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[Sewanu Godonu](#) *

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Review

Engineered *Escherichia coli* Strains as Therapeutic Agents in Reactive Oxygen Species (ROS)-Mediated Glioblastoma Treatment: A Systematic Review of Mechanisms, Efficacy, and Challenges

Sewanu Stephen Godonu

godonusewanu80@gmail.com or sewanustephen_godonu@aaims.edu.jm

Abstract

Glioblastoma multiforme (GBM) remains one of the most aggressive and treatment-resistant brain tumors, characterized by a hostile microenvironment and poor prognosis. Recent advances in synthetic biology have led to the engineering of *Escherichia coli* (E. coli) strains as living therapeutics capable of targeting GBM via reactive oxygen species (ROS)-mediated mechanisms. This systematic review evaluates the mechanisms by which engineered *E. coli* modulate ROS, their therapeutic efficacy in preclinical models, and challenges in delivery, safety, and regulation. Engineered strains show promise through prodrug conversion, direct ROS production, and immune activation, especially when combined with nanocarriers and immune checkpoint inhibitors. However, tumor heterogeneity, biosafety concerns, and regulatory complexities remain significant hurdles. Advancing toward clinical translation will require robust biocontainment systems, adaptable genetic circuits, and validation in humanized preclinical models.

Keywords: Glioblastoma Multiforme (GBM) Engineered *Escherichia coli* Reactive Oxygen Species (ROS) Synthetic Biology Therapeutics

1. Introduction

Glioblastoma multiforme (GBM) is the most aggressive and lethal primary brain tumor in adults, characterized by rapid proliferation, extensive infiltration into surrounding brain tissue, marked genetic heterogeneity, and resistance to conventional therapies (Lim et al., 2018). Notwithstanding the existing standard of treatment, comprising maximum surgical resection, subsequent concomitant radiation, and temozolomide chemotherapy, median survival remains bleak, often fluctuating between 12 and 15 months (Stupp et al., 2005; Ostrom et al., 2023). This poor prognosis is largely attributed to the tumor's capacity to evade immune surveillance, its inherent radioresistance, and the protection offered by the blood-brain barrier (BBB), which restricts the delivery of many therapeutic agents (Abbott et al., 2010). Another critical factor influencing GBM progression and therapy resistance is the unique tumor microenvironment (TME). The GBM TME is marked by a high level of oxidative stress, chronic inflammation, hypoxia, and elevated levels of reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) (Guan et al., 2023). While excessive ROS levels can be cytotoxic and induce cell death, GBM cells often exploit moderate ROS signaling to support proliferation, angiogenesis, and immune evasion (Hayes et al., 2020). Consequently, addressing redox homeostasis is a dual-faceted therapeutic approach: Elevating reactive oxygen species (ROS) beyond lethal limits might induce apoptosis, while antioxidant systems can safeguard non-tumor tissue from unintended harm. In this context, synthetic biology has emerged as a powerful tool for designing precision therapeutics. One of the most promising applications involves engineering bacterial strains, particularly *Escherichia coli* (E. coli), to serve as live therapeutic agents. These strains can be genetically engineered to target tumor tissues, react to

tumor-specific signals (such as hypoxia or lactate concentrations), and administer therapeutic agents, including ROS-generating enzymes, prodrug-converting systems, or immunomodulatory factors, in a regulated and localized fashion (Din et al., 2016; Chowdhury et al., 2019). Engineered *E. coli* strains have shown the ability to penetrate hypoxic tumor regions and maintain stable colonization, allowing for sustained, site-specific therapeutic activity with minimal systemic toxicity (Zhao et al., 2023). Recent studies demonstrate that such strains can be programmed to produce ROS directly within the tumor milieu or to convert inert prodrugs into ROS-generating cytotoxins, selectively killing GBM cells (Chen et al., 2022). Additionally, certain bacterial strains may possess genetic safety circuits, such as quorum-sensing systems or inducible death switches, to mitigate the risk of uncontrolled proliferation or systemic infection (Wang et al., 2022). Thus, the convergence of microbial engineering and ROS-based therapeutic strategies offer a highly innovative and targeted approach to overcoming some of the major limitations of current GBM treatments. The therapeutic potential of *E. coli*-based systems in glioblastoma multiforme remains in the nascent phase of research and needs thorough assessment of effectiveness, delivery, safety, and regulatory adherence prior to clinical use.

2. Methods

A systematic literature review was conducted following PRISMA 2020 guidelines. Databases including PubMed, Scopus, and Web of Science were searched using combinations of keywords: "*Escherichia coli*," "glioblastoma," "reactive oxygen species," "synthetic biology," and "bacterial therapy." Inclusion criteria were (1) peer-reviewed studies published between 2018 and 2025, (2) studies reporting experimental or computational data on *E. coli*-based ROS modulation in GBM models, and (3) English-language publications. Exclusion criteria were review papers, non-cancer models, or non-*E. coli*-based systems. Data were extracted on strain type, engineering strategy, delivery mechanisms, therapeutic outcomes, and adverse effects. Quality assessment was performed using the SYRCLE risk of bias tool for in vivo studies and the GRADE system for in vitro and translational studies.

3. Mechanisms of Action

Genetically modified *Escherichia coli* (*E. coli*) strains serve as versatile therapeutic platforms that enable targeted, programmable interventions in the glioblastoma (GBM) tumor microenvironment. These engineered bacteria leverage two principal mechanistic strategies: (1) ROS amplification via prodrug conversion and (2) direct ROS generation coupled with immune activation. Both approaches aim to exploit GBM's redox imbalance, increase tumor-specific oxidative stress, and enhance immunogenic cell death while minimizing damage to healthy tissues.

3.1. ROS Amplification Via Prodrug Conversion

One of the earliest and most established synthetic biology approaches in bacterial cancer therapy involves arming *E. coli* with enzymes capable of prodrug conversion. Strains engineered to express cytosine deaminase (CD) convert the non-toxic prodrug 5-fluorocytosine (5-FC) into the cytotoxic chemotherapeutic 5-fluorouracil (5-FU) (Zhao et al., 2023). The resultant medication promotes apoptosis by inhibiting thymidylate synthase, causing DNA strand breaks, and inducing excessive reactive oxygen species production, a mechanism especially potent in redox-dysregulated malignancies such as glioblastoma multiforme (GBM). Another potent prodrug strategy involves nitroreductase (NTR) enzymes. NTRs catalyze the reduction of nitroaromatic compounds such as CB1954 into cytotoxic intermediates, producing ROS as collateral products (Guan et al., 2023). The increased local ROS production causes mitochondrial malfunction, DNA double-strand breaks, and activation of intrinsic apoptotic pathways in GBM cells. These effects are particularly amplified in the hypoxic core of GBM, where *E. coli* thrives due to its facultative anaerobic nature (Chen et al., 2022). Prodrug conversion strategies benefit from spatial control, as gene expression in *E. coli* can be restricted to hypoxic or lactate-rich environments via tumor-specific promoters (Din et al., 2016).

Additionally, quorum-sensing systems allow the bacterial population to sense its density before releasing the prodrug-converting enzymes, reducing the risk of premature activation or systemic toxicity (Wang et al., 2022).

3.2. Direct ROS Generation and Immune Activation

A more recent and highly innovative strategy involves engineering *E. coli* to directly generate ROS within the tumor microenvironment. This is typically achieved by introducing genes encoding NADPH oxidase, glucose oxidase, or other redox enzymes that catalyze the production of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) in situ (Li et al., 2024). These ROS molecules accumulate in the TME, overwhelming antioxidant defenses such as glutathione and catalase, leading to irreversible damage to proteins, lipids, and nucleic acids in GBM cells (Hayes et al., 2020). Moreover, ROS-induced damage is not purely cytotoxic but also immunogenic. Oxidative stress in tumor cells causes immunogenic cell death (ICD), which is defined by the release of damage-associated molecular patterns (DAMPs) such as HMGB1 and ATP, as well as the surface expression of calreticulin (Wang et al., 2022). These signals activate dendritic cells, which then transmit tumor antigens to cytotoxic T lymphocytes, increasing systemic antitumor immunity. In certain studies, combining ROS-producing *E. coli* with immune checkpoint inhibitors such as anti-PD-1 or anti-CTLA-4 antibodies resulted in synergistic therapeutic effects (Xu et al., 2023). Furthermore, ROS-producing strains can be designed with genetic logic gates that ensure precise activation only in specific microenvironmental conditions, such as low oxygen or acidic pH. This enhances tumor selectivity and limits damage to surrounding healthy brain tissue (Zhao et al., 2023). Encapsulation in lipid-based vesicles or biocompatible hydrogels has also been explored to shield the bacteria from immune clearance before reaching the tumor, thereby improving efficacy (Chen et al., 2022). Collectively, these approaches establish modified *E. coli* as both ROS-delivery systems and immune adjuvants, transforming immunologically “cold” GBM tumors into “hot,” inflamed tumors that respond to immunotherapy (Li et al., 2024).

4. Therapeutic Efficacy

Preclinical studies using murine models of glioblastoma (GBM) have provided compelling evidence for the therapeutic efficacy of engineered *Escherichia coli* (*E. coli*) strains, particularly when designed to modulate the tumor redox environment through localized ROS production. These bacteria-mediated therapies have shown the potential to drastically reduce tumor burden, extend longevity, and even synergize with immune-based therapies, putting them forward as attractive candidates for next-generation GBM treatments. One of the most robust demonstrations of efficacy comes from Chen et al. (2022), who utilized a synthetic biology platform to engineer *E. coli* strains capable of producing reactive oxygen species (ROS) within GBM tumors. In an orthotopic xenograft mouse model of GBM, intracranial injection of ROS-producing *E. coli* resulted in a 45% improvement in median survival when compared to control mice were given either saline or non-engineered bacterial strains. Tumor volume, as determined by bioluminescence imaging and histology, was considerably decreased in the therapy group, showing robust anticancer action specific to glioma tissue. Beyond monotherapy, combining these bacterial systems with immune checkpoint inhibitors, such as anti-PD-1 or anti-CTLA-4 antibodies, has shown synergistic therapeutic benefits. Xu et al. (2023) reported that co-administration of engineered *E. coli* and PD-1 blockade in immunocompetent mouse models not only extended survival but also led to increased infiltration of CD8⁺ cytotoxic T cells and a reduction in immunosuppressive tumor-associated macrophages. These effects were linked to the immunogenic cell death caused by ROS, which liberated tumor antigens and activated dendritic cells in the tumor microenvironment. Importantly, the efficacy of these strains was not limited to intracranial administration. Zhao et al. (2023) found that when ROS-producing *E. coli* strains were administered systemically by intravenous injection, they preferentially homed to hypoxic tumor cores inside GBM due to their anaerobic tropism. This resulted in significant tumor growth inhibition without observable off-target effects or systemic toxicity, which was attributed to

the use of tumor-specific genetic promoters and suicide-switch circuits that restricted bacterial activity to the tumor site. In another study, Wang et al. (2022) utilized a strain of *E. coli* programmed to secrete glucose oxidase, which converts glucose into hydrogen peroxide, a potent ROS in the tumor niche. In addition to reducing tumor development, this therapy altered the local immunological landscape by increasing antigen presentation and T cell activation, lending credence to the developing notion of bacterial agents as immune-sensitizing adjuvants in “cold” malignancies such as GBM. Furthermore, long-term follow-up in animal models has shown minimal relapse rates and, in some cases, complete tumor regression, particularly when engineered bacteria were used as part of a combination therapy regimen. This includes approaches that integrate bacterial ROS modulation with radiotherapy or autophagy inhibitors, which amplify the oxidative stress to lethal thresholds in GBM cells (Li et al., 2024). Collectively, these findings underscore the potential of engineered *E. coli* strains as effective, tumor-targeted biotherapeutics capable of disrupting redox homeostasis in GBM while also promoting adaptive antitumor immune responses. Continued optimization of bacterial chassis, payload delivery systems, and combination protocols is expected to further enhance efficacy and support translational progression into early-phase clinical trials.

Table 1. Summary of representative preclinical studies using engineered *E. coli* in GBM models.

Study	Engineered strain	Mechanism	Model used	Main outcome
Chen et al., 2022	<i>E. coli</i> Nissle	ROS via CD enzyme	GBM mouse (orthotopic)	↑ Survival (45%)
Zhao et al., 2023	Hypoxia-inducible <i>E. coli</i>	Direct ROS (NADPH oxidase)	IV GBM model	Tumor targeting without systemic toxicity
Xu et al., 2023	ROS- <i>E. coli</i> + anti-PD1	ROS + immune activation	Immunocompetent mice	Synergistic tumor regression
Li et al., 2024	Glucose oxidase <i>E. coli</i>	H ₂ O ₂ -based ROS release	Subcutaneous GBM model	↑ Antigen presentation, ↓ tumor volume

5. Delivery and Targeting Strategies

Recent breakthroughs in synthetic biology and biomaterials engineering have markedly improved precise control over modified *E. coli*, allowing safer and more effective ROS-based glioblastoma (GBM) treatment through intricate transport and targeting mechanisms.

5.1. Bio-Orthogonal Genetic Circuits and Safety Switches

Tumor microenvironment-responsive genetic circuits have become integral for spatial and temporal control over *E. coli* behavior. Hypoxia-inducible promoters (e.g., *nirB*, *fdhF*) activate therapeutic gene expression selectively within GBM’s hypoxic core, minimizing off-target activity (Zhao et al., 2023). Quorum sensing modules, derived from *luxI/luxR* or *agr* systems, provide synchronized lysis or reactive oxygen species release alone upon surpassing a predetermined bacterial density threshold, thereby mitigating systemic dissemination hazards (Din et al., 2016; Wang et al., 2022). Synthetic kill-switch designs, including protease-based inducible circuits and auxotrophy-based systems, guarantee the dependable self-destruction of bacteria post-treatment (Gao et al., 2021; Cai et al., 2022).

5.2. Hydrogel Microspheres and Nanogels for Instrumental Delivery

Hydrogel microspheres provide a biocompatible scaffold for local bacterial delivery post-surgical resection, enabling sustained ROS release at the tumor interface. Poly(ethylene glycol)-polycaprolactone (PEG-PCL) or PCLA-PEG-PCLA hydrogels, which encapsulate *E. coli*, successfully infiltrate brain tissue, sustain vitality, and disintegrate in a predictable manner, therefore facilitating regulated therapeutic delivery (Turner et al., 2023; Huang et al., 2024). Nanogel platforms (~30–200 nm) combine hydrophilic polymers and encapsulated nanoparticles to traverse the blood-brain barrier (BBB) via receptor-mediated transcytosis, subsequently releasing payloads under tumor acidic or hypoxic stimuli (Singh et al., 2022; Zhao et al., 2021). These nanogels protect *E. coli* from immune clearance while promoting sustained colonization in GBM.

5.3. Lipid-Based and Polymer Nanocarriers for Systemic Administration

For minimally invasive systemic approaches, *E. coli* or bacterial outer membrane vesicles (OMVs) are encapsulated in PEGylated liposomes (~100–150 nm) or polymeric nanoparticles such as PLGA. These carriers use surface ligands such as transferrin, angiopep-2, or iRGD to enhance BBB transcytosis via receptor-mediated pathways (Shah et al., 2019; Kong et al., 2016; Smith et al., 2023). In vivo models using transferrin-PEG-liposomes have shown a 13-fold increase in brain accumulation of encapsulated bacteria-derived vesicles compared to non-targeted controls (Cohen et al., 2020). Hybrid OMV-liposome systems developed from *E. coli* K1 have used OmpA-gp96 interactions to enable targeted BBB penetration and intratumoral bacterial colonization in breast cancer brain metastasis models, indicating translational potential for GBM (Zhang et al., 2024).

5.4. Active Targeting Ligands and Biomimetic Membranes

Surface functionalization significantly enhances tumor targeting and BBB penetration. Nanocarriers coated with angiopep-2, iRGD peptides, or transferrin show markedly improved uptake across the BBB and tumor accumulation (Singh et al., 2022; Luo et al., 2021). Additionally, biomimetic cloaks, such as membranes that imitate erythrocytes, macrophages, or gliomas, alter immune recognition and enable homotypic targeting of GBM (Liang et al., 2023; Ma et al., 2024). In murine GBM models, red blood cell-coated nanoparticles loaded with bacterial OMVs increased median survival and reduced systemic inflammation (Dong et al., 2023).

5.5. Focused Ultrasound-Mediated BBB Modulation

Focused ultrasound (FUS), combined with microbubbles, enables site-specific BBB disruption, creating transient permeability windows without surgery. Studies using FUS-mediated delivery of therapeutic nanocarriers show up to two-fold enhanced tumor penetration, with extended treatment zones and no measurable off-target damage (Meng et al., 2018; Burgess & Hynynen, 2013). When applied to *E. coli* therapies, FUS allows precise temporal synchronization between BBB opening and microbial delivery, improving colonization and efficacy in deep-seated GBM (Rogers et al., 2022).

Through the integration of advanced excisional biomaterials, responsive genetic circuits, stealthy carrier systems, and BBB-facilitated methods, the delivery of engineered *E. coli* has evolved into a sophisticated, multimodal platform. These strategies have successfully improved tumor targeting, safety, and therapeutic outcomes in preclinical GBM models and set the foundation for future clinical translation.

Table 2. Key delivery and targeting platforms for engineered *E. coli* in GBM therapy.

Strategy	Delivery method	Targeting feature	Example
Hydrogel microsphere	Intracranial/local	Sustained ROS release	Turner et al., 2023
Nanogel	IV systemic	pH/hypoxia-triggered release	Singh et al., 2022
Lipid-based OMV	IV systemic	Transferrin/iRDG-mediated BBB crossing	Shah et al., 2019
FUS-enhanced	IV + ultrasound	Temporal BBB opening	Rogers et al., 2022

6. Challenges and Future Perspectives

While ROS-producing *Escherichia coli* (*E. coli*) therapies have shown great promise in preclinical glioblastoma (GBM) models, many important hurdles must be overcome in order to assure safe and successful clinical translation.

6.1. Safety Concerns and Biosafety Mechanisms

One of the foremost challenges in deploying live bacterial therapeutics is biosafety. Engineered *E. coli* strains can potentially cause septicemia or colonize off-target tissues, especially in immunocompromised individuals (Chen et al., 2022). To address this, researchers have developed layered biocontainment systems that include metabolic auxotrophy, inducible kill switches, and CRISPR-based genome editing safeguards (Gurbatri et al., 2020; Wang et al., 2022). For instance, auxotrophic bacteria can only survive in the tumor microenvironment where specific nutrients are present, thereby limiting off-site proliferation (Gao et al., 2021). Kill switches triggered by temperature, pH, or quorum-sensing signals can also enforce programmed bacterial death after treatment (Cai et al., 2022). Nonetheless, evolutionary instability in these circuits is a worry. Mutations can deactivate death switches or restore auxotrophy, allowing germs to survive beyond the planned therapeutic window (Hayashi et al., 2024). Future efforts must prioritize strong circuit integration into chromosomal DNA and redundancy in biocontainment mechanisms.

6.2. Regulatory and Ethical Barriers

Live microbial therapeutics, especially those genetically engineered, face strict regulatory scrutiny under GMO (genetically modified organism) policies. Regulatory agencies such as the FDA and EMA require detailed data on horizontal gene transfer risk, ecological release, and immune effects (Singh et al., 2022). Unlike inert drugs, live therapies must meet dual standards: for efficacy and ecological safety. Furthermore, ethical issues about the purposeful release of modified organisms, particularly in the brain, need public openness, patient informed consent standards, and a thorough risk-benefit analysis (Li et al., 2024). Clear regulatory frameworks and validated biosafety data are needed to streamline clinical trial approval.

6.3. Tumour Heterogeneity and Microenvironmental Complexity

GBM is characterized by profound inter- and intratumoral heterogeneity, with regional differences in oxygenation, acidity, and immune infiltration (Zhao et al., 2023). Consequently, a single-mode bacterial therapy may be insufficient. Engineered *E. coli* strains must be adaptable, equipped with logic-gated circuits that respond to multiple tumor microenvironmental cues (e.g., hypoxia, lactate, pH) (Li et al., 2024). Dual-input promoters and conditional payload systems are being investigated to enable precision delivery of ROS in context-specific niches (Xu et al., 2023). To truly address this heterogeneity, preclinical models must better recapitulate human tumor

complexity. Standard rodent models are insufficient. Humanized PDX (patient-derived xenograft) models and 3D GBM organoids represent promising alternatives (Huang et al., 2024).

6.4. Immune Systems Interactions

ROS-producing *E. coli* can induce immunogenic cell death in tumor tissues, potentially acting as adjuvants to immunotherapy (Wang et al., 2022). However, this immune activation can also trigger excessive inflammation or autoimmunity. For instance, lipopolysaccharide (LPS) on *E. coli* surfaces can provoke Toll-like receptor (TLR)-mediated cytokine storms (Zhao et al., 2023). Fine-tuning the immunostimulatory profile, such as by LPS modification or strain reduction, is critical for maximizing therapeutic effect while minimizing risk (Cai et al., 2022).

6.5. Manufacturing, Scalability, and Delivery

Unlike conventional drugs, engineered *E. coli* must be cultured, preserved, and delivered without losing function. Maintaining plasmid stability, viability, and biosafety features during large-scale manufacturing poses logistical hurdles (Gao et al., 2021). Freeze-drying (lyophilization), encapsulation in protective hydrogels, or storage under anaerobic conditions are being tested (Huang et al., 2024). Moreover, *E. coli* transport to intracranial tumors via the blood-brain barrier (BBB) is ineffective. Focused ultrasound (FUS)-assisted BBB opening, hydrogel microsphere delivery, and lipid nanocarriers are also being investigated to increase targeted delivery and retention (Turner et al., 2023).

7. Future Perspectives

To overcome the constraints preventing clinical translation of modified *Escherichia coli* (*E. coli*) therapeutics for glioblastoma, future research should target five crucial domains:

7.1. Clinical-Grade Biocontainment

Ensuring patient safety is critical when delivering live bacterial treatments. Advanced biocontainment systems that include numerous safety layers, such as auxotrophy, quorum-sensing death switches, and CRISPR-based genetic confinement, must be adjusted for evolutionary stability and performance in vivo (Hayashi et al., 2024). These protections will reduce the hazards of bacterial escape, systemic infection, and horizontal gene transfer.

7.2. Logic-Gated Payload Control

Next-generation therapeutic strains should be equipped with logic-gated genetic circuits that trigger ROS generation or drug release only in response to specific tumor microenvironmental cues, such as hypoxia, lactate accumulation, or acidic pH. This dual- or multi-signal gating improves selectivity and reduces off-target effects (Xu et al., 2023).

7.3. Combination Therapeutic Approaches

The integration of engineered *E. coli* therapies with existing modalities, such as immune checkpoint inhibitors, radiotherapy, and temozolomide, offers a synergistic strategy to overcome tumor resistance and enhance antitumor immunity. Such combinatorial approaches can exploit ROS-induced immunogenic cell death to prime the tumor for immune engagement (Wang et al., 2022).

7.4. Advanced Preclinical Models

Translational fidelity remains a major gap. Current rodent models inadequately mimic human GBM heterogeneity and immune complexity. Therefore, humanized animal models, 3D tumor organoids, and patient-derived xenograft (PDX) systems are essential to assess efficacy, immunotoxicity, and spatially variable responses (Huang et al., 2024).

7.5. Regulatory Collaboration

Establishing clear regulatory frameworks tailored to live microbial therapeutics is essential. Ongoing dialogue between researchers, regulatory bodies, and bioethicists will be required to develop approval pathways that address unique concerns associated with genetically modified organisms (GMOs), including biocontainment, long-term monitoring, and environmental impact (Singh et al., 2022).

By investing in these strategic areas, the field can advance from proof-of-concept to viable clinical applications, potentially offering transformative therapies for patients with GBM.

8. Conclusions

Engineered *Escherichia coli* strains represent a groundbreaking approach in glioblastoma therapy, offering precision-targeted delivery of reactive oxygen species (ROS) for selective tumor destruction and immune activation. Preclinical studies have shown encouraging efficacy, especially when combined with immunotherapy or advanced delivery systems. However, significant translational barriers remain—including biosafety, tumor heterogeneity, immune compatibility, and regulatory hurdles. Addressing these challenges through robust biocontainment strategies, adaptive genetic circuits, advanced preclinical models, and collaborative regulatory frameworks are essential. With continued innovation and validation, *E. coli*-based ROS therapeutics may soon emerge as a clinically viable, next-generation treatment for glioblastoma.

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