THE UNIVERSITY OF MANITOBA

A STUDY OF THE METABOLISM OF A NOVEL DINUCLEOSIDE POLYPHOSPHATE (HS3) FOUND IN MAMMALIAN CELLS: POSSIBLE REGULATION OF NUCLEIC ACID BIOSYNTHESIS BY HS3 DURING STEP-DOWN GROWTH CONDITIONS

BY

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To Siew See and my parents

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ABSTRACT

A novel dinucleoside polyphosphate (HS3) prevously found in various fungi has now been detected in numerous cultured mammalian cell lines. Physical and chemical analyses of HS3 (McNaughton et. al. 1978) show that the molecule consists of a glutamyl-ADP-sugar moiety which is covalently linked to a UDP-mannitol-tetraphosphate.

Physiological studies demonstrated that as long as de novo purine biosynthesis was blocked (by nutrient deprivation, drugs or mutation) and no exogenous purines (and some pyrimidines) were supplied, HS3 accumulation took place. For example, the addition of de novo purine biosynthesis inhibitors [azaserine (50 μg/ml) or MTX (1 μM)] resulted in HS3 synthesis as did the withdrawal of glutamine (2 mM). The addition of hypoxanthine, inosine, adenine, adenosine, guanosine, uridine and cytidine, but not thymidine, (all at 0.3 mM) strongly suppressed HS3 accumulation in glutamine-starved CHO cells. CHO purine salvage mutants ($HGPRT^-$) failed to do likewise when supplied with high concentrations of hypoxanthine, guanosine and inosine (0.3-2.0 mM). A CHO mutant (GAT^--auxotrophic for adenosine, thymidine and glycine) with blocked de novo purine biosynthesis, accumulated HS3 only when adenosine (0.1 mM), but not glutamine, was withdrawn from its growth medium. Thus

HS3 accumulation may be related to a lack of either precursors for purine nucleotide biosynthesis or of purine nucleotides themselves.

The rate of HS3 synthesis in CHO WT cells increased 5-6 fold shortly after glutamine withdrawal and was maintained at that level for at least 6 hr before declining slowly back to the control rate by 22 hr. The pool sizes of HS3 increased and decreased in concert with rate changes. The replenishment of glutamine and adenosine to starved CHO WT and CHO GAT$^-$ cells respectively, resulted in an immediate depletion of the accumulated HS3.

An inverse relationship was observed between nucleic acid (DNA and RNA) synthesis and HS3 accumulation. When DNA and RNA synthesis decreased, HS3 synthesis increased and vice versa. No such correlation was apparent for protein synthesis.

The inhibition of protein synthesis by either puromycin (100 µg/ml) or cycloheximide (10 µg/ml) or that of RNA synthesis by actinomycin D (1 µg/ml), stimulated HS3 depletion in glutamine-starved CHO WT cells.

Both fungal and mammalian HS3 were found to be equally potent inhibitors of in vitro and in vivo

mammalian RNA synthesis. Also, studies by Lewis et. al. (1977) showed that HS3 strongly inhibited partially purified ribonucleotide reductase from CHO cells. Thus the decrease in DNA synthesis during periods of rapid HS3 accumualtion may be the indirect result of a deficiency in deoxyribonucleotides. The data suggest that HS3 may be involved in the regulation of nucleic acid biosynthesis in mammalian cells.

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ABBREVIATIONS

ADA	adenosine deaminase
ADP	adenosine 5'-diphosphate
α-MEM	alpha-minimal essential medium
AMP	adenosine 5'-monophosphate
$APRT^-$	adenine phosphoribosyltransferase deficient
ATP	adenosine 5'-triphosphate
cAMP	cyclic 3',5'-adenosine monophosphate
cGMP	cyclic 3',5'-guanosine monophosphate
CHO	Chinese hamster ovary
cm	centimetre
cpm(CPM)	counts per minute
dATP	deoxyadenosine 5'-triphosphate
dCDP	deoxycytidine 5'-diphosphate
DFCS	dialysed fetal calf serum
dGTP	deoxyguanosine 5'-triphosphate
FCS	fetal calf serum
5-FdUMP	5-fluorodeoxyuridine 5'-monophosphate
fig.	figure
5-FU	5-fluorouracil
GDP	guanosine 5'-diphosphate
gln	glutamine

GMP	guanosine 5'-monophosphate
GTP	guanosine 5'-triphosphate
HCl	hydrochloric acid
$HGPRT^-$	hypoxanthine/guanine phosphoribosyltransferase deficient
hr	hour
IMP	inosine 5'-monophosphate
M	molar
min	minute(s)
ml	millilitre
mM	millimolar
m mol	millimole
MTX	methotrexate
MW	molecular weight
OMP	orotidine 5'-monophosphate
PEI	polyethyleneimine
Pi	inorganic phosphate
ppGpp	guanosine 3'-diphosphate, 5'-diphosphate (MS I)
pppGpp	guanosine 3'-triphosphate, 5'-diphosphate (MS II)
PRA	phosphoribosylamine
PRPP	5 —phosphoribosyl-1-pyrophosphate
TCA	trichloroacetic acid
TdR	thymidine

TEAB	triethylammonium bicarbonate
Tris	tris (hydroxymethyl) amino methane
uCi	microcurie
UDP	uridine 5'-diphosphate
ug	microgram
UMP	uridine 5'-monophosphate
UTP	uridine 5'-triphosphate
UV	ultraviolet
WT	'wild type'
(+)	supplemented
(-)	not supplemented

INTRODUCTION

It is apparent now that complex biochemical regulatory functions, at the enzyme level, have been evolved in living organisms. Yet it is evidently desirable that the diverse cellular activities should be coordinated in some fashion so as to ensure balanced growth during periods of rapid nutrient changes in the organism's growth environment. Since the isolation and characterization of various small yet unique nucleotides from both procaryotes and eucaryotes, there has been an increasing interest in attempting to understand the functions of these molecules in the regulation of growth.

The data on the role(s) of cyclic adenosine monophosphate and bacterial guanosine nucleotides (ppGpp and pppGpp) in procaryotic growth regulation is somewhat convincing. The involvement of ppGpp and pppGpp in regulating transcription during the bacterial 'stringent response' is clear. To date, however, no definitive data have been presented to demonstrate the existence of ppGpp and pppGpp in cultured mammalian cells. Cyclic adenosine monophosphate and cyclic guanosine monophosphate have been implicated in regulating eucaryotic growth. Other