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# The unsolved mystery of human ADP-dependent glucokinase

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## **Abstract**

Human ADP-dependent glucokinase (hADP-GK) is the most recently discovered glycolytic enzyme that has been shown experimentally to phosphorylate glucose to glucose-6-phosphate. This reaction is catalysed using ADP as the phosphoryl donor, which is uniquely different from the traditional ATP-dependent hexokinase type I-IV enzymes that were initially shown to carry out the first step of glycolysis.

The functional role of hADP-GK within the cell and the significance of utilizing an ADP-dependent glucokinase have yet to be elucidated experimentally. It has been hypothesised that the unique characteristic of ADP utilisation may provide cellular advantages during times of limited energy and oxygen (hypoxia), as ATP is able to be conserved during anaerobic glycolysis. Cellular survival and sustainability may be increased during disease states as ADP is invested into the first step of glycolysis instead of ATP. AMP is also produced during this reaction, and may activate AMP-activated protein kinase (AMPK), an energy sensor within the cell, which may regulate energy metabolism during times of low energy and/or hypoxia.

Prior to this research no experimental work had been carried out on the regulation of hADP-GK at the transcriptional level. The main objectives of this project were to investigate the effect of glucose concentration on the expression of hADP-GK at the transcriptional level, and to investigate the promoter and potential transcription factors responsible for promoter activity. The cellular localisation of hADP-GK was also briefly investigated through microscopy. This experimental work has opened up a new path for future research, and therefore the continuation of this project would be important in understanding the role of hADP-GK.

## Abbreviations

<b>ADP-GK</b>	ADP-dependent glucokinase
<b>Amp</b>	Ampicillin
<b>AMP</b>	Adenosine monophosphate
<b>APS</b>	Ammonium persulfate
<b>ATP</b>	Adenosine triphosphate
<b>bp</b>	Base pairs
<b>BSA</b>	Bovine serum albumin
<b>cDNA</b>	Complimentary DNA
<b>CDTA</b>	1,2-disminocyclohexane-N,N,N',N-tetraacetic acid
<b>ChREBP</b>	Carbohydrate response element-binding protein
<b>CRE</b>	cAMP response element
<b>CREB</b>	cAMP response element binding protein
<b>cpm</b>	counts per minute
<b>Cq/Ct values</b>	PCR crossing points
<b>CV</b>	Coefficient of variance
<b>DAPI</b>	4',6-diamidino-2-phenylindole
<b>DEPC</b>	Diethylpyrocarbonate
<b>DMSO</b>	Dimethyl sulfoxide
<b>DNA</b>	Deoxyribose nucleic acid
<b>dNTP</b>	Deoxynucleoside triphosphate (dATP, dCTP, dGTP, dTTP)
<b>DTT</b>	Dithiothreitol
<b>E</b>	Amplification efficiency
<b><i>E. coli</i></b>	<i>Escherichia coli</i>
<b>EDTA</b>	Ethylene diamine tetra-acetic acid
<b>EMSA</b>	Electrophoretic Mobility Shift Assay

<b>ER</b>	Endoplasmic reticulum
<b>FCS</b>	Fetal calf serum
<b>FITC</b>	Fluorescein-5-isothiocyanate
<b>GAPDH</b>	Glyceraldehyde 3-phosphate dehydrogenase
<b>gDNA</b>	Genomic DNA
<b>GSB</b>	Gel shift buffer
<b>GR</b>	Glucocorticoid receptor
<b>hADP-GK</b>	Human ADP-dependent glucokinase
<b>HCl</b>	Hydrochloric acid
<b>HeLa</b>	Human cervical cancer cell line
<b>HEPES</b>	N-[2-hydroxyethyl]piperazine-N'-[2-ethane sulfonic acid]
<b>HRP</b>	Horse radish peroxidase
<b>IgG</b>	Immunoglobulin G
<b>IPTG</b>	Isopropyl $\beta$ -D-thiogalactoside
<b>KCl</b>	Potassium chloride
<b>kDa</b>	Kilodaltons
<b>LB</b>	Luria Bertani bacteriological media
<b>mADP-GK</b>	Mouse ADP-dependent glucokinase
<b>mM</b>	Milli Molar
<b><math>\mu</math>L</b>	Micro Litre
<b><math>\mu</math>g</b>	Micro Gram
<b>mRNA</b>	Messenger RNA
<b><i>mt</i></b>	Mutant
<b>Mw</b>	Molecular weight
<b>NaCl</b>	Sodium chloride
<b>NADH</b>	Nicotinamide adenine dinucleotide
<b>ng</b>	Nano Gram

<b>NS</b>	Non-specific
<b>NTC</b>	Non-template control
<b>ONPG</b>	o-nitrophenyl 1- $\beta$ -D- galactopyranoside
<b>PAGE</b>	Polyacrylamide gel electrophoresis
<b>PBS</b>	Phosphate buffered saline
<b>PCR</b>	Polymerase chain reaction
<b>PDI</b>	Protein Disulfide Isomerase
<b>PM</b>	Plasma membrane
<b>RNA</b>	Ribonucleic acid
<b>RNase</b>	Ribonuclease
<b>rpm</b>	revolutions per minute
<b>RT-qPCR</b>	Reverse transcription-quantification polymerase chain reaction
<b>SDS</b>	Sodium dodecyl sulfate
<b>SDS-PAGE</b>	SDS-polyacrylamide gel electrophoresis
<b>Sp1</b>	Specificity factor 1
<b>TAE</b>	Tris acetate EDTA buffer
<b>TBE</b>	Tris borate EDTA
<b>TBST</b>	Tris-buffered saline-Tween 20
<b>TE</b>	Tris-EDTA buffer
<b>TEMED</b>	N,N,N',N'-Tetramethylethylenediamin
<b>Tm</b>	Melting temperature
<b>Tris</b>	Tris (hydroxymethyl)-aminomethane
<b>TRITC</b>	Tetramethyl Rhodamine Isothiocyanate
<b>TSP</b>	Transcription start point
<b>UV</b>	Ultra violet
<b>Wt</b>	Wildtype
<b>X-gal</b>	5-bromo-4-chloro-3-indoyl- $\beta$ -D-galactosidase

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