes [27].

To overcome the promoter availability limitation, there is an urgent need for compact, well-characterised promoter libraries with known strengths, characterised performance across species, improved tissue-specificity or inducibility with limited leakage to enable the reconstruction and programmable control of metabolic pathways [17,18]. Massively Parallel Reporter Assays (MPRAs), which enable high-throughput, simultaneous evaluation of thousands of regulatory sequences, have successfully been employed to characterise the strength of plant enhancers, silencers, insulators, and promoters [20,21,28] (Figure 1d). Despite the large datasets generated, these remain dispersed across supplementary materials due to the lack of a standardised repository, limiting reuse. Nevertheless, expanding MPRA datasets combined with machine learning show great promise for designing effective synthetic promoters, overcoming the limitations of mining naturally occurring sequences [17,28].

Beyond transcriptional control, repression of specific genes can alleviate metabolic bottlenecks and redirect flux towards desired pathways. The most established approach is RNA interference (RNAi) [29], enabling post-transcriptional gene silencing through two main strategies: expression of double-stranded RNA (dsRNA) or hairpin RNA (hpRNA) that is processed into small interfering RNAs (siRNAs) (Figure 1e), or use of artificial microRNAs (amiRNAs) that co-opt the endogenous miRNA biogenesis machinery to silence a target transcript

Figure 1



Multi-layered synthetic biology toolkit for programmable control of plant metabolism. (a) Constitutive promoters drive broad expression and can be fine-tuned through *cis*-regulatory elements such as TATA boxes (part of the promoter), silencers or enhancers. (b) Inducible promoters drive expression upon addition of external stimuli such as heat, light, or small molecules. (c) Tissue-specific promoters drive expression in defined cell types or developmental stages. (d) Massively Parallel Reporter Assays enable simultaneous evaluation of thousands of regulatory sequences, identifying fine-tuned expression. Machine learning further identifies key regulatory nucleotides. (e) RNA interference mediates post-transcriptional gene silencing through dsRNA or hpRNA. (f) Protein binders can inhibit or modulate protein function upon binding. (g) Channelrhodopsin (ChR) activity is modulated by light at specific wavelengths. (h) Calcium (Ca^{2+})-transporting ChRs enable the modulation of Ca^{2+} signalling by light, affecting multiple proteins. (i) Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)–CRISPR associated protein (Cas) systems enable gene knockout or, when fused to other protein domains, base editing or prime editing. (j) CRISPR proteins can be harnessed to modulate expression as CRISPR activation or CRISPR interference. (k) Anti-CRISPR (Acr) proteins inhibit Cas function. (l) Cas13 targets RNA rather than DNA, enabling transcript knockdown. Created in BioRender https://BioRender.com/7tyu5mv.

[29]. Transient RNAi silencing can also be achieved through foliar application of dsRNA [30] or virus-induced gene silencing [31], allowing rapid but temporary modulation of metabolic pathways. This is particularly useful for species with long growth periods, where generating stable transgenic lines is difficult and time-consuming. Despite the versatility of RNAi-based gene silencing strategies, their implementation still presents important limitations, mainly the potential for off-target effects arising from partial sequence complementarity with unintended transcripts and variable knockdown efficiency that may be insufficient for complete gene repression [32].

Protein function can also be regulated post-transcriptionally. Protein binders are engineered proteins or peptides designed to bind a target protein, inhibiting catalytic activity without modifying genomic sequences or transcripts [33,34] (Figure 1f). Recent deep learning-based tools show potential to greatly improve success rates of synthetic protein binder design [33,34]. While the application of protein binders in plants has been proposed [34], its potential remains to be validated. We also anticipate binders capable of modifying rather than inhibiting protein function, as well as *de novo* protein design and improvement of core metabolic enzymes. In addition to protein binders, post-translational control can be achieved using light-gated channelrhodopsins (ChRs), whose activity is modulated by light at specific wavelengths [35] (Figure 1g). The function of these proteins can therefore be controlled with light. A deeper understanding of how light modulates ChRs' activity may enable the engineering of other proteins to respond to light, expanding the tools available for metabolic control *in planta*.

At a higher level of organisation, plant metabolism can also be modulated through signalling pathways coordinating multiple proteins. Since ChRs transport ions such as calcium and protons, ChRs-mediated ion fluxes have been proposed as tools to induce signalling pathways, including calcium-dependent signalling cascades [14,36] (Figure 1h). Signalling outputs can also be modulated via engineered control of protein turnover. For example, a synthetic PEST-containing protein was used to transiently interfere with CULLIN3-RING E3 ligase activity in *Brassica napus*, resulting in stabilisation of endogenous regulatory proteins normally targeted for degradation [15]. While not a signalling system per se, this approach provides a means to alter signalling pathways by tuning the lifetime of key regulators, thereby altering downstream stress and hormonal responses. In addition, the mitogen-activated protein kinase (MAPK) signalling pathway, which affects multiple processes of plant growth including development and plant adaptation, has been altered to improve yield and stress tolerance [37]. Together, these approaches illustrate complementary routes for controlling signalling, spanning upstream signal induction, modulation of protein turnover, and direct rewiring of signalling cascades.

The advent of more advanced genetic engineering tools, represented by the CRISPR—Cas systems, simplifies programmable control of metabolism-associated genes. Besides irreversible CRISPR-mediated mutagenesis and gene knockouts (Figure 1i), catalytically inactive Cas proteins (dead Cas) serve as DNA binders to recruit activator and repressor domains, enabling targeted transcriptional regulation [38,39] (Figure 1j). This capability becomes particularly powerful in metabolic engineering when multiplexed, using CRISPR guide RNAs (gRNAs) to simultaneously target, regulate, and synchronise transcription of multiple genes within or across pathways. Anti-CRISPR (Acr) proteins, shown to inhibit CRISPR—Cas systems in plants [40], provide additional conditional switches (Figure 1k).

Ultimately, the rapidly expanding functionality of CRISPR-based toolkits, integrated with developments in synthetic and conditional promoter elements, could facilitate highly versatile engineering and even accurate design of plant metabolism, where each enzymatic component and output would be precisely programmed and tuned as desired. These layers of control can be integrated in synthetic genetic circuits [41], including feedback-responsive biosensors that sense metabolites or internal signals to adjust expression or activity [42]. Combined with controlled environment agriculture for precise external cue delivery [43], this opens the possibility of closed-loop metabolic control. The following sections illustrate how this expanding toolkit is being deployed to reshape plant cell wall biosynthesis and glycan metabolism, pathways that exemplify the limitations of static engineering and the potential for programmable control.

Reprogramming cell wall composition and properties

Cell wall engineering has diverse applications, ranging from enhancing wood quality in trees and reducing lodging in broadacre crops to improving forage quality [44]. A major focus of recent investment has been the development of sustainable biomass feedstock crops required to support an economically viable bioeconomy [45]. The bioeconomy refers to an economic system based on renewable resources from biological origin used to produce industrial goods, including energy in the form of biofuels [46]. Plants are uniquely suited for biofuel production due to their photosynthetic capacity, which enables direct conversion of solar energy into chemical energy which is stored as biomass [47]. Since the cell wall constitutes the majority of crops biomass, improving biofuel production through cell wall engineering without compromising growth or yield is essential to achieve economically viable biofuels [47].

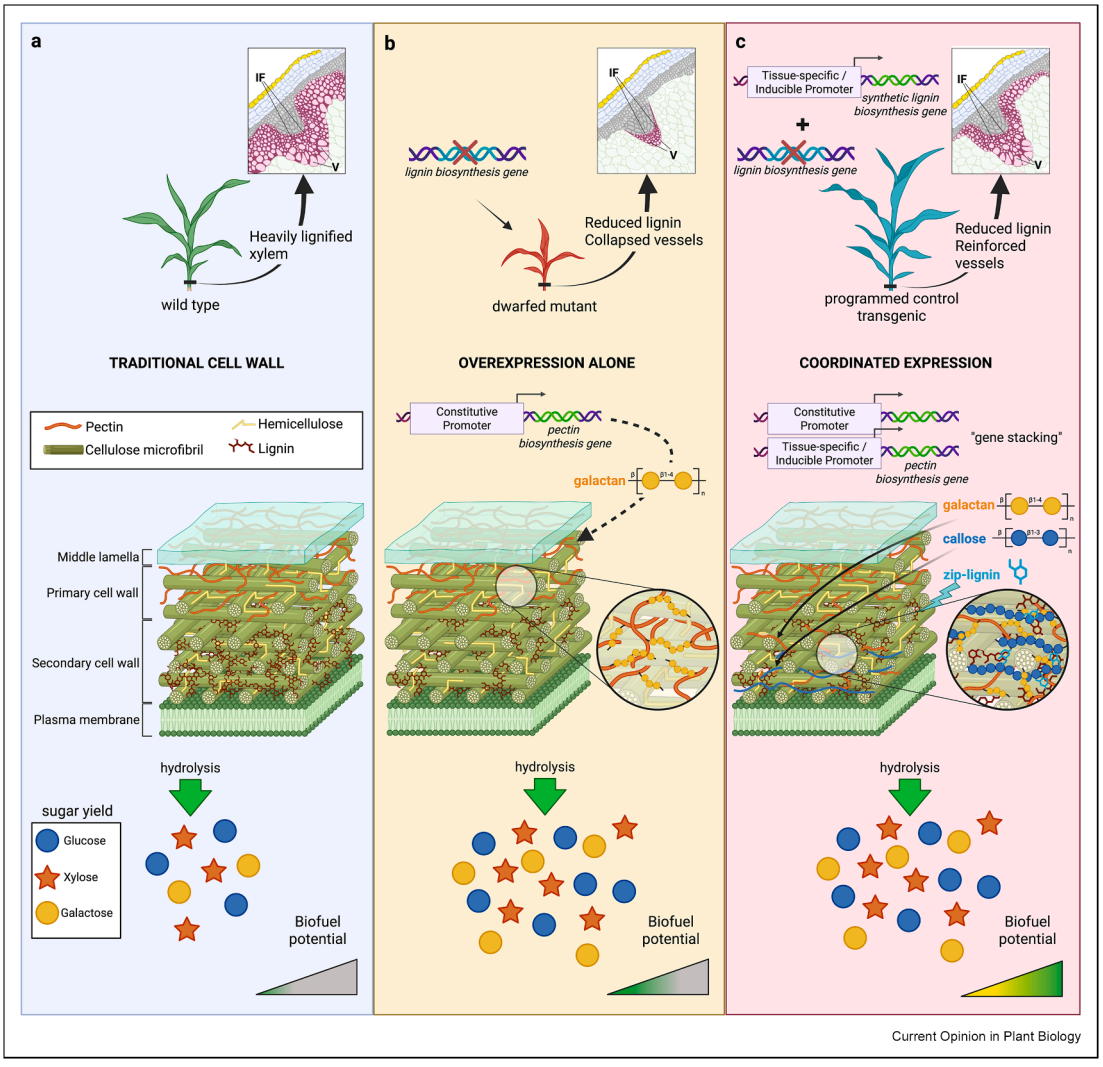
SynBio has the potential not only to accelerate conventional breeding approaches but also to deliver improvements beyond their usual bounds, overcoming restrictions such as the cell-type-specific essentiality of some polymers. Yet the complexity and essentiality of the cell wall make it particularly recalcitrant to static engineering, and programmable spatiotemporal control offers a route through this constraint, enabling precise modification of individual components without sacrificing plant fitness.

Cell wall polysaccharides primarily consist of a mixture of hexose (C6) and pentose (C5) sugars, but most

industrial microbes preferentially metabolise hexose sugars (Figure 2a) [48]. Increasing the proportion of fermentable hexose sugars in the cell wall is therefore a central goal for bioeconomy applications. However, achieving an increase in hexose proportion without growth penalties is surprisingly non-trivial, often because constitutive modification of specific polymers triggers CWI sensing or disrupts cell-type-specific functions (Figure 2b). For example, constitutive overexpression of glucomannan synthetases under the 35S promoter impairs embryo viability and development [49], while callose overaccumulation affects growth [50]. Consequently, more complex multi-component approaches, as exemplified below, have proven more successful for altering the plant cell wall.

While constitutive overexpression of callose synthetases is deleterious, targeted deposition using the secondary

Figure 2



Transition from static defects to programmable metabolic control of the cell wall. SynBio enables a shift from constitutive "always-on" modifications to logic-gated systems that treat the cell wall as a responsive metabolic interface. (a) Wild Type: Shows native secondary cell wall architecture with heavily lignified xylem vessels (V) and interfascicular fibres (IF). This provides structural resilience but creates a formidable industrial bottleneck, limiting sugar release during hydrolysis. (b) Static Engineering: Conventional approaches often rely on constitutive knockouts of lignin biosynthesis genes or overexpression of glycosyltransferase genes. While lignin reduction increases digestibility, this often leads to severe growth-defence trade-offs, including dwarfism and xylem collapse (flattened vessels). Furthermore, single-gene overexpression often fails to significantly alter wall composition due to limited substrate availability or lack of enzyme activity. (c) Programmable SynBio Control: Precision restructuring utilises "knockout-and-rescue" and "gene stacking" strategies to decouple structural integrity from digestibility. Spatio-temporal Rescue: Reintroducing a modified lignin biosynthesis gene via the vessel-specific *ProSNBE* promoter restores reinforcement specifically in the xylem vessels (V), preserving hydraulic integrity while maintaining low-lignin fibres (IF). Metabolic Tailoring: Alternative strategies include coordinated expression of biosynthetic enzymes alongside transporters and sugar-epimerases for successful overexpression and integration of glycans. Incorporating "zip-lignin" (chemically labile ester bonds) or unconventional polysaccharides, like callose, allows the wall to "unzip" or restructure on demand via inducible systems or mild pre-treatment to bypass growth penalties.

cell wall-specific *IRX8* promoter reduced lignin content in poplar without an associated growth penalty [51]. Extension of this work to glucomannan and galactan, with the secondary cell wall-specific *IRX3*, *IRX5*, *IRX8*, and *C4H* promoters, led to an unexpected finding: introduction of additional polysaccharides can loosen wall architecture, enhancing saccharification [51–53]. Engineering galactan, a galactose-rich pectin side chain, proved insufficient after overexpression of the biosynthetic galactosyltransferase alone [54]. Success required co-expression with a nucleotide sugar epimerase and transporter to expand the UDP-galactose pool in the correct subcellular compartment [52,55], demonstrating that glycan engineering demands coordinated control of substrate supply, not just enzymatic activity.

Another instructive case is mixed linkage glucan (MLG), where constitutive or xylem-specific MLG-synthase expression caused severe growth defects, whereas the senescence-induced *SAG12* promoter enabled up to a four-fold accumulation increase [56]. MLG undergoes diurnal turnover, meaning that it is degraded during the night and accumulated in the cell wall during the day, so the full potential of this approach likely requires simultaneous repression of degrading enzymes [57]. Together, these examples highlight how SynBio can improve saccharification by introducing additional polysaccharides, by coordinating substrate availability, biosynthetic timing, spatial specificity, and polymer turnover (Figure 2c).

Although lignin is a phenolic compound rather than a glycan, its close interaction with cell wall polysaccharides warrants its inclusion in this review. Reduced lignin content enhances saccharification [58], which benefits the production of cellulosic biofuels such as bioethanol. In parallel, lignin is also considered relevant for advanced biofuels due to its high energy density [59]. Thus, modulating lignin content increases the versatility of biomass feedstocks for different biofuel applications. Programmable control promises to be equally transformative for fine-tuning lignin content, where spatial and temporal precision has enabled engineering strategies that were previously inaccessible.

Fibre-specific suppression of lignin deposition improves lignocellulosic biomass utilisation while avoiding the weakening of xylem vessel walls and their subsequent collapse seen in global knockouts [60]. An elegant example of tuned spatial control is the xylem-targeted reintroduction of the lignin biosynthesis gene *CCR1* under the control of a synthetic secondary wall NAC binding element (*ProSNBE*) promoter [61]. By strategically directing expression only where needed, rescued Arabidopsis *ccr1* mutants achieved up to 59% increase in biomass, and a four-fold increase in total sugar yield, compared to wild-type plants. This xylem-specific complementation strategy, when combined with CRISPR–Cas9-mediated knockout of native *CCR1*, provides a rapid tool for generating desirable plants with a single "knockout-and-rescue" transformation event [62].

Lignin engineering has also proved to be amenable to the incorporation of alternative lignin monomers [63] into the wall to enhance post-harvest deconstruction. One prominent example is the "zip-lignin" toolkit, which introduces monolignol-ferulate (ML-FA) conjugates into the lignin backbone. These chemically labile ester bonds make the wall "unzip" more readily during chemical deconstruction with no consequence to plant health, and can be synthesised *in planta* [64]. Alternatively, expression of microbe-derived enzymes, such as QsuB (3-dehydroshikimate dehydratase) from *Corynebacterium glutamicum*, can be used to alter flux through key substrate pathways, such as the shikimate pathway [65]. The introduction of QsuB not only reduces lignin content by as much as 20% in the bioenergy crop switchgrass [66] and 33% in hybrid poplar [67], yielding respective biomass saccharification efficiencies of 30% and 40%, but it also results in the accumulation of protocatechuic acid, a valuable biomanufacturing precursor. These strategies would become even more effective when combined with programmable modifications of the cell wall polysaccharides (Figure 2c).

Beyond engineering individual cell wall components, the natural diversity in cell wall composition and metabolism across the plant kingdom provides a valuable framework for feedstock improvement. Comparative analyses have revealed fundamental architectural differences between Type I and Type II cell walls [68]. Type I cell walls, which are pectin-rich with xyloglucan as the primary hemicellulose, are typical of dicots and non-commelinid monocots [68]. By contrast, Type II cell walls are found in commelinid monocots, and are instead enriched in glucuronoarabinoxylan, hydroxycinnamates, and variable mixed-linkage glucan, with reduced pectin content [68]. This divergence has direct implications for biomass recalcitrance and the enzymatic strategies required for efficient deconstruction, especially because most grass-based bioenergy feedstocks are commelinid monocots [45].

Together, these examples establish that programmable control transforms the cell wall from a recalcitrant barrier into a tuneable interface, a principle that extends to other complex plant metabolism.

Controlled production of cross-kingdom bioactive glycans in plants

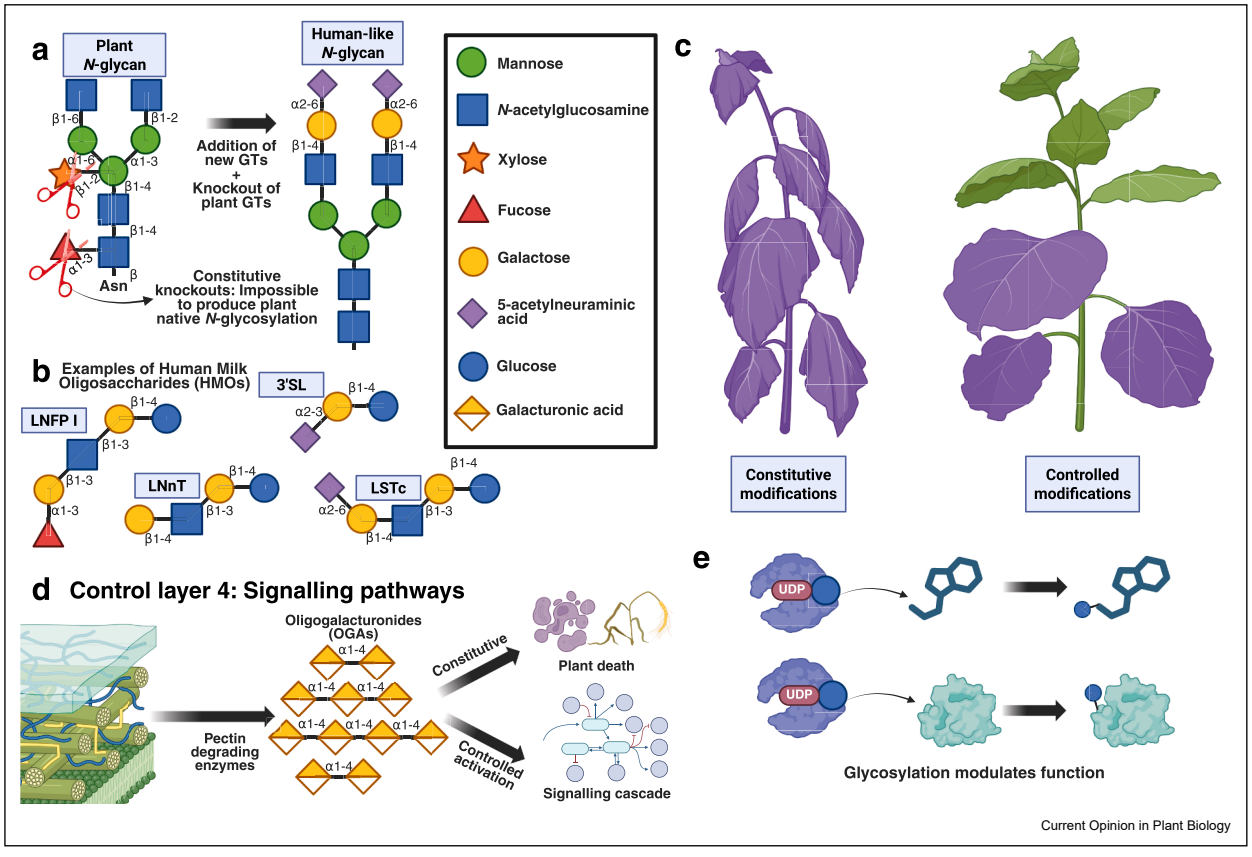
Beyond the cell wall, functional glycosylation extends to proteins, lipids, and metabolites, where programmable control offers equally transformative opportunities. Many of these pathways are specific to eukaryotes, limiting bacterial production systems due to the

absence of organelles and complex post-translational modification machinery [69]. Plants have long been identified as a promising alternative to mammalian cells to produce glycosylated therapeutics because of their lower cost, faster production time, capacity for larger genetic constructs, and lack of zoonotic pathogens [70]. However, the production of these therapeutics in plants requires glycosylation patterns to be modified to more closely resemble those found in humans [71].

Humanisation of protein glycosylation in plants has been achieved by removing fucose and xylose from plant *N*-glycans through knockout of the corresponding glycosyltransferases (GTs) [2], and overexpressing mammalian galactosylation and sialylation enzymes to add in galactose and sialic acid (Figure 3a) [3]. However, these static modifications have recently been reported to compromise fitness in stably transformed *Nicotiana benthamiana* [72], highlighting the limitations of constitutive perturbation of plant glycan metabolism. In this study, two different promoters were used to overexpress a galactosyltransferase: ubiquitin promoter (higher expression) and octopine synthase promoter (lower expression). The construct with lower expression was selected for detailed analyses but still exhibited retarded growth rate and reduced seed production [72].

Human milk oligosaccharides (HMOs), a diverse family of bioactive glycans essential for infant development and microbiome establishment, represent another class of non-native glycans of high biotechnological interest (Figure 3b) [73]. While only a limited subset is currently used in synthetic milk formulations, recent work has demonstrated the production of complex HMOs in plants [74]. These results support the potential for scalable production of HMOs in plants, although challenges such as downstream processing costs and batch variability remain [75]. Spatiotemporal regulation of pathway components provides a means to balance metabolic burden with yield, enabling the production of

Figure 3



Programmable plant glycoengineering across molecular farming, signalling, and functional glycosylation. (a) Structures of plant native and humanised *N*-glycans, including necessary modifications and the limitation of constitutive engineering. (b) Structure of selected Human Milk Oligosaccharides. (c) Constitutive modifications for molecular farming compromise plant health by disrupting native metabolism, whereas programmable control, here exemplified by fully-formed leaf-specific expression, enables the production of glycosylated compounds without affecting plant viability. (d) Controlled pectin degradation enables inducible oligogalacturonide signalling, targeting a pathway where constitutive activation would be lethal, introducing a new layer of programmable control. (e) UDP-glycosyltransferases (UGTs) glycosylate diverse substrates including proteins, hormones, and even herbicides, modulating their function. Created in BioRender https://BioRender.com/kjuc26w.

these bioactive glycans without compromising plant fitness (Figure 3c).

SynBio now offers opportunities to make glycosylated compound production in plants more efficient while minimising associated growth trade-offs through programmable control. The synthesis of these compounds can be restricted spatially and temporally using cell-specific and inducible promoters, while plant-specific glycosylation can be eliminated or downregulated conditionally. These strategies have the potential to reduce unintended growth stress and allow glycoengineering to be decoupled from plant integrity. Thus, programmable regulation offers not only efficiency enhancement but also platform stabilisation for molecular farming, and opens further opportunities for controlling plant glycan metabolism.

Emerging opportunities for the control of plant glycan metabolism

Beyond the established applications of plant glycoengineering discussed above, programmable control of glycan metabolism opens additional design spaces where static engineering is particularly challenging. In these essential, sensitive, and context-dependent metabolic pathways, SynBio enables intervention in a spatial, temporal, and conditional manner, extending control to processes such as signalling or herbicide tolerance.

Cell wall polysaccharides possess the potential to become signalling molecules through partial degradation. For example, oligogalacturonides (OGs) derived from pectin degradation are signalling molecules involved in different processes such as defence and development [8,76]. *Arabidopsis thaliana* POLYGALACTURONASE LATERAL ROOT (PGLR) and ARABIDOPSIS DEHISCENCE ZONE POLYGALACTURONASE2 (ADPG2) generate OGs of different sizes [77]. Exogenous application of these enzymes to roots produced different phenotypes: PGLR caused only minor reductions in cell size, whereas ADPG2 caused root growth inhibition and root cap detachment [77]. Although constitutive overexpression studies are lacking, continuous pectin degradation into OGs would likely compromise cell adhesion and viability. Inducible degradation therefore provides a more viable strategy to modulate signalling sources in a controlled manner (Figure 3d).

Beyond enzymatic control, small-molecule interventions also show promise for inducing signalling pathways. Recently, a bacterial iminosugar that mimics galactose, glycosyrin, was shown to rewire plant glycan metabolism [78]. Rather than targeting individual enzymes, such cross-kingdom metabolites illustrate how whole-pathway inputs can reshape glycan metabolism. While these approaches require careful calibration, SynBio expands the design space for manipulating signalling sources.

Programmable control may also provide new strategies to develop herbicide-tolerant crops [79]. Protein engineering has recently generated glyphosate-insensitive variants of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme required for aromatic amino acid biosynthesis whose glyphosate-mediated inhibition is lethal in wild-type plants [80]. Given that the short- and long-term human health effects of many commonly used herbicides remain unresolved [81], cellulose biosynthesis inhibitors (CBIs) represent a mechanistically distinct alternative target class with reduced off-target risk compared to broad spectrum herbicides [82]. However, cellulose biosynthesis represents a particularly complex aspect of glycan metabolism, since it is polymerised by the Cellulose Synthase Complex (CSC), a heteromeric assembly of Cellulose Synthase A (CESA) enzymes [9]. Two major challenges would arise from static overexpression of resistant variants: overexpression of a specific CESA subunit could disrupt CSC stoichiometry, and multiple CESAs could be targeted by CBIs [82]. Potential disruption of CSC stoichiometry could be reduced by expressing engineered CESA variants only in tissues or developmental stages particularly vulnerable to the herbicide, thereby restricting perturbation of CSC stoichiometry to those tissues while still potentially conferring CBI tolerance. In parallel, resistance to broad-spectrum CBIs would likely require coordinated expression of multiple engineered proteins. These examples illustrate how SynBio could enable protection while minimising unintended perturbation of cellulose biosynthesis.

At the interface of the processes discussed above, UDP-glycosyltransferases (UGTs) from the GT1 family can glycosylate signalling hormones, defence metabolites, and xenobiotics such as herbicides (Figure 3e) [83]. Controlling the expression of these enzymes opens the possibility of modulating or spatially restricting these processes. However, as in previous examples, constitutive expression of UGTs is suboptimal, causing broad signalling alterations rather than targeted control when required [84]. Compounding this, UGTs often have broad substrate specificity and limited predictability for novel acceptors [85]. Restricting activity to specific tissues or times could minimise off-target effects. Although still underexplored, the GT1 family's vast potential makes programmable control particularly promising for revealing and engineering novel glycosylation pathways.

From signalling triggers to herbicide targets to small-molecule glycosylation, SynBio transforms recalcitrant engineering into programmable engineering beyond cell wall biosynthesis and the production of heterologous glycosylated compounds. By replacing constitutive

perturbations with programmable control, these strategies show potential to redesign metabolism without compromising plant fitness or function.

Concluding remarks

Plant metabolic pathways, particularly cell wall and glycan metabolism, resist static engineering due to their essentiality, developmental integration, or dosage sensitivity. We have illustrated how SynBio strategies enable programmable control of metabolism, circumventing these constraints across structural, signalling, and therapeutic applications. Future advances in transcription regulators and *de novo* protein design will further enhance precision reprogramming of metabolism while balancing productivity and plant fitness. By moving from static engineering to programmable control, SynBio is reshaping what is possible in plant metabolic engineering. The next frontier is not engineering individual pathways, but designing self-regulating metabolic systems that sense, adapt, and optimise autonomously, transforming plants into living, programmable biofactories.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

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