From *In Vitro* to *In Vivo*

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Abstract: The purpose of this review is to compare and contrast eukaryotic cell culture experiments with ecological microcosms and will emphasize the areas where the two approaches overlap. Cell culture experiments involve cultivating cells outside their natural setting to study cellular behavior, disease mechanisms, and drug responses. Conversely, microcosms replicate natural ecosystems in controlled environments, providing insights into ecological processes and substance fate. While cell culture focuses on cellular and molecular biology, microcosms lean towards ecological and environmental studies. However, both techniques overlap, especially in environmental toxicology, where cell culture findings are validated in microcosms to assess ecological impacts. Advancements in whole genome sequencing, metagenomics, and metabolomics have enabled the linkage of cell culture-based studies and microcosms to investigate molecular biology. Cell culture experiments contribute significantly to biomedical research, drug development, and regenerative medicine, while microcosms are valuable for understanding ecosystem dynamics and assessing environmental risks. The review discusses the historical development of cell culture and microcosms, highlighting key milestones such as the creation of the first human cell line (HeLa cells) and the emergence of stem cells and organoids. It also explores the future applications of cell culture, including cell-based screening for drug testing and the transition from 2-D to 3-D cell screening techniques for more accurate results. Addressing global health and environmental challenges through small-scale experiments using microcosms or "within the glass" (*in vitro*) cell line models is essential. Despite the time-consuming nature of these experiments, they contribute to developing theories and practical solutions for responding to climate change and emerging diseases. In summary, cell culture experiments and microcosms are indispensable in scientific research, offering unique insights into biology and ecology and hold immense potential for addressing pressing challenges in various fields.
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Introduction

Eukaryotic cell culture-based experiments and microcosms are both *in vitro* techniques used in scientific research, but they serve different purposes and are used in other contexts. Cell culture experiments involve growing cells in a controlled environment outside their natural setting, typically in a laboratory. The first report of eukaryotic cells cultured *in vitro* came from Harris and colleagues in 1906, who adapted techniques that Robert Koch used for bacterial cell culturing that he developed in the later 1800s to culture frog tissues *ex vivo* [1]. This technique allowed researchers to study cellular behavior, responses to stimuli, and disease mechanisms in a controlled and reproducible manner. However, having a stable and immortal human cell line capable of generating reproducible data would come much later and be essential in uncovering the unknown biology of human cells. Henrietta Lacks suffered from a severe form of cervical adenocarcinoma. During her diagnostic evaluation, a tissue biopsy was taken, providing extra samples for Dr. George O. Gey's tissue culture lab at Johns Hopkins in Baltimore, Maryland. Though Lacks passed away from cancer in 1951, the cancer cells, known as HeLa cells, quickly proliferated in cell culture and became the first established human cell line, still used today [2]. Cell culture experiments are often used in biomedical research, drug development, and regenerative medicine, among other fields.

While cell culture systems attempt to replicate organismal function, microcosms are miniature ecosystems contained within vessels designed to replicate natural environments on a smaller scale. Microcosms are used to study ecological processes, such as nutrient cycling, species interactions, and the effects of environmental stressors. Although microcosms have likely been in use by early scientists for centuries, if not millennia, the earliest recorded instance of microcosms in scientific literature occurred in 1851 [3]. In a meticulously balanced aquatic micro-ecosystem housed within a twelve-gallon glass aquarium, Robert Warington demonstrated the interaction between goldfish, snails, Vallisneria plants, and a multitude of attendant microorganisms. From his later work, we gain insight into the intricate roles played by each organism in the cyclical flow of materials within this aquatic community [3]. They provide a simplified yet realistic representation of natural ecosystems, allowing researchers to manipulate variables and study ecological dynamics in a controlled setting and reproducible manner.

While cell culture experiments focus on cellular and molecular biology, microcosms are more oriented toward ecological and environmental studies. However, there can be overlap between the two techniques, especially in areas such as environmental toxicology, where researchers may use cell culture experiments to study the effects of pollutants on cellular function and then validate their findings in microcosm experiments to assess their ecological impacts. With the recent improvements in metataxonomic, metagenomics, and metabolomics and increased affordability of whole genome sequencing, cell culture-based studies and microcosms can be linked to a better understanding of molecular biology at the species and community levels [4]. Tumor cells exist within an ecosystem that includes both the tumor cells themselves and their surrounding microenvironment. Emerging spatial genomic, transcriptomic, and proteomic technologies provide new methods for studying cancer evolution with detailed molecular and spatial insights [5]. Overall, both cell culture experiments and microcosms are valuable tools in scientific research, each offering unique insights into different aspects of biology and ecology.

Research conducted *in vitro* allows scientists to conduct experiments that might be impossible or unethical to conduct on whole organisms or ecosystems. Using eukaryotic cell culture to mimic models of human disease has profoundly contributed to our understanding of factors influencing everything from cancer to molecular evolution. For example, current drug candidates and toxicity screening processes depend on early-stage *in vitro* cell-based assays, which are anticipated to accurately reflect key aspects of *in vivo* (under normal conditions in a living organism) pharmacology and toxicology [6]. *In vitro* models have recently attracted attention as methods that might reduce and eventually replace the use of animals in research. Advancements in 3D bioprinting, spheroids, organoids of human tissues, and microfluidics have significantly progressed in replicating human physiology, which may one day allow for the replacement of animal models entirely [7, 8]. Practical applications relying on cell culture techniques have emerged across diverse domains, encompassing the evaluation of new drug efficacy and toxicity, the production of vaccines and biopharmaceuticals, and the advancement of assisted reproductive technologies. With recent advancements enabling the reprogramming of somatic cells, researchers worldwide are engaged in intense competition to spearhead progress in regenerative medicine. Similarly, within this field, cell culture technology is recognized as a cornerstone for continued advancement and widespread adoption.

Similarly, microcosms are *in vitro* models of natural ecosystems contained within vessels and originated to bring nature's intricacies into educational and domestic settings worldwide. Over time, these compact "worlds" have evolved into a significant research instrument. For instance, they help address complex questions like the impact of biodiversity on ecosystems. A Rutgers study on decomposer bacteria revealed that decomposition rates increased with greater bacterial species diversity compared to the higher abundance of a single species [9]. Such broad-scale questions are challenging to investigate in a controlled setting without using microcosms. Microcosms are particularly valuable because they offer a method to study the effects of various factors on entire ecosystems using simplified models, and their replicability allows for cost-effective experimental studies.

The following review provides historical context for the development and implementation of eukaryotic cell culture-based systems and microcosms and provides insight into how recent advances in the field make these approaches more powerful tools for understanding the impact of pollution at the species and community levels.

Discussion

What is Cell culture?

Cell culture is the practice of maintaining and continuous propagation of cells outside of their natural environment. Under *in vivo* conditions, the homeostatic regulation of metabolism and gene expression in tissues would be governed via cell signaling, and the tissue's microenvironment would promote appropriate growth. Under cell culture conditions *in vitro*, cells are given the proper nutrients and growth factors to be maintained outside of the organism. Today, we use this practice to investigate the response of eukaryotic cells to various conditions. For example, if a contaminant is released into a community, and little is known about what it does to mammalian cells, cell culture-modeled experiments allow us to study its effects under controlled conditions. This process is also used to screen drugs to determine their efficacy and toxicity [3].

The Origin of Cell Culture and Common Applications

Attempts to culture cells *in vitro* began very early in history, in 1885, when Wilhelm Roux isolated a chicken embryo and sustained it outside its normal conditions [10]. *In vitro* stems from the Latin term "in glass," which refers to the act of removing a cell line and placing it into a glass container. Later, in 1910, Carrel, Burrows, and Montrose improved the technique by holding tissue samples for 2-3 months [11]. The American Type Culture Collection (ATCC) later crafted the regulatory language for cell culture to fit certain standard assessments, which was the backbone for developing the much-needed aseptic techniques to ensure samples' purity and improve cell preservation. In 1949, American virologists John F. Enders, Thomas H. Weller, and Frederick C. Robbins revolutionized virology when they successfully cultured the three poliomyelitis viruses *in vitro* using non-nervous cell cultures [12]. This groundbreaking achievement earned them the Nobel Prize in Physiology and Medicine in 1954. Their discovery quickly led to the development of effective poliomyelitis vaccines by J. E. Salk in 1953 [13]. From 1952 to 1955, Gey created the first human cell line from HeLa cells as mentioned previously [2]. Establishing a stable, immortal human cell line ushered in a new era of biomedical research. This section will discuss the utility of HeLa, CHO, and HEK 293 cells.

HeLa cells were the first human cell line able to be propagated indefinitely in culture. Henrietta Lacks died from an aggressive form of Cervical Cancer in 1951. During her prognosis and treatment, her cells were preserved without her existing family's consent. This led to many ethical debates among the scientific community and the rules and regulations regarding the patient's consent and privacy. The Department of Health also enforces its model for patient consent depending on the situation, and acting professionals can be persecuted when performing research on patients or their Biospecimens [14]. Ethical questions notwithstanding, research using HeLa cells continues to pay dividends to the scientific world. In fact, "Knowledge of almost every process that occurs in human cells has been obtained using HeLa cells" making them perhaps the most historically valuable cell line [15].

CHO cells, also known as Chinese Hamster Ovary cells, are a mammalian cell line that is easy and affordable to mass culture, which is why they are used to produce 70% of therapeutic proteins [16].

Recently, CHO cells have begun to play a more significant role in antibody production for therapeutic applications [16]. How they respond in cell-based drug assays are a good indicator of how other mammalian cells react when treated with medication, exposed to a chemical, or transfected with a pathogen [8].

HEK293, or human embryonic kidney-derived epithelial cells, are among the most frequently utilized cell lines in cell biology research. This cell line is a frequent choice for research and therapeutics because of its rapid division rate, robustness, and proficiency in post-translational modification of heterologously expressed proteins [17]. Additionally, HEK293 cells are highly amenable to transfection, making them a preferred choice for transient and stable transformation experiments, protein expression and production, and even electrophysiological studies [17].

Stem cells and Organoids

Stem cells are nonspecific human cells that can be taken from an adult or embryo and manipulated to differentiated into various human cell types, depending on the origin of the stem cell. Though not cell lines, their *in vitro* research and clinical applications make them a powerful tool. These cells can be classified into three categories for simplicity's sake, which will be introduced when narrowing the applications. The first classification would be Totipotent, which can create both embryonic and extra-embryonic structures, an example being a zygote. The second classification would be Pluripotent, which can form only embryonic structures such as organelles and be gathered from the Blastocyst inner cell mass four days after development. Lastly, multipotent stem cells can only generate specific cell lines, such as generating macrophages from hematopoietic stem cells. The discovery of embryonic and adult stem cells was revolutionary because of their potential in regenerative medicine. Currently, stem cell-derived organs and organoids have been successfully demonstrated in mice from mammary to prostate glands [18]. Not only can it have major beneficial effects in regrowing already existing damaged organs, but it can also theoretically create whole organs, also known as organoids, which can be risk-free from rejection, unlike organs donated from other humans, which we use in transplants today [19]. Organoids are artificially created organs from existing stem cells to perform the function of organs. Though our understanding of organogenesis is quite limited within the organoids' microenvironment, the potential for organoid structures to faithfully mimic organs may change the future as we know about transplant procedures, organ donors, and cell-to-cell interactions within organ systems [20].

Limitations of Cell Culture Models

Various cell lines are used in scientific research, yet they come with certain disadvantages and limitations, especially in drug development. One such limitation is the acquisition of additional genetic aberrations associated with increasing passage numbers. As most cell lines are transformed or derived from cancer cells, they possess mutations in cancer genes, including those associated with genomic instability which may result in genetic changes with the passage of cells [21]. Even without severe genetic changes, genotypic and phenotypic drift in continuous culture can shift cellular responses over time, which can differ significantly from patient responses to the same drugs [21]. Additionally, there are disparities between the microenvironments of original tumors and cancer cell cultures (both 2D and 3D) and cross-contamination with HeLa cell lines. Culture conditions can alter morphology, gene expression, and several cellular pathways, while mycoplasma infections can change culture properties [21]. Establishing long-term cancer cell lines for specific tumor types can be challenging, and the cell culture environment differs from the original tumor's. Furthermore, the natural heterogeneity of the tumor or tissue, the interplay of multiple cell types influencing each other, is often lost in cell culture environments which are monocultures.

The Future of Cell Culture Applications

Due to the increasing need for treatment, cell-based screening methods have become more efficient and cost-effective for drug testing than animal testing. Animal testing is a solid model for understanding the effects of drugs on mammals. However, it creates multiple problems, including the cost and ethical dilemmas of harming animals [21]. Furthermore, the drug action in animal models might differ greatly from how it acts in clinical trials, showing animal models and cell culture models will require improvements to represent human physiology better. After decades of performing anti-cancer drug screening in mice, the National Cancer Institute (NCI) significantly shifted to a novel approach: screening 10,000 diverse compounds per year against a panel of 60 human tumor cell lines *in vitro*. Alley and colleagues introduced this new "NCI-60" screening method in a landmark 1988 Cancer Research article, showcasing the technological foundation for this large-scale microculture screen [22]. The core idea was that the NCI-60 screen would accelerate the discovery of innovative cancer drugs, particularly those effective against specific solid cancers, which was not previously feasible. Currently employed in modern molecular target-based drug discovery, these panels are revolutionizing biomarker-driven precision oncology and improving the lives of cancer patients [23]. Clearly, cell-based screening creates less of an ethical dilemma and can be more accurate in determining the actual outcome of a particular drug treatment [24].

2-D vs 3-D cell Screening

Currently, 2-D cell screening is the most popular technique; however, 3-D cell screening will allow for more accurate drug treatment effects *in vivo*. 2-D screening is a more cost-effective and convenient form of *in vitro* model for screening; in its more simplistic forms, it tends to be made of a monolayer cell culture, added with molecules to perform a biological function also known as a biological library and the use of a microplate reader or microscope. 3D cell screening is more complex and tends to be a replica of the environment in which the cell resides; thus, it tends to be more costly but is more comparable to *in vivo*. Recently, a cancer research study showed that certain breast cancer cell lines developed dense 3D multicellular spheroids (MCSs), and these spheroids exhibited reduced sensitivity to the chemotherapeutic drugs doxorubicin (DXR) and paclitaxel (PTX) compared to cells cultured in 2D environments [25]. This demonstrates how 3D culturing of cells will produce more reliable data on the future efficacy and toxicity of cancer drugs. Researchers suggest that their downstream applications will take advantage of the 3-D model but with the manufacturing cost of the 2-D model [16].

History of Microcosms and Their Applications

Ecological microcosms are small ecosystems held in containers used as a research tool for studying the way ecosystems work and determining what happens to different substances in ecosystems. They are a simplified way to model whole ecosystems and can be replicated for experimental studies at reasonable cost [26]. Basically, microcosms are of different shapes, sizes, and compositions, but they possess some or all of the following properties: origin, isolation, size, genotypic heterogeneity, spatial heterogeneity, and temporal heterogeneity [27]. Microcosms find their origin in the natural world, and are isolated systems of reasonable size, no longer in contact with the natural world. Microcosms may be as small as a few milliliters or as large as a building. Genotypic heterogeneity refers to the fact that microcosms typically exist as mixed populations of organisms. Spatial and Temporal heterogeneity implies that the microcosms differ at places within the system and change over time. Ultimately, due to the advantages of controllability, replicability, and low cost, microcosm experiments have been used in practically every area of modern ecology, terrestrial and aquatic [26].

Modern biological science regards ecological microcosms as a relatively new research technique. However, Robert Warington introduced his work on a balanced aquarium in 1857, considered one of the first scientific papers on microcosms. He pointed out the interaction of producers, consumers, and decomposers, laying the basis for the aquarium concept, with its contained organisms functioning as a unit [27]. Microcosms vary greatly in their construction and contents but tend to follow at least a few general criteria. They typically contain multiple species to reflect the systems interactions within ecosystems, they contain artificial boundaries created by experimenters and not seen in nature, and the experimental system is at least partially isolated from the natural world [26]. Therefore, we can see the great value in using a closed system where all of the variables can be controlled or manipulated in order to gain insight into the workings of actual ecosystems throughout the world.

Since the adoption of microcosms as models for use in the laboratory, there have been great strides in their complexity and utility. Natural ecosystems, besides being challenging to define precisely, frequently exhibit considerable size and are influenced by a range of environmental factors affecting their boundaries. These factors include variations in light intensity and duration, fluctuations in temperature encompassing both average conditions and extreme events, the presence of suspended or dissolved materials in the surrounding fluid medium, and the dynamics of importing and exporting non-living materials and organisms [28]. If one is unable to control the boundaries of an experiment, determining the relationship between the independent and dependent variables becomes nearly impossible. It is also important to note that to purposefully alter an ecosystem for experimental purposes could prove quite destabilizing and result in the death of many living things, an unethical prospect. Therefore, the microcosm serves as a way not only to better control the variables in an experiment but also to avoid ethical quandaries about tampering with natural ecosystems.

The microcosm approach enables the creation of replicated ecosystems, even those that are rare, such as California vernal pools or nonexistent on Earth (Mars soil, for example), and the replicates provide better statistical veracity to the data generated from the experiments. To achieve replication, living and non-living components from a natural ecosystem are transferred into a laboratory setting and maintained under conditions closely resembling those of the original environment [28]. These replicated samples are distributed across multiple containers. Although variation due to the seeding of microcosms occurs, a greater number of replicates helps to eliminate statistical outliers and provide robust data sets.

Microcosms have been adapted to a wide variety of experimental subjects over time. Historically, theoretical ecology, biogeochemical cycles, radioactivity tracing, life support systems, and sanitary engineering have been studied using these systems [26]. One of the earliest papers on nutrient influx and aquatic ecosystem productivity used a concrete pond microcosm seeded with nitrates to determine their effect of productivity [29]. Shortly after, we see the first attempts to outline the microcosm method as a distinct approach, building on earlier work where scientists utilized microcosm experiments to demonstrate the presence of balanced aquatic environments [30]. Subsequent to the world entering the Atomic Age, microcosms were employed in radioactive tracer investigations [31] for primarily two reasons. Firstly, they offered a more manageable experimental environment compared to the complexities of controlling variables in natural ecosystems. Secondly, they helped circumvent potential pollution issues. Later, the Space Race and population explosion ushered in an era of microcosm research to address the burgeoning population and survival of humans in space. In space, the challenge revolves around sustaining human life outside of Earth's biosphere. Meanwhile, on our planet, the expanding population necessitates more effective waste management solutions to handle the growing volume of waste generated. Microcosm research began to demonstrate how closed systems could be balanced to sustain human life in space travel and how the same approach could be used to manage the production of vast amounts of human waste in the industrialized world [32, 33].

Aquatic mesocosms and microcosms are experimental systems designed to replicate natural ecosystems, such as small freshwater ponds or wetlands, in a controlled environment. They contain physical, chemical, and biological components similar to those of the natural ecosystem. These systems consist of populations and communities of organisms at multiple trophic levels, including algae, plants, invertebrates, and fish, that interact with their environment and each other in complex ways.
In ecotoxicology, microcosms are used to examine the effects of environmental stressors, including chemicals, on the ecosystem. Specifically, they have been used to study the impact of chemical pollutants on amphibian and insect metamorphosis as well as other physical and biological components of the ecosystem. These effects can include changes in nutrient cycling, primary productivity, species interactions, and community structure. Microcosms can also be used to investigate the direct and indirect effects of chemicals and other stressors on ecosystem structure and function, the recovery process from these effects, and to inform ecological risk assessment.

Using microcosms, researchers can examine how chemicals and other stressors impact ecosystems in a way that is impossible in the natural environment. For example, the exposure of plankton microcosms to Nickel (Ni) demonstrated noticeable effects on a few individual species, which allowed the researchers to determine that environmental threshold concentrations for nickel in European environmental laws are adequate [34]. They can also conduct experiments over a shorter time frame than would be required in the field, allowing them to understand the mechanisms behind the observed effects better. Overall, microcosms are a valuable tool for ecotoxicologists and other researchers interested in understanding the complex interactions between organisms and their environment [35].

Furthermore, in order to increase the accuracy and realism in the ecological risk assessment of chemicals, multispecies experiments are carried out. These experiments have certain advantages over laboratory single-species tests that enable them to provide more comprehensive and reliable data. For example, they evaluate more realistic exposure regimes, assess the effects of chemicals on populations rather than individuals, and allow the assessment of recovery of populations that have been affected by chemical exposure. Additionally, multispecies tests include food netting interactions between populations, which are essential in understanding the broader ecological impacts of chemicals.

Multispecies tests come in a wide range of experimental designs, ranging from relatively simple indoor multispecies assemblages to more complex model ecosystems and field monitoring studies. These designs are tailored to the specific needs of the experiment and the complexity of the ecosystem being studied. For instance, simple indoor multispecies assemblages are useful for preliminary screenings of chemical toxicity. Meanwhile, model ecosystems and field monitoring studies are more appropriate for assessing the long-term effects of chemicals on ecosystems.

In recent decades, significant progress has been made in the development of multispecies experimental designs. For example, a multispecies experiment demonstrated that the spatial organization of Pseudomonas putida and Acinetobacter was driven by metabolic cross-feeding, findings that would not be observed in monoculture [36]. Studies indicate that wastewater treatment systems consist of over a thousand species-level operational taxonomic units (OTUs), microbial mats contain more than 750 species, and oral biofilms harbor hundreds of different organisms, including both bacteria and eukaryotes [37]. The acid mine drainage (AMD) ecosystem, known for its low pH and high concentrations of toxic metals and sulfate, is one of the most comprehensively studied natural communities. 'Omics' approaches have significantly advanced our understanding of AMD, revealing that a few species dominate the community. However, rare taxa play crucial roles in processes like nitrogen fixation and sulfur oxidation [38]. This complexity underscores the importance of considering both the dominant and rare species to fully understand ecosystem function. These designs have become more sophisticated and have incorporated new technologies that allow for more precise and detailed data collection. As a result, multispecies experiments have become an essential tool in ecological risk assessment, enabling scientists to gain a deeper understanding of the potential impacts of chemicals on ecosystems [37].

Nonetheless, global environmental problems that affect the entire planet can be difficult to address with traditional scientific experiments; thus, conducting small-scale experiments using "model organisms" in microcosms or mesocosms can be a helpful approach to tackling these seemingly insurmountable issues. By experimenting on a smaller scale, different scenarios can be tested out. For example, how ecosystems respond to climate change or how to manage biodiversity through nature reserves. This experimental approach can also aid in the development of theories and inspire further research, ultimately leading to a better understanding of the issues and practical solutions. Although this process has been influential in the past, it can be time-consuming. Therefore, addressing global issues with significant policy implications would be a key move [39].

Additionally, when conducting microcosm studies, it is crucial to consider the experiment's duration. Typically, microcosm experiments only last for several weeks to months. However, it is essential to ensure that the experiment's duration is long enough to evaluate the effects on slow-responding organisms or processes. Like natural ecosystems, microcosm conditions can change over time, and these changes should be assessed throughout the study. As the experiment's duration increases, there is a higher likelihood of greater variability developing between replicates due to natural divergence. Time series sampling can be included in the experimental design to monitor changes. However, the impact of any repetitive sampling of components should be carefully considered. At the start of the experiment, a large number of microcosm replicates can be established to enable complete sampling of a subset of replicates at designated time intervals. In the laboratory, natural diurnal variations can be simulated to some extent by using controlled light-dark cycling of artificial lights. For shorter experiments, seasonal variability can be taken into account by repeating experiments on a seasonal basis. Additionally, ecological processes that occur over longer periods, such as succession, predator-prey cycles, and extinction, can be studied in microcosms with short real-time duration using organisms with very short generation times, such as microorganisms. This approach is known as the biological accelerator approach [40].

Conclusion

Cell 1 variables and study ecological dynamics in a reproducible manner. Both approaches serve to recreate the natural world in the laboratory from whole organisms in the case of cell culture and from an entire ecosystem in the case of microcosms.

While cell culture experiments primarily focus on cellular and molecular biology and microcosms are oriented towards ecological and environmental studies, there exists an overlap between the two techniques, especially in areas like environmental toxicology. Researchers may use cell culture experiments to study the effects of pollutants on cellular function and then validate their findings in microcosm experiments to assess ecological impacts. Moreover, recent advancements in whole genome sequencing, metagenomics, and metabolomics have facilitated linking cell culture-based studies and microcosms to investigate molecular biology at both the species and community levels [4].

In conclusion, both cell culture experiments and microcosms are indispensable tools in scientific research, offering unique insights into different aspects of biology and ecology. Their continued development and integration with emerging technologies hold immense potential for addressing pressing challenges in fields ranging from medicine to environmental science.

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