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Hypoxia-Inducible Factor-1α mRNA Contains An Internal Ribosome Entry Site That Allows Efficient Translation During Normoxia And Hypoxia

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Summary

HIF-1α is the regulated subunit of the HIF-1 transcription factor, which induces transcription of a number of genes involved in the cellular response to hypoxia. The HIF-1α protein is rapidly degraded in cells supplied with adequate oxygen, but is stabilised in hypoxic cells. Using polysome profile analysis, I found that translation of HIF-1α mRNA in NIH3T3 cells is spared the general reduction in translation rate that occurs during hypoxia. To assess whether the 5'UTR of the HIF-1α mRNA contains an internal ribosome entry site (IRES), I constructed a dicistronic reporter with the HIF-1α 5'UTR inserted between the two reporter coding regions. I found the HIF-1α 5'UTR promoted translation of the downstream reporter, indicating the presence of an IRES. IRES activity was not affected by hypoxic conditions that caused a reduction in cap-dependent translation, and IRES activity was less affected by serum-starvation than was cap-dependent translation. This indicates that the presence of the IRES in the HIF-1α 5'UTR is to allow translation to be maintained under conditions that are inhibitory to cap-dependent translation.

Several reports have implicated the 5'UTR to be important for HIF-1α protein synthesis upon activation of the phosphatidylinositol 3-kinase (PI3K) signal transduction pathway. I hypothesised that this specific induction of HIF-1α protein synthesis is mediated by the HIF-1α IRES. I found HIF-1α IRES activity was increased in response to activation of the PI3K signaling pathway, with a concomitant increase in cap-dependent translation. Results also indicate that activation of the IRES is independent of Akt (a downstream target of PI3K), implicating other signaling pathways in the regulation of HIF-1α IRES function.

I also demonstrate that GC rich 5'UTRs containing an IRES may stimulate mRNA destabilisation. This mode of regulation was found in three IRESs tested (vascular endothelial growth factor, HIF-1α and c-myc), but is not a mechanism of all cellular IRESs, as the X-linked inhibitor of apoptosis 5'UTR could not destabilise a reporter

mRNA. Data presented in this thesis indicates that the HIF-1α 5'UTR is subject to multiple modes of regulation, including translation by an IRES, and this is regulated by signal transduction pathways, and mRNA instability.

Statement of Originality

This thesis contains no material that has been accepted for the award of any degree or diploma by any other university. To the best of my knowledge it contains no material that has previously been published by any other person, except where due reference has been made in the text. I consent to this thesis, when deposited in the university library, being available for photocopying and loan.

Kenneth Lang

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Publications arising from work presented in this thesis

Lang, K.J.D., Goodall, G.J. (2001) Translation of hypoxia-inducible factor-1α is mediated by an internal ribosome entry site *13th Lorne Cancer Conference.*

Lang, K.J.D., Kappel, A., Goodall, G.J. (2002) Hypoxia inducible factor-1α mRNA contains an internal ribosome entry site that allows efficient translation during hypoxia *23rd Lorne Genome Conference on the Organisation and Expression of the Genome.*

Lang, K.J.D., Kappel, A., Goodall, G.J. (2002) Hypoxia inducible factor-1α mRNA contains an internal ribosome entry site that allows efficient translation during normoxia and hypoxia *Mol Biol Cell* **13**: 1792-1801.

Lang, K.J.D., Goodall, G.J. (2002) HIF-1α mRNA contains an IRES that allows efficient translation during normoxia and hypoxia *Australian Society for Medical Research*

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ABBREVIATIONS

All abbreviations used throughout this thesis are in accordance with those described in the *Journal of Biological Chemistry*; additional and alternate abbreviations are shown below.

APS	ammonium persulphate
Amp	ampicillin
ARE	AU-rich element
ATP	adenosine triphosphate
bp	base pairs
BSA	bovine serum albumin
CHX	cycloheximide
CIP	calf intestinal phosphatase
DEPC	diethyl pyrocarbonate
DMEM	Dulbecco's modified Eagle's Medium
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
DTT	dithiothreitol
EDTA	ethylenediaminetetra-acetic acid
eIF	eukaryotic initiation factor
FCS	foetal calf serum
FF	firefly
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid
HIF	hypoxia-inducible factor
IRES	Internal ribosome entry site
kDa	kilodalton
mA	milliamps
MEN	Mops EDTA sodium acetate
Met	methionine
mins	minutes
mRNA	messenger RNA
NP-40	Nonident ® P 40
nt	nucleotides
OD	optical density
PAGE	polyacrylamide gel electrophoresis

PBS	phosphate buffered saline
PCR	polymerase chain reaction
PPT	polypyrimidine tract
RL	renilla
RNA	ribonucleic acid
RNase A	ribonuclease A
rpm	revolutions per minute
rRNA	ribosomal RNA
SeAP	Secreted alkaline phosphatase
SDS	sodium dodecyl sulphate
secs	seconds
SEM	standard error of the mean
SSC	Saline sodium citrate
TAE	tris-acetic acid EDTA
TBE	tris-boric acid EDTA
TE	tris-EDTA
TEMED	N,N,N',N'-tetramethyl-ethene-diamine
tRNA	transfer RNA
UTR	untranslated region
UV	ultra-violet
VEGF	vascular endothelial growth factor
VHL	von-Hippel Lindau protein
XIAP	X-linked inhibitor of apoptosis