Biotechnology Advances 35 (2017) 805–814

Contents lists available at ScienceDirect

Biotechnology Advances

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

Research review paper

Channeling in native microbial pathways: Implications and challenges for metabolic engineering

Mary H. Abernathy, Lian He, Yinjie J. Tang*

Department of Energy, Environmental and Chemical Engineering, Washington University, St. Louis, MO 63130, USA

ARTICLE INFO

Keywords:
Compartmentalization
Flux
Glycolysis
Isotopic labeling
Thermodynamics
Tunneling

ABSTRACT

Intracellular enzymes can be organized into a variety of assemblies, shuttling intermediates from one active site to the next. Eukaryotic compartmentalization within mitochondria and peroxisomes and substrate tunneling within multi-enzyme complexes have been well recognized. Intriguingly, the central pathways in prokaryotes may also form extensive channels, including the heavily branched glycolysis pathway. *In vivo* channeling through cascade enzymes is difficult to directly measure, but can be inferred from *in vitro* tests, reaction thermodynamics, transport/reaction modeling, analysis of molecular diffusion and protein interactions, or steady state/dynamic isotopic labeling. Channeling presents challenges but also opportunities for metabolic engineering applications. It rigidifies fluxes in native pathways by trapping or excluding metabolites for bioconversions, causing substrate catabolite repressions or inferior efficiencies in engineered pathways. Channeling is an overlooked regulatory mechanism used to control flux responses under environmental/genetic perturbations. The heterogeneous distribution of intracellular enzymes also confounds kinetic modeling and multiple-omics analyses. Understanding the scope and mechanisms of channeling in central pathways may improve our interpretation of robust fluxomic topology throughout metabolic networks and lead to better design and engineering of heterologous pathways.

1. Introduction

Metabolic flux is controlled by both enzyme kinetics and substrate diffusions. Under *in vitro* enzyme biochemistry, purified enzymes are often dissociated and metabolites are well-mixed; so enzymatic reactions have no spatial effect, and the homogenous system can be described by Michaelis–Menten models. However, macromolecular crowding in cells has been shown to promote enzyme associations and structural organizations within the cytoplasm (Mourão et al., 2014; Spitzer, 2011). Meanwhile, fluidity in the cytoplasm has been found to be closely related to metabolic activity (Parry et al., 2014). Since *in vivo* environments may significantly impede enzyme kinetics (Minton, 2001), enzymes may organize to pass the product of one reaction site to an adjacent site without releasing into the bulk phase. Such a mechanism not only improves substrate diffusion and reaction-equilibrium, but also benefits other cellular functions, including protection of unstable intermediates, forestallment of substrate competition or inhibition among different pathways, and maintenance of a stable enzymatic microenvironment (Zhang, 2011).

Several mechanisms of enzyme complex channeling for cascade reactions have been recognized (Wheeldon et al., 2016). Intramolecular tunnels, as evidenced in tryptophan synthase, connect two active sites (Miles, 2001). Activated chemical swing arms can pass intermediates between active sites as seen in fatty acid synthase (type I) and pyruvate dehydrogenase complex. Moreover, electrostatic guidance uses complementary charges between protein residues spanning two active sites to direct intermediates as confirmed in malate dehydrogenase and citrate synthase (Wu and Minteer, 2015). Additionally, eukaryotes can compartmentalize enzymes in cellular organelles—*e.g.*, the TCA cycle is carried out in the mitochondrion. On the other hand, evidences have shown that cascade enzymes in central and secondary pathways in prokaryotes, as well as eukaryotes, are also organized extensively (Table 1). For example, glycolytic enzymes may form glycolysisosomes, which suggests intracellular enzymes are not randomly distributed in the cytosol. This hypothesis is not yet generally accepted because central metabolism enzymes often have high copy numbers and, metabolite diffusion is believed to be sufficiently fast (Wheeldon et al., 2016). In this review, we update evidences and scopes of natural channels in microbial metabolism as well as methods to measure metabolite channeling through cascade enzymes. We further discuss how channeling influences metabolic fluxes in central pathways. Validation and understanding of natural metabolite channeling through cascade

* Corresponding author.
E-mail address: yinjie.tang@seas.wustl.edu (Y.J. Tang).

http://dx.doi.org/10.1016/j.biotechadv.2017.06.004
Received 4 April 2017; Received in revised form 8 June 2017; Accepted 10 June 2017
Available online 13 June 2017
0734-9750/

Table 1
Evidences of enzyme tunneling or channeling.

Enzyme reactions	Detection method	System employed	Species studied	Citation
Polyfunctional enzymes forming molecular tunnels	Kinetic studies, enzyme cross linking, LC-MS/MS, SDS-PAGE, and X-ray structural analysis	*In vitro*	Purified enzymes	(Huang et al., 2001; Miles, 2001; Dunn, 2012)
Oxidative pentose phosphate pathway	Isotopic dilution of ^{14}C-labeled OPPP intermediates by unlabeled intermediates	*In vitro*	Purified yeast enzymes, yeast extract, pea and soybean nodule extracts	(Debnam et al., 1997)
Glycolysis to lactate	Isotopic dilution with ^{13}C labeled substrates and steady-state MFA	*In vivo*	Human Cell line AGE1.HN	(Niklas et al., 2011)
TCA cycle	Isotopic dilution using [3-^{13}C]propionate and observed carbon orientation using ^{13}C NMR	*In vivo*	*Saccharomyces cerevisiae*	(Sumegi et al., 1990)
Calvin cycle	Isotopic dilution using $^{13}CO_2$ and INST-MFA	*In vivo*	*Synechocystis* sp. PCC 6803	(Young et al., 2011)
EMPP	Isotopic dilution with challenging substrates	*In vivo*	*Escherichia coli*	(Shearer et al., 2005)
TCA cycle (from malate to citrate)	LC-MS/MS and SDS-PAGE; Site-directed mutagenesis; kinetic modeling	*In vivo*, *in vitro*, and *in silico*	Recombinant enzymes from *E. coli* or *Saccharomyces cerevisiae*	(Bulutoglu et al., 2016; Vélot and Srere, 2000)
TCA cycle (fumarate to citrate; malate to citrate)	Isotopic dilution, NMR (^{13}C and ^{1}H) and steady-state MFA	*In vivo*	*Arabidopsis thaliana*	(Williams et al., 2011)
TCA cycle (from ketoglutarate to succinate/fumarate	Isotopic labeling with ^{13}C glutamate or ^{13}C acetate; ^{13}C NMR analysis	*In vivo*	*Saccharomyces cerevisiae*	(Sumegi et al., 1993)
Glycolysis (3PG to PEP)	Isotopic dilution using $^{13}CO_2$ and INST-MFA	*In vivo*	*Synechocystis* sp. PCC 6803	(Huege et al., 2011)
PEP to PYR to lactate	Isotopic dilution with [U-^{13}C]glucose and INST-MFA	*In vivo*	Chinese Hamster Ovary-K1 cell line	(Nicolae et al., 2014)
Glycolysis	Proteomic analysis of mitochondrial fractions and ^{13}C-glucose studies	*In vitro*	*Arabidopsis thaliana*	(Giegé et al., 2003)
PCB-degradation pathway	Kinetic studies compared between channeled complex and individual complexes	*In vitro*	*Burkholderia xenovorans*	(Baker et al., 2009)
CoA channeling through fatty acid synthase	Substrate pool size quantification and comparative enzyme kinetics	*In vitro*	Purified chloroplasts from spinach and pea shoots	(Roughan, 1997)
Fatty acid β-oxidation cycle	Protein crystallization, X-ray diffraction, and homology modeling	*In vitro*	*Escherichia coli*	(Ishikawa et al., 2004)
Polyketide pathway	Kinetic analysis of modular enzymes	*In vitro*	*Saccharopolyspora erythraea*	(Wu et al., 2001)
Electron transport chain complexes	Analysis of enzyme activities in the presence of electron donors and inhibitors along with genetically modified supercomplexes	*In vivo*	Mitochondria from mouse fibroblasts	(Lapuente-Brun et al., 2013)
Photosynthesis (light-harvesting complexes)	Spectroscopic analyses of isolated protein complexes and genetically modified complexes	*In vitro*	*Chlamydomonas reinhardtii*	(Iwai et al., 2010)