Genetic response to metabolic fluctuations: correlation between central carbon metabolism and DNA replication in *Escherichia coli*

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**Abstract**

**Background:** Until now, the direct link between central carbon metabolism and DNA replication has been demonstrated only in *Bacillus subtilis*. Therefore, we asked if this is a specific phenomenon, characteristic for this bacterium and perhaps for its close relatives, or a more general biological rule.

**Results:** We found that temperature-sensitivity of mutants in particular genes coding for replication proteins could be suppressed by deletions of certain genes coding for enzymes of the central carbon metabolism. Namely, the effects of *dnaA46*(ts) mutation could be suppressed by dysfunction of *pta* or *ackA*, effects of *dnaB*(ts) by dysfunction of *pgi* or *pta*, effects of *dnaE486*(ts) by dysfunction of *tktB*, effects of *dnaG*(ts) by dysfunction of *gpmA*, *pta* or *ackA*, and effects of *dnaN159*(ts) by dysfunction of *pta* or *ackA*. The observed suppression effects were not caused by a decrease in bacterial growth rate.

**Conclusions:** The genetic correlation exists between central carbon metabolism and DNA replication in the model Gram-negative bacterium, *E. coli*. This link exists at the steps of initiation and elongation of DNA replication, indicating the important global correlation between metabolic status of the cell and the events leading to cell reproduction.

**Background**

When considering a bacterial cell as a microbial factory, producing various macromolecules either natural or formed due to introduction of recombinant genes, several biochemical processes must be taken into consideration. Among them, there are two basic processes ensuring that more specialized reactions (like transcription of particular genes and translation of particular mRNAs on ribosomes as well as enzyme-mediated production of various compounds) can occur. These two processes are central carbon metabolism (for a review see ref. [1]) and DNA replication (for a review see ref. [2]). The former one provides energy from nutrients, which is absolutely necessary to all life functions of cells. The latter one, although consuming cellular energy, ensures integrity of genetic material and its inheritance by daughter cells after each cell division, providing the source of information about biological structures and functions of macromolecules.

The central carbon metabolism (CCM) is generally recognized as a set of biochemical pathways devoted to transport and oxidation of main carbon sources in the cell [1]. In a model Gram-negative bacterium, *Escherichia coli*, it consists of the phosphotransferase system, glycolysis, gluconeogenesis, pentose-monophosphate bypass with Entner-Dudoroff pathway, Krebs cycle with glyoxylate bypass and the respiration chain [3]. Biochemical reactions of these pathways ensure the optimal energy production and usage in the cell at particular growth conditions, in order to keep homeostasis.

DNA replication is a process of genetic information duplication, which is necessary to equal and precise distribution of the genetic material to both daughter cells after each cell division [2]. The process of replicative DNA synthesis requires large cellular machinery, which in *E. coli* consists of DNA polymerase III holoenzyme (containing at least 10 subunits) and other essential proteins, including DnaB helicase and DnaG primase.

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Additional proteins (DnaA, DnaC) are required for DNA replication initiation at a specific genome region, called oriC [2,4]. Although it was observed previously that regulation of DNA replication may depend on bacterial cell metabolism, it was generally assumed that this dependency is indirect. For example, it might result from different availability of cellular energy and/or precursors of macromolecules [5,6] or from production of specific alarmons, like cyclic AMP (cAMP) [7,8] or guanosine tetraphosphate (ppGpp) [9-12], in response to nutritional deprivations. However, it was reported recently that DNA replication may be directly linked to central carbon metabolism, particularly glycolysis, in a model Gram-positive bacterium, Bacillus subtilis [13]. Namely, specific suppression of conditionally-lethal (temperature-sensitive, ts) mutations in genes coding for replication proteins (DnaE, a DNA polymerase involved in lagging strand synthesis, DnaC, a helicase - homologue of *E. coli* DnaB protein, and DnaG, the primase) by dysfunction of certain genes coding for enzymes involved in glycolysis, was observed. An indirect suppression mechanism (e.g. by slowing down of bacterial growth rate) was excluded, strongly suggesting a real link between glycolysis and DNA replication. Thus, the existence of such a link should be considered in any studies on both these processes, as well as when constructing and using biotechnological systems for efficient production of desired compounds.

Until now, the direct link between central carbon metabolism and DNA replication has been demonstrated only in *B. subtilis* [13]. Therefore, we asked if this is a specific phenomenon, characteristic for this bacterium and perhaps for its close relatives, or a more general biological rule. Since *E. coli* is both a model Gram-negative bacterium and a widely used host for production of recombinant proteins, in our studies, which were performed to answer the above question, we employed strains of this species.

**Methods**

**Bacterial strains, plasmids and bacteriophages**

*E. coli* strains used in this work are listed in Table 1. Plasmids and bacteriophages are described in Table 2. New bacterial strains and plasmids were constructed according to standard procedures of P1 transduction and molecular cloning, respectively [14].

**Oligonucleotides**

Oligonucleotides are described in Table 3.

**Growth conditions**

Luria-Bertani (LB) medium, and minimal media M9 and MM, were used [14]. Solid media contained 1.5% of bacteriological agar. For liquid cultures, bacteria were grown in various media in shake flasks, with aeration (by shaking). Overnight cultures were diluted in LB and grown to OD<sub>600</sub> = 0.3. Then, 100 μl of the culture or its dilution was plated on solid media. The plates were then incubated at indicated temperatures for indicated time. CFU (colony forming units) were calculated from plates where colony number was between 100 and 1000.

**Results**

We have employed six *E. coli* temperature-sensitive mutants in following genes coding for proteins necessary for chromosomal DNA replication: dnaA (coding for the replication initiator protein that binds to the oriC region and forms a specific nucleoprotein structure; this is the first step in the DNA replication initiation), dnaB (coding for the main DNA helicase, the enzyme necessary to melt DNA during the replication process), dnaC (coding for the protein which delivers DnaB helicase to the DnaA protein bound to oriC), dnaE (coding for the α subunit of DNA polymerase III, the catalytic subunit of this enzyme), dnaG (coding for primase, an enzyme necessary to synthesize RNA primers during DNA replication) and dnaN (coding for the β subunit of DNA polymerase III, a protein forming the sliding clamp and allowing DNA polymerase III to be kept on the template DNA strand when synthesizing new polynucleotide strand) [for more detailed information on these genes and their products, see ref. 2]. These mutants are described in Table 1.

To test whether mutations (particularly deletion-insertion mutations) in genes coding for enzymes from central carbon metabolism (CCM) may suppress temperature sensitivity of the replication mutants, we have determined the sensitivity profiles of all tested conditionally lethal mutants. This was necessary to chose temperatures that severely restricted growth of mutant cells, however, which still allowed observing some viability of tested strains; otherwise detection of any suppression would be impossible, as observed in the *B. subtilis* study [13]. The profiles of temperature-sensitivity of dnaA, dnaB, dnaC, dnaE, dnaG and dnaN mutants in LB medium are shown in Figure 1.

A series of double mutants, bearing mutations in one of the replication genes and in one of genes coding for CCM enzyme, has been constructed by P1 transduction (Table 1). For these constructions, deletion-insertion mutants in following genes were employed: gapC, pykF, tpiA, pgi, fbaB, gpmA, pck, zwf, tkbB, pta, ackA, aceB, acnB, and icd. Enzymes encoded by these genes are listed in Table 4, and locations (in particular biochemical pathways) of reactions catalyzed by them are marked on the scheme depicting the central carbon metabolism in *E. coli* (Figure 2).
### Table 1  *E. coli* strains used in this work

| Strain                  | Relevant characteristics                                                                 | Reference or source |
|-------------------------|------------------------------------------------------------------------------------------|---------------------|
| JCB809 (PC8)            | dnaB8(ts) Cm<sup>F</sup> F<sup>2</sup> leuB6 thyA47 deoC3 rps153 l2                      | [21]                |
| PC2                     | dnaC(ts) thy leu rpsL                                                                    | [21]                |
| PC3                     | dnaG(ts) Hfr leu thy rpsL                                                                 | [22]                |
| MG1655                  | F<sup>-</sup> ilvG::rpsE rpsE::rpsL                                                     | [23]                |
| MG1655ΔdnaA46           | F<sup>-</sup> ilvG::rpsE rpsE::rpsL                                                     | [24]                |
| DH5α                    | F<sup>-</sup> g80lacZMD15 Δ(lacZYA-argF)U169 deoR recA1 endA1 hisR17(rK, mK+) phoA supE44 | [25]                |
| BW25113                 | ΔaroA-ara8)567, ΔlacZ4787:mmB-3, λ<sup>-</sup>, rph-1, Δ(araD-ara8)567, hisD514          | [26]                |
| JW1122                  | Same as BW25113 but Δcdc::kan                                                            | [27]                |
| JW1413                  | Same as BW25113 but ΔgacP::kan                                                           | [27]                |
| JW1666                  | Same as BW25113 but ΔgacP::kan                                                           | [27]                |
| JW1841                  | Same as BW25113 but ΔgacB::kan                                                           | [27]                |
| JW2449                  | Same as BW25113 but ΔgacB::kan                                                           | [27]                |
| JW3666                  | Same as BW25113 but ΔgacC::kan                                                           | [27]                |
| JW3890                  | Same as BW25113 but ΔgacA::kan                                                           | [27]                |
| JW3974                  | Same as BW25113 but ΔgacB::kan                                                           | [27]                |
| JW3985                  | Same as BW25113 but ΔgacA::kan                                                           | [27]                |
| JW2294                  | Same as BW25113 but ΔgacA::kan                                                           | [27]                |
| JW2293                  | Same as BW25113 but ΔgacA::kan                                                           | [27]                |
| JW5173                  | Same as BW25113 but ΔgacC::kan                                                           | [27]                |
| JW5344                  | Same as BW25113 but ΔgacB::kan                                                           | [27]                |
| JW0738                  | Same as BW25113 but ΔgacB::kan                                                           | [27]                |
| NR13339                 | Same as KA796 with dnaN159(Ts) zid501::Tn10                                               | [28]                |
| NR7651                  | Same as MC4100 lacZ104 ΔdnaA46::kan                                                     | [28]                |
| AS701                   | MG1655 ΔdnaA46 Δacr::kan                                                                 | This study, by P1 transduction from JW0114 |
| AS702                   | MG1655 ΔdnaA46 Δcdc::kan                                                                 | This study, by P1 transduction from JW1122 |
| AS703                   | MG1655 ΔdnaA46 ΔgacP::kan                                                                 | This study, by P1 transduction from JW1413 |
| AS704                   | MG1655 ΔdnaA46 ΔgacP::kan                                                                 | This study, by P1 transduction from JW1666 |
| AS705                   | MG1655 ΔdnaA46 ΔgacP::kan                                                                 | This study, by P1 transduction from JW1841 |
| AS706                   | MG1655 ΔdnaA46 ΔgacB::kan                                                                  | This study, by P1 transduction from JW2449 |
| AS707                   | MG1655 ΔdnaA46 ΔgacC::kan                                                                  | This study, by P1 transduction from JW3366 |
| AS708                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW3890 |
| AS709                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW3974 |
| AS710                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW3985 |
| AS711                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW2294 |
| AS712                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW2293 |
| AS713                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW5173 |
| AS714                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW5344 |
| AS715                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW3890 |
| AS716                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW5173 |
| AS717                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW5344 |
| AS718                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW3890 |
| AS719                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW3890 |
| AS720                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW3890 |
| AS721                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW3890 |
| AS722                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW3890 |
| AS723                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW3890 |
| AS724                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW3890 |
| AS725                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW3890 |
| AS726                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW3890 |
| AS727                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW3890 |
| AS728                   | MG1655 ΔdnaA46 ΔgacA::kan                                                                  | This study, by P1 transduction from JW3890 |
Table 1 *E. coli* strains used in this work (Continued)

| Strain   | Mutation | Source                          |
|----------|----------|---------------------------------|
| AS779    | MG1655 dnaA88 ΔacDC::kan        | This study, by P1 transduction from JW5173 |
| AS780    | MG1655 dnaA88 ΔdcbB::kan         | This study, by P1 transduction from JW5344 |
| AS781    | MG1655 dnaA88 ΔgmnA::kan         | This study, by P1 transduction from JW0738 |
| AS750    | PC2 dnaC(ts) Δacn::kan           | This study, by P1 transduction from JW0114 |
| AS751    | PC2 dnaC(ts) Δcd::kan            | This study, by P1 transduction from JW1122 |
| AS752    | PC2 dnaC(ts) ΔgapC::kan          | This study, by P1 transduction from JW1413 |
| AS753    | PC2 dnaC(ts) ΔpykF::kan          | This study, by P1 transduction from JW1666 |
| AS754    | PC2 dnaC(ts) Δwfs::kan           | This study, by P1 transduction from JW1841 |
| AS755    | PC2 dnaC(ts) ΔlkbB::kan          | This study, by P1 transduction from JW2449 |
| AS756    | PC2 dnaC(ts) Δpck::kan           | This study, by P1 transduction from JW3366 |
| AS757    | PC2 dnaC(ts) ΔpsiA::kan          | This study, by P1 transduction from JW3890 |
| AS758    | PC2 dnaC(ts) ΔaceB::kan          | This study, by P1 transduction from JW3974 |
| AS759    | PC2 dnaC(ts) ΔpsiG::kan          | This study, by P1 transduction from JW3985 |
| AS760    | PC2 dnaC(ts) Δpta::kan           | This study, by P1 transduction from JW2294 |
| AS761    | PC2 dnaC(ts) ΔackA::kan          | This study, by P1 transduction from JW2293 |
| AS762    | PC2 dnaC(ts) ΔacDC::kan          | This study, by P1 transduction from JW5173 |
| AS763    | PC2 dnaC(ts) ΔsbaB::kan          | This study, by P1 transduction from JW5344 |
| AS764    | PC2 dnaC(ts) ΔgmnA::kan          | This study, by P1 transduction from JW0738 |
| AS783    | PC3 dnaS(ts) Δacn::kan           | This study, by P1 transduction from JW0114 |
| AS784    | PC3 dnaS(ts) Δcd::kan            | This study, by P1 transduction from JW1122 |
| AS785    | PC3 dnaS(ts) ΔgapC::kan          | This study, by P1 transduction from JW1413 |
| AS786    | PC3 dnaS(ts) ΔpykF::kan          | This study, by P1 transduction from JW1666 |
| AS787    | PC3 dnaS(ts) Δwfs::kan           | This study, by P1 transduction from JW1841 |
| AS788    | PC3 dnaS(ts) ΔlkbB::kan          | This study, by P1 transduction from JW2449 |
| AS789    | PC3 dnaS(ts) Δpck::kan           | This study, by P1 transduction from JW3366 |
| AS790    | PC3 dnaS(ts) ΔpsiA::kan          | This study, by P1 transduction from JW3890 |
| AS791    | PC3 dnaS(ts) ΔaceB::kan          | This study, by P1 transduction from JW3974 |
| AS792    | PC3 dnaS(ts) ΔpsiG::kan          | This study, by P1 transduction from JW3985 |
| AS793    | PC3 dnaS(ts) Δpta::kan           | This study, by P1 transduction from JW2294 |
| AS794    | PC3 dnaS(ts) ΔackA::kan          | This study, by P1 transduction from JW2293 |
| AS795    | PC3 dnaS(ts) ΔacDC::kan          | This study, by P1 transduction from JW7173 |
| AS796    | PC3 dnaS(ts) ΔsbaB::kan          | This study, by P1 transduction from JW5344 |
| AS797    | PC3 dnaS(ts) ΔgmnA::kan          | This study, by P1 transduction from JW0738 |
| AS718    | MG1655 dnaA486 Δacn              | This study, by P1 transduction from JW0114 |
| AS719    | MG1655 dnaA486 Δcd               | This study, by P1 transduction from JW1122 |
| AS720    | MG1655 dnaA486 ΔgapC             | This study, by P1 transduction from JW1413 |
| AS721    | MG1655 dnaA486 ΔpykF             | This study, by P1 transduction from JW1666 |
| AS722    | MG1655 dnaA486 Δwfs              | This study, by P1 transduction from JW1841 |
| AS723    | MG1655 dnaA486 ΔlkbB             | This study, by P1 transduction from JW2449 |
| AS724    | MG1655 dnaA486 Δpck              | This study, by P1 transduction from JW3366 |
| AS725    | MG1655 dnaA486 ΔpsiA             | This study, by P1 transduction from JW3890 |
| AS726    | MG1655 dnaA486 ΔaceB             | This study, by P1 transduction from JW3974 |
| AS728    | MG1655 dnaA486 Δpsi              | This study, by P1 transduction from JW3985 |
| AS729    | MG1655 dnaA486 Δpta              | This study, by P1 transduction from JW2294 |
| AS730    | MG1655 dnaA486 ΔackA             | This study, by P1 transduction from JW2293 |
| AS731    | MG1655 dnaA486 ΔcdC              | This study, by P1 transduction from JW5173 |
| AS732    | MG1655 dnaA486 ΔsbaB             | This study, by P1 transduction from JW5344 |
| AS733    | MG1655 dnaA486 ΔgmnA             | This study, by P1 transduction from JW0738 |
| AS734    | MG1655 dnaN159 ΔacnN8::kan       | This study, by P1 transduction from JW0114 |
| AS735    | MG1655 dnaN159 Δcd::kan          | This study, by P1 transduction from JW1122 |
| AS736    | MG1655 dnaN159 ΔgapC::kan        | This study, by P1 transduction from JW1413 |
| AS737    | MG1655 dnaN159 ΔpykF::kan        | This study, by P1 transduction from JW1666 |
We have tested whether mutations in the CCM genes can suppress temperature sensitivity of bacteria caused by mutations in the replication genes. In this test, bacteria were plated at sublethal temperatures (i.e. temperatures causing a decrease in the efficiency of plating for several orders of magnitude, but still allowing survival of a small fraction of mutant cells), selected on the basis of temperature-sensitivity profiles determined as shown in Figure 1 (in control experiments, the temperature permissive to all strains, 30°C, was used). These following sublethal temperatures were chosen for particular replication mutants: 39°C for dnaA46(ts), 41°C for dnaB8(ts), 35°C for dnaC(ts), 36.5°C for dnaE486(ts), 34°C for dnaG(ts) and 37.5°C for dnaN159(ts). The effects of dnaN159(ts) mutation could be increased by at least one order of magnitude (often considerably more) at sublethal temperatures in the presence of particular mutations in genes coding for enzymes from CCM (Figure 3). The effects of dnaA46(ts) mutation could be suppressed by dysfunction of pta or ackA, effects of dnaB8(ts) by dysfunction of pgI or pta, effects of dnaE486(ts) by dysfunction of tktB, effects of dnaG(ts) by dysfunction of gpmA, pta or ackA, and effects of dnaN159(ts) by dysfunction of pta or ackA. Most of the suppression phenomena were not complete, i.e. the efficiency of survival of the ts mutants in the sublethal temperature was between 1 and 10% of that in the permissive temperature, though still it was 10 to 100 times higher than that of the ts mutant without suppressor mutation at the sublethal temperature (Figure 4).

Table 2 Plasmids employed and constructed in this study

| Plasmid  | Relevant characteristics | Reference |
|----------|--------------------------|-----------|
| pBAD24   | ori pBR322; bla+ P_BAD  | [29]      |
| pAS101   | pBAD24 bearing the ackA gene under of pBAD control | This study, by cloning of a PCR amplified fragment of E. coli MG1655 chromosome, obtained with primers ackaF and ackaR (Table 3), into the SmaI side of pBAD24 |
| pAS102   | pBAD24 bearing the pgI gene under of pBAD control | This study, by cloning of a PCR amplified fragment of E. coli MG1655 chromosome fragment obtained with primers pgIF and pgIR (Table 3), into the SmaI side of pBAD24 |
| pAS103   | pBAD24 bearing the fbaB gene under of pBAD control | This study, by cloning of a PCR amplified fragment of E. coli MG1655 chromosome fragment obtained with primers fbaBF and fbaBR (Table 3), into the KpnI side of pBAD24 |
| pAS104   | pBAD24 bearing the tktB gene under of pBAD control | This study, by cloning of a PCR amplified fragment of E. coli MG1655 chromosome fragment obtained with primers tktBF and tktBR (Table 3), into the KpnI side of pBAD24 |
| pAS105   | pBAD24 bearing the pta gene under of pBAD control | This study, by cloning of a PCR amplified fragment of E. coli MG1655 chromosome fragment obtained with primers ptaF and ptaR (Table 3), into the KpnI side of pBAD24 |
| pAS106   | pBAD24 bearing the gpm gene under of pBAD control | This study, by cloning of a PCR amplified fragment of E. coli MG1655 chromosome fragment obtained with primers gpmAF and gpmAR (Table 3), into the KpnI side of pBAD24 |
| pAS107   | pBAD24 bearing the aceB gene under of pBAD control | This study, by cloning of a PCR amplified fragment of E. coli MG1655 chromosome fragment obtained with primers aceBF and aceBR (Table 3), into the KpnI side of pBAD24 |
This correlates with the previous findings on the \textit{B. subtilis} model [13]. Interestingly, the only exceptions were \textit{dnaA46} suppressors, restoring 100\% growth relative to that under permissive conditions. It is worth noting that \textit{dnaA} mutants of \textit{B. subtilis} were not tested in the previous work, mentioned above [13].

To test whether suppressions depicted in Figure 3 were specific, plasmids bearing wild-type copies of disrupted metabolic genes (Table 2) have been introduced into cells of the double mutants. The wild-type alleles were under control of the pBAD promoter, which could be stimulated by addition of L-arabinose into growth medium. We found that for \textit{dnaA46} (ts), \textit{dnaB8} (ts), \textit{dnaE486} (ts), \textit{dnaG} (ts) and \textit{dnaN159} (ts) hosts, expression of appropriate wild-type allele of CCM gene reversed effects of temperature sensitivity suppression by the corresponding mutant allele (Figure 4). Therefore, we conclude that the suppression effects depicted in Figure 3 are specific for certain mutations.

We asked whether the suppression of temperature sensitivity of mutants in the replication genes by dysfunction of particular genes coding for CCM enzymes could be caused by decreased growth rates of double mutants. This question was substantiated by the fact that DNA replication regulation is known to be dependent on bacterial growth rate [2]. However, we found that although in most cases (excluding the \textit{dnaA46} mutants) at 30\textdegreeC the growth rates of the double mutants revealing suppression of the temperature sensitivity were lower than in wild-type bacteria, a similar or lower decrease in the growth rate was observed also in double mutants which did not suppress the temperature sensitivity (Figure 5). Therefore, we conclude that the observed suppression effects could not be caused simply by a decrease in bacterial growth rate.

We have also tested whether the suppression can be caused by growth of the replication mutants in media containing various carbon sources, which also allow for different growth rates. Therefore, we have plated \textit{dnaA46} (ts), \textit{dnaB8} (ts), \textit{dnaC} (ts), \textit{dnaE486} (ts), \textit{dnaG} (ts) and \textit{dnaN159} (ts) mutants on plates containing a minimal medium supplemented with various carbon sources: glucose, glycerol, maleic acid or sodium acetate. However, in these experiments, we did not observe any improvement in viability of these mutants at the sub-lethal temperatures (data not shown). These results corroborate the results of experiments with growth rate measurement, and support our conclusion that the suppression of temperature sensitivity of the replication mutants cannot be explained by lower growth rates of bacteria.

### Discussion

The approach to understand cellular processes as a network of complex relations becomes more appreciated...
only nowadays. Two major processes responsible for maintenance and reproduction of the cell (i.e. energy metabolism and DNA replication) were studied mostly independently until recently. A direct link between DNA replication and central carbon metabolism (CCM) has been demonstrated solely for one species of Gram-positive bacterium, B. subtilis [13]. This finding was a breakthrough in considering these processes as interrelated. Thus, it was crucial to address the question whether such a phenomenon occurs only in the specific strain or it is more general. Here we present evidence that such a link exists also in E. coli, a model Gram-negative bacterium.

Despite the general similarity, there are important differences between suppression of effects of mutations in replication genes by dysfunction of genes coding for enzymes of CCM in E. coli and B. subtilis. According to previous report [13], in B. subtilis, the temperature-sensitivity suppression was detected for only three genes: dnaE, dnaC (an equivalent of the E. coli dnaB gene, coding for helicase) and dnaG. Temperature-sensitive mutants in these genes could grow at elevated temperatures in the presence of additional mutations in gapA, pgk, pgm, eno or pykA. These five genes code for enzymes acting at the late stages of glycolysis and gluconeogenesis. In E. coli, we were able to observe suppression of effects of temperature-sensitive mutations not only in dnaE, dnaB and dnaG genes (like in B. subtilis), but also in dnaN and - perhaps the most surprisingly - in dnaA. Moreover, growth at sublethal temperatures of these mutants was observed under conditions of a lack of enzymes involved not only in glycolysis and gluconeogenesis (pgi and gpmA), but also in other regimens of CCM, namely the pentose phosphate pathway (tktB gene) and the overflow pathway (pta and ackA genes). This suggests that in E. coli the link between DNA replication and CCM may be broader than in B. subtilis. Alternatively, the observed differences might result from a partial exploration of a complex system (only some replication and metabolic genes were tested due to technical reasons, namely unavailability of viable mutants).

For B. subtilis, the target of the regulation by metabolic-related signals was shown to be mostly the elongation of the DNA replication process, though some suppressed replication mutations affected also replication initiation [13]. In E. coli, the evidence presented here shows the link between CCM and replication elongation (represented by enzymes involved in the replication complex), and initiation. One of indispensable regulators of the latter process in E. coli is DnaA protein [15,4]. Thus, the finding of the suppression of dnaA46(ts) conditionally-lethal phenotype by mutants in genes involved in CCM suggests the presence of as yet unidentified correlation. Moreover, the observed suppression was complete (100% survival at sublethal temperature relative to survival at permissive temperature), contrary to those noted for other mutants in replication genes. Both suppressors of the dnaA46(ts) phenotype map in the overflow pathway of CCM. This and the presence of the suppressors in genes of enzymes from other pathways beside glycolysis, linking energy turnover and DNA replication, thus,
**Figure 2** A scheme for CCM including main pathways - glycolysis/gluconeogenesis, penthaphosphate pathway, citrate cycle, overflow pathway. Mutants tested in this work are indicated by following colours: blue - non suppressor mutants, red - suppressors of replication genes mutants. Metabolites abbreviations: 1,3-BGP, 1,3-biphosphoglycerate; 2PG, 2-phophoglycerate; 3PG, 3-phosphoglycerate; 6PGLN, 6-phosphoglucono-δ-lactone; 6PGNT, 6-phophogluconate; GLC, glucose; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FUM, fumarate; MAL, malate; OXA, oxaloacetate; PBP, fructose-1,6-biphosphate; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde 3-phosphate; PEP, phosphoenolpyruvate; PYR, pyruvate; Ru5P, ribulose-5-phosphate; S7P, sedoheptulose-7-phosphate; E4P, erythrose-4-phosphate; Ac-CoA, acetyl coenzyme A; Ac-P, acetyl phosphate; Ac-AMP, acetyl-AMP; CIT, citrate; ICT, isocitrate; GOX, glyoxylate; α-KG, α-ketoglutarate; SUC-CoA, succinyl-coenzyme A; SUC, succinate; Xu5P, xylulose-5-phosphate.

**Figure 3** Suppression pattern of double mutants in CCM and replication genes. Red - full suppression, yellow - incomplete suppression. Suppressions were observed in sublethal temperatures.
Figure 4: Complementation of suppression phenotypes in double replication/CCM mutants by the overproduction of the metabolic enzymes. The experiments were performed in sublethal temperatures (relevant for each strain). Mutations as indicated above the graphs were employed. Panel A. Bacterial growth measured in CFU. Empty columns - growth in the presence of 0.2% arabinose, shaded columns - growth in the presence of 0.1% glucose. Efficiencies of plating (CFU/ml) of the replication mutants at 30°C are indicated by a dashed line at each graph. Panel B and C. The growth of temperature-sensitive dnaA46-derivatives in permissive and sublethal temperature. B - dnaA46Δpta, C - dnaA46ΔackA. Panels A, B and C. 1 - temperature-sensitive replication mutants, 2 - double mutants in replication and CCM genes, 3 - double mutants in replication and CCM genes complemented with the relevant metabolic gene under the control of arabinose-inducible pBAD promoter.
it may benefit from more metabolic sensors. Similarly to *B. subtilis*, the suppression observed in *E. coli* was not caused by a decrease in the growth rate. Moreover, the increase in the doubling time of replication mutants (by growth on the minimal media containing various carbon sources, including very poor ones, like maleic acid or acetate) did not improve their viability at sublethal temperatures.

The proposed mechanism of the regulation of DNA replication by CCM in *B. subtilis* involves a putative metabolic linker which can cause conformational changes in replication proteins to modulate replisome properties [13]. This hypothesis may be supported by the role of acetyl phosphate which can accumulate in the overflow pathway mutants. Acetyl phosphate has been proposed to function as a global signal that fits into various two-compound systems [16,17]. This may require the second, as yet unknown, protein modulating replication proteins, or the mechanism can rely on autophosphorylation. The role of acetyl phosphate in protein folding and stability has been proposed as well [18]. In this light it is interesting that AckA and Pta reduce the production of double-stranded breaks in DNA [19]. Moreover, DiaA, a DnaA-binding protein, contains a SIS motif that might bind phosphosugars [20]. These facts may provide a start point to further works on understanding the link between CCM and DNA replication.

It is worth noting that since we have used deletion-insertion mutants in genes coding for CCM enzymes, the suppressions of the temperature-sensitivity phenotypes of the replication mutants cannot be explained by direct protein-protein interactions. Indeed, numerous and large-scale interactions between replication proteins and CCM enzymes seemed unlikely, which led us to use a set of deletion mutants in tested genes. On the other hand, the use of such mutants ensured that particular enzymatic functions were absent in mutant cells, which excluded potential problems with putative partial inactivation of CCM enzymes caused by point mutations.

One should also take into consideration a possibility that changes in chemical composition of the cells caused by a lack of particular CCM enzymes might alleviate temperature sensitivity of mutants in genes coding for replication proteins. In fact, we cannot exclude that increased concentrations of some substances that accumulate due to metabolic blocks at certain steps of CCM might stabilize the temperature-sensitive replication proteins and allow them to function at higher temperatures. If so, CCM could have no effects on wild-type replication proteins and the DNA replication process in wild-type cells. However, to accept such a hypothesis it would be necessary to assume that there are at least several compounds (metabolites) able to interact specifically with several different temperature-sensitive variants of the replication proteins, resulting in their stabilization at elevated temperatures. Although still possible, such a scenario seems unlikely, therefore, we prefer the hypothesis that there is a link between CCM and DNA replication in bacterial cells.

**Conclusions**

We show the genetic correlation between central carbon metabolism and DNA replication in the model Gram-negative bacterium, *E. coli*. Therefore, one might suggest

| Replication mutants | Mutation in metabolic gene |
|---------------------|---------------------------|
|                     | None | tpiA | pgI | fbaB | gpmA | tktB | pta | ackA | aceB |
| dnaA46              | 52 ± 7.6 | - | 35 ± 0.0 | - | - | - | 38 ± 7.6 | 37 ± 3.8 | - |
| dnaB8               | 56 ± 5.8 | 46 ± 6.4 | 115 ± 0.0 | - | - | - | 50 ± 0.0 | - | - |
| dnaC(Ts)            | 48 ± 0.7 | - | 42 ± 2.1 | 75 ± 0.0 | 45 ± 8.3 | 65 ± 7.0 | - | - | - |
| dnaE486             | 37 ± 2.8 | - | 24 ± 5.0 | - | - | 42 ± 4.2 | - | - | - |
| dnaG(Ts)            | 43 ± 3.4 | 55 ± 7.6 | - | - | 44 ± 12.8 | - | 53 ± 6.6 | 52 ± 7.1 | 41 ± 5.3 |
| dnaN159             | 43 ± 2.5 | - | - | 73 ± 3.5 | - | - | 46 ± 8.5 | 50 ± 7.0 | - |

**Figure 5** Generation times of double mutants in replication and CCM genes. Bacteria were grown at 30°C in LB and doubling time (values presented in the boxes ± SD) was assessed in the exponential growth phase. The doubling time for the wild-type strain (MG1655) was 48 ± 0.7 min. The colors represent genotypes in which suppressions were observed at sublethal temperatures (red - full suppression, yellow - incomplete suppression). Dash - the generation time was not determined.
that the existence of such a link is a general phenomenon rather than an event occurring very specifically in a small group of organisms. This link exists at the steps of initiation and elongation of DNA replication, indicating the important global correlation between metabolic status of the cell and the events leading to cell reproduction.

List of abbreviations
CFU: colony forming unit; CCM: central carbon metabolism; PPP: pentose phosphate pathway; ts: temperature-sensitivity.

Acknowledgements and Funding
We are grateful to Dr. Benedict Michel and Dr. Ivona Fjalkowska for replication mutant strains. The mutants in the CCM genes were obtained from the keio collection (25, National BioResource Project (NIG, Japan): E. coli). This work was supported by Ministry of Science and Higher Education (Poland) (project grant no. N N301 467234 to GW).

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Authors’ contributions
MM and DN performed all experiments. LJ was the initiator of the project and contributed to experimental design and data analysis. ASP supervised the experiments and participated in preparation of the manuscript. GW was a project leader, supervised the work and drafted the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Received: 11 February 2011 Accepted: 31 March 2011 Published: 31 March 2011

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