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REVIEW ARTICLE

# ARCHAEOSOMES: A ROBUST LIPOSOME

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**ABSTRACT**

Archaeosomes have proved to be an add-on to the liposomal delivery. Natural archaeal membrane lipids and/or synthetic lipid analogues have been grafted in the form of vesicle so as to incarnate archaeosomes. Archaeal lipids are unique and distinct from those encountered in *Eukarya* and *Bacteria as* these are polyether derivatives. Archaeal type lipids consist of archaeol (diether) and/or caldarchaeol (tetraether) core structures wherein regularly branched and usually fully saturated phytanyl chains (20-40 carbons in lengths), are attached via ether bonds to the sn-2,3 carbons of the glycerol backbone. The cardinal feature of archaeosomes includes; relatively higher stabilities to oxidative stress, high temperature, pH, action of phospholipases, bile salts, and serum proteins. In this review, some of the fascinating features of archaeosomes are enumerated with its applications and some general aspects.

## INTRODUCTION

Drug delivery systems have been continuously refined to derogate the one which is already available. The loopholes with one that is available have been resolved with some added advantages and increased specificity. Vesicular drug delivery is one such delivery system which has provided an alternative niche to the formulation scientist. With large no of patents and commercial products family is continuously growing with more than 30 polymersomes are reported in various literature.[(1)] The evolution begins with liposome; an artificial, spherical, closed vesicles consisting of one or more lipid bilayer(s). Liposomes made from ester phospholipids have been studied extensively over the last 3 decades including their use as diagnostic reagents, as carrier vehicles in vaccine formulations, or as delivery systems for drugs, genes, or cancer imaging agents.[(2)] The structure of liposomes can differ widely depending on both composition and process conditions. They may also contain either one (unilamellar vesicles, ULV) or several (multilamellar vesicles, MLV) bilayer structures.[(3)] One of the major limitations with them is poor circulation time and in ability administer by extravascular route. Attempts are being made either by incorporation of high quantities of cholesterol or by coating the liposome surface with hydrophilic polyethylene glycol polymers to resolve such issues but have led to limited success.

The study of archaeobacteria has become eminent as a realization of the potential commercial applications of their unique metabolic properties. For example, uses are found for thermostable enzymes such as DNA polymerases, bacteriorhodopsin for production of computer chips, methanogenic bacteria in the treatment of organic wastes and for their symbiotic role in degradation of recalcitrant aromatic compounds, and production of specifically labeled cell metabolites.[(4, 5)] The potential use of their polar ether phospholipids in delivery rather than conventional ester phospholipid represents another new application that should spur the continued interest in these organisms. These lipids are unique and distinct from those encountered in *Eukarya* and *Bacteria*. The liposomes made from these ether lipids are termed as archaeosomes. Polar glycerolipids make up the bulk of the membrane lipids, with the remaining neutral lipids being primarily squalenes and other hydrocarbons.(See **Fig. 1**) The polar lipids consist of regularly branched, and usually fully saturated, phytanyl chains of 20, 25, or 40 carbon length, with the 20 and 40 being most common. The phytanyl chains are attached via ether bonds to the *sn*-2,3 carbons of the glycerol backbone(s). Diether and tetraether lipid derivatives made monolayer and bilayer structure respectively. The cardinal feature of archaeosomes includes; relatively higher stabilities to oxidative stress, high temperature, pH, action of phospholipases, bile salts, and serum proteins.[(6)]



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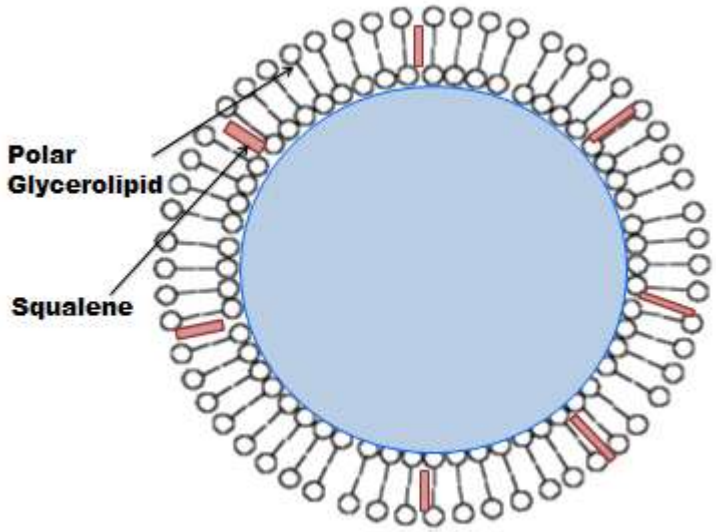


**Figure 1: Structure of Archaeosomes**

## ADVANTAGES [6-8]

- ✓ The ether linkages of archaeal lipids are more stable than esters over a wide range of pH, and the branching methyl groups help both to reduce crystallization (membrane lipids in the liquid crystalline state at ambient temperature) and membrane permeability (steric hindrance of the methyl side groups)
- ✓ The saturated alkyl chains would impart stability towards oxidative degradation
- ✓ It doesn't require addition of cholesterol
- ✓ The unusual stereochemistry of the glycerol backbone (opposite to mesophilic organisms) would ensure resistance to attack by phospholipases released by other organisms
- ✓ The bipolar lipids span the membranes and enhance their stability properties hence they can be prepared and stored in the presence of air/oxygen without any degradation.
- ✓ Some archaeosome formulations can be sterilized by autoclaving, without problems such as fusion or aggregation of the vesicles
- ✓ The addition of cyclic structures (in particular five membered rings) in the Trans membrane portion of the lipids appears to be a thermo adaptive response, resulting in enhanced membrane packing and reduced membrane fluidity.
- ✓ Archaeal lipid ensure membrane functions even in harsh destabilizing environmental conditions (high or low temperatures, high salinity, acidic media, anaerobic atmosphere, high pressure) which provide a boon for thermo labile compounds.
- ✓ The uptake of archaeosomes by phagocytic cells can be up to 50-fold greater than that of conventional liposome formulations which ensures good adjuvant activity in vaccine delivery with good memory
- ✓ It can be utilized for specific organ targeting
- ✓ The synthetic archaeal lipid derivative provides more stability to the hydrolysis with phospholipases (See **Fig. 2**)

## FORMULATION

The archaeosomal anatomy mainly depends on the polar lipids from which they are prepared. Extensive research has been devoted in this field not only to isolate various polar lipid fractions from various bacterial species but also to synthesize some of semi synthetic and totally synthetic analogues.

### 1. Natural polar lipid

Archaeobacteria ("Archaea") is considered to be a Domain of prokaryotes that is distinct from Eukarya and Bacteria domains as it requires harsh condition for optimum growth.[9] one of the cardinal features of their membrane structure is the ethereal glycerolipids what it contains (5% of cell dry wt). The structures of archaeal lipids are closely related to the organisms from which they are extracted. In general, these lipid containes a polar head group usually glycerophosphate or ethanolamine phosphate with branched, 5-carbon repeating units forming phytanyl chains (fully saturated with few exceptions) that are linked via ether bonds to the *sn*-2,3 carbons of the glycerol backbone. Basically, these consist of the ubiquitous standard monopolar archaeol (2,3-di-*O*-diphytanyl-*sn*glycerol or standard diether) consisting of $C_{20,20}$ (20 carbons per alkyl chain, attached to the *sn*-3 and *sn*-2 glycerol carbons, respectively) and/or the standard acidophilic bipolar caldarchaeol (2,2',3,3'-tetra-*O*-dibiphytanyl-*sn* diglycerol or standard tetraether) with $C_{40,40}$ carbon chains.[7]



 

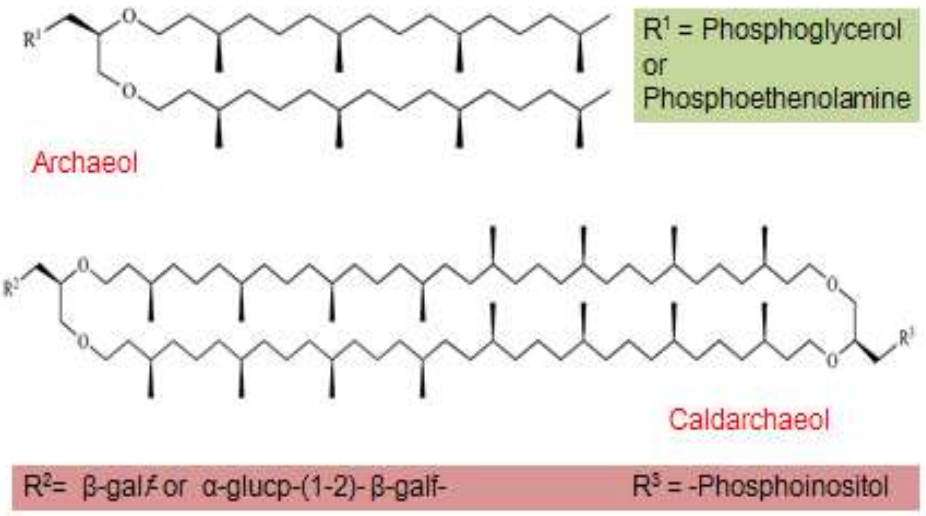


**Figure 2: Structure of natural archaeal lipid**

Some archaeobacteria such as *Halobacterium cutirubrum* and *Methanosarcina mazei* contain only archaeol lipids, thermoacidophilc archaeobacteria such as *Thermoplasma acidophilum* and *Sulfolobus acidocaldarius* consist of predominantly caldarchaeol lipids.[10] Several archaea organisms such as methanogens have developed mixtures of both diether and tetraether type polar lipids.

## 2. Semi synthetic or chemically modified natural lipid

Clearly, the polar lipids of archaeobacteria are unique and distinct from those found in other natural sources. The polar head group in natural moiety is sensitive to acid hydrolysis hence hydrophilic polymers (PEG), aminated derivatives (spermine), phosphogroups (phosphate, phosphoethanolamine, phosphocholine), biotin and cell recognition ligands were tried to solve it.[8]

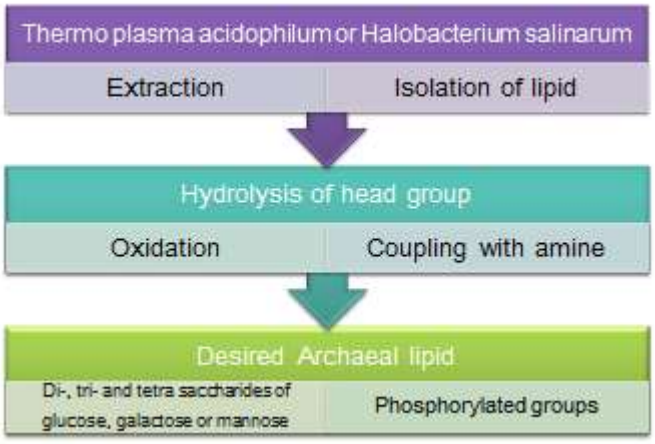


**Figure 3: Hemi-synthetic archaeal lipid preparation**

## 3. Synthetic archaeal lipid

One of the major drawback of natural lipid is its sensitivity towards phospholipases (PL) especially PL C hence to resolve it total synthesis of archaeal lipid analogues was investigated by several academic or company research teams.[11, 12] Symmetrical 1,3-cyclopentane ring bearing synthetic archaeal lipid analogues with two alkyl (X = (CH2)2) or alkoxy (X = O) chains linked to two glycerol units were reported.[13] Caldarchaeol (tetraethers) analogues with quasimacrocyclic backbones are based on a C32 chain core bearing two branched methyl groups within the middle of the bridging chain and two phytanyl or linear C16 arms were nurtured.[14] Both caldarchaeol and isocaldarchaeol analogues were designed and functionalized by several polar head groups such as a PEG chain, aminated groups or phosphorylated groups. Still more no of such synthetic analogues with better properties will come in future.



 

**PREPARATION METHODS**

The lipid extraction is commenced from suitable archaeobacterial species. The total natural archaeal lipid extract (TLE) composed of total polar lipids (TPL) and neutral lipids like Squalene and other hydrocarbons. Chloroform/methanol/water extraction from freeze thawed biomass coming from the selected archaea species provides such TLE.[15] From TLE, TPL and neutral lipids are separated by precipitation of TPL using acetone. These TPL made from isoprenoid ether lipids of opposite sn-2,3 stereochemistry can be stored as chloroform or chloroform/methanol (2:1) solutions without special conditions. It is worth to mention that only glycol lipid sulfate and phospatidylglycerophosphate fraction form the vesicle.[16] Pure archaeal lipids can be further obtained by chromatography, either column chromatography or preparative thin layer chromatography.[15, 17, 18] The resulting pure archaeal lipids can be chemically modified in order to introduce specific head groups. One can also synthesize the archaeal lipid. Phosphatidylmyoinositol as one polar head group and either glucopyranose or galactopyranosyl-glucopyranose as the other polar head group is essential for structural stability. Further it is very much difficult to hydrate such lipids.[19] Starting from natural, chemically modified or synthetic archaeal lipids, it was possible to prepare archaeosomes formulations and encapsulate/associate hydrophilic or hydrophobic compounds using methods developed for the preparation of conventional liposomes.[20-22]

**1.Lipid hydration method**

In this method, lipid mixture are dissolved in solvent mixture of chloroform : methanol (2:1) in rotary evaporator flask and dried thin film of lipid is made using rotary evaporator above the phase transition temperature of the bulk lipid (60 rpm, ≈ 30ºC, and about 15 min). Hydration of lipid is done by adding 5ml of saline phosphate buffer containing drug/solute to be encapsulated and again use of rotary evaporator for making homogeneous milky white suspension. The archaeobacterial polar ether lipids are in a liquid crystalline-like or fluid state at ambient temperature, and hence they can be hydrated at room temperature, if need be. It is allowed to stand for 2 h at RT/above Tc for complete swelling process. This will give MLVs. Archaeosomes can be annealed even at refrigeration temperatures.[23-26]

**2.Detergent dialysis method**

In this method, the ethereal phospholipids are brought into intimate contact with the aqueous phase via the intermediary of detergents, which associate with phospholipid molecules and serve to screen the hydrophobic portions of the molecule from water. Detergent depletion is achieved by four following approaches:

*A. Dialysis:* The dialysis can be performed in dialysis bags immersed in large detergent free buffers (equilibrium dialysis) or by using continuous flow cells, diafiltration and cross filtration.

*B. Gel filtration:* In this method the detergent is depleted by size exclusive chromatography. Sephadex G-50, Sephadex G-100, Sepharose 2B-6B and Sephacryl S200-S1000 can be used for gel filtration. The archaeosomes do not penetrate into the pores of the beads packed in a column.

*C. Adsorption using biobeads:* Detergent adsorption is achieved by shaking of mixed micelle solution with beaded organic polystyrene adsorbers such as XAD-2 beads and Bio-beads SM2. The great advantage of the using detergent adsorbers is that they can remove detergents with a very low critical micelle concentration (CMC) which are not completely depleted by dialysis or gel filtration methods.

*D. Dilution:* Upon dilution of aqueous mixed micellar solution of detergent and phospholipids with buffer the micellar size and the polydispersity increases dramatically, and, as the system is diluted beyond the mixed micellar phase boundary, a spontaneous transition from polydisperse micelles to monodisperse vesicles occurs.

The detergent/dialysis method can result in poor entrapment due to the leakage of loaded molecules during the dialysis process.[17, 27]

**3.Reverse phase evaporation**

The essential feature of this method is the removal of solvent from emulsion by evaporation. In this method, polar lipids are dissolved in organic solvents that are sonicated by bath sonication which form emulsion (w/o) and then emulsion is dried down to a semi solid gel using rotary evaporator under reduced pressure. The next step is to bring about the collapse of a certain proportion of water droplets by vigorous mechanical shaking with a vortex mixer. This will give LUVs.[18, 28]

**4.Membrane extrusion**

Size of prepared archaeosomes is reduced by gentley passing them through membrane filter of defined pore size and this can be achieved at much lower pressure. In this process, the vesicles content are extruded with the dispersion medium during breaking and resealing of polar phospholipids as they pass through the polycarbonate membrane in order to achieve high entrapment. The archaeosomes produced by this method have been termed as LUVETs and 30% encapsulation can be obtained using high lipid concentration.[22, 29]



 

### 5. Freeze-thaw method

This method is based on freezing of unilamellar dispersion and thawing (melting) by standing at RT for 15 min. and finally subjected to a sonication cycle. This process ruptures and refuses SUVs during which the solute equilibrates between inside and outside, and archaeosomes themselves fuse and markedly increase in size. The second step of the sonication considerably reduces the permeability of the archaeosomes membrane, by accelerating the rate at which the packing defects are eliminated. For producing giant vesicles of diameter having 10 – 50 µm, the sonication step is replaced by the dialysis against hypo-osmolar buffer. In this case, SUVs are mixed with salt solution followed by freeze thawing. During this dialysis, the large vesicles formed by freeze thawing swell and rupture as a result of the osmotic lysis, where the fuse and prepare as giant vesicles.[29, 30]

### 6. Sonication

Archaeosomes could also be formed from the polar lipid fraction 'PLE' (apparently equivalent the total polar lipids) of S. solfataricus, without the need for exogenous lipid supplementation, by sonication at 60°C.[31] Sonication at 0°C was also successful to produce liposomes from S. acidocaldarius polar lipids.[32]

### 7. French pressure cell extrusion

In this method, liquid sample of preformed MLVs are introduced into the sample cavity, then the position of piston and pressure is set up to fill sample upto the outlet hole. Then power is switched on. At high pressure (2000 psi) and at 40ºC, MLVs are extruded through small orifice, which is collected in suitable container. This technique yields uni- or oligo lamellar archaeosome of intermediate size. More stable than they obtained by sonication method and also leakage of the content from the archaeosomes are lesser.[33]

## EVALUATION

The large variety of lipid structures reflects the need for Archaea to adjust their core lipid structures in order to be able to ensure membrane functions despite harsh destabilizing environmental conditions (high or low temperatures, high salinity, acidic media, anaerobic atmosphere, high pressure). Empty and/or drug-loaded archaeosomes are characterized by methods enumerated in **Table 1.**[13, 17, 18, 23, 34]

**Table 1: Characterization of archaeosomes**

| Assay | | Methodology |
|---|---|---|
| **Physical Characterization/Stability** | | |
| 1 | Vesicle size, surface morphology and size distribution | Transmission electron microscopy (TEM), freeze fracture electron microscopy, Dynamic light scattering, TEM, Contrast and fluorescence microscopy, Confocal Microscopy |
| 2 | Osmotic pressure | Osmometer |
| 3 | Phase behavior | Differential scanning calorimetry |
| 4 | Lamellarity | Small angle X-ray scattering, $^{31}$P-NMR |
| 5 | Surface charge | Free-flow electrophoresis |
| 6 | Entrapment efficiency | SDS gel electrophoresis. |
| 7 | Percentage leakage | Exposure to Phospholipases in simulated condition or radioactivity leakage |
| 9 | *In vitro* release | Dialysis through a semipermeable membrane and its measurement using suitable analytical method |
| **Chemical Characterization/Stability** | | |
| 1 | pH | pH meter |
| 2 | Concentration of TPL | HPLC, TLC |
| 3 | Enzyme stability | Using various phospholipases |
| **Biological Characterization/Stability** | | |
| 1 | Sterility | Aerobic and Anaerobic culture |
| 2 | Pyrogenicity | SAM or LAL test |
| 3 | Immuno-adjuvanticity | ELISA |
| 3 | Animal toxicity | Monitor survival, histology and pathology |



 

## APPLICATIONS

Mononuclear phagocytes are one of the natural targets for archaeosomes hence can be utilized in vaccine delivery as well as an adjuvant. From the last three or five year so it has gain huge attention not only for vaccine delivery but for gene/drug delivery also. Many patents are registered to adjudicate such therapy.

### 1. Archaeosomes as stable antigen carriers and/or Adjuvants for Vaccine

Because liposomes naturally target to the cells of the mononuclear phagocytic system, it would be expected that they would be ideally suited for delivery of antigens, either as a carrier system and/or directly as adjuvants that stimulate the immune system. An adjuvant is defined as a substance or a material which when administered together or in conjunction with an immunogen (antigen) increases the amount and quality of the immune response to that immunogen in the vaccinated/immunized host. Some of these interventions are depicted in **Table 2.**

**Table 2: Archaeosomes as stable antigen carriers and/or Adjuvants for Vaccine**

| Author | Title | Remark | Reference |
|---|---|---|---|
| He et al., (2013) | Kinetics of Adeno-Associated Virus Serotype 2 (AAV2) and AAV8 Capsid Antigen Presentation In Vivo Are Identical | Effective strategies to prevent capsid-specific $CD8^+$ T cell-mediated elimination of AAV-transduced target cells | (35) |
| Sprott et al., (2012) | Synthetic Archaeosome Vaccines Containing Triglycosylarchaeols Can Provide Additive and Long-Lasting Immune Responses That Are Enhanced by Archaetidylserine | Vaccines giving best protection against solid tumor growth | (36) |
| Li et al., (2011) | Archaeosomes with encapsulated antigens for oral vaccine delivery | Facilitated antigen specific $CD8^+$ T cell proliferation with IgG response | (37) |
| Sprott et al., (2008) | Archaeosomes varying in lipid composition differ in receptor-mediated endocytosis and differentially adjuvant immune responses to entrapped antigen | Modulate antigen delivery and dendritic cell activation | (38) |
| Patel et al., (2008) | Archaeal polar lipid aggregates for administration to animals | Vaccine therapy | (28) |
| Rowland et al., (2008) | Isoprenoid compounds, their isolation and use | Improve stability | (26) |
| Sprott et al., (2008) | Synthetic Archaeal Glycolipid Adjuvants | Showing good response | (25) |
| Patel et al., (2007) | Mucosal and systemic immune responses by intranasal immunization using archaeal lipid-adjuvanted vaccines | Proved to be non-replicating mucosal adjuvant | (39) |
| Sprott et al., (2001) | Archaeosomes as immunomodulating carriers for acellular vaccines to induce cytotoxic T lymphocyte (CTL) responses and protect the vaccinated host against intracellular pathogens and cancer | Improved response | (24) |
| Sprott et al., (1997) | Archaeosomes, archaeosomes containing coenzyme Q10, and other types of liposomes containing coenzyme Q10 as adjuvants and as delivery vehicles | Good adjuvant activity | (18) |



 

## 2. Archaeosomes for drug/gene delivery

It was well described in literature that archaeosomes are more stable then liposome and can bear harsh condition also. Much work has not been done using it as a carrier of drug or gene as compared to its exploration in the delivery of vaccine. **Table 3** gives the brief regarding its drug/gene delivery applications.

**Table 3: Archaeosomes for drug/gene delivery**

| Author | Title | Remark | Reference |
|---|---|---|---|
| Ulrih et al., (2013) | Cytotoxicity and uptake of archaeosomes prepared from Aeropyrum pernix lipids | The uptake of archaeosomes and the release of loaded calcein are more prominent in EA.hy926 cells, which is in line with high toxicity toward these cells. | (40) |
| Barbeu et al., (2011) | Preparation and Characterization of Stealth Archaeosomes Based on a Synthetic PEGylated Archaeal Tetraether Lipid | Slower release of the dye encapsulated into PEGylated archaeosomes with enhanced stability | (41) |
| Benvegnu et al., (2006) | Compounds analogous to membrane lipids in archaebacteria and liposomal compositions including said compounds | Efficient pCMVluc plasmid delivery by such cationic archaeosome | (13) |
| Richardsen et al., (2004) | Liposome-forming compositions | PEGylated Archaeosome preparation which improves stability for peptide delivery | (30) |
| Khul et al., (2006) | Tetraether lipid derivatives and liposomes containing tetraether lipid derivatives and lipid agglomerates and the use thereof | Efficient oral Octride delivery | (27) |
| Freisleben et al., (1999) | Tetraether lipid derivatives and liposomes and lipid agglomerates containing tetraether-lipid derivatives, and the use thereof | Efficient Drug/gene delivery using modified tetraether derivative with quaternary ammonium salt as head group | (23) |

## FUTURE PROSPECTS

Despite of the advancement made in this field there is no information on their safety for use in humans. Another issue that will require resolution is the scale-up for the growth of large quantities of archaeobacteria. There are many patent available which sets one's thinking to graft archaeosone based nanovectors capable of overcoming the various biological, biophysical, and biomedical barriers that the body stages against drug/gene or vaccine therapies. It also provides potential avenues for gene and drug delivery. Cost effective and safe therapy with it will leads to ultimate commercial success for sure.

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