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شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

BIOCHEMICAL AND GENETIC STUDIES ON TRYPANOSOMES

THESIS

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ABSTRACT

BIOCHEMICAL AND GENETIC STUDIES ON TRYPANOSOMES

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African trypanosomes are pathogenic kinetoplastid protozoa. They cause a fatal disease in humans called *Sleeping Sickness*. African trypanosomes are infamous for their ability to evade the immune responses by periodically switching their variant surface glycoprotein, a phenomenon called antigenic variation. In trypanosomes, controlled protein degradation by proteasome plays an important role in regulating the cellular development.

In this study an *in vitro* translation system from bloodstream forms *T. brucei* was used to study trypanosomes-specific post-translational modifications of VGS. The results revealed that the trypanosome lysate system could not synthesize VSG117 after addition of either VSG117-mRNA or VSG117-total RNA and had high rate of endogenous translation. The role of proteasomes in *T. brucei* was also studied by the use of transgenic trypanosomes expressing protein "PPαF-GPI221". Western blotting analysis proved that "PPαF-GPI221" was glycosylated and had no GPI anchor. Immunofluorescence and immunoelectron microscopy revealed that "PPαF-GPI221" was localized in the cytoplasm and could not be expressed at the transgenic trypanosome surface. Western blotting analysis showed "PPαF-GPI221" to be a short-lived protein and its degradation was mediated by

the proteasome because it was sensitive to the proteasome inhibitors LLnV and lactacystin as well as NEM. Whereas LLnV and lactacystin had incomplete inhibitory effect, NEM completely inhibited the degradation of "PPαF-GPI221". Also, in this study, it was proved that transgenic trypanosomes could be cultivated axenically in modified MEM medium for long-term by daily replacements of the modified MEM medium.

Keywords:

T. brucei – *in vitro* translation system – GPI anchor – glycosylation – proteasome – proteasomal inhibitors.

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CONTENTS

ABBREVIATIONS

ATP	Adenosine triphosphate
BBB	Blood brain barrier
BF	Bloodstream forms
BHC	Benzene hexachloride
CATT	Card agglutination test for trypanosomiasis
cDNA	Complementary DNA
CIATT	Card indirect agglutination test for trypanosomiasis
CRD	Cross reacting determinant
CSF	Cerebrospinal fluid
Cys	Cysteine
DDT	Chlorophenothane
DEAE	Diethylaminoethyl
DEPC	Diethyl pyrocarbonate
DFMO	γ-Difluromethylornithine
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleoside triphosphate
dT	Deoxythymidine
E-64	Trans-epoxy succinyl-L-leucylamido-(4 guanidino) butane
EDTA	Ethylene diamine tetraacetate
EEF	Exoerythrocytic forms
EGTA	Ethylene glycol-bis(2-aminoethylether)-N,N` tetraacetate
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
ERAD	Endoplasmic reticulum associated degradation
ES	Expression site
ESAG	Expression site-associated gene
FAZ	Flagellar attachment zone
FP	Flagellar pocket
FITC	Fluorescein isothiocyanate
G	Applied centrifugal field

ABBREVIATIONS (continue)

G1	First 'resting' stage of interphase
G2	Second 'resting' stage of interphase
GERL	Golgi endoplasmic reticulum lysosome
GAM	Goat anti-mouse
GPI	Glycosylphosphatidylinositol
GPI-PLC	Glycosylphosphatidylinositol- phospholipase C
Ha	Hectare
HA	Haemagglutination tag-epitope
HCT	Haematocrit centrifuge technique
HDL	High density lipoprotein
IFA	Indirect fluorescent antibody test
IFN	Interferon
IL	Interleuken
Kb	Kilobase
kBq	Kilobecquerels
kDa	Kilodalton
LDL	Low density lipoprotein
LLnV	N-Carbobenzoxy-L-Leucyl-L-Leucyl-L-Norvalinal
M	Mitosis
mAECT	Miniature anion exchange centrifugation technique
MEM	Minimum essential medium
mfVSG	membrane form VSG
MITat	Molteno Institute Trypanozoon antigenic type
ml	Milliliter
mm	Millimeter
mRNA	Messenger RNA
MVAT	Metacyclic variant antigen type
µg	Microgram
µl	Microliter
µm	Micrometer

ABBREVIATIONS (continue)

NEM	N-ethylmaleinimide
nm	Nanometer
NP-40	Nonidet
PCR	Polymerase chain reaction
PFR	Paraflagellar rod
PLC	Phospholipase C
PMSF	Phenylmethylsulfonyl fluoride
PPαF	Prepro-α-Factor
QBC	Quantitative buffy coat
RER	Rough endoplasmic reticulum
RLO	Rickettesia-like organisms
RNA	Ribonucleic Acid
rNTP	Ribonucleoside triphosphate
rRNA	Ribosomal RNA
S	DNA replication
SDS	Sodium dodecyle sulphate
SDS-PAGE	SDS Polyacrylamide Gel Electrophoresis
SIF	Stumpy induction factor
SRA	Serum resistance associated gene
sVSG	Soluble form VSG
Thr	Threonine
Tht	Trypanosome hexose transporter
TLCK	Tosyl-lysine chloromethylketone
TLF	Trypanosome lytic factor
TNF	Tumor necrosis factor
tRNA	Transfer RNA
VAT	Variant antigen type
VSG	Variant surface glycoprotein
VSG ES	VSG Expression Site
°C	Degree(s) Celsius
%	Percent

LIST OF FIGURES