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Engineering of non-model eukaryotes for bioenergy and biochemical production

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The prospect of leveraging naturally occurring phenotypes to overcome bottlenecks constraining the bioeconomy has marshalled increased exploration of nonconventional organisms. This review discusses the status of non-model eukaryotic species in bioproduction, the evaluation criteria for effectively matching a candidate host to a biosynthetic process, and the genetic engineering tools needed for host domestication. We present breakthroughs in genome editing and heterologous pathway design, delving into innovative spatiotemporal modulation strategies that potentiate more refined engineering capabilities. We cover current understanding of genetic instability and its ramifications for industrial scale-up, highlighting key factors and possible remedies. Finally, we propose future opportunities to expand the current collection of available hosts and provide guidance to benefit the broader bioeconomy.

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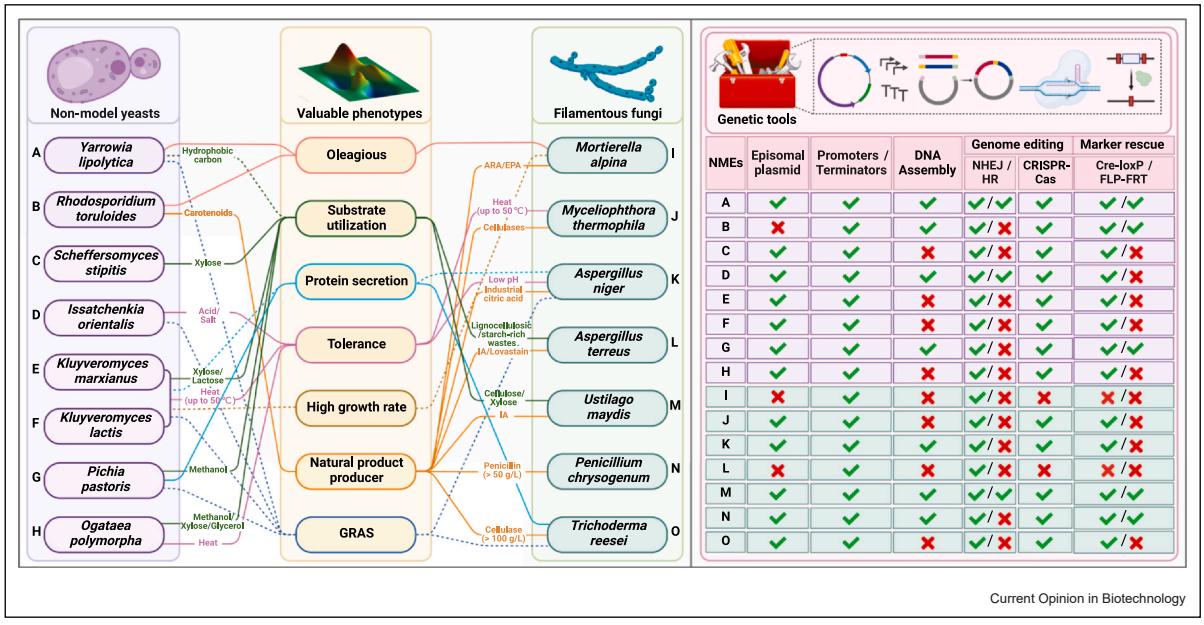
Introduction

Poor scaling of bioprocesses has precluded the bioeconomy from supplanting the petrochemical industry. The diverse set of challenging and chaotic conditions in industrial fermentation necessitates an equally diverse repertoire of microbial factories. Non-model eukaryotes (NMEs) provide a rich supply of evolutionary and phenotypic diversity, ideal for preselecting a physiology best equipped for the task at-hand [1,2•]. Feedstock, product, and fermentation condition represent critical 'process attributes' that direct rational microbial host selection. In this review, we prioritize discussions on unicellular fungi (i.e. yeasts). We also highlight works in filamentous fungi (FF) to formulate a more global perspective on NME engineering and illuminate facets of FF that could aid yeast researchers. A few strategies in *Saccharomyces cerevisiae* we deem relevant to tackling universal problems in engineering NMEs in terms of enhancing fitness and genetic instability are also included.

Streamlining strain selection

The highly dynamic relationship between a microbial host and the bioprocess employing it emphasizes that pragmatic selection of an appropriate host should precede any laborious strain development. Such 'host preselection' requires defining the key parameters of a desired bioprocess, including i) feedstock (e.g. lignocellulosic hydrolysates, biorefinery/industrial side streams, waste cooking oil (WCO), and C_1 substrates such as CO_2, CO, and methanol), ii) target product (e.g. precursor availability, acidity/basicity, hydrophobicity, or other modes of cytotoxicity), and iii) process parameters (e.g. temperature, pH, oxygen, and mixing-induced shear stress). Concurrent evaluation of a candidate host's innate capabilities and domestication potential maximizes the probability of engineering a system with the desired outcome. For example, oleaginous yeasts *Yarrowia lipolytica* and *Rhodosporidium toruloides* have enabled efficient production of fatty acid-derived biodiesels and value-added biochemicals due to their high content of CoA precursors [3,8,12••]. Well-characterized xylose metabolism and high cellular flux toward erythrose-4-phosphate in *Scheffersomyces stipitis* facilitates the production of aromatic products [15••] and ethanol fermentation from lignocellulosic feedstock [44]. A representative acid-tolerant yeast *Issatchenkia orientalis* produces organic acids at low pH and grows on several carbon substrates [16]. *Kluyveromyces marxianus* and *Kluyveromyces lactis* possess multiple advantages, including thermotolerance, rapid growth rate, and broad substrate spectrum (e.g. lactose and xylose), which have been deployed in production of industrial enzymes and

Figure 1



Overview of selected NMEs, their representative valuable phenotypes, and the existing genetic tools. Some of the NMEs are also known by other names: *Rhodosporidium toruloides, Rhodotorula toruloides*; *Scheffersomyces stipitis, Pichia stipitis*; *Issatchenkia orientalis*, *Pichia kudriavzevii*; *Pichia pastoris*, *Komagataella phaffii*; *Ogataea polymorpha*, *Hansenula polymorpha*; *Penicillium chrysogenum*, *Penicillium rubens*; *Trichoderma reesei*, *Hypocrea jecorina*. On the left side, the solid connecting lines represent the most representative phenotypes, and the dashed connecting lines represent other valuable phenotypes. Abbreviations: GRAS, Generally Recognized As Safe; NHEJ, non-homologous end-joining; HR, homologous recombination; CRISPR-Cas, Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR associated protein; IA, itaconic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid.

proteins [45]. Methylotrophic yeasts *Pichia pastoris* and *Ogataea polymorpha* efficiently glycosylate and secrete heterologous proteins, which have been utilized for the commercial synthesis of various human proteins [23,46,47] and industrial enzymes. Recent advances in these two yeasts achieved high-titer synthesis of oleochemicals and natural products from the C1 substrate, methanol [24,26,28]. The discovery of these highly relevant properties promoted rapid development of various genetic tools to overcome the inherent difficulties in domesticating these yeasts (e.g. proficient non-homologous end-joining (NHEJ) DNA repair, lack of well-characterized genetic elements). Figure 1 summarizes the unique features of some selected NMEs and the status of their corresponding tool development. Table 1 compiles the most representative biofuel and biochemical productions in the selected NMEs reported in recent years.

Whole-genome sequence, elaborate annotation and prediction of functional parts, and omics data (e.g. genomics, transcriptomics, proteomics, and metabolomics) set foundational understanding of a candidate host's physiology and metabolism to properly assess its amenability to the objective. More advanced computational tools can simulate metabolic networks to discover novel valuable chemicals and the associated bioprocesses to design cost-effective solutions with high titer, productivity rate, and yield (TRY) [48]. Genome-scale metabolic models (GEMs) coupled with constraint-based models that evaluate cell metabolic potential and analyze gene-protein-reaction associations yield refined genome annotations and metabolic flux distributions that enhance genotype–phenotype prediction. GEM-based metabolic engineering and flux balance analysis also enable identification of pathway bottlenecks/imbalances and prediction of genetic interventions to boost TRY (reviewed here [49•]). For instance, reconstructing GEM toward a specific microbial system, product, and/or culture scenario guided rational metabolic engineering, enhancing production of organic acids, lipids, and recombinant proteins in non-model yeasts [2•]. Moreover, advances in computational synthetic microbial co-cultures expanded the scope of host preselection by alleviating the constraints of complex feedstock catabolism or scavenging of toxic compounds from the environment [50,51].

Filamentous fungi: emerging leaders of the fungus kingdom

Despite the erratic rheology of FF-submerged cultures and associated mass/heat transfer limitations (attributable to dynamic, unpredictable FF mycelial macromorphologies [52]), FF reign supreme relative to their yeast counterparts in multiple biochemical pipelines (Table 1). Industrial fermentation of FF dates back to

Table 1

Summary of representative biofuel and biochemical productions in NMEs.

Non-model eukaryotes	Product of interest	Representative compound	Carbon source	Titer/enzyme activity (scale)	Strategy	Ref
Yeasts						
Y. lipolytica	Oleochemicals, terpenoids, polyketides, organic acids, and proteins	FAMEs	Glucose	98.9 g/L (3-L bioreactor)	Balancing cytosolic redox potential	[3]
		TAG	Glucose	66.5 g/L (3-L bioreactor)	Fine-tuning of metabolic pathway	[4]
		Fatty alcohols	Glucose	5.8 g/L (2-L bioreactor)	Fine-tuning of pathway	[5]
		Wax esters	WCO	7.6 g/L (flask)	Fine-tuning of pathway	[6]
		TAL	Glucose	35.9 g/L (3-L bioreactor)	Improving precursor pool	[7]
		β-carotene	Glucose	39.5 g/L (3-L bioreactor)	Structure-based enzyme engineering and fine-tuning of metabolic flux	[8••]
		Lycopene		17.6 g/L (3-L bioreactor)	Fine-tuning of metabolic flux	
		Succinic acid	Glucose	101.4 g/L (1-L bioreactor)	Transporter engineering and fine-tuning of metabolic pathway	[9]
		Lipase	Glucose	90 500 U/mL (15-L bioreactor)	Multiple integration of the endogenous lipase gene *lip2*	[10]
R. toruloides	Oleochemicals, polyketides, carotenoids, and terpenes	Lipids	Glucose	89.4 g/L (1-L bioreactor)	High-density fed-batch fermentation	[11]
		TAL	Glucose	28 g/L (1-L bioreactor)	Fine-tuning of metabolic pathway	[12]
			Oilcane juice	23 g/L (1-L bioreactor)		
		Monoterpene (1,8-Cineole) Sesquiterpene (α-Bisabolene)	Corn stover hydrolysates	1.4 g/L (2-L bioreactor) 2.6 g/L (2-L bioreactor)	Fine-tuning of metabolic pathway and fermentation process optimization	[13]
S. stipitis	Organic acid and aromatic products	Fumaric acid	Xylose	4.67 g/L (flask)	Fine-tuning of metabolic pathway	[14]
		Shikimate	Glucose	3.4 g/L (culture tube)	Fine-tuning of metabolic pathway	[15••]
I. orientalis	Organic acids	IA	Glucose	1.23 g/L (1-L bioreactor)	Fine-tuning of metabolic pathway	[16]
		2-PE	Glucose	5.09 g/L (flask)	Fermentation process optimization	[17]
K. marxianus	Recombinant proteins and aromatic products	Inulinase	Inulin	896.1 U/mL (10-L bioreactor)	High-cell-density fed-batch fermentation	[18]
		TAL	Xylose	1.24 g/L (culture tube)	Fine-tuning of metabolic pathway	[19]
		2-PE	Whey permeate	2.59 g/L (4.8-L bioreactor)	Fermentation process optimization	[20]
K. lactis	Industrial enzymes and organic acid	Lactase	Glucose	150 000 U/L (1-L bioreactor)	Fermentation process optimization	[21]
		Hyaluronic acid	Glucose	1.89 g/L (1.3-L bioreactor)	Chassis construction and fine-tuning of metabolic pathway	[22]
P. pastoris	Recombinant proteins, terpenoids, polyketides, and oleochemicals	α-Amylase	Methanol	3700 U/mL (flask)	Fine-tuning of metabolic pathway	[23]
		Monacolin J	Methanol	593.9 mg/L (5-L bioreactor)	Fine-tuning of metabolic pathway and coculture of *P. pastoris–P. pastoris*	[24]
		Lovastatin		250.8 mg/L (5-L bioreactor)		
		2,3-Butanediol	Glucose	74.5 g/L (5-L bioreactor)	Fine-tuning of metabolic pathway and fermentation medium optimization	[25]
		FFA	Methanol	23.4 g/L (1-L bioreactor)	Rewiring of metabolic pathway	[26]
		Fatty alcohols				

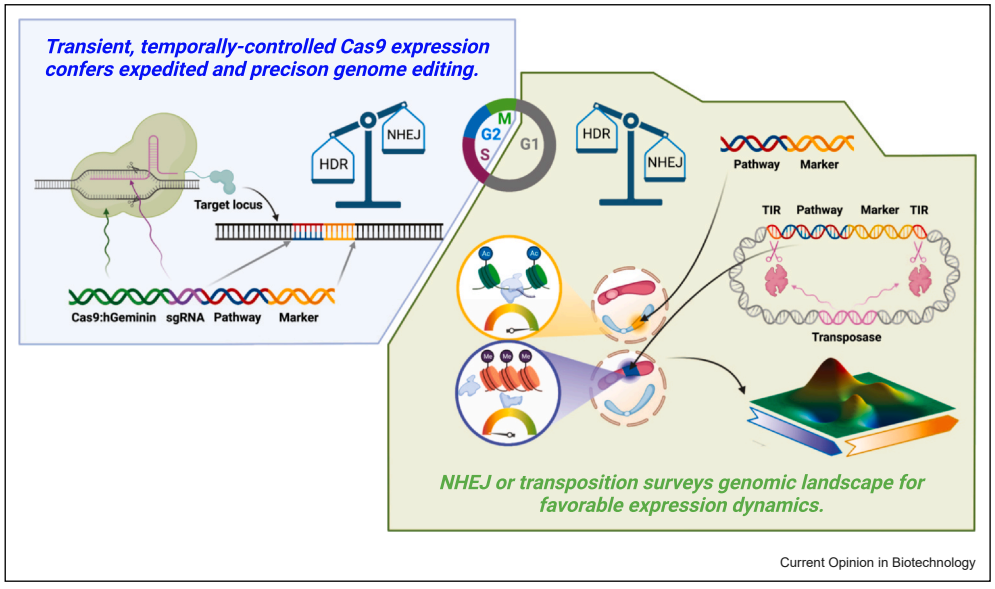
Table 1 (*continued*)

Non-model eukaryotes	Product of interest	Representative compound	Carbon source	Titer/enzyme activity (scale)	Strategy	Ref
				2.0 g/L (1-L bioreactor)		
O. polymorpha	Recombinant proteins and oleochemicals	RV VP6	Methanol	3350.71 mg/L (1.3-L bioreactor)	Chassis construction and fed-batch fermentation	[27]
		FFA	Methanol	15.9 g/L (1-L bioreactor)	Adaptive laboratory evolution, multiomics analysis, and cellular metabolism rewiring	[28••]
Filamentous fungi						
M. alpina	PUFAs	ARA	Glucose	19.02 g/L (5-L bioreactor)	UV mutagenesis and optimized mycelia aging technology	[29]
M. thermophila	Cellulases and organic acids	Malic acid	Crystalline cellulose	181 g/L (5-L bioreactor)	Chassis construction	[30]
		Ethanol	Cellobiose	11.3 g/L (flask)	Fine-tuning of metabolic pathway	[31]
A. niger	Organic acids, antibiotics, and natural products	Citric acid	Glucose	169.1 g/L (flask)	Regulation of intracellular NADH reoxidation and ATP generation	[32]
		Malic acid	Glucose	201.13 g/L (2-L bioreactor)	Fine-tuning of metabolic pathway	[33]
		Enniatin	Glucose	4.5 g/L (6.6-L bioreactor)	Chassis construction and fine-tuning of metabolic pathway	[34]
A. terreus	Organic acids and lovastatin	IA	Glucose	160 g/L (1.5-L bioreactor)	Fermentation process optimization	[35]
		Monacolin J	Glucose	5.5 g/L (flask)	Fine-tuning of metabolic pathway	[36]
U. maydis	Organic acids, polyols, and glycolipids	IA	Glucose	220 g/L (2-L bioreactor)	Fine-tuning of metabolic pathway, morphology engineering, and fermentation process engineering	[37]
			Glucose	205.6 g/L (2-L bioreactor)	Fine-tuning of metabolic pathway and morphology engineering	[38]
		Erythritol	Glucose	100 g/L (3-L bioreactor)	Fermentation process optimization	[39]
		Glycolipids	Crude glycerol	32.1 g/L (2-L bioreactor)	Fermentation process optimization	[40]
P. chrysogenum	Penicillin and secondary metabolites	Penicillin	Unknown	50 g/L (industrial scale)	Fed-batch fermentation	[41]
		Pravastatin	Glucose	6 g/L (10-L bioreactor)	Chassis construction and reprogramming of metabolic pathway	[42]
T. reesei	Cellulase and heterologous proteins	Extracellular protein	Sugarcane molasses	80.6 g/L (3-L bioreactor)	Fine-tuning of metabolic pathway	[43]

Abbreviations: FAMEs, fatty acid methyl esters; TAG, triacylglycerol; TAL, triacetic acid lactone; IA, itaconic acid; 2-PE: 2-phenylethanol; FFA, free fatty acids; RV VP6: rotavirus VP6 protein; PUFAs, polyunsaturated fatty acids; ARA, arachidonic acid; NADH, reduced form of nicotinamide adenine dinucleotide; ATP, adenosine triphosphate.

1919, with Pfizer employing *Aspergillus niger* to produce citric acid, accounting for the bulk of current production [53]. The proceeding century has witnessed adoption of FF for production of more sophisticated biomolecules. The discovery of cryptic biosynthetic gene clusters in FF [54] indicates that we have merely scratched the surface, inspiring efforts to explore, understand, and manipulate FF. These endeavors spawned novel transformation protocols [55], MoClo building blocks [56] compatible with the universal FF Autonomous Maintenance in Aspergillus (AMA1) vector [57], and clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein (Cas9) systems [58,59]. The direct porting of FF components to yeast (e.g. organelle localization signals [60] and light-responsive elements [61]) concomitant with yeast and FF residing in the fungus kingdom implies cross-compatibility. The apparent conservation of several engineering

Figure 2



Exploiting transient expression of Cas9 and transposase to achieve precision genome editing and genomic landscape surveying in the NHEJ-prominent species with expedited workflows.

modalities across phyla of fungi supports the notion of universality: i) the prominent function of introns for heterologous expression in *Ganoderma lucidum* [62] parallels the strong, constitutive gene Translation elongation factor EF-1 alpha (*TEF1*) intron-containing promoter employed in *Y. lipolytica*; ii) transient expression of Cas9 in *Fusarium veneatum* [63] and cell cycle synchronization in *Aspergillus oryzae* [58] enhanced genome editing, two strategies we employed to boost genome editing in nonconventional yeasts [64••]; and iii) targeted morphology engineering [52] to bias unicellular morphologies bolstered performance in the FF *Ustilago maydis* [38] and *Y. lipolytica* [65].

Blending yeast and FF synthetic biology potentiates emergence of general system principles, creating a positive feedback loop where advances in one species augment others. For example, penicillin production in industrial strains of *Penicillium chrysogenum* depends primarily on complex regulatory network variances [66] with negligible dependence on gene cluster copy number [67], an insight that merits reevaluating the emphasis on gene dosage versus gene regulation in yeast engineering. The literal blending of yeasts and FFs in plug-and-play consortia synergizing the complementary strong suits of yeasts (hydrolysate detoxification) and FFs (lignocellulose hydrolysis/assimilation) represents a beautiful manifestation of fully harnessing the biotechnological potential of the fungus kingdom [68]. However, engineering a sophisticated biological system such as a consortium requires a suite of genetic manipulation tools for each constituent.

Promoting genome editing via temporally modulating nuclease activity

The availability of genetic manipulation tools for a prospective host governs the feasibility of harnessing that host's potential. Figure 1 summarizes the current state of fundamental genetic tools in NMEs. Although plasmids enable rapid proof of concept, heterogeneity and/or instability necessitate genomic integration for industrial applications. Plasmids and chromosomes reside in varying nanoscale transcriptional environments, indicating suboptimal extrapolation of pathway behavior with plasmid assays. Recent insights highlight the elegance of genomic context: topological differences between circular and linear DNA and their cognate transcription-induced supercoiling patterns result in varied pathway transcriptional dynamics [69•]. Such considerations reinforce early-stage genomic integration to effectively evaluate pathways for end-stage performance. In addition to the conventional CRISPR-based genome editing skeleton, tuning Cas9p/single guide RNA (sgRNA) expression via promoter engineering reliably confers Cas9 DNA binding and/or cleavage in NMEs (reviewed here [70]), potentiating simple knockout. However, the ubiquitous propensity of NMEs to NHEJ in DNA double-strand break (DSB) repair has long constrained targeted integration of large payloads. We strongly advocate researchers re-evaluate conventional NHEJ inhibition strategies and the potential trade-off between genome editing efficiency and host robustness [71]. Alternatively, encoding Cas9/sgRNA elements on a linear, nonpropagative DNA, opposed to conventional encoding on plasmids, restricts expression

to freshly transformed, actively dividing cells (Figure 2). The dampened genotoxicity from short nuclease exposure coupled with synchronized DSB induction in mitotically active cells boosted integration (67–100% efficiencies) without inhibiting NHEJ in nonconventional yeasts, preserving host TRY [64••]. Analogously, nonreplicable vector delivery of a transposase for pathway integration dampened the frequency of transposition escapers, slashing the workflow by half [15••]. Both strategies emphasize leveraging random integration (via NHEJ or transposition) to identify 'target-specific production hotspots', whose unique spatiotemporal genomic architectures and transcriptional patterns harmonize heterologous expression with relevant endogenous pathways. Elucidating 'target-specific production hotspots' via sequencing strains with desirable and/or intriguing phenotypes could potentially expand empirical knowledge of genomic architecture–phenotype relationships to guide a priori selection of pathway-specific integration loci in the future. The newfound ability to integrate large payloads in NMEs potentiates adaptation of techniques developed in model organisms to confer robust pathway performance. Accordingly, the proceeding sections will include a few strategies in *S. cerevisiae* now accessible to NMEs to combat universal challenges in strain engineering.

Fitness heterogeneity reinforces genetic instability in both model and non-model hosts

Irrespective of whether working in a model organism or NME, successfully integrating pathways represents only a portion of the battle genetic engineers face. As the metabolic engineering field advances from laboratory demonstration to industrial production, genetic instability of the inserted payload in progeny stands out as an outstanding hurdle (Figure 3). Independent of scale or application, microbial culture is always subject to fitness-based competition exploiting the inherent stochasticity of cellular populations to amplify the genetic and/or phenotypic anomalies that confer a fitness advantage. This promotion of cellular heterogeneity spells trouble for bioproduction: low-producing/non-producing cells can partition more cellular resources to growth relative to their high-producing counterparts, enabling the former to dominate the culture. 'Load of production' (i.e. the percentage decrease in specific growth rate attributed to heterologous expression [72]) quantifies the replacement rate of a high-producing strain by undesired mutants in process scale-up. While pathway tuning strategies (e.g. dynamic balancing of expression, redox, and adenosine triphosphate (ATP)) [73] can attenuate immediate/obvious threats to fitness, only more rigorous omics approaches can diagnose more nuanced inhibitory influences. Deep sequencing to investigate the types and frequencies of mutations and the pattern of function loss over time provides another vector to combat genetic instability. Recoding sequences prone to DNA polymerase slippage, recombination, transpositions, and base substitutions could attenuate genetic heterogeneity [72] (Figure 3).

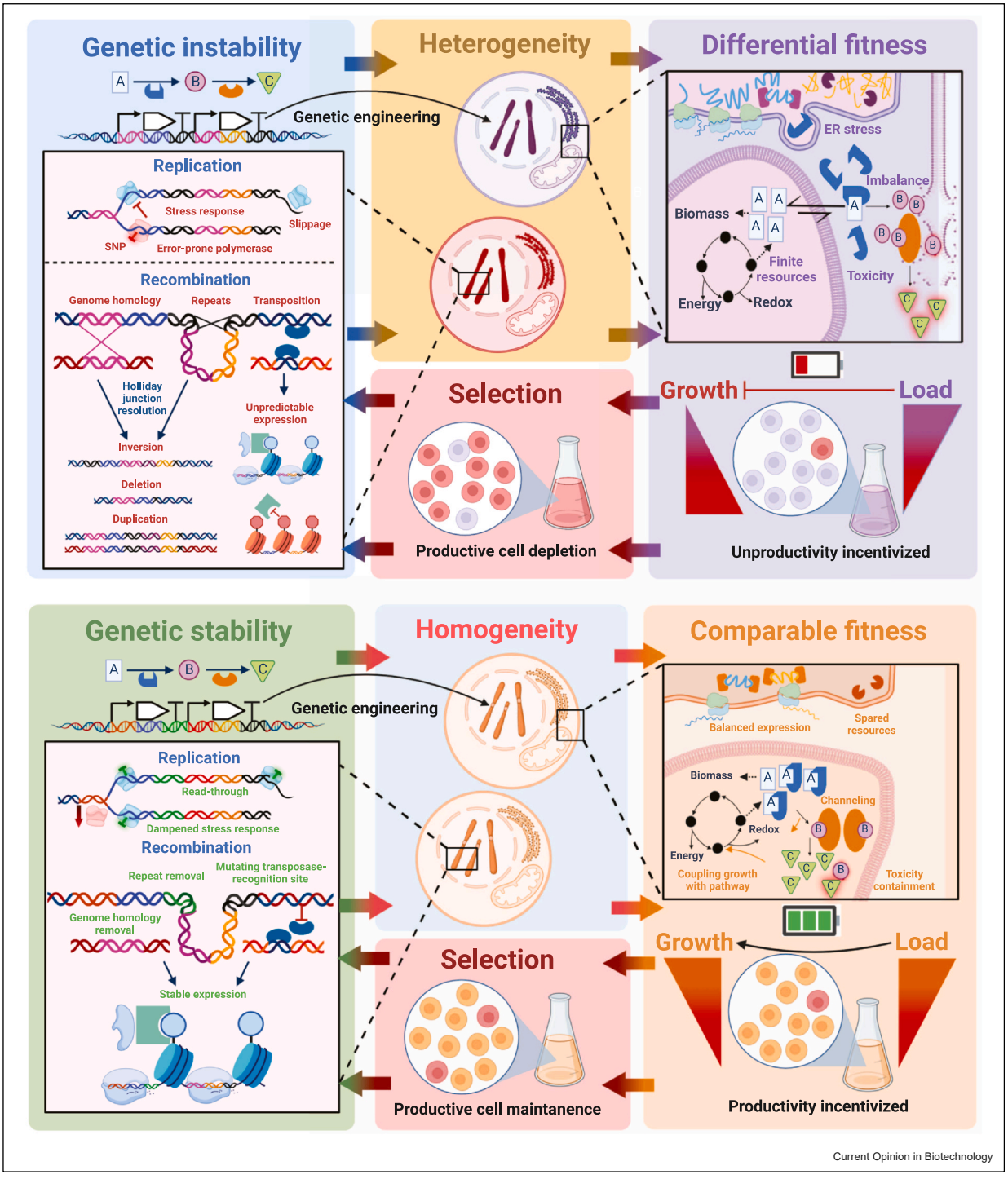
To combat the fitness advantage gained by siphoning resources otherwise devoted to the heterologous pathway, enforcing a punishment to low-/non-producing subpopulations can coax the culture in becoming 'pathway or product addictive' [74•]. One viable strategy is to couple target accumulation to the transcription of an essential gene or selection marker, provided that an orthogonal transcriptional regulator responsive to the target exists. Alternatively, inserting a pathway gene directly into the N-terminus of an essential gene's open reading frame (ORF) couples their expression. This coupling promotes sequence fidelity by prohibiting pathway gene frameshifts, as such mutagenesis also disturbs the essential gene's ORF. The integration locus also influences genetic instability. A recent development in *S. cerevisiae* demonstrated that genomic regions harboring essential genes, intrinsically safeguarded from mutagenesis, provide stable integration loci for long heterologous pathways [75••]. Mixing and matching the outlined strategies provides multiple layers of defense to thwart cells' attempts to escape carrying their load of production.

The continuously decreasing cost of sequencing fosters feasible monitoring of genetic stability of top producers after each iteration of strain engineering. Such rigor becomes paramount when switching from laboratory media (i.e. selective, defined substrates) to industrial media (i.e. usually nonselective, raw hydrolysates). Remaining cognizant of environment-specific fitness disadvantages encountered throughout each stage of the strain development process will maximize retention of engineered functions. Enhancing host genetic stability and robustness has recently garnered more attention. Although most of the published works demonstrate success in model organisms, integrating them with the foundational genetic engineering tools in NMEs opens the possibility for more refined strategies to harness NMEs.

Engineering redox homeostasis and pathway compartmentalization to dampen load

Zooming out from pathway-specific considerations on fitness and genetic instability, the indispensable role cellular and subcellular redox plays in homeostasis indicates that modulating redox could promote genetic stability (Figure 3). Generally, the cytosol, mitochondrial matrix, and peroxisome harbor relatively reducing environments, whereas the endoplasmic reticulum and mitochondrial inner membrane space harbor oxidative environments, permitting protein folding and maturation

Figure 3



Production-associated impacts on growth cause cellular heterogeneity, leading non- or low-performing variants to dominate the culture. Addressing genetic instability will enhance the scalability of microbial cell factories.

that require disulfide bond formation [76]. To meet the demand of industrial production, optimizing the carbon flux toward a target product should seriously consider the associated fluctuations in redox, as nicotinamide adenine dinucleotide phosphate NAD(P)H/NAD(P)$^+$ cofactor pairs participate in numerous biochemical reactions. Specifically, cofactor engineering strategies developed mainly for model microorganisms [77] examine pathway-relevant subcellular contexts and account for host-specific microenvironments to accommodate the redox requirements of the associated compartments in NMEs.

In addition to redox cofactors, cells partition metabolites, proteins/enzymes, and ions across organelles in accordance with each's physiological role. Consolidating heterologous pathways in a compartment with a favorable biochemical environment (e.g. higher local enzyme/metabolite concentrations or pH and redox levels suitable for enzymatic activities) promotes substrate channeling [78,79], orthogonality with global metabolism/regulation, and clearance of toxic intermediates (Figure 3). Accordingly, localization to peroxisomes, mitochondria, endoplasmic reticulum, Golgi, and lipid bodies in NMEs can bolster production [80,81]. *In silico* prediction tools can expedite identification of signaling motifs to effectively compartmentalize pathways in novel NMEs.

These clever engineering strategies in the spatial domain accompany equally bright solutions in the time domain. Temporal induction of heterologous pathways after sufficient accrual of biomass and cellular resources supports genetic stability and improves TRY by decoupling production of load and growth. Optogenetic control of fermentation via irradiating bioreactors has emerged as a promising technology in yeast fermentation [82•]. To facilitate the translation of this technology to NMEs, researchers should remain cognizant that the operational wavelength/intensity represents a principal component influencing host preselection, as light interacts intricately with the host (i.e. host-specific tolerance to wavelength/intensity and expression of the optogenetic components) and bioreactor contents (i.e. fermentation broth influences penetrance and power emission influences temperature). Establishing universal heuristics will aid in transforming optogenetic conceptualizations to industrial technologies.

Conclusions and future perspectives

Successful adaptation of some/all the highlighted developments potentiates construction of elegant pathways tunable at multiple layers of execution. Efficient allocation of cellular resources bestowed by more sophisticated logic could alleviate the impact of poor heterologous enzyme expression, a feature especially relevant in NMEs that lack multicopy integration tools used to increase dosage of poorly expressed genes. Beyond enhancing TRY of the current nonconventional yeast-based processes, we encourage nonconventional yeast groups to 'sporulate' to other phyla and perhaps tackle FF as a biotechnological equivalent of hedging one's bets. Deepening knowledge in FF biology could benefit other aspects of the bioindustry pipeline. Characterizing mycelial growth to enhance fermentation reproducibility augments fungicide development, critical for safe and sustainable preservation of feedstock [83].

Independent of microbial hosts, common laboratory practices typically save only a few 'top producers'. Considering a high producer in one experimental condition/iteration might not perform as well in other conditions/subsequent iterations, saving a collection of strains in initial screening serves as a contingency for unanticipated phenomena commonly observed in scale-up. Taking out a strain 'insurance policy' has the added benefit of elucidating genetic mosaicisms underlying second-tier and 'low-producing' strains [84]. Such a workflow maximizes the 'Learn' component of the DBTL cycle, justifying the laborious nature of the 'Build' component and fully leveraging the power of computational biology guided metabolic engineering.

Advancements in next-generation sequencing, computational power, and digital storage coupled with the cognate generation of enormous data quantities has catalyzed the emergence of computational biology. However, many scientists contributing to these datasets lack essential data/computer science and/or statistics backgrounds to maintain the quality and standardization necessary for cross-comparison and AI model training. Moreover, the current incentive structure predicated on publishing novel, intriguing results in high-impact-factor journals exacerbates data inaccessibility via omission of vitally important, albeit 'unglamorous' results deemed unsuitable for publication. Incentivizing the reporting of valuable 'poor' results in publications saves other labs precious time and resources spent exploring dead-end hypotheses. This incentive structure could also dampen temptations to compromise research integrity in pursuit of prestige and monetary benefit, as highlighted by the recent beta-amyloid controversy. Such a paradigm shift could permeate well beyond biotechnology, aiding the re-establishment of trust within and across scientific, industrial, and public circles. To effectively commune with biological systems, we must first do so with our fellow scientists.

Data Availability

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

Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest.

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