On the question of activity of oxidative branch of Pentose Phosphate shunt in *pgl* mutant of *E. coli*

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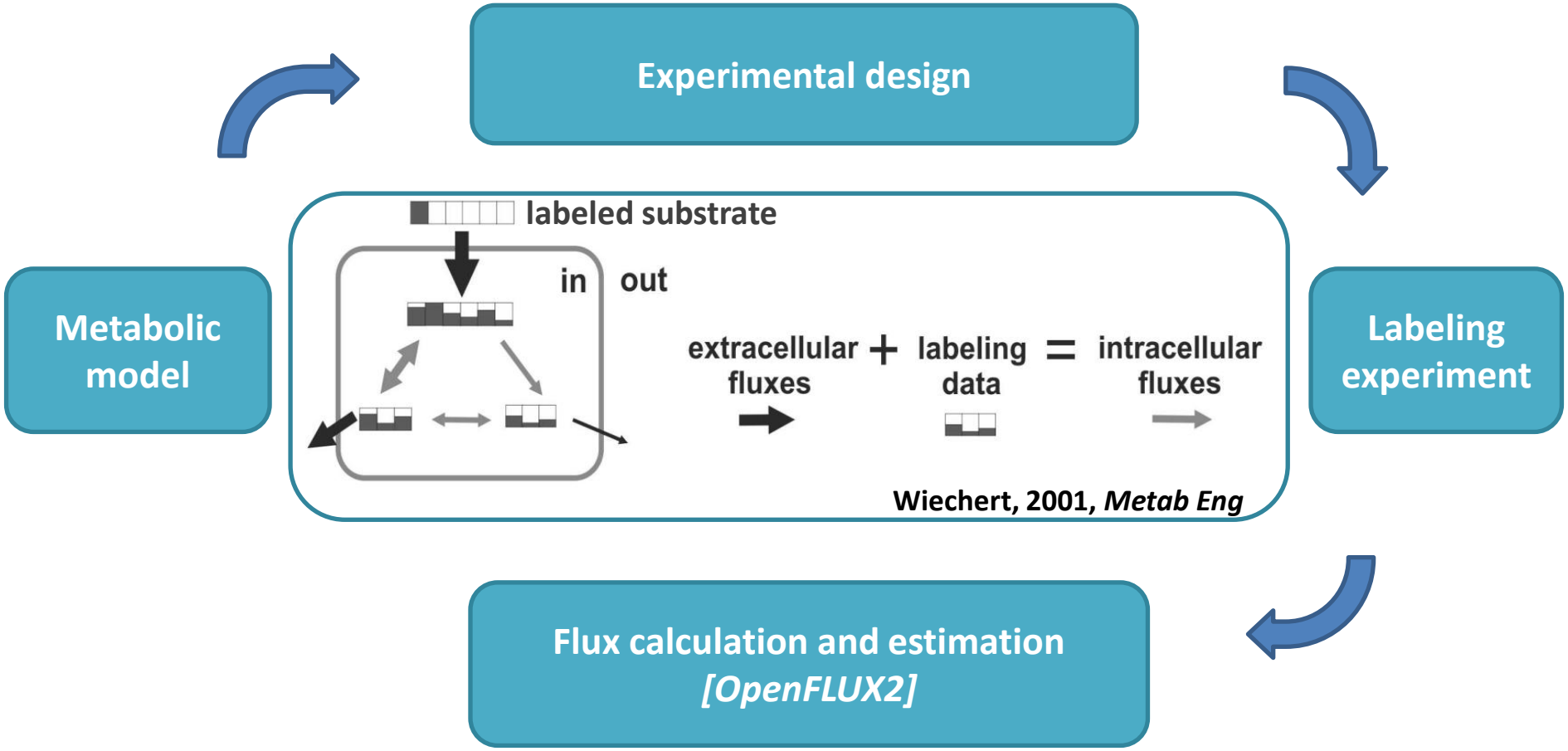
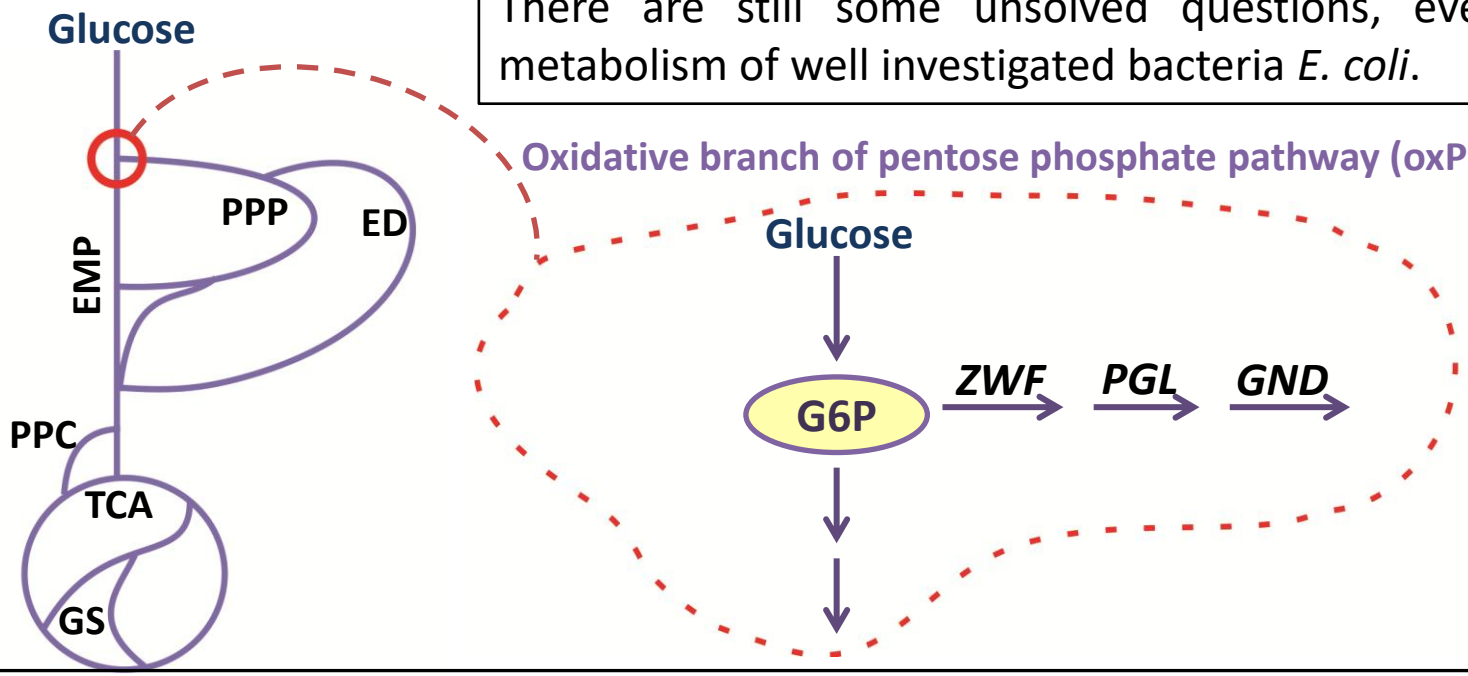
The principle scheme of ^{13}C-MFA (^{13}C metabolic flux analysis)

^{13}C metabolic flux analysis (^{13}C-MFA) is complex method for metabolism investigations allowing quantitative estimation of actual carbon metabolic fluxes distribution *in vivo*.



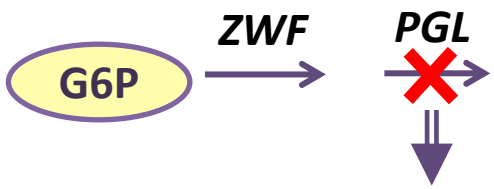
The role of PGL reaction in *E. coli* central metabolism

There are still some unsolved questions, even about central metabolism of well investigated bacteria *E. coli*.



Flux distribution in *E. coli* BL21 or MG1655 *Δpgl*

Flux balance analysis (FBA)*

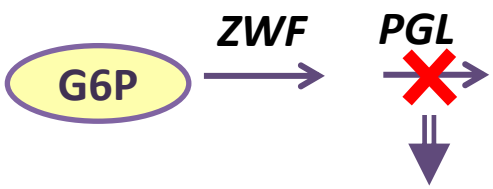


flux through oxPPP = 0

What's the true

?

^{13}C metabolic flux analysis**



flux through oxPPP is only insignificantly decreased

*Juan C. Aon et al., 2008, *Appl Environ Microbiol*

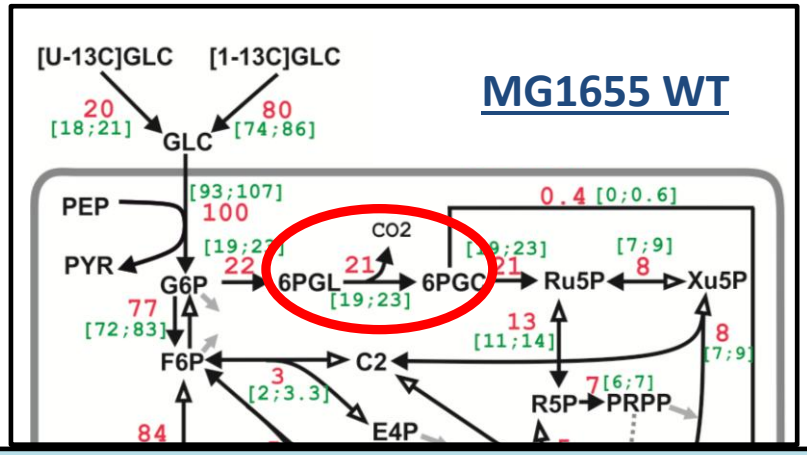
*Jonathan M. Monk et al., 2016, *Cell Syst*

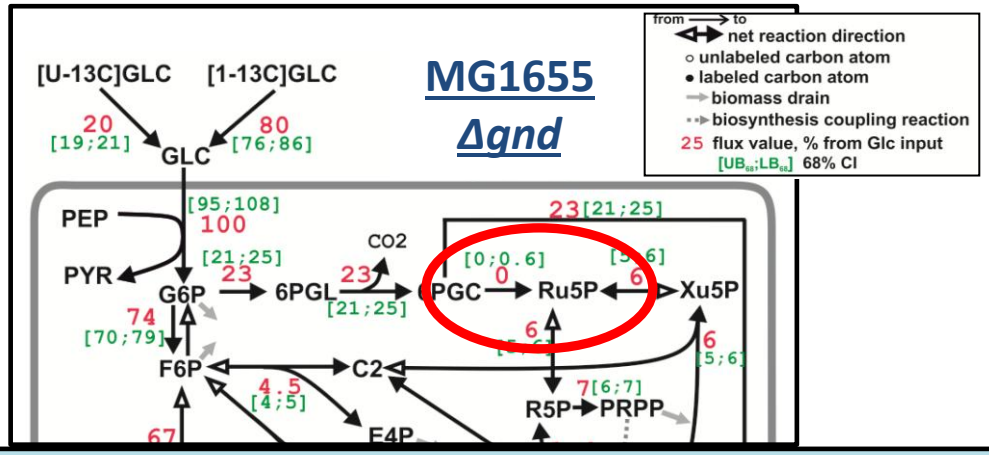
** C. P. Long and M. R. Antoniewicz, 2019, *Metab Eng*

** Christopher P Long et al., 2017, *Metab Eng*

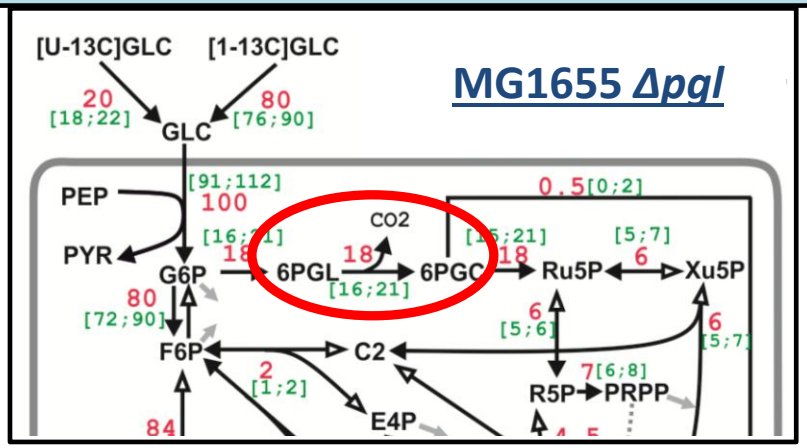
** this work

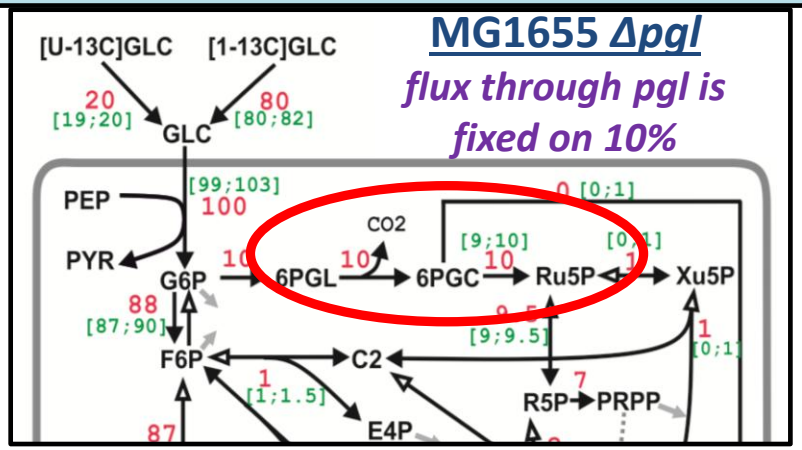
^{13}C-MFA of *E. coli* oxPPP mutants





The flux solution with the best accordance with experimental data for *Δpgl* mutant corresponds to carbon flux through the oxPPP of **18%** of input glucose in contrast with **25%** in parent strain. In flux calculations minimal value of SSR is commonly used as conditional criterion. Formally, any flux distribution providing SSR value which passes χ^2-criterion can be recognized as acceptable. However, flux calculation under assumption that *pgl* inactivation blocks oxPPP led to only statistically unacceptable solution. We determined that flux through PGL reaction responded to χ^2-criterion on minimal 10% level. It means that the the flux through the oxPPP decreases by no more than 2.5-times in the *Δpgl* mutant.

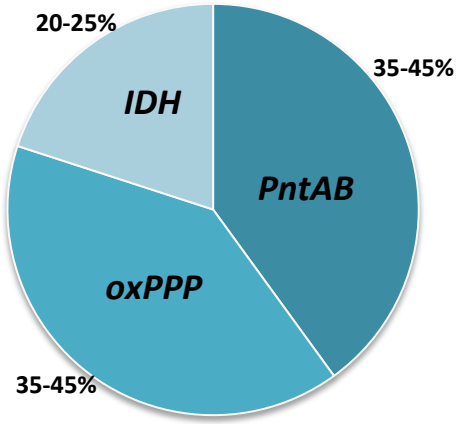


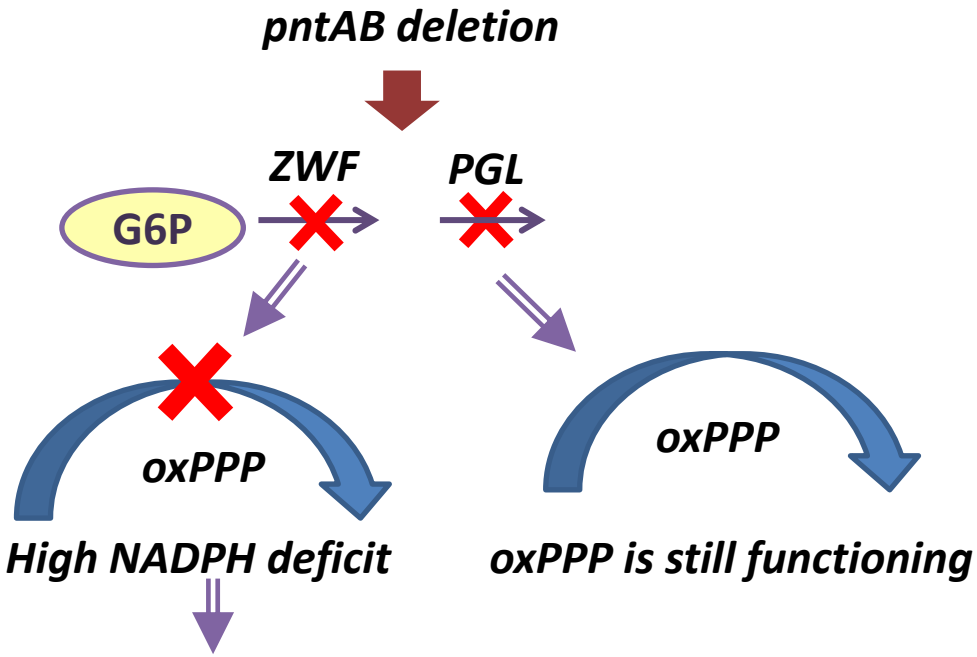


Role of oxPPP in NADPH supplementation

According to Sauer et al.*, the main sources of NADPH in *E. coli* are oxidative pentose phosphate pathway (oxPPP), transhydrogenase PntAB and isocitrate dehydrogenase (IDH).

Reactions contribution to production of NADPH required for biosynthesis





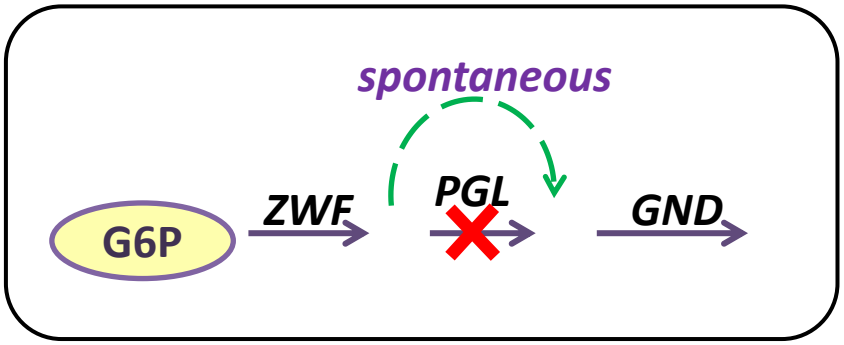
Strain	Growth rate, h^{-1}
MG1655	0.63
MG1655 Δzwf	0.55
MG1655 Δzwf ΔpntAB	0.09
MG1655 Δpgl	0.48
MG1655 Δpgl ΔpntAB	0.38
MG1655 Δgnd	0.56
MG1655 Δgnd ΔpntAB	0.33

*U. Sauer et al., 2004, *J Biol Chem*

The *ΔpglΔpntAB* and *ΔgndΔpntAB* mutants demonstrated decrease of growth rate in 1.3 and 1.7 times, respectively. This additionally confirms that *pgl* inactivation do not abolish activity of oxPPP as in case of *zwf* inactivation. The interesting fact that mutant *ΔgndΔpntAB* grows 1.3 times slower than *ΔpglΔpntAB* mutant despite of activity of Entner-Doudoroff bypass, indicating, probably, that the first strain has more restriction in NADPH supplementation.

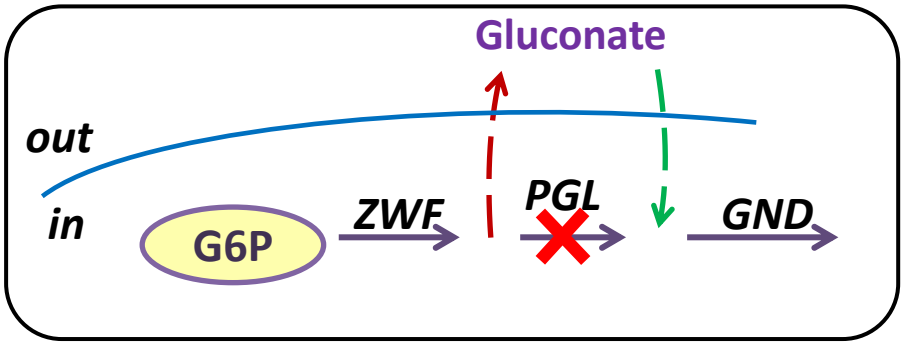
Role of gluconate bypass in oxPPP fluxes maintaining

Possible ways of oxPPP functioning in *Δpgl* mutant



Full compensation by spontaneous hydrolysis of 6-phosphogluconolactone.

It looks doubtful



Bypass through gluconate

Testing of hypothesis

We analyzed the growth of mutant with inactivation of gluconate utilization genes, *gntT*, *gntKU* and *gntP*, on minimal medium with glucose. Simultaneous inactivation of these genes reduces growth of Δ*pgl* and *ΔpglΔpntAB* mutants on glucose. These observations allow us to suppose that in MG1655Δ*pgl* strain the PGL reaction can be at least partly bypassed by the dephosphorylation of the substrate of PGL reaction, 6-phosphogluconolactone, and its conversion to gluconate, which then is consumed by the cells.