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Next-generation biocontainment systems for engineered organisms

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The increasing use of engineered organisms for industrial, clinical, and environmental applications poses a growing risk of spreading hazardous biological entities into the environment. To address this biosafety issue, significant effort has been invested in creating ways to confine these organisms and transgenic materials. Emerging technologies in synthetic biology involving genetic circuit engineering, genome editing, and gene expression regulation have led to the development of novel biocontainment systems. In this perspective, we highlight recent advances in biocontainment and suggest a number of approaches for future development, which may be applied to overcome remaining challenges in safeguard implementation.

A major goal of synthetic biology is to design and construct genetically modified organisms (GMOs) with novel functions and biological behaviors that can be used for biomedical, industrial, and environmental applications[1–3]. In addition to their potential use in technological applications, GMOs serve as powerful research tools for studying biological processes and principles and have become prevalent in many science and engineering fields. Depending on the purpose, GMO release into the environment may be deliberate, such as in waste treatment, or unintentional. While many GMOs are safe for release, as their engineered functions reduce cellular fitness and render the spread of transgenic material unfavorable, biocontainment measures become necessary to constrain proliferation when GMOs are able to outcompete natural organisms and negatively affect the environment and human health.

Since the 1970s, scientists and the general public have voiced concern that the intentional or accidental release of GMOs may lead to an unexpected impact on the environment[4–6]. Although there are no reports of biological hazards caused by GMOs thus far, many studies have shown that the use of engineered organisms can result in the invasion of transgenes into the natural environment[7–12]. One alarming example regards the spread of antibiotic-resistance genes that are often incorporated into GMOs. A report revealed that resistance genes were found in water polluted by farmland fecal waste and could be transferred to natural bacterial species by bacteriophages[13]. Such transgene leakage can directly impact human health by increasing drug resistance among pathogens.

With the rapid rise in the design and deployment of GMOs in recent years, the chance of engineered organisms influencing ecosystems and human health is expected to increase[14,15]. Therefore, continuous effort has been invested in designing safeguard measures to prevent GMO escape[14,16,17] (Fig. 1). The resulting biocontainment strategies focus on the engineered prevention of self-replication, synthetic auxotrophism, genetic circuit–actuated killing, and horizontal gene-transfer blocking involving a wide range of cellular mechanisms.

This Perspective article joins other recent reports[16–18] in an effort to capture the many challenges of GMO safeguarding and further offers directions for overcoming them. Here, the main focus is to discuss how emerging technologies may be harnessed to create new biocontainment methods that complement existing systems in order to improve the safety of GMO practices. We first detail the general requirements of an effective biocontainment system and present a number of synthetic biology approaches that are leading the race toward effective GMO safeguarding while discussing their advantages and limitations. We then outline the latest developments and apply insight gathered from existing approaches to propose strategies for building next-generation biocontainment systems.

Criteria for effective biocontainment

Key safeguard requirements must be met to prevent the release and proliferation of GMOs in natural environments and their ensuing interaction with ecosystems. GMOs are living entities that can grow and self-replicate; therefore, any embedded biocontainment system must be extremely robust to prevent the release of even a small fraction of organisms that could grow to dominate an ecosystem. The National Institute of Health has presented a guideline stating that a GMO escapee rate below 1 in 10^8 cells is considered to be acceptably safe[6], and a number of existing biocontainment systems have met this goal. However, with an ever-increasing scale in GMO deployment, the containment and/or killing efficiencies of these systems may not be sufficient to prevent a buildup of GMOs in the environment. Therefore, continuous improvements in system robustness and efficiency are needed to ensure biosafety.

Long-term system stability is another key criterion for effective safeguarding, as GMOs are often designed and expected to replicate through many generations and different growth phases. To maintain robustness and stability, genetic safeguard systems must take into account the potential undermining effects of genetic silencing mechanisms, such as DNA recombination and mutagenesis. Moreover, the biocontainment systems themselves often place a metabolic burden on engineered hosts or cause background

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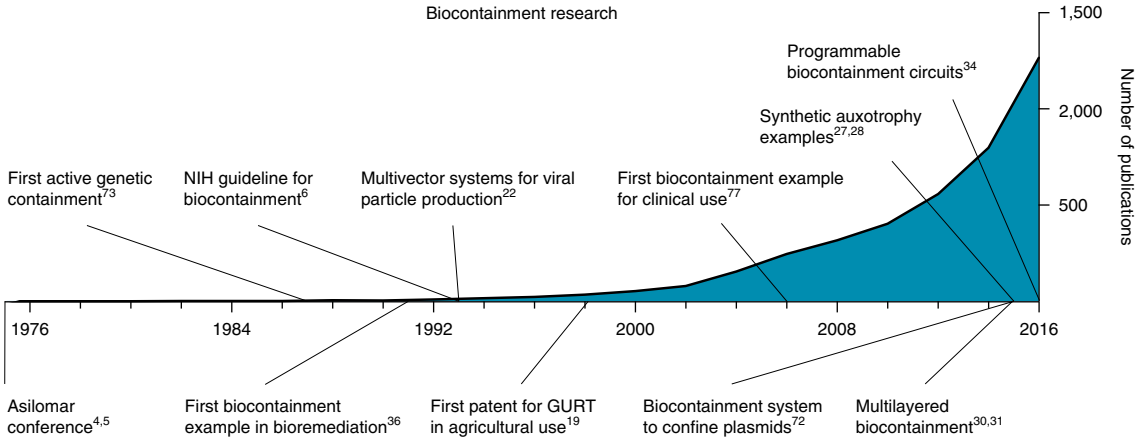

Fig. 1 | Biocontainment research timeline. A chart representing the number of published articles found on PubMed using the search terms "biocontainment," "biosafety," and "biosecurity".

toxicity, which may limit the desired cellular performance and further increase the selective pressure to disrupt the biocontainment apparatus. To address this problem, safeguard systems must be tightly regulated when GMOs are under permissive conditions. Lastly, as engineered cells may be required to function in a variety of environments for different applications, rapid customization is a beneficial trait. To meet these criteria, various promising biocontainment strategies have been developed, as described below.

Pioneering biocontainment strategies

Biocontainment via engineered prevention of self-replication. Some of the earliest examples of biocontainment focused on the concept of blocking self-replication in GMOs. One such example, Genetic Use Restriction Technology (GURT), was developed in the late 1990s to restrict the unintended and unauthorized spread of engineered plants by disabling their reproductive ability[19]. Generally, this approach involves placing seed development genes under the control of chemically inducible promoters such that when the engineered crops are grown in open environments the lack of inducing chemicals leads to infertile seed formation. Of note, GURTs are still broadly used in the agricultural industry.

Genetic separation is a second replication-prevention strategy that is commonly employed in virus engineering and was established to prevent the uncontrolled replication of engineered lentiviruses that serve as gene delivery tools[20–22]. In this approach, essential genes involved in viral particle synthesis are distributed among multiple vectors, and only the transgene-containing vector is packaged into the resulting replication-null viral particles. Though such engineered infertility and controlled replication have been effective safeguard approaches in plants and viruses, they are not applicable for microorganisms that reproduce through independent self-replication.

Biocontainment through auxotrophy. One of the most common biocontainment approaches is engineering GMOs to become dependent on an exogenous supply of a metabolite for growth and survival (Box 1). To facilitate this, auxotrophic GMOs have been generated through the deletion of essential genes responsible for producing key metabolites, such as nucleosides[23,24] and amino acids[25] (Fig. 2a). However, this auxotrophic strategy is not foolproof, as the organisms may still survive in natural environments by obtaining essential metabolites generated by other organisms. Additionally, GMOs may regain deleted essential genes via horizontal gene transfer, which incapacitates the biocontainment system.

The strength of auxotrophy-based biocontainment has been improved upon by the recent development of synthetic auxotrophic GMOs that depend on noncoding amino acids (ncAA) to survive. In these strains, all naturally occurring amber stop codon sites (UAG) have been replaced with an ochre stop codon (UAA). The strains express a non-native tRNA and aminoacyl-tRNA synthetase pair that incorporates an ncAA into amber codons that have been strategically placed within the coding sequences of essential genes. The maintenance of critical cellular activities therefore requires the presence of the ncAA, which is only supplied in the designated environment[26,27] (Fig. 2b). To restrict GMOs from circumventing this mechanism by nonspecifically incorporating natural amino acids into the amber stop codon, Mandell et al. structurally redesigned essential enzymes to depend on the ncAA's residue for enzymatic activity[28]. This strict synthetic-molecule dependency leaves the resulting auxotrophs with extremely low escapee frequencies (10^{-12}). Unfortunately, implementing this type of strategy requires extensive genome editing in the microbial strain, which limits its availability. Additionally, synthetic auxotrophy cannot prevent the release of transgenic elements through various biological activities such as horizontal gene transfer.

Biocontainment using synthetic gene circuits. Another major strategy for biocontainment involves the use of synthetic gene circuits to control cell proliferation based on the presence of environmental signals, which are detected by allosterically regulated transcription factors that modulate gene expression[29]. In some designs, an exogenous supply of specific molecules is required to induce the transcription of genes that are essential for cell survival[30–32]. In others, endogenous signaling molecules support survival. For example, an *Escherichia coli* strain was engineered such that essential gene expression was dependent on the presence of the quorum-sensing molecule acyl-homoserine lactone (AHL) at a concentration that is only achievable in high-cell-density environments[33]; thus, escapee proliferation is prevented because of low levels of AHL in the nonpermissive environment (Fig. 3a).

Other biocontainment gene circuits use a two-layered gene expression regulation architecture, in which the first inducible regulatory element represses the expression of a second regulator that is responsible for repressing a toxic output that triggers cell death[34–36] (Fig. 3b). Cell survival requires the controlled, constant presence of the first regulatory element's inducer to repress expression of the toxic output. An important benefit of using genetic circuit–based biocontainment systems is the ability to tune the mechanism and employ different small-molecule sensors to control confinement conditions for each specific application. A recently reported circuit-based system known as the Passcode switch implements such reprogrammability by using hybrid transcription factors that can rewire

Box 1 | Signaling molecules that actuate biocontainment systems

Different biocontainment strategies are designed to use specific sets of factors to define biocontainment conditions because of the varying biological properties and environmental settings found in each GMO application. One main category of biocontainment circuits depends on the use of allosterically regulated transcription factors to detect signaling molecules in designated environments. In these systems, the signaling molecules modulate transcription factor behavior, and an absence of signal leads to cell death via the activation of toxin expression or the inhibition of essential gene expression. Such 'signaling molecule–transcription factor' pairs include tryptophan-TrpR[73], IPTG–LacI[34,74–76], benzoate derivatives–XylS[25,36], ATc–TetR[30,34], galactose–GalR[30,34], estradiol–estrogen receptor hybrid protein[31], cellobiose–CelR[34], and homoserine lactone–luxR[33]. Another common biocontainment strategy involves the engineering of cellular dependence on an exogenous supply of an essential metabolite. Traditionally, this is achieved by knocking out genes that are involved in the biosynthesis of an essential metabolite. Such nutrient-essential gene pairs include thymine/thymidine–*thyA*[23,77] and biotin–*bioA*[30]. A recent study demonstrated a different form of synthetic auxotrophy by using synthetic amino acids that are not common in natural systems, including L-4,4′-biphenylalanine[28], *p*-acetyl-L-phenylalanine[27], *p*-iodo-L-pheylalanine[27], and *p*-azido-L-phenylalanine[27]. Other efforts have expanded the collection of potential biocontainment molecules by creating hybrid transcription factors[78,79], engineering transcription regulators for a new ligand[80], and developing new ncAAs[50].

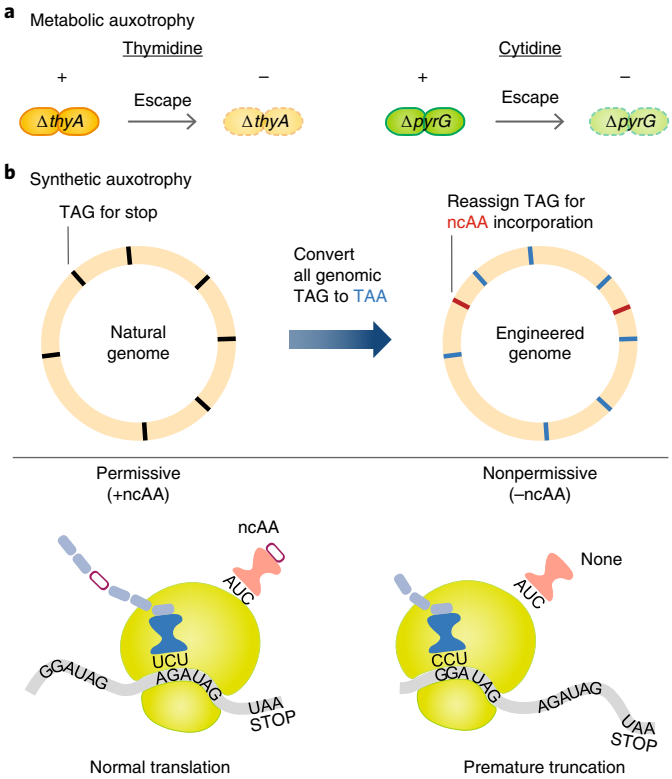

Fig. 2 | Biocontainment through auxotrophy. a, Two examples of metabolic auxotrophy: deletion of the *thyA* gene (left) or *pyrG* gene (right) in *L. lactis* limits engineered cells to environments containing an exogenous supply of thiamine or cytidine, respectively[23,24]. **b**, A synthetic, auxotrophic strain was developed by replacing all amber stop codons (TAG) with ochre stop codons (TAA) and using the amber codon sequence to incorporate an ncAA into essential enzymes in the permissive environment[27,28]. Lack of the supplemented ncAA leads to lethal truncation of the enzyme in escapee GMOs.

input–output connections[34]. This circuit confines engineered cells to environments that must contain two small-molecule signals but lack a third. The respective roles of the three signals can be shuffled by changing the positions of the hybrid transcription factors (Fig. 3b). Thus far, however, GMOs equipped with circuit-based containment systems have displayed escapee frequencies (10^{-6} to 10^{-8}) that are higher than the ideal as a result of circuit deactivation by genetic recombination and mutagenesis.

Prevention of horizontal gene transfer. Many prokaryotes have developed pathways for horizontal gene transfer. In the context of GMOs, this leads to the risk of distributing engineered genes to natural organisms. Therefore, in addition to the development of biological measures to control GMO proliferation, several strategies have been implemented to prevent the transfer of transgenic materials. One strategy is to use toxin–antitoxin pairing systems[37–39], in which a toxin gene is incorporated into a plasmid whose recipients must express the appropriate antitoxin. Another strategy is to use a conditional origin of replication that only allows plasmid replication by bacteria with a specific set of replication initiation proteins[40,41]. These technologies can be used to minimize the release of genes encoded in a plasmid or other mobile DNA elements, but not within chromosomal DNA.

Future directions for biocontainment

As described above, many pioneering approaches have been devised to address the issue of biocontainment. However, these approaches all have limitations that prevent them from serving as highly efficient safeguards, which leads us to explore additional mechanisms that could be developed to further enhance biosafety. Below, we describe a number of strategies—transgene inactivation, gene-integrity surveillance, mutagenesis reduction, transgene compartmentalization, genetic-code encryption, cell-free production, and functional redundancy—that may complement these methods and offer improvements on robustness and efficiency.

Transgene-specific targeting to reduce fitness cost to hosts. Many biocontainment strategies aim to kill escapees or inhibit their growth, which often leads to background cytotoxicity even under confined conditions, reducing their growth rate and cell fitness. Potentially, this fitness burden can be reduced by targeting engineered genes for removal instead of eliminating the entire organism. CRISPR technologies allow for in vivo, sequence-specific DNA editing, which could be harnessed for target-specific degradation of transgenes incorporated in GMOs[42] to revert engineered cells to their wild-type states. This approach prevents the spread of transgenic materials but does not hamper cell fitness, as transgene degradation does not impede natural biological activities. Furthermore, this strategy might enhance genetic stability because it minimizes the burden on the GMOs and any resulting evolutionary pressure to deactivate the biocontainment system. Although this CRISPR-based safeguard has important benefits, it may only be suitable for GMOs harboring a transgene function that exhibits a low fitness cost. CRISPR-mediated transgene removal in GMOs containing a transgene function that exacts a high fitness cost may accelerate the loss of transgene function in the entire GMO population due to takeover via natural selection.

Monitoring genetic integrity for kill-switch maintenance. Our previous work has shown that mutagenesis is one of the major

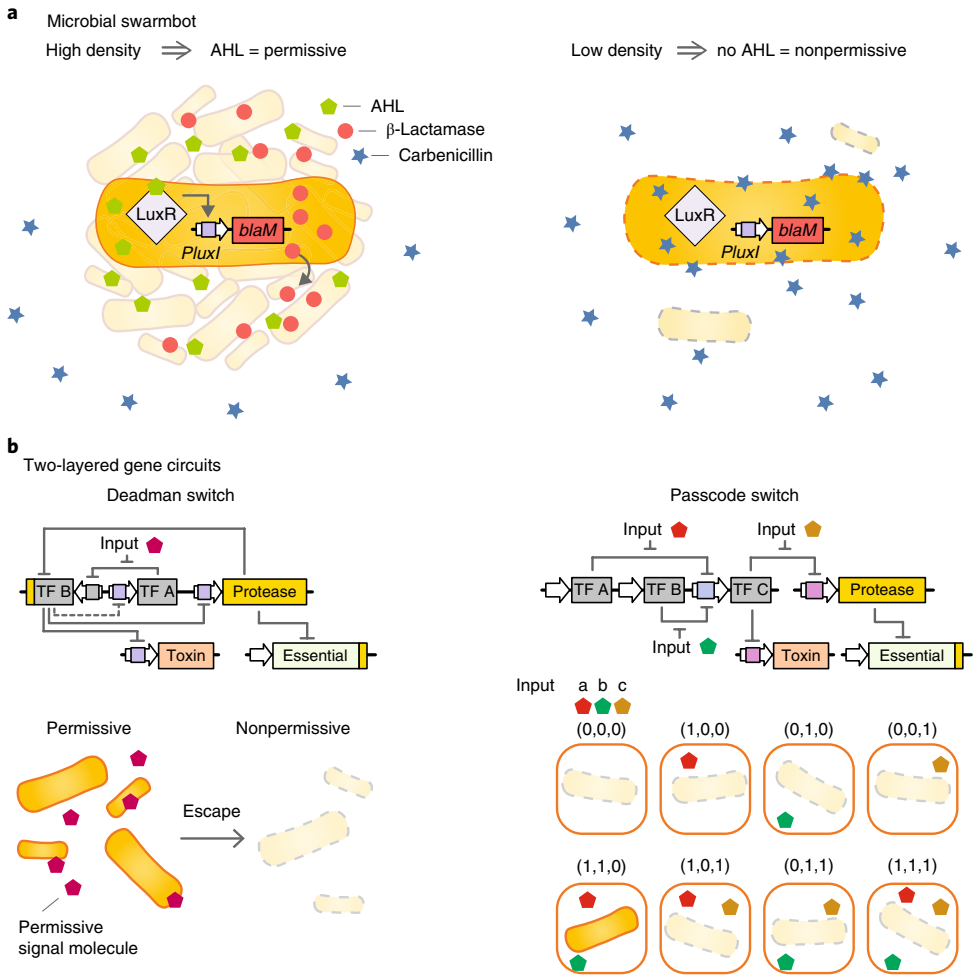

Fig. 3 | Biocontainment using synthetic gene circuits. a, Schematic of a microbial swarmbot[33]. High cell density leads to accumulation of quorum-sensing molecules (AHL) in the confined environment, inducing expression of β-lactamase, which confers resistance to carbenicillin. At low cell density, β-lactamase is not expressed, leading to cell death by carbenicillin. **b**, Schematic of two-layered kill switches[34]. In the Deadman switch (left), the absence of a permissive signal allows the repression of transcription factor (TF) B by TF A, thereby allowing toxin expression, as well the expression of a protease that targets an essential enzyme (and any remaining TF B), causing cell death. In the Passcode switch (right), hybrid TFs are applied to rewire input–output connections in the genetic circuit such that a specific input combination (eight different passcodes shown below) determines whether there will be cell survival or death. In the presented case, cell survival occurs only if two signals, a and b, are present and a third, signal c, is absent.

causes of safeguard-system breakdown[34], and thus the robustness of biocontainment could be improved by restoring mutated circuits back to their original genetic sequences. Recently, Chavez et al. developed a mutation recovery system in which Cas9 is used to monitor specific changes in a DNA sequence and convert the sequence back to a preset composition, preventing undesired mutations[43]. The researchers demonstrated that this system has the programmable flexibility to protect selected sequences in a specific gene and is capable of simultaneously monitoring multiple mutations. Potentially, this platform could be applied to target mutation hotspots in a biocontainment circuit, facilitating long-term maintenance of the system.

Reduced host mutagenesis in synthetic organisms. As mentioned above, innate mutagenesis mechanisms commonly lead to the breakdown of genetic safeguard systems in GMOs. Appropriate modifications of intrinsic cellular DNA-repair and mutagenesis pathways can thus complement all biocontainment approaches by decreasing the escapee rate[27,31]. For example, in a genetic circuit–based biocontainment study, deletion of genome-encoded insertion-sequence elements, mutagenic DNA polymerases, and other genome repair machinery in *E. coli* reduced the escapee rate by 3–5 orders of magnitude[34]. This result suggests that the stability of biocontainment systems could be further improved by eliminating all mutation-related factors from the host genome, though there may be yet unknown contributors. A more extreme, future approach might be to construct a synthetic cell containing a minimal and well-defined set of genes of which none are involved in mutagenesis. Supporting this concept, a synthetic cell with 473 well-characterized genes was recently created[44], and the minimal genome was used as a research platform to accurately predict how changes in genetic composition affect biological activities. Adopting a synthetic cell strategy to eliminate mutagenesis may improve the stability and effectiveness of genetic safeguard systems.

Multispecies consortia as a biocontainment strategy. The emerging understanding of microbial signaling pathways has begun to allow the design and engineering of customizable intercellular interactions and the development of multispecies communities for novel technological applications[45,46]. One may also envision the possibility of harnessing synthetic microbial consortia to confine synthetic biological functions. Many synthetic biological applications, such as the biosynthesis

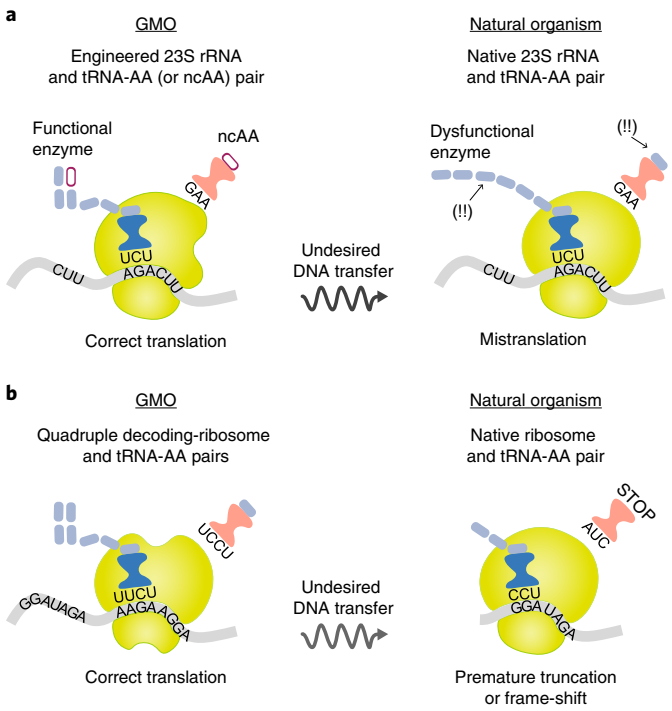

Fig. 4 | Orthogonal translation to confine transgenic functions. **a**,**b**, A schematic showing how orthogonal translation systems can only function in the intended, engineered hosts (left sides), preventing undesired DNA transfer and gain-of-synthetic function in natural organisms (right sides). Examples include (**a**) the use of engineered 23S rRNA and tRNA pairs to reassign amino acids to existing codons[49] and (**b**) the use of engineered tRNAs that utilize four nucleobases in a single codon[47,48].

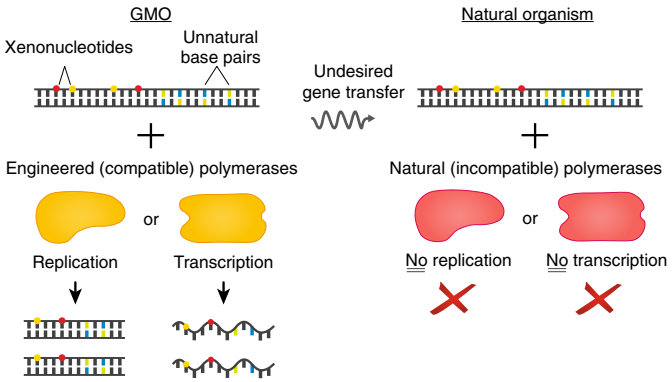

Fig. 5 | Unnatural nucleotides prevent access to transgenic information. A schematic showing how unnatural nucleotides are replicated or transcribed in the engineered hosts (left sides), prohibiting undesired gene transfer and gain-of-synthetic function in natural organisms (right sides). Examples include the use of xenonucleotide sequences of noncanonical sugar backbones or unnatural base pairs paired with their engineered, compatible polymerases, such that the synthetic nucleotide fragments cannot be replicated or transcribed by natural organisms.

of chemicals and pharmaceuticals, involve multiple synthetic genes that only produce an end product when combined. It may be possible to prevent biosynthetic products from influencing the environment by compartmentalizing the individual necessary biological activities into different strains, with product generation only occurring if the complete multispecies consortium is present. Careful pathway design and species subgrouping would prevent exposure to potentially toxic intermediaries. This approach could be further bolstered by introducing an element of auxotrophy, wherein different engineered strains are forced to coexist through mutual dependency by designing each strain to supply an essential nutrient to the community[46]. Because these engineered auxotrophic organisms require multiple nutrients, each supplied by a different member of the consortium, obtaining the entire collection of essential nutrients after leaving their designated environment would be extremely unlikely.

The concept of multispecies consortia could also potentially be harnessed to improve the genetic stability of biocontainment circuits without sacrificing the fitness of hosts. Though eliminating mutagenic activities in hosts could prevent circuit breakdown, it may also block many cellular stress-response pathways, which in turn could reduce cellular growth together with the engineered activities required for a designated application. A consortium approach may be used to avoid this problem by separating engineered biological processes and biocontainment activities into different hosts. One strain with a low mutation rate may host the biocontainment circuit and actively donate the circuit to companion strains via horizontal gene transfer. With such a design, the low-mutation strain would serve as a stable resource that provides frequent replenishment of the safeguard measures to other members of the consortia.

Biocontainment using additional xenobiological approaches. Xenobiology aims to completely control the flow of genetic information by creating organisms whose survival is dependent on unnatural synthetic molecules, such as nucleic acid analogs and synthetic amino acids. This approach eliminates the possibility of escape, proliferation, and cross-feeding, and renders gene transfer impossible, as the xenobiological genes cannot be used in natural organisms. Several studies have shown that engineered tRNA–aminoacyl-tRNA-synthetase pairs and ribosomes can be used to massively modulate codon interpretation, including codon reassignments and the generation of quadruplet codons[47–50] (Fig. 4a,b). Xenobiology-based synthetic auxotrophs have been established at the protein level and may also be extended to the DNA and RNA levels. Marliere et al. adaptively evolved a thymine biosynthesis-deficient *E. coli* strain that can substitute chlorouracil for thymine[51]. The engineered cells can survive in the presence of chlorouracil by integrating A, G, C, and chlorouracil into their DNA. In another study, researchers showed that 'xeno-nucleic acid (XNA)' polymers containing various sugar-molecule backbones can functionally replace DNA and RNA containing deoxyribose and ribose, respectively, in order to store retrievable genetic information that is unreadable in natural cellular entities[52] (Fig. 5). Another group developed 'unnatural base pairs' that can be replicated and amplified by engineered DNA polymerases both in vivo and in vitro, and demonstrated that the base pairs cannot be removed by natural DNA-repair mechanisms[53] (Fig. 5).

These successes show much promise for the use of orthogonal biological systems as an effective strategy for biocontainment. With future developments on XNA-based replication and propagation for in vivo use, one may envision nucleic-acid-level xenobiological safeguard systems in which engineered hosts can utilize unnatural nucleic acids to encode genetic information that directs functional activities. Any released XNA fragments cannot be replicated or transcribed by natural organisms, and XNA-based cells similarly cannot read genetic codes in canonical nucleic acid sequences. Of note, Although such orthogonality in processing nucleic acid information would provide a unique advantage in genome-wide prevention of horizontal gene transfer, this concept is currently limited by a technological barrier. Similar to the unnatural amino-acid-based synthetic auxotroph approach, building xenobiological systems requires extensive genetic modification, and the challenge of engineering specific survival conditions restricts both the scalability and flexibility.

Cell-free systems to avoid transgene release. One way to prevent the unintentional spread of transgenic components is to entirely decouple biological operation from the living cell by using cell-free systems, which can generate biological molecules and compounds while remaining innately abiotic and sterile. Recent studies have demonstrated the use of this format for molecular diagnostics and therapeutic biomanufacturing, suggesting that in many instances, cell-free systems can replace living organisms conventionally used in biological applications[50,54–59]. Unlike GMOs, which are intrinsically linked to genetic duplication, mutagenesis, and cell division, cell-free systems lack such activities. Recently, Adamala et al. encapsulated cell-free transcription and translation into small lipid vesicles to enable compartmentalization of genetic circuits and cascades, which improves modularity and controllability of genetic circuits[54]. The use of cell-free systems encapsulated in a well-isolated environment indicates their potential to replace living cells in broader practical applications. Cell-free systems may even be coupled with xenobiological approaches to avoid any possible uptake of transgenic functions by natural organisms (Fig. 5). Additionally, researchers have been using cell-free systems as test beds to incorporate ncAAs into target proteins via codon reassignment[47–50,53,60]. A cell-free system possessing reassigned codon usage would further prevent natural organisms from implementing genetic information encoded in its transgenes. Overall, for appropriate bioprocesses that can be implemented by cell-free systems, this strategy has the potential to avoid the risk of GMO escape as well as the undesired transfer of transgenic components.

Appropriating GMO biocontainment tactics to bioremediate natural organisms. Researchers have begun to develop systems that appropriate genetic circuit–based concepts intended for biocontainment to modulate the proliferation of unmodified organisms in their natural environments. These bioremediation systems generally involve two major components: a genetic killing module and a vector that effectively targets the desired organism. For example, bacteriophage-based vectors have been used to kill pathogenic bacteria by delivering synthetic gene circuits that express antimicrobial peptides[61,62]. Other bioremediation approaches deliver CRISPR-based devices that abolish antibiotic-resistance genes or regulons involved in pathogenic activities[63–66]. Recently, researchers have presented a novel approach called "gene drive," which uses CRISPR–Cas9, toxin–antitoxin pairs, or RNA interference (RNAi) to control the populations of target organisms[67]. This method is based on the biased inheritance of a particular gene, which, once dispersed, can manipulate the behavior of an entire population. Highly efficient CRISPR–Cas9-based gene-drive systems have been demonstrated in yeast, fruit flies, and mosquitoes[68–71]. As these biocontainment technologies continue to improve, they will likely see much utility in the bioremediation space.

Functional redundancy to enhance system performance. Biological systems are intrinsically dynamic, as genomes undergo continuous evolution through various mutagenesis pathways. This property poses a challenge for the long-term maintenance of genetic safeguards in GMOs. Studies have suggested that introducing redundancy into engineered biological functions can enhance the stability and robustness of biocontainment systems. For instance, a plasmid-based biocontainment system called "GeneGuard" prevents horizontal gene transfer by implementing host–plasmid mutual dependency using multiple strategies in parallel[72]. Such concurrent approaches include the use of a unique origin of replication for selective host replication and the expression of a broadly effective toxin gene that can only be countered by an antitoxin present in the desired host. In another example of redundancy, researchers presented a biological safeguard that confines engineered cells by controlling the expression of essential genes through both transcriptional regulation and DNA recombination[31]. This two-layer expression-control design reduces the escapee rate to $<10^{-10}$, which is significantly lower than the rate observed in systems that utilize just one of the two layers of control (10^{-6}).

Redundancy-based biocontainment improvement is governed by the rationale that engineered cells must obtain genomic or epigenomic mutations at multiple specific loci to deactivate all parallel safeguard pathways and escape. With several independent biocontainment pathways in place, both the amount of time and the number of generations required for cells to incapacitate the system increase, and the escapee rate is substantially reduced. Though early biocontainment systems have employed one or two safeguard approaches, future systems may include multiparallel pathways to synergistically reduce the escapee rate of GMOs.

Concluding remarks

Advances in molecular and synthetic biology have contributed immensely to rapid biotechnological development and have fueled the development of GMOs for many purposes, but this comes with the risk of unintentional spread and the responsibility to prevent it. Fortunately, these same advances have provided us with the means to create modular and multilayered biocontainment systems, and their implementation is now at a critical stage, as technologies are being transferred from laboratories to real-world use. Moving forward, researchers will need to learn how to introduce biocontainment systems that were originally demonstrated in standard laboratory strains into GMOs of interest. Biocontainment efficiency will also need to be evaluated in GMO strains under a broad range of environmental conditions, as the behavior of any biological entity will likely change in different circumstances. The potential impact of synthetic biocontainment systems on the environment and on human health must also be evaluated, as some biocontainment strategies, such as xenobiology, involve noncanonical genetic processes that could unexpectedly interact with natural species. Together, this outlook will allow us to continue to benefit from technological advances and GMOs while maintaining robust safeguarding of their containment.

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Competing interests

The authors declare no competing interests.

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