An Exploratory Research on the NanoVectors for Drug Delivery and for Gene Therapy

TANG SHAO-PU
School of Pharmacy,
Taipei Medical University
250 Wu-Xing Street, Taipei City, TAIWAN 110
TAIPEI TAIWAN, ROC.
wangsj@ms78.hinet.net

Abstract:-The purpose of this study is to explore the application of nanotechnology to prepare optimal vectors for drug delivery systems and for gene therapy delivery. We first review various types of drug delivery systems as vectors and then to explore nanovecotrs in gene therapy. By reviewing relative literature, we found out that nano drug vectors can be administered by all possible routes of administration and will revolutionize both gene therapy and the in vivo delivery of drugs. Three relative cases, inclusive of C Montemagno's study on a biomolecular motor in Cornell University, were explored. The findings show that applying nanotechnology will provide preparing nano vectors for both drug delivery and gene therapy another promising implication.

Key-Words: - Nanotechnology, Nanovectors, Biomolecular, Drug delivery, Gene therapy.

1 Introduction

Nanotechnology, known as the fourth industry revolution, is the creation and utilization of materials, devices, and systems through the control of matter at the dimension of roughly 1 to 100 nano meters. Nanotechnology, amongst the most promising technologies of 21st century and one of the hottest cross-disciplinary field of research areas [20], could be applied to lots of fields, including science, engineering, medicine, electron, photonics, superconductor, especially in bionanotechnology and nanomedicine. For instance, nanotechnology holds great potential for the delivery of precisely targeted medical procedures that will minimize collateral tissue damage—to a far greater degree than current cancer therapies, and all of the various nanotechnology techniques can be customized for killing different types of cancer[3]. Nanotechnology will markedly improve the implants, gene therapy, and tissue engineering approaches as well.

Nanomedicine is an application of nanotechnology to the prevention and treatment of disease in the human body. Nanomedicine exploration is the biologically motivated discovery and development that will incorporate nanotechnology tools, devices and processes to provide fundamental insights into cellular function and dysfunction, and leading to therapeutic interventions for disease. Nanomedicine is now within the realm of reality starting with nanodiagnostics and drug delivery facilitated by nanobiotechnology. Miniature devices such as nanorobots could carry out integrated diagnosis and therapy by refined and minimally invasive procedures, nanosurgery, as an alternative to crude surgery. Take gene therapy as an example.

Some of the earliest applications are in molecular diagnostics utilizing nanoparticles.

Various nanodiagnostics that have been explored will improve the sensitivity and extend the present limits of molecular diagnostics. Other applications are in cancer imaging and targeted therapy, which could potentially improve both the delivery of anticancer drug and the localized killing of cancerous and pre-cancerous cells[29].

Currently an increasing use of nanotechnology by the pharmaceutical and biotechnology industries is anticipated [25, 30]. Bionanotechnology, an integration of physical sciences, molecular engineering, biology, chemistry and biotechnology holds considerable promise of advances in pharmaceuticals and healthcare. So far the most important pharmaceutical applications are in drug delivery. Nanotechnology will be applied at all stages of drug development from formulations for optimal delivery to diagnostic applications in clinical trials. In addition to providing a solution to solubility problems, nanobiotechnology offers an intracellular delivery possibility and has the potential to provide controlled release devices with autonomous operation guided by the needs [25, 30]. For instance, by applying nanobiotechnology, Chitosan can be applied to prepare nanoparticle drug vectors, while methyl methacrylate and ethyl cyanoacrylate can be applied to prepare nanocapsules. In the past decade, with the help of the nanobiotechnology, a great deal of scientists have been devoted in developing optimal vectors using particulate delivery systems as carriers for small and large molecules to deliver drugs or therapeutic genes.

This research will review the nanoparticle-based vectors which have been explored for both drug delivery and gene therapy, and will discuss in more detail three cases.

2 Literature

Most therapeutic drugs distribute to the whole body, which brings about generally toxicity and poor patient's acceptance of the treatments. Since transporting across the membrane is one of the key obstacles, drug molecules must overcome to effectively function in the cell. Potential drugs should therefore be designed taking into account these specific membrane-transport properties[34]. Although a variety of drug delivery systems as vectors have been designed with their own advantages and limitations, scientists are learning to optimalize drug delivery to enhance the bioavailablility of the drugs towards diseased cells, promoting the required response while minimizing side-effects. Therefore in the field of targeted therapies or in gene therapy how to develop chemically derivatized drugs or drug delivery vectors which are able to target defined cells by means of specific recognition mechanisms and also able to overcome biological barriers has become a common goal the scientists try to reach[20].

2.1 Nanovectors

What is a nanovector? Nanovectors are multifunctional organic and inorganic nanoparticles, nanowires, and nanotubes. In the laboratory setting, nanovectors could potentially improve both the targeted delivery of anticancer drugs and the targeted localized killing of cancerous and precancerous cells[3]. Nanovectors also offer us a hope that nanosurgical tools may be integrated with micro- and macro-surgical tools for surgical treatment of cancer in the future[3]. Moreover, nanovectors (in development today) have the potential to be a generic platform for different types of cancer treatments.

While rapid advances are being made, nanovectors do have some barriers: (1) nanoparticles may aggregate, potentially blocking arteries and veins or even blocking the kidneys; (2) nanovectors might also trigger sensitization reactions; (3) nanovectors might cause increased osmotic pressures in malignant cancer tissues; and (4) nanovectors might cause some kind of toxicity. (註103) Therefore, new solutions are necessary for overcoming these problems.

2.2 Nanovectors for Drug Delivery

Nanoparticles are a class of artificially engineered materials in use today as nanovectors. Their miniscule dimensions imbue nanoparticles with unique physical proper- ties that offer enticing possibilities for cancer therapeutics. The surface of nanoparticle-based vectors designed for drug delivery can be adorned with various targeting agents or biological materials, such as antibodies, drugs, imaging agents, or reporters, which can be used to target a specific receptor in malignant cells. Such vectors have the ability to deliver a multiphase attack against malignant cells. Most nanoparticles are

hollow, which provides a central reservoir that can also be loaded with anticancer drugs, detection agents, imaging agents, chemicals known as reporters that can signal if a drug is having a therapeutic effect, or therapeutic agents that "deploy" when reaching their target malignant cells. Since most nanoparticles are constructed to be small enough to pass through blood capillaries, nano drug-vectors may be injected directly into cancer sites or into the blood to travel to their target malignant cells. Once its therapeutic drug payload has been delivered, a nanovector can be destroyed using external energy sources such as optics or magnetics[3].

To date, various types of nanovectors have been proposed. Liposomes are one of the most widely used nanovectors for different types of drug delivery modalities in the fight against cancer[28]. Liposomes uses overexpressions of fenestrations in cancer neovasculature to increase drug concentration at tumor sites[2]. So far only liposomes and fullerene-based derivatives have been translated into the clinic. Most other nanovectors are still being used in the laboratory, and it may take another five to ten years before these can be translated from the lab to clinical advances[3].

2.3 Types of Drug Delivery Vectors

Before exploring the delivery vectors in gene therapy, we will first review various vectors designed for drug delivery. Numerous drug delivery systems as carriers have been used for drug delivery research, particularly biodegradable ones, including liposomes, microspheres made of the biodegrable poly (lactic-co-glycolic) acid, albumin microspheres, synthetic polymers, protein-DNA complexes, protein conjugates, and erythrocytes [31]. Apart from micron drug delivery vectors, over the past few decades, there has been extensive interest in developing sub-micron drug delivery vectors, such as nanoparticles.

Currently nanoparticles have served as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. There are many reports of the artificial biomineralization of inorganic nanoparticles (NPs) in the hollow cavities of cage-shaped protein supuramolecules. Genetic modification of the outer surfaces of these cages makes it possible to deliver the conjugates, protein shells and NPs to designated positions and align them into higher ordered structures[16]. Nanoparticles have been used in vivo systemically or locally to protect the drug entity in the systemic circulation, restrict access of the drug to the targeted sites and to deliver the drug at a controlled and sustained rate to the targeted cells. Various polymers have been used in the formulation of nanoparticles for drug delivery research [29], particularly biodegradable polymeric nanoparticles, such as liposomes, micelles, nanospheres, nanoparticles and nanocapsules. They have been applied to drug targeting, cancer chemotherapy [6], intracellular antibiotherapy [28], gene therapy [5], ocular and oral drug delivery [4] , oral drug delivery [8, 30], etc.

By using different preparing methods, nanospheres, nanoparticles and nanocapsules can be obtained. Nanocapsules are one of the sub-micron drug delivery vectors in which the drug is confined to a cavity surrounded by a unique polymer membrane; nanospheres on the other hand are matrix vectors in which the drug is physically and uniformly dispersed [29]. Generally, drug-loaded nanocapsules are prepared with methyl methacrylate (MMA) or ethyl cyanoacrylate (ECA). To prepare drug-loaded nanocapsules, ECA is a better monomer compared to MMA because nanocapsules prepared with ECA has a better interface assembly property. Such nanocapsules posses the properties of circulating for a prolonged period time before reaching the site, of protecting drugs from degradation, of sustaining release of the drug during the transportation, and of altering subsequent clearance of the drug[3]. Chitosan-coated Fe3O4, a kind of magnetic nanoparticles as vectors of epirubicin for cancer therapy, can increase the ability of reducing cytotoxicity. Furthermore, nano-inorganic-biomoleculars can serve as drug vectors, biosensors, bioreactors, contrast medium for magnetic drug targeting MRI contrast agent [1, 7, 26].

Many studies have showed that nanoparticles of sub-micron size have a lot of advantages over microparticles as a drug delivery system 24. Generally nanoparticles have relatively higher intracellular uptake compared to microparticles and available to a wider range of biological targets due to their small size and relative mobility. The

properties of nanoparticles as a drug delivery vectors are: (1) drug targeting can be easily controlled passively and actively; (2) drug sustain release can be controlled during the transportation; (3) by choosing optimal matrix constituents, drug release and particle degradation can be modulated. Drug can be easily loaded and merged into the systems without any chemical reaction; (4) by attaching targeting ligands to surface of particles or use of magnetic guidance, site-specific targeting can be achieved; and (5) can be used for various routes of administration including oral, nasal, parenteral, intra-ocular, etc [29]. For example, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly (ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery vectors because of their ability to circulate for a prolonged period time target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes [3, 17, 18, 19].

Nanoparticulate drug vectors include a class of particles made of polymers or lipids that — because of their size and chemical composition — permit systemic and local treatment. Synthetic polymers offer an almost infinite array of chemical composition and structure combinations. Polyesters, including polylactide (PLA) derivatives and polyepsiloncaprolactone (PCL), polyalkylcyanoacrylate (PACA) and corresponding copolymers with polyethylene glycol (PEG), polysaccharides and polyethylenimine, are a few of the polymers with the requirements that make them useful as nanoparticulate drug carriers[9].

Depending on the materials of preparation, nanoparticles have been prepared most frequently by three methods: (1) dispersion of preformed polymers; (2) polymerization of monomers; and (3) ionic gelation or coacervation of hydrophilic polymers [29]. The applications of nanoparticulate delivery systems include: for tumor targeting; for oral delivery of peptides and proteins; for targeting of nanoparticles to epithelial cells in the GI tract using ligands; for gene delivery; and for drug delivery into the brain, etc.

In spite of these advantages, nanoparticles have some limitations, such as difficult physical handling in liquid and dry forms, limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available.

After reviewing various drug delivery vectors, in the following, we will introduce some realistic studies. Currently the most common one for cancer therapy is high molecular ploymer matrix drug system. For instance, enteric-coated tablets can be utilized for oral administration, while biodegrable polymer such as polylactide can be used for injection or subcutaneous plantation. (Bechgard and Cadefoged 1981); Milovanoric and Norin 1986; Jeyanth and Panduranga 1987; Gupta & Johnson and Allexon 1993). M. D. Blanco in Spain utilized PHEMA [Poly (2-hydroxyethy 1 methacrylate and Poly (acrylamide-co-monomethy litaconate) to control the speed of drug release [25,30]. Y. E. Fang in China exploited Chitosan/Gelatin Hybrid Menbrane as drug vector of 5-Fluorouracil (5-FU) for the therapy of colon cancer so as to control the speed of drug release and enhance the efficiency of restraining cancer[33]. Meeltem Gokce in Turkey successfully designed a drug vector which could control drug release more precisely by using PHEMA to deliver 5-FU with the help of Monolithic Device and Reservior-like Device [33]. Fuh in Taiwan designed another drug delivery system by using polylatide and poly (D, L-lactide-co-golycolide) as a polymeric matrix so as to control the release of anticancer drugs[10].

Unlike organic drug vectors, in 1980 Camazano & Sanchez and Vicenteu utilized montmorillonite (MMT), one of clay minerals, as drug carrier in which drugs are intercalated into layers to control the drug release under different sites. In 2002 Jian & Lin and Lee utilized pillared-montmorillonite to develop a new carrier for sulfasalazine (SAZ) for the sustained release purpose. Such a complex of SAZ-pillared-MMT, will exhibit a high durability as adduct, because its SAZ may be released slowly from pillared-Mont molecular interlayer [15].

Earlier in 1976 Kremkau, et al, combining the characteristics of anticancer drugs and ultrasound, developed an ultrasound drug delivery system. The properties of ultrasound drug vector are: (i) able to overcome biological barriers more efficiently; (ii) help the drug infiltrate the membrane more easily; and (iii) be able to change the chemical properties of drugs to achieve special treatment purpose. In 1991 Tachibana and Tachibana successfully used ultrasound as a drug delivery vector of high molecular weight insulin. Gaber, et al., 1996, Huang,

1994 confirmed that thermotherapy can increase the dynamics and porousness of vessels so as to intensify the absorption and effect of liposomes in cancer chemotherapy. In 1995 Tachibana and Tachibana verified that by combining albumins and ultrasound, the effect of thrombolytic agents can be enhanced [11, 13, 27].

2.4 Vectors for Gene Therapy

In recent years, gene therapy is opening up a possibility for treating some incurable diseases. Broadly defining, gene therapy is the insertion of genes into an individual's cells and tissues to treat a disease, such as a hereditary disease in which a deleterious mutant allele is replaced with a functional one. Although the technology is still in its beginning, it has been used with some success [31] and has the potential to change medical science dramatically in the future.

Gene therapy covers a broad range of applications, from gene replacement and knockdown for genetic or acquired diseases such as cancer, to vaccination, each with different requirements for gene delivery. In most gene therapy, a "correct copy" or "wild type" gene is provided or inserted into the genome; therefore a carrier called a vector must be used to deliver the therapeutic gene to the patient's target cells. Therefore in gene therapy, an optimal gene delivery vector plays a very important role. Currently, the most common vectors in gene therapy involve three types: (i) viral methods, such as viruses, retroviruses, adenoviruses, adeno-associated viruses. These viral vectors show great promise for the development of "magic bullet" gene therapy. ; (ii) non-viral methods, such as naked DNA, oligonucleotides, and lipoplexes and polyplexes, and (iii) hybrid methods, such as virosomes and dendrimers[31]. Both viral vectors and synthetic liposomes have limitations and risks, including complexity of production, limited packaging capacity, and unfavorable immunological features.

Viruses are the most common type of vectors used in gene therapy, involving a way of encapsulating and delivering their genes to human cells in a pathogenic manner. This ability is harnessed to remove disease-causing genes and insert therapeutic ones. Retroviruses are a type of virus used as a vector in gene therapy. It was the first vectors to be used for such a purpose. These vectors show great promise for the development of "magic bullet" gene therapy [31].

Non-viral methods, on the other hand, with simple large scale production and low host immunogenicity being just two, show certain advantages over viral methods and have good therapeutic potentials. To improve the delivery of the new DNA into the cell, the DNA must be protected from damage and its entry into the cell must be facilitated. To this purpose, new moleculars, lipoplexes and polyplexes, have been created that have the ability of transfection efficiencies similar to those of viruses and of protecting the DNA from undesirable degradation during the transfection process [31].

Due to every method of gene delivery having drawbacks, some hybrid methods have been developed that combine two or more techniques, such as dendrimers that can be specially constructed to carry a DNA or RNA payload that transfects cells at a high efficiency with little or no toxicity [31].

Conventional means of gene delivery using viral vectors because of the high efficiency of gene transfer, however, has undesirable side effects such as insertion of mutational viral gene into the host genome and development of replication competent viruses. On the other hand, among non-viral gene delivery methods, polymeric nanoparticles are increasingly becoming popular as vectors of choice. The major limitation of these nanoparticles is poor transfection efficiency at the target site after systemic administration due to uptake by the cells of reticuloendothelial system (RES) [18].

In a study of hepatitis B virus in humans-- hepatitis B virus envelope L particles form hollow nanoparticles displaying a peptide that is indispensable for liver-specific infection by hepatitis B virus in humans-- the researchers demonstrate the use of L particles for the efficient and specific transfer of a gene or drug into human hepatocytes both in culture and in a mouse xenograft model. The yeast-derived L particle is free of viral genomes, highly specific to human liver cells and able to accommodate drugs as well as genes. These advantages should facilitate targeted delivery of genes and drugs to the human liver [23].

In a 2007's nonviral study, researchers describe experiments to elucidate the structure of a ternary, targeted, lipopolyplex synthetic vector, the LID complex. Fluorophore-labeled lipid, peptide, and

DNA components were used to formulate the vector, and the stoichiometry of the particles was established by fluorescence correlation spectroscopy (FCS). The results showed that the cationic portion of the peptide, I, interacts with the plasmid DNA, resulting in a tightly condensed DNA-peptide inner core; this is surrounded by a disordered lipid layer, from which the integrin-targeting sequence of the peptide partially protrudes [21].

In a 2009's study, the researchers review the prospects for nonviral biological delivery vehicles as gene therapy vectors with focus on their unique evolved biological properties and respective limitations and potential applications. The potential of these nonviral biological entities to act as clinical gene therapy delivery vehicles has already been shown in clinical trials using bacteria-mediated gene transfer, and with sufficient development, these entities will complement the established delivery techniques for gene therapy applications [32].

3 Case Exploration

In Nov 2000 a team of nanobiotechnologists led by Professor C. Montemagno from Cornell University reported a breakthrough for vector technology in which they successfully built an inorganic nanodevice powered with a biomolecular motor which are said to herald a new generation of ultrasmall, robotic, medical devices.

This device consists of three primary elements: (i) engineered, nanofabricated substrate of neckle (Ni) post; (ii) recombinant F1-ATPase biomolecular motors specially engineered to selectively interface with nanofabricated structures; and (iii) engineered nanopropellers. (Ricky K. Soong, et al., http//www.sciencemag.org, 2009) This technology opens the door to hybrid nanodevices. The properties of this device include: (1) serve as "nanonurses" that move about the body, ministering to its needs, or as "smart pharmacies" that detect chemical signals from body cells, calculate the dose and precisely dispense drugs.; (2) fueled by adenosine triphosphate (ATP, the so-called energy of cellular life), spinning nickel propellers, and made of ATPase enzyme; and (3) can be assembled, maintained and repaired using the physiology of life[14]. This device is still in its infancy; therefore, Cornell nanobiotechnologists are learning to clean away caustic chemicals left over from the nanofabrication processes with inorganic materials so that delicate living molecules are not hindered, and would like to engineer the device to run on light energy, with photons instead of ATP.

In 2007, Gold nanoparticles are first being investigated as carriers for drugs such as Paclitaxel In the research, Jacob D. Gibson, Bishnu P. Khanal, and Eugene R. Zubarev* describe the first example of 2 nm gold nanoparticles (Au NPs) covalently functionalized with a chemotherapeutic drug, paclitaxel. The synthetic strategy involves the attachment of a flexible hexaethylene glycol linker at the C-7 position of paclitaxel followed by coupling of the resulting linear analogue to phenol-terminated gold nanocrystals. The research demonstrates that organic molecules with exceedingly complex structures can be covalently attached to gold nanocrystals in a controlled manner and fully characterized by traditional analytical techniques. Besides, this method offers a new alternative for the design of nanosized drug-delivery systems [15].

Gold nanoparticle (Au NP) is again utilized in a research in 2008 July. Yu Cheng and Clemens Burda, researchers at Case Western Reserve University, have developed a technique that has the potential to deliver cancer-fighting drugs to diseased areas within hours, as opposed to the two days it currently takes for existing delivery systems. The system uses gold nanoparticle (Au NP) vectors to deliver photodynamic therapy (PDT) drugs through the bloodstream to cancerous sites since gold is chemically inert with a versatile surface chemistry, large surface-to-volume ratio and variable size and shape, and nontoxic to the human body. The diameter of an Au NP is only 5 nm and the addition of PEG ligands expands the total diameter to 32 nm, larger than some other nanoparticles currently in use, but still small enough to pass unencumbered through the bloodstream. Looking like a "Hair Ball", each Au NP is coated with polyethylene glycol (PEG) ligands. The advantages of PEG molecules over other materials are: they are soluble in fats and water, don't interact with proteins in the bloodstream and help protect the drug, keeping it safe and stable until delivery to the cancer site.

When tested on the mice, the drug is uploading into the diseased area within minutes. When tested on human cells called HeLa — a line of laboratory-grown human cells used in testing — most of the drug is uploaded within one hour. However, testing on human beings may not begin for

some time. The Researchers are being engaged in minimizing the amount of material and drug load needed for effective interaction with cancer cells; optimizing potential targeting systems on the PEG ligands for faster, even more specific placement in diseased areas; and increasing the overall effectiveness of nanoparticle enhanced therapy[34].

4. Findings and Discussion

From the foregoing literature review, we can find out that depending on the materials or the methods of preparation, and the mechanisms or the surface size of the particles, the drug delivery vectors can categorized into virus or non-virus, organic or inorganic, micron or sub-micron, particles or nanoparticles, biodegradable or non-biodegradable, polymers or non-polymers, including bacteria, virus, liposomes, microspheres made of the biodegrable poly(lactic-co-glycolic) acid, albumin microspheres, synthetic polymers, protein-DNA complexes, protein conjugates, and erythrocytes. They, in other sense, can be divided into four types: (1) organic high molecular, (2) inorganic mineral clay montmorillonite; (3) ultrasound, and (4) nanoparticulates. Among them, nanoparticulates are the most potential one. Particle size and size distribution are the most important characteristics of nanoparticle delivery vectors. They influence the in vivo distribution, biological fate, toxicity and the targeting ability of nanovectors. In addition, they can also influence the drug loading, drug release and stability of nanoparticles.

Comparing the nanoparticle-based vectors with the traditional ones, we found out that nanoparticle-based vectors are capable of ferrying large doses of chemotherapeutic agents, therapeutic genes, or detection agents which are known as reporters that can signal if a drug is having a therapeutic effect., into malignant cells while sparing healthy cells, greatly minimizing drug degradation and inactivation upon administration, reducing or eliminating the undesirable side effects that accompany many traditional therapies, and increasing bioavailability[29]. This review also shows that nanoparticulate technology can prove to be very useful in various therapies allowing for effective and targeted drug delivery by overcoming the many biological, biophysical and biomedical barriers that the body stages against a standard intervention.

Currently, there are lots of deadly diseases which are still incurable. To break through in the fighting against incurable diseases, potential future research projects should look at providing a time-release administration of the drug rather than a more all-at-once release, including minimizing the amount of material and drug load needed for effective interaction with cancer cells; optimizing potential targeting systems, even more specific placement in diseased areas; and increasing the overall effectiveness of nanovectors enhanced therapy.

Although a variety of drug delivery systems have been designed with their own advantages and limitations, the common goal is to optimize drug delivery vectors to increase the bioavailability of the drugs towards targeted diseased cells via specific recognition mechanisms, promoting the required response and overcoming biological barriers while minimizing side-effects. The review in this article shows that nanovectors have great potentials though they do have limitations, such as difficult physical handling in liquid and dry forms, limited drug loading and burst release. These practical problems have to be overcome before nanoparticle drug carriers can be used clinically or made commercially available. To make drug delivery system more optimal, greater understanding of the different mechanisms of biological interactions, and nanoparticle engineering, is still required. Further improvements are needed in order to turn the concept of nanoparticle technology into a realistic practical application.

5. Conclusion

Nanovectors, an integration of physical nanotechnology, molecular engineering, biology, chemistry and biotechnology, though still in its infancy and though there is some concern about the safety of nanovectors introduced in the human body and released into the environment, are highly anticipated, offer various possibilities for treating incurable diseases, and holds considerable promise of advances in pharmaceuticals and healthcare.

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