Gene 650 (2018) 1–6

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Double silencing of relevant genes suggests the existence of the direct link between DNA replication/repair and central carbon metabolism in human fibroblasts

Aneta Wieczorek[1], Karolina Fornalewicz[1], Łukasz Mocarski, Robert Łyżeń, Grzegorz Węgrzyn*

Department of Molecular Biology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland

ARTICLE INFO

Keywords:
DNA replication
DNA repair
Central carbon metabolism
Gene silencing
Cell cycle

ABSTRACT

Genetic evidence for a link between DNA replication and glycolysis has been demonstrated a decade ago in *Bacillus subtilis*, where temperature-sensitive mutations in genes coding for replication proteins could be suppressed by mutations in genes of glycolytic enzymes. Then, a strong influence of dysfunctions of particular enzymes from the central carbon metabolism (CCM) on DNA replication and repair in *Escherichia coli* was reported. Therefore, we asked if such a link occurs only in bacteria or it is a more general phenomenon. Here, we demonstrate that effects of silencing (provoked by siRNA) of expression of genes coding for proteins involved in DNA replication and repair (primase, DNA polymerase ι, ligase IV, and topoisomerase IIIβ) on these processes (less efficient entry into the S phase of the cell cycle and decreased level of DNA synthesis) could be suppressed by silencing of specific genes of enzymes from CMM. Silencing of other pairs of replication/repair and CMM genes resulted in enhancement of the negative effects of lower expression levels of replication/repair genes. We suggest that these results may be proposed as a genetic evidence for the link between DNA replication/repair and CMM in human cells, indicating that it is a common biological phenomenon, occurring from bacteria to humans.

1. Introduction

DNA replication and repair are basic processes occurring in every cellular organism. To ensure the organism survival, these processes must be strictly regulated. Although it appears obvious that duplication of the genetic material and DNA repair should depend on the metabolic status of the cell, until recently, it was assumed that DNA synthesis is only indirectly connected to cellular metabolism, mainly in the sense of availability of energy and precursors for macromolecules' production (for a review, see (Barańska et al., 2013)). Only a decade ago, a genetic evidence for the direct link between DNA replication and glycolysis has been demonstrated experimentally for the first time (Jannière et al., 2007). In a bacterium *Bacillus subtilis*, effects of thermosensitive mutations in genes coding for major replication enzymes, the lagging strand DNA polymerase, the primase and the helicase, could be fully suppressed by mutations in specific genes coding for glycolytic enzymes. Therefore, it appeared that the central carbon metabolism (CCM) is directly involved in the control of DNA replication (Jannière et al., 2007). Subsequent studies indicated that a similar link exists in *Escherichia coli*, where analogous suppressions could be found, however,

Abbreviations: **P**, primase (product of the *PRIM* gene); **D**, DNA polymerase ι (product of the *POLI* gene); **L**, ligase IV (product of the *LIG4* gene); **T**, topoisomerase (DNA) IIIβ (product of the *TOP3B* gene); ACO, aconitase; ALDO, fructose-bisphosphate aldolase; CS, citrate synthase; DERA, deoxyribose-phosphate aldolase; ENO, enolase; FH, fumarase; G6PD, glucose-6-phosphate dehydrogenase; GAPDH, glyceraldehyde phosphate dehydrogenase; GPI, glucose-6-phosphate isomerase; H6PD, hexose-6-phosphate dehydrogenase; HK, hexokinase; IDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; OGDH, α-ketoglutarate dehydrogenase; PC, pyruvate carboxylase; PDH, puryvate dehydrogenase; PFK, phosphofructokinase; PGAM, phosphoglycerate mutase; PGD, 6-phosphogluconate dehydrogenase; PGI, phosphoglucose isomerase; PGLS, phosphogluconolactonase; PGK, phosphoglycerate kinase; PGM, phosphoglucomutase; PKM, pyruvate kinase; PRPS, ribose-phosphate pyrophosphokinase; RBKS, ribokinase; RPE, ribulose-5-phosphate epimerase; RPI, ribulose-5-phosphate isomerase; SUCL, succinyl-CoA synthetase; SDH, succinate dehydrogenase; TALDO, transaldolase; TKT, transketolase; TPI, triosephosphate isomerase

Abbreviations of metabolite names: 1,3BPG, 1,3-bisphosphoglycerate; 2d-D-Rib, 2deoxy-D-ribose; 2d-D-Rib-1P, 2deoxy-D-ribose 1-phosphate; 2d-D-Rib-5P, 2deoxy-D-ribose 5-phosphate; 2PG, 2-phosphoglycerate; 3-P-G, 3-phosphoglycerate; 6PG, 6-phosphogluconate; 6PGL, 6-phosphogluconlactone; AC-CoA, acetyl-CoA; CIT, citrate; DHAP, dihydroxyacetone phosphate; D-Rib, D-ribose; D-Rib-1P, D-ribose 1-phosphate; E4P, erythrose 4-phosphate; F1,6BP, fructose 1,6-bisphosphate; F6P, fructose 6-phosphate; FUM, fumarate; G3P, glyceraldehyde 3-phosphate; G6P, glucose 6-phophate; GADP, glyceraldehyde 3-phosphate; ICT, isocitrate; MAL, malate; OXA, oxaloacetate; PEP, phosphoenolpyruvate; PRPP, phosphoribosyl-pyrophosphate; Pyr, pyruvate; R5P, ribose 5-phosphate; Ru5P, ribulose 5-phosphate; S7P, sedoheptulose 7-phosphate; SUC, succinate; SUC-CoA, α-ketoglutarate; Xu5P, xylulose 5-phosphate

* Corresponding author.
E-mail addresses: aneta.szczepanska@biol.ug.edu.pl (A. Wieczorek), karolina.sawiuk@biol.ug.edu.pl (K. Fornalewicz), robert.lyzen@biol.ug.edu.pl (R. Łyżeń), grzegorz.wegrzyn@biol.ug.edu.pl (G. Węgrzyn).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.gene.2018.01.068
Received 21 October 2017; Received in revised form 17 January 2018; Accepted 19 January 2018
Available online 31 January 2018

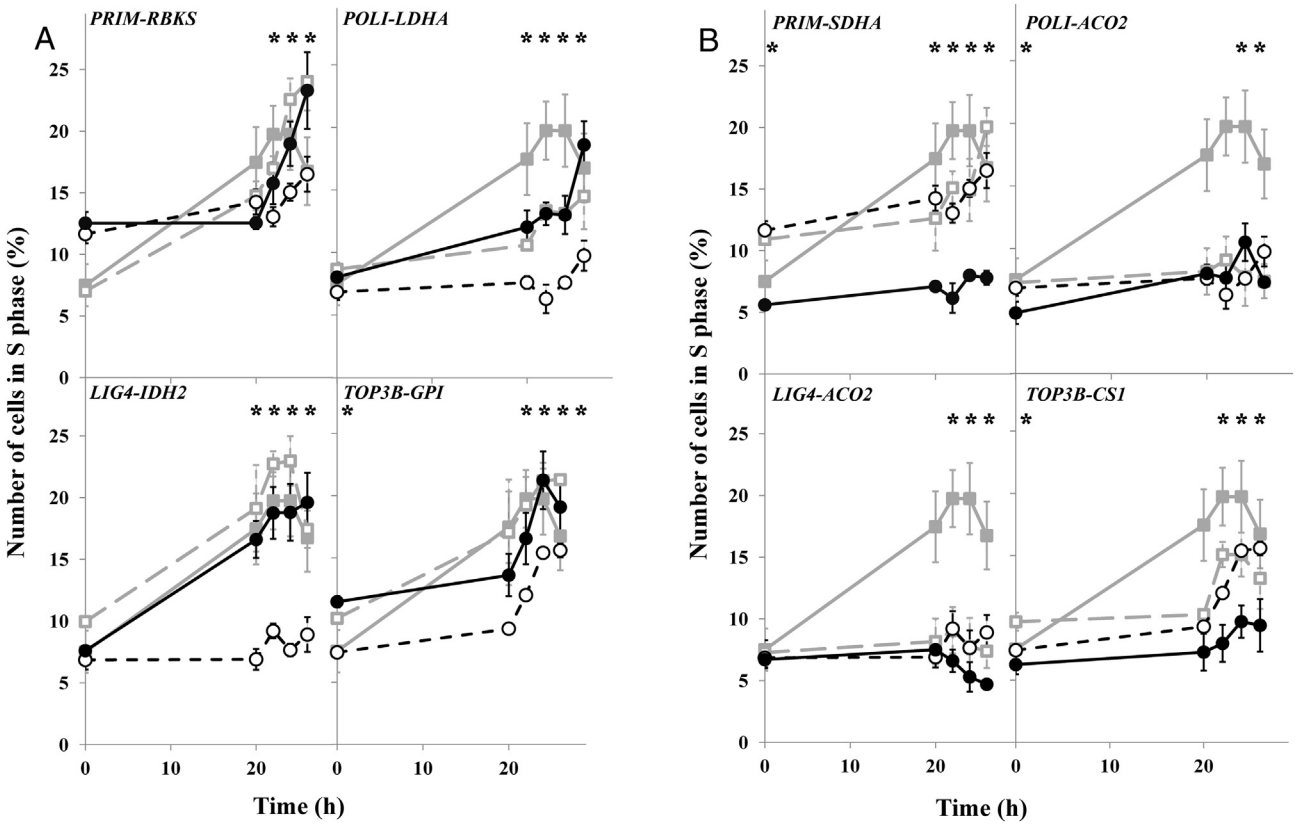

Fig. 1. Effects of simultaneous siRNA-mediated silencing of one of replication/repair genes and one of CCM genes on the enterance of human fibroblasts into the S phase of the cell cycle; examples of suppression of effects of silencing of a replication/repair gene by impaired expression of a CCM gene (A), and either no significant changes or enhancement of such effects by impaired expression of a CMM gene (B). Cells were seeded on Petri dishes, transfected with siRNAs specific for indicated genes and synchronized. After cell cycle releasing, the cells were collected every 2 h, starting from 14 h, and analyzed by flow cytometry. Following experimental systems are presented in each panel: no gene silencing due to absence of specific siRNA (open grey squares), silencing of only CCM gene (closed grey squares), silencing of only replication/repair gene (open black circles), silencing of both replication/repair and CCM genes (closed black circles). Presented results are mean values from at least four independent experiments, with error bars indicating SD. Statistically significant differences between results of experiments with silencing of only replication/repair gene and with silencing of both genes are indicated by asterisks.

some other genes coding for replication and CCM proteins were involved, including the initiator DnaA protein and enzymes from gluconeogenesis, pentose phosphate pathway and overflow pathway (Maciąg et al., 2011). This suppression included defects in nucleoid position and cell division of the replication mutants (Maciąg-Dorszyńska et al., 2012). Interestingly, in *E. coli*, mutations in genes coding for CCM enzymes influenced significantly not only control of DNA replication, but also fidelity of this process, affecting DNA repair (Maciąg et al., 2012). Recent study suggested that signals connecting CCM and DNA replication might consist of metabolites that accumulate in the cells when particular metabolic pathways are impaired due to specific mutations; an example of pyruvate accumulation that was able to suppress effects of the *dnaA46* mutation has been investigated in detail (Tymecka-Mulik et al., 2017). General summary on the regulation of DNA replication in bacteria and its connection to CMM has been presented recently (Glinkowska et al., 2015).

Since the link between DNA replication/repair and CCM has been discovered in bacteria, the question appeared whether this kind of regulation occurs also in eukaryotic organisms, including humans. On the basis of analyses of previously published reports, it was hypothesized that some CCM enzymes might be linkers between transcription and DNA replication (Konieczna et al., 2015a). Moreover, certain metabolic perturbations might directly influence DNA replication control and fidelity, thus facilitating carcinogenesis (Konieczna et al., 2015a). To test experimentally if CCM may directly influence DNA synthesis (crucial for both DNA replication and repair) in normal human cells, effects of partial silencing of genes coding for enzymes from glycolysis and tricarboxylic acid cycle (Krebs cycle) (Konieczna et al., 2015b), as well as from pentose phosphate pathway (Fornalewicz et al., 2017), on the entry of human fibroblasts to the S phase were tested. It was found that impaired expression of some genes coding for CCM enzymes either delayed entry of cells into the S phase of the cell cycle or decreased number of cells in this phase. This concerned some genes which encode enzymes involved in glycolysis (*HK2*, *PFKM*, *TPI*, *GAPDH*, *ENO1*, *LDHA*), as well as certain genes responsible for production of enzymes from the Krebs cycle (tricarboxylic acid cycle) (*CS1*, *ACO2*, *SUCLG2*, *SDHA*, *FH*, *MDH2*) (Konieczna et al., 2015b). Recent studies indicated that this is also true for particular genes coding for enzymes from the pentose phosphate pathway, namely *H6PD*, *PRPS1*, and *RPE* (Fornalewicz et al., 2017). These results suggested the influence of CCM on DNA replication/repair, but an experimental proof for a direct link between these processes was lacking. Thus, in this work, we aimed to test if effects of partial silencing of expression of genes coding for DNA replication and repair proteins can be suppressed by down regulation of expression of CCM genes. We assumed that positive answer to such a question would provide genetic evidence for the direct link between DNA replication/repair and CCM in normal human cells, while negative answer would reject the hypothesis.

2. Materials and methods

Methods employed in this work were described previously in detail (Konieczna et al., 2015b). Thus, they are presented only briefly in subsequent subsections (although they were also described in such a short form in our recent article (Fornalewicz et al., 2017), we provide this material here for clarity of the paper).