microbial opioids, or facilitate conversion of alimentary oligopeptides into potent MOR ligands? Microbial MOR agonists have been identified before, such as the ultrapotent dermorphins or deltorphins, opioid agonists up to 40 times more potent than morphine that have been isolated from the skin of giant leaf frogs of the genus *Phyllomedusa*. Albeit highly speculative, such a potential interaction of our gut flora and the nutropioid gut-brain circuitry could add interesting future perspectives to an already fascinating research field.

The work by Duraffourd et al. (2012) may not replace the currently accepted, classical model of opioidergic food intake control, which suggests the central release of endogenous opioids as the major stimulus for a hedonic drive to consume food. Nonetheless, Duraffourd et al. (2012) expand a CNS-centric view to a model that also allows direct peripheral action of alimentary opioid antagonists on periportal MORs, followed by the (IGP-dependent) decrease in food consumption. The strength of this new model lays in the integration of both worlds; direct effects of alimentary ''nutropioids'' could be followed by—or compete—with a concomitant increase in endogenous opioids, such as β-endorphin (which could be released by both the GI tract and the CNS). Thus, exogenous and endogenous opioids could orchestrate hedonic feeding mechanisms at multiple sites, both in the periphery and in the CNS.

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Nrf2 Orchestrates Fuel Partitioning for Cell Proliferation

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Why upregulation of the transcription factor Nrf2 increases tumor cell proliferation is unclear. Mitsuishi et al. (2012) now provide evidence that Nrf2 augments purine nucleotide synthesis, thus supporting tissue hypertrophy. This change in cellular metabolism requires loss of Nrf2 repression by Keap1 as well as costimulation via the PI3K-Akt pathway.

NF-E2 p45-related factor 2 (Nrf2, also called Nfe2l2) is a cap 'n' collar (CNC) basic-region leucine zipper (bZIP) transcription factor that allows cells to adapt to oxidative stress and electrophiles by mediating induction of a battery of cytoprotective genes, including those encoding enzymes involved in the synthesis of reduced glutathione (GSH), repair of oxidized protein thiols, scavenging of reactive oxygen species (ROS), and drug metabolism (Hayes et al., 2010). The stress-responsive activity of Nrf2 is dictated at the level of protein stability, which is controlled by the ubiquitin ligase substrate adaptor Kelch-like ECH-associated protein (Keap1). In normal unstressed cells, Nrf2 protein is rapidly turned over in a Keap1-dependent manner through Cul3-Rbx1 ubiquitylation and proteasomal degradation. However, Keap1 is inactivated by ROS and electrophiles, and such agents stabilize Nrf2 protein and cause induction of Nrf2-target genes (McMahon et al., 2010). By contrast with normal cells, Nrf2 protein is constitutively upregulated in many tumors due to somatic mutations in the *Keap1* or *Nrf2* genes. Tumors that overexpress Nrf2 exhibit increased resistance to chemotherapeutic drugs and higher rates of proliferation (Zhang et al., 2010), though it is unclear why the latter occurs. It has been suggested that Nrf2 might increase phosphorylation of retinoblastoma protein and prevent cell-cycle arrest (Homma et al., 2009) or that Nrf2 might allow tumors to survive the high levels of ROS that K-Ras, B-Raf, and Myc oncogenes produce (DeNicola et al., 2011). Given that cancer cells are subject to metabolic reprogramming to support rapid synthesis of macromolecules (Ward and Thompson, 2012), it is also possible that Nrf2 promotes tumor cell proliferation by influencing intermediary metabolism. In a recent, groundbreaking study, Mitsuishi et al. (2012)

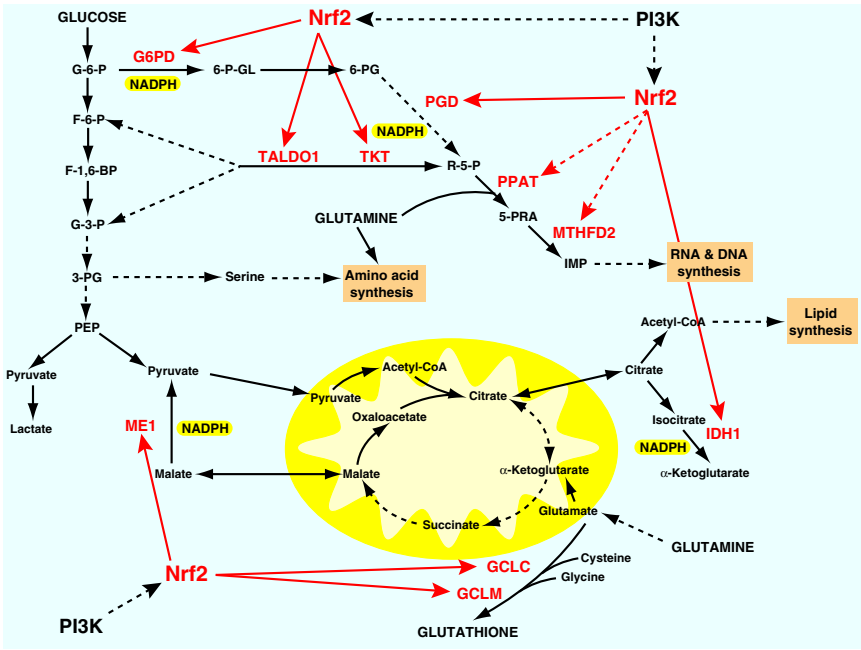

Figure 1. Metabolic Pathways and Their Regulation in Proliferating Cells by the Transcription Factor Nrf2

The diagram depicts the relationship between glycolysis, the TCA cycle, the pentose phosphate pathway, glutamine metabolism, and glutathione synthesis. It also indicates, with a solid arrow or a dashed arrow, respectively, genes that are directly or indirectly regulated by Nrf2. The inclusion of a dashed arrow from PI3K to Nrf2 indicates that activation of the PI3K-Akt pathway substantially augments induction of metabolic genes by the CNC-bZIP factor, though the mechanism has not been established. As shown, Nrf2 controls the expression of glucose-6-phosphate dehydrogenase (G6PD) and phosphogluconate dehydrogenase (PGD) in the oxidative arm of the pentose phosphate pathway, as well as transaldolase 1 (TALDO1) and transketolase (TKT) in the nonoxidative arm of the pentose phosphate pathway. In addition, Nrf2 regulates malic enzyme 1 (ME1) and isocitrate dehydrogenase 1 (IDH1), which generate NADPH to support synthesis of macromolecules. It also regulates the expression of glutamate-cysteine ligase catalytic (GCLC) and modifier (GCLM) subunits, which together combine to catalyze the rate-limiting step in glutathione synthesis. Both ME1 and GCLC-GCLM contribute to the utilization of glutamine in cancer cells. Lastly, Nrf2 indirectly regulates phosphoribosyl pyrophosphate amidotransferase (PPAT, also known as amidophosphoribosyltransferase [ATase] or glutamine phosphoribosylpyrophosphate amidotransferase [GPAT]) and methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) that are involved in purine synthesis. Abbreviations: G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; F-1,6-BP, fructose-1,6-bisphosphate; G-3-P, glyceraldehyde-3-phosphate; 3-PG, 3-phosphoglycerate; PEP, phosphoenolpyruvate; 6-P-GL, 6-phosphogluconolactone; 6-PG, 6-phosphogluconate; R-5-P, ribulose-5-phosphate; 5-PRA, 5-phosphoribosylamine; IMP, inosine monophosphate.

show that Nrf2 orchestrates profound metabolic changes by directing glucose and glutamine along anabolic pathways that augment purine synthesis and demonstrate that its influence on the pentose phosphate pathway (PPP) contributes to cell proliferation.

Human lung A549 cells constitutively overexpress Nrf2 because they harbor mutant *Keap1* (Singh et al., 2006). Following knockdown of Nrf2 in A549 cells, Mitsuishi et al. (2012) find, using microarray analysis, that Nrf2 regulates the expression of all four enzymes responsible for NADPH synthesis (G6PD, PGD, ME1, and IDH1), consistent with the notion that Nrf2 activity supports synthesis of macromolecules (Figure 1). Of interest, G6PD and PGD catalyze reactions in the oxidative arm of the PPP, and the finding by Mitsuishi et al. (2012) that Nrf2 also regulates TKT and TALDO1, which catalyze reactions in the nonoxidative arm of the PPP, suggests the transcription factor represents a dominant controller of metabolic flux through the PPP. Lastly, Nrf2 increases expression of the nucleotide synthesis enzymes PPAT and MTHFD2. ChIP-Seq analyses indicate that the *G6PD*, *PGD*, *ME1*, *IDH1*, *TKT*, and *TALDO1* genes are regulated directly by Nrf2, whereas *PPAT* and *MTHFD2* are regulated indirectly. Consistent with the microarray and ChIP-Seq data, metabolomic experiments indicate that upregulation of Nrf2 shifts glucose metabolism from glycolysis toward purine nucleotide synthesis. Thus, knockdown of Nrf2 in A549 cells increases the levels of both glycolytic and PPP intermediates while decreasing intermediates involved in purine synthesis. Furthermore, tracer studies indicated that upregulation of Nrf2 augments utilization of glutamine by increasing GSH synthesis as a consequence of inducing glutamate-cysteine ligase (GCL, which comprises GCLC and GCLM subunits) and by increasing the decarboxylation of malate as a consequence of inducing ME1 (Figure 1).

Mitsuishi et al. then test the hypothesis that constitutive upregulation of Nrf2 in tumors increases cell proliferation because the transcription factor redirects glucose metabolism from glycolysis through the PPP to purine synthesis. Using siRNA knockdown, the authors show that loss of G6PD and TKT greatly impedes growth of A549 cells in xenograft experiments. Moreover, forced expression of Nrf2 in 293T cells increases their rate of proliferation, and this can be recapitulated by expression of ectopic G6PD or TKT. Thus, cell proliferation that accompanies upregulation of Nrf2 appears, at least in part, to be due to increased G6PD and TKT activity.

Mitsuishi et al. (2012) find that glucose metabolism also shifts toward purine nucleotide synthesis in immortalized Keap1 null mouse embryonic fibroblasts, raising the question of whether upregulation of Nrf2 might support cell proliferation in nontransformed tissues as well as in tumors. Comparison of wild-type mice and those with low levels of Keap1 (i.e., $Keap1^{loxP/-}$ mice) revealed that *G6pd*, *Tkt*, *Taldo1*, *Pgd*, and *Me1* are significantly overexpressed in the forestomach of the mutant animals, whereas no significant change was observed in the liver. Furthermore, the increased expression of these Nrf2-target genes in the forestomach of $Keap1^{loxP/-}$ mice is associated with increased epithelial cell proliferation, leading to hypertrophy. As forestomach epithelial cells normally turnover rapidly, whereas hepatocytes are normally quiescent, it seems likely that loss of Keap1 activity per se is not sufficient to redirect

glucose metabolism through the PPP to purine synthesis. Mitsuishi et al. (2012) therefore argue that a proliferative signal provided by the PI3K-Akt pathway is required to increase flux through the PPP in an Nrf2-dependent fashion and provide evidence that Akt is activated in mouse forestomach. The idea that increased PI3K-Akt signaling in the liver allows Nrf2 to induce *G6pd*, *Tkt*, *Taldo1*, *Pgd*, and *Me1* was confirmed by making liver-specific Pten and Keap1 double-knockout mice.

In summary, Mitsuishi et al. show that under the direction of PI3K-Akt signaling, Nrf2 regulates the expression of genes involved in the PPP, generation of NADPH, and synthesis of purine nucleotides. They have expanded our knowledge of Nrf2 functions from increasing cytoprotection to the regulation of metabolism and the synthesis of macromolecules, thereby providing an explanation of how Nrf2 supports cell proliferation. A number of critical issues remain unresolved. The mechanism by which Nrf2-mediated induction of metabolic genes requires both inhibition of Keap1 and activation of PI3K-Akt, as opposed to induction of antioxidant and detoxication genes, which requires only inhibition of Keap1, is unclear. It is also not known how Nrf2 cooperates with other transcription factors implicated in metabolic reprogramming in tumor cells, including p53, Myc, and HIF1α (Kroemer and Pouyssegur, 2008; Ward and Thompson, 2012), to maintain high rates of glycolysis and increase cell proliferation. It remains to be established whether activation of Nrf2 along with the PI3K-Akt pathway is sufficient to promote carcinogenesis. Lastly, while inhibition of Nrf2 as a cancer chemotherapeutic strategy is an attractive proposition, it may be unwise to reduce PPP metabolic flux, given that the pathway provides an important defense for neurons and astrocytes against oxidative damage and neuronal death (Fernandez-Fernandez et al., 2012). Accordingly, it is becoming clear that Nrf2, in combination with growth factor signaling, provides a critical interface between oxidative stress sensing and metabolic reprogramming of cells.

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The Mitochondrial Pyruvate Carrier: Has It Been Unearthed at Last?

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The mitochondrial pyruvate carrier (MPC) is essential for several major pathways of carbohydrate, fat, and amino acid metabolism, yet its molecular identity has remained elusive. Two recent papers in *Science* (Herzig et al., 2012; Bricker et al., 2012) implicate three newly identified inner mitochondrial membrane proteins as MPC components.

Pyruvate lies at the heart of carbohydrate, fat, and amino acid metabolism and is usually produced in the cytoplasm before being transported into the mitochondria for further metabolism (Figure 1). Transport is mediated by the mitochondrial pyruvate carrier (MPC) whose existence was confirmed in 1974 by the discovery of a potent and specific inhibitor, α-cyano-4-hydroxycinnamate (CHC). Subsequently, its substrate and inhibitor specificity and roles in metabolism were extensively investigated (Halestrap et al., 1980). Transport of most metabolites across the inner mitochondrial membrane (IMM) involves members of the mitochondrial carrier family (MCF; 53 members in humans), which are usually 30–35 kDa in size and have six transmembrane domains (TMDs) (Palmieri and Pierri, 2010). In 1981 the CHC analog UK5099 (K_i 5 nM)