Glycomics and Lipidopics

Extended Abstract

Evaluation of metabolic redox homeostasis in prokaryotes

Sanna Kreula

Abstract:

The work focuses on cellular redox homeostasis in prokaryotic micro-organisms, and specifically on factors associated with nicotinamide adenine cofactor [NADP(H) and NAD(H)] metabolism in *E. coli* and photoautotrophic cyanobacterium *Synechocystis sp*. PCC 6803. These cofactors participate in numerous electron transfer reactions in the cell, linking enzymatic reactions with the overall energy metabolism with biosynthetic reactions and housekeeping functions. Obtaining a comprehensive view of the interactions and the regulatory circuits is thus of central importance in understanding the adaptation to different environmental conditions, such as those involved with the transition between autotrophic and heterotrophic growth modes in cyanobacteria. The principal objective is to study the role of the proton gradient-coupled pyridine nucleotide transhydrogenase PntAB. Functional characterization combined with structral modelling of PntAB in *Synechocystis sp*. PCC 6803 has been carried out, and information-rich networks have been created to identify identify novel candidates involved in the NADP(H)-regulation in different organisms. In addition, PntAB is studied through deletion and over-expression mutants under anaerobic fermentative conditions and under different pH's in *E. coli*. Specifically, the initiative is to elucidate to what extent the regulation of the cofactor redox balance takes place at the level of alternative catabolic routes in glucose breakdown, and what is the role of PntAB under these specific conditions. The approach is to generate *pntAB* over-expression and knock-out strains, and to compare them in phenotypic growth properties as well as in respect to changes in the central carbon metabolism by analyzing the distribution of local ratios of amino acids using C^{13} labelled glucose as a probe. Redox chemistry is an intrinsic part of plant metabolism. The cellular redox state is determined by oxidation or reduction of various redox-active species, which are involved in a large number of metabolic reactions. In the chloroplast, reductants such as ferredoxin (Fdx) and NADPH are produced by the photosynthetic electron transport chain, and along with ATP, used to generate sugar-phosphates, amino acids, and many other metabolites that are supplied to the rest of the cell. In addition to this, NAD(P)H metabolism is involved in central processes such as glycolysis, fermentation, and oxidative pentose phosphate pathway (OPP) in the cytosol, tricarboxylic acid (TCA) cycle, respiratory electron transport, and biosynthetic processes in mitochondria, and photorespiration in plastids, mitochondria, and peroxisomes. In addition to being an intrinsic part of metabolism, redox status plays an active role in metabolic regulation. In this regard, the redox status operates as a major integrator of cellular metabolism and is simultaneously regulated itself by metabolic processes. This enables readjustment of global metabolic pathways and redox status homeostasis in response to changes in environmental conditions, involving reprogramming of gene expression and post-translational modification of target enzymes by thiol-disulfide modulations. The underlying signaling pathways have only partially been discovered in the previous years. While much is known about redox status signals involved in light activation of photosynthetic processes, little is known about redox regulation of other metabolic pathways in the plastid and of extra-plastidial metabolism. While recent studies provide evidence for the existence of redox signals coordinating metabolism and gene expression between different organelles, such as plastid, mitochondrion, and nucleus, their nature has not yet been clarified. In this review, we will describe the redox status control of metabolism and the metabolic control of redox status at both the cellular and subcellular levels, mainly focusing on post-translational mechanisms. Despite the vastness of literature concerned with redox status-regulated gene expression, we will only describe this in passing given that it is the subject of a couple of excellent recent reviews. In the first part, redox status-related metabolic processes will be described within their subcellular context, with regard to redox status-regulatory properties and intra-organellar signals involved in their co-ordination. In this regard, our major focus is placed on organelles such as plastids, mitochondria, and peroxisomes with readers being referred to other comprehensive reviews for details on both cytosolic and apoplastic aspects of redox status with only a broad overview of the most important features in the context of cellular metabolism and function being provided here. In the second part, we will discuss the integration at the cellular level while mainly focusing on inter-organellar signals co-ordinating redox status regulation of metabolism between different subcellular compartments. Plastidial Redox Status Biology Chloroplasts are plant-specific organelles with important properties, the most prominent

Sanna Kreula

University of Turku, Finland

being their ability to carry out oxygenic photosynthesis. During this process, light energy is absorbed by photosystems I (PS I) and II (PS II) located in the thylakoid membrane and used to activate photosynthetic electron transport. Linear electron flow requires both photosystems working in series, leading to electron transfer from water to $NADP^+$ to generate NADPH as a reducing power and a trans-thylakoid proton gradient that drives ATP synthesis *via* CF_0F_1ATPase. This involves electron transfer from PS II to PS I *via* plastoquinone (PQ), the cytochrome b_6f complex, and plastocyanin as additional redox carriers. At the stromal side of PS I, electrons are subsequently donated to Fdx, which functions as a mobile electron carrier distributing electrons to $NADP^+$ *via* Fdx-NADP-reductase (FNR) to produce NADPH or directly to specific processes located in the stroma, such as S and N assimilation, the synthesis of chlorophyll and fatty acids, and reactions involved in chloroplast redox regulation. In the latter, electrons are transferred from Fdx to thioredoxins (Trxs) *via* Fdx-Trx-reductase (FTR). Trxs are small regulatory proteins containing a redox-active disulfide group that controls the thiol-disulfide exchange of target proteins . In plants, Trxs comprise a medium-sized gene family with 10 different isoforms (*f*1–2, *m*1–4, *x*, *y*1–2 and *z*) being located in the chloroplast of Arabidopsis, while other isoforms are located in the cytosol and mitochondria. *In vitro* studies using purified proteins indicate Trxs *f* and *m* to be involved in the regulation of stromal metabolism, while x-, y-, and z-types serve as reducing substrates for antioxidant enzymes. More recently, genetic studies have been used to further dissect the specific roles of different Trxs *in vivo*, providing evidence for different isoforms of Trxs *f* and *m* having different functions in plants.

Sanna Kreula

University of Turku, Finland