

THE INTERNALIZATION AND FATE OF HPMA  
COPOLYMERS AND ANTISENSE-HPMA  
COPOLYMER CONJUGATES  
IN HEP G2 CELLS

by

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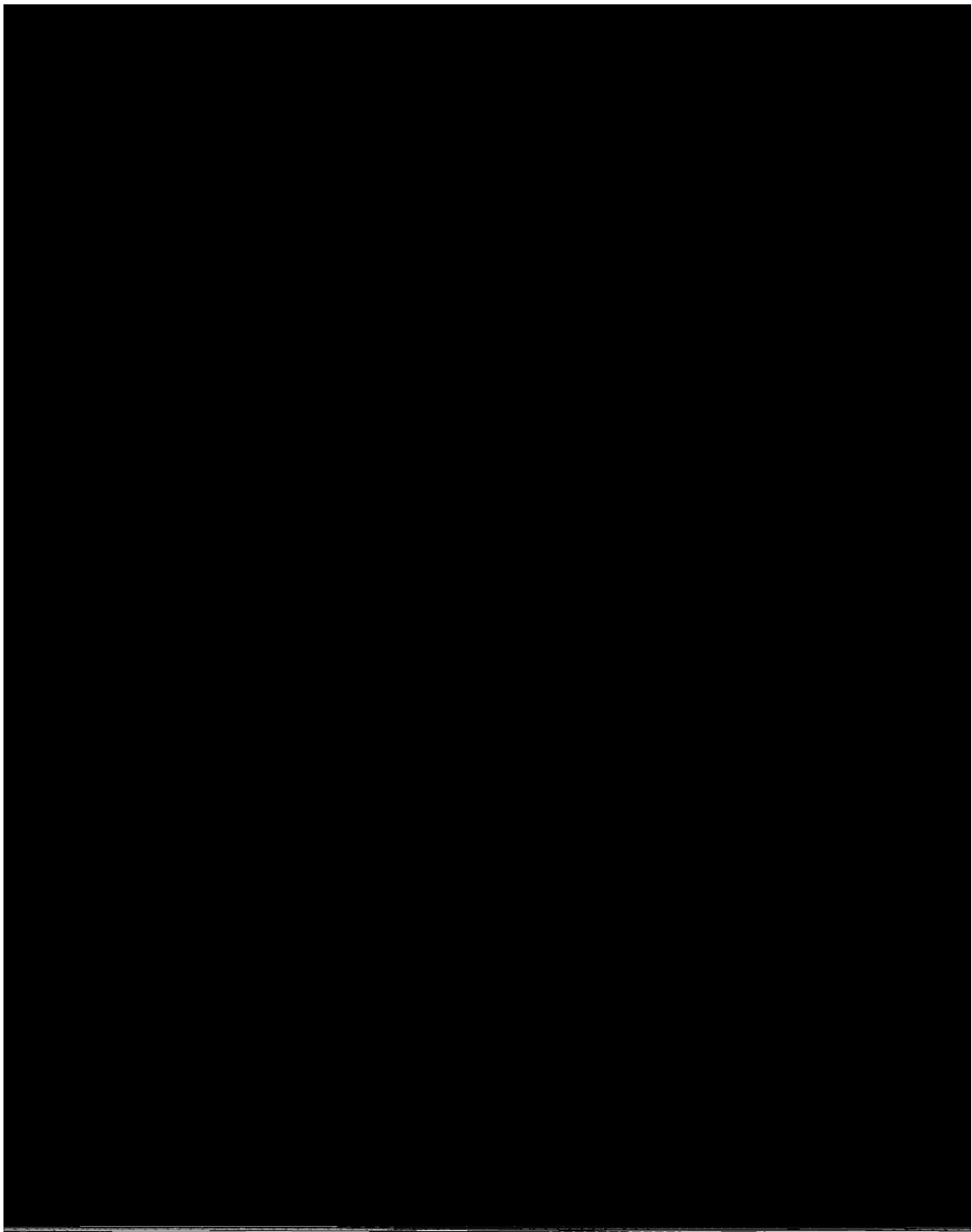


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## ABSTRACT

To better understand the fate of macromolecules in cells and begin to alter that fate, we studied an antisense oligonucleotide designed to inhibit the hepatitis B virus. Copolymers of N-(2-hydroxypropyl)methacrylamide (HPMA) were initially used as a model compound and later they were used as a delivery carrier for the oligonucleotides. The subcellular fate of fluorescently labeled polymers was monitored by confocal microscopy and fluorescent spectrophotometry in Hep G2 cells (hepatocellular carcinoma).

Semiquantitative fluorometry confirmed that the targeting moiety galactose was an effective ligand for receptor-mediated endocytosis for Hep G2 cells. The rate of internalization of a galactose-targeted copolymer was almost two orders of magnitude greater than that of the nontargeted copolymer. Confocal microscopy of both fixed and live cells revealed that the polymer entered the cells by endocytosis. After longer incubation times (typically >8 hours), polymer escaped from small vesicles and was distributed throughout the cytoplasm and nuclei of the cells. Polymer that entered the cytoplasm (after incubation or microinjection) accumulated in the nucleus. To examine the effect of the fluorescent dye on the intracellular fate, polymers with fluorescein, Oregon Green 488, Lissamine rhodamine B, and doxorubicin were tested; no significant differences were observed.

To better understand the subcellular fate of oligonucleotides and HPMA copolymer-oligonucleotide conjugates, we studied their internalization and subcellular trafficking. A fraction of the free oligonucleotides were internalized, escaped into the cytoplasm and nucleus of Hep G2 cells, but were not active antiviral agents. Covalently attaching the oligonucleotides to the HPMA copolymers via non-degradable GG spacers resulted in sequestering the oligonucleotide in vesicles after internalization. Conjugation of the oligonucleotides to an HPMA copolymer via a degradable GFLG spacer resulted in the release of the oligonucleotide in the lysosome and subsequent translocation into the cytoplasm and nucleus of the cells. The HPMA copolymer-oligonucleotide conjugate possessed antiviral activity, indicating that phosphorothioate oligonucleotides released from the carrier in the lysosome were able to escape into the cytoplasm and nucleus and remain active. The Hep G2 cells appeared to actively internalize the phosphorothioate oligonucleotides as oligonucleotide-HPMA copolymer conjugates were internalized to a greater extent than unconjugated polymers.

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## LIST OF ABBREVIATIONS

AIBN:	2,2'-azo-bis-isobutyronitrile
DCC:	N,N'-dicyclohexylcarbodiimide
DCU:	dicyclohexyl urea
DMF:	dimethylformamide
DMSO:	dimethyl sulfoxide
DOX:	doxorubicin
DPBS:	Dulbecco's phosphate buffered saline
EPR:	enhanced permeability and retention effect
FBS:	fetal bovine serum
F:	phenylalanine
FITC:	fluorescein isothiocyanate
Fl:	fluorescein
G, Gly:	glycine
GI:	gastrointestinal
GalN:	N-acyl galactosamine
HCl:	hydrochloric acid
Hep G2:	human hepatocellular carcinoma cell line designation
HEPES:	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC:	high pressure liquid chromatography

HPMA:	N-(2-hydroxypropyl)methacrylamide
iv:	Intravenous
$K_d$ :	dissociation constant
$K_i$ :	constant of inhibition
$KHSO_4$ :	potassium hydrogen sulfate
L:	leucine
LG-OMe:	leucylglycyl methyl ester
LR:	Lissamine rhodamine B
MA:	methacryloyl
MA-GF:	N-methacryloylglycylphenylalanine
MA-GFLG-OMe:	N-methacryloylglycylphenylalanylleucylglycyl methyl ester
MA-GFLG-ONp:	N-methacryloylglycylphenylalanylleucylglycyl p-nitrophenyl ester
MA-GG:	methacryloylglycylglycine
MA-GG-ONp:	N-methacryloylglycylglycine p-nitrophenyl ester
MA-GG-GalN:	N-methacryloylglycylglycyl galactosamine
MEM- $\alpha$ :	minimum essential media-alpha modification
MeOH:	methanol
$M_n$ :	number average molecular weight
$M_w$ :	weight average molecular weight
$NaHCO_3$ :	sodium bicarbonate
NaOH:	sodium hydroxide
OG:	Oregon Green 488

ONp:	p-nitrophenoxy
PEG:	polyethylene glycol
PNA:	peptide nucleic acids
PVP:	polyvinyl-pyrrolidone
SEC:	size exclusion chromatography
TEA:	triethylamine
TGN:	trans-Golgi network
THF:	tetrahydrofuran
TLC:	thin-layer chromatography
VEGF:	vascular endothelial growth factor

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