Annu. Rev. Nutr. 2002. 22:87–105
doi: 10.1146/annurev.nutr.22.110801.140547

REGULATION OF ENZYMES OF THE UREA CYCLE AND ARGININE METABOLISM

Sidney M. Morris, Jr.
Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261; e-mail: smorris@pitt.edu

Key Words arginase, ornithine, polyamines, nitric oxide, liver, macrophage, transcription

■ **Abstract** The urea cycle is comprised of five enzymes but also requires other enzymes and mitochondrial amino acid transporters to function fully. The complete urea cycle is expressed in liver and to a small degree also in enterocytes. However, highly regulated expression of several enzymes present in the urea cycle occurs also in many other tissues, where these enzymes are involved in synthesis of nitric oxide, polyamines, proline and glutamate. Glucagon, insulin, and glucocorticoids are major regulators of the expression of urea cycle enzymes in liver. In contrast, the "urea cycle" enzymes in nonhepatic cells are regulated by a wide range of pro- and antiinflammatory cytokines and other agents. Regulation of these enzymes is largely transcriptional in virtually all cell types. This review emphasizes recent information regarding roles and regulation of urea cycle and arginine metabolic enzymes in liver and other cell types.

CONTENTS

INTRODUCTION

The urea cycle (58), an essential pathway for the disposal of ammonia in mammals and many amphibian species, is comprised of five enzymes: carbamyl phosphate synthetase-I (CPS-I), ornithine transcarbamylase, argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL), and arginase. In addition to these five

enzymes, several other proteins are required for efficient functioning of the pathway in vivo, including hepatic glutaminase (reviewed in 29, 122), N-acetylglutamate synthetase, mitochondrial ornithine/citrulline transporter(s), and the mitochondrial aspartate/glutamate transporter (48, 57, 94). With the exception of the ornithine/citrulline transporter ORNT1, these other enzymes are not considered here.

The overview of mammalian arginine metabolic enzymes in Figure 1 shows that the urea cycle is but one aspect of mammalian amino acid metabolism centered around the amino acid L-arginine and that several other pathways involving arginine synthesis or catabolism include enzymes also present within the urea cycle. This simplified scheme does not indicate subcellular localization of the individual enzymes or the roles of transport proteins. It should be emphasized that the ornithine produced by arginase within the urea cycle (Figure 1) is highly restricted to recycling within the urea cycle and probably is not a significant source of substrate for ornithine decarboxylase or ornithine aminotransferase. Although it is possible that all of the metabolic activities depicted in Figure 1 may be found within a single organ such as the liver, it is highly unlikely that all of them are simultaneously expressed within any single cell type. With the exception of arginine decarboxylase, all enzymes listed in Figure 1 [including agmatinase (66)] have been cloned, thus facilitating studies to elucidate their physiologic roles. In addition to the urea

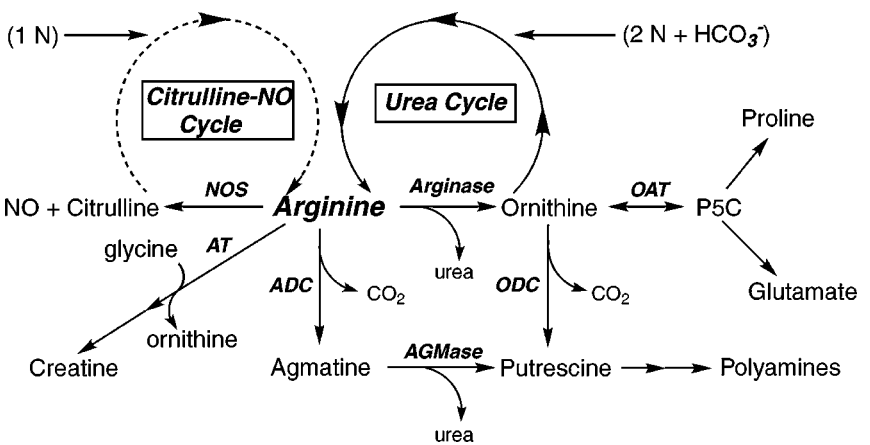

Figure 1 Overview of mammalian arginine metabolism. Not all enzymes shown are expressed in all cells or tissues. Selected enzymes are indicated: NOS, nitric oxide synthase; AT, L-arginine:glycine amidinotransferase; ADC, arginine decarboxylase; AGMase, agmatinase; ODC, ornithine decarboxylase; OAT, ornithine aminotransferase. For the sake of simplicity, not all reactants or cofactors are shown. N^G-OH-arginine, an intermediate in NO synthesis, is a potent inhibitor of arginase activity (29). Stoichiometry of nitrogen atoms and bicarbonate needed to regenerate arginine in the two cycles is shown. The dashed line indicates that efficiency of the citrulline-NO cycle is variable and not quantitative.

cycle itself, this review briefly considers other arginine metabolic pathways that involve urea cycle enzymes. More comprehensive reviews of mammalian arginine metabolism have been presented elsewhere (30, 128). Developmental changes in expression are not reviewed here.

THE UREA CYCLE

Ureagenesis and Urea Cycle Enzymes

The urea cycle is expressed within periportal hepatocytes of the liver, and there is evidence for a low level of urea cycle activity in enterocytes also (125). CPS-I and ornithine transcarbamylase are located within the mitochondrial matrix, whereas the other urea cycle enzymes are cytosolic. Efficiency of the urea cycle is enhanced by tight metabolic channeling of reaction products between successive enzymes within this pathway (21, 25, 121).

The capacity of the liver for ureagenesis is sufficiently in excess of usual rates of urea production (e.g., 7) that it can immediately respond to acute increases in waste nitrogen load. Substrate availability is probably the most important determinant of short-term changes in rates of ureagenesis. CPS-I, which catalyzes the initial, committed step in ureagenesis, has an absolute requirement for N-acetyl-glutamate as an allosteric activator. This compound is synthesized within the mitochondrion by N-acetyl-glutamate synthetase, and its levels within liver are variable (7, 71, 87, 107). Whether N-acetyl-glutamate is merely a cofactor of CPS-I or plays a major role as a short-term regulator of the rate of ureagenesis via its effect on CPS-I activity remains a point of some controversy (reviewed in 120). The interrelationship between N-acetyl-glutamate concentration and substrate supply in regulating rates of ureagenesis is still incompletely understood.

Longer-term changes in activities of the urea cycle enzymes occur in response to alterations in amino acid nitrogen flux resulting from changes in dietary protein intake or catabolism of endogenous protein. Glucagon, insulin, and glucocorticoids play major roles in mediating changes in activity of the urea cycle enzymes under these conditions, and altered rates of transcription represent a major portion of changes in expression of the urea cycle enzymes (72, 109). Nevertheless, mechanisms that operate to ensure that changes in levels of the urea cycle enzymes in the liver are well-coordinated are still far from clear. For example, responses of the individual urea cycle enzyme mRNAs to cyclic AMP and glucocorticoids in vivo or in cultured hepatocytes are quite different (78, 84). As noted below, recent investigations of certain disorders of fatty acid metabolism have revealed a link with the urea cycle. The following discussions of individual urea cycle enzymes and the mitochondrial ornithine/citrulline transporter emphasize results obtained since previous reviews (72, 109).

Expression of the urea cycle enzymes, including CPS-I, is reduced in juvenile visceral steatosis (JVS) mice, an animal model with a defect in carnitine transport that results in fatty liver, hyperammonemia, hypoglycemia and growth retardation

(reviewed in 100). Saheki's group demonstrated that transcription of urea cycle enzyme genes was reduced in JVS mice (112) and provided evidence that this involved accumulation of long-chain fatty acids in liver (114), resulting in activation of AP-1 (113), which in turn inhibited activation of CPS-I transcription by glucocorticoids. This was not a general effect on glucocorticoid-regulated genes in liver because expression of the phosphoenolpyruvate carboxykinase (PEPCK) gene was not significantly affected under these conditions (112). The effect on CPS-I expression was further shown to involve an AP-1 site within the CPS-I enhancer region located at approximately 6.3 kb upstream of the transcription start site (81) (see discussion of CPS-I enhancer below). The same mechanism presumably acts to reduce expression of the other urea cycle enzymes in JVS mice, but the DNA regulatory elements involved in the effect on these genes have not been identified. These studies not only elucidated a new link between fatty acid metabolism and the urea cycle but also underscored the importance of glucocorticoids in maintaining urea cycle enzyme activity. Interestingly, acylation of CPS-I has recently been found to inhibit its activity (27a), providing additional support for a link between these metabolic pathways.

Another recent finding is that the expression of CPS-I and glutaminase in mouse liver is elevated by caloric restriction (33, 34, 111), a dietary condition that delays many age-related physiological changes. Increases in CPS-I enzyme levels reflected increased transcription of the CPS-I gene (111). The elevated expression is consistent with the notion that calorically restricted mice have higher rates of protein catabolism than do control-fed mice. Although not measured in these studies, it is likely that expression of the other urea cycle enzymes was similarly elevated. The hormones or metabolites that signal the reductions in hepatic CPS-I expression in calorically restricted mice have not been identified.

Apart from studies using the JVS mice, relatively little new information is available regarding nutritional or hormonal regulation of hepatic ornithine transcarbamylase, ASS, and ASL expression since earlier reviews. Expression of ASL was recently reported to be reduced by hypoxia, probably at the transcriptional level (59). This may contribute to the zonation of ASL expression within the liver.

Arginase is the only urea cycle enzyme that exists as two distinct isozymes. The isozymes, designated type I (liver-type) and type II (kidney-type), are encoded by different genes (52). Arginase I is highly expressed in liver as a component of the urea cycle. Metabolic studies using perfused liver suggest that some arginase II may also be coexpressed with ornithine aminotransferase in perivenous hepatocytes to facilitate synthesis of glutamine by these cells (93). Whereas arginase I in healthy adult mammals is expressed almost exclusively in liver, it has recently been found to be highly induced in many tissues and cell types following exposure to a variety of cytokines and other agents (69, 74) (see "Arginine Catabolism," below).

Ornithine and citrulline must be able to readily traverse the mitochondrial membrane for the urea cycle to function efficiently. This occurs to a considerable extent via the ornithine/citrulline transporter (ORNT1), as deduced from the hyperornithinemia-hyperammonenia-homocitrullinemia (HHH) syndrome that

occurs in individuals with defects in this transporter (16, 115). The properties of ORNT1, which was cloned in 1999 (16), indicate that it corresponds to an ornithine/citrulline carrier purified from rat liver mitochondria (49). However, it has not been established that ORNT1 represents the sole transporter of ornithine and citrulline across the mitochondrial membrane. Although ORNT1 is not listed as a component of the urea cycle in many texts, its properties and strong expression in liver indicate that it should be included in descriptions of this pathway.

Consistent with its role in the urea cycle, ORNT1 expression as estimated by Northern blotting is highest in liver of both mice and humans (16). Levels of ORNT1 mRNA became elevated in livers of mice fed a high-protein diet (16), similar to the dietary regulation of urea cycle enzyme mRNAs (78). In addition, ORNT1 mRNA is strongly induced by cAMP and dexamethasone in cultured rat hepatocytes (D. Kepka-Lenhart & S. Morris, unpublished results), a response pattern very similar to that of urea cycle enzyme mRNAs (84). Although these results indicate that it is likely that changes in ornithine/citrulline transporter activity are coordinated with changes in activity of the urea cycle enzymes, this remains to be determined.

In addition to liver, ORNT1 is highly expressed in several other tissues such as kidney and brain of mouse and pancreas of humans, and lower levels of expression were detected in almost all other tissues surveyed. Thus, ORNT1 may play a role in other aspects of arginine metabolism in nonhepatic tissues (see Figure 2). Determinations of ORNT1 protein levels in liver and other tissues await the development of specific antibodies.

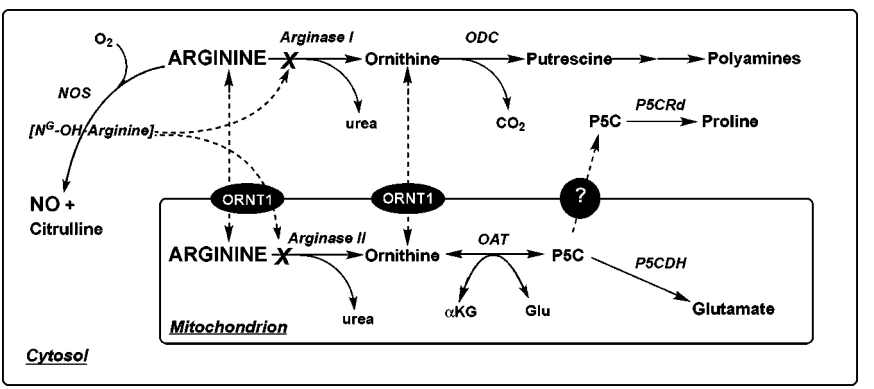

Figure 2 Metabolic roles of the arginases. Abbreviations: NOS, nitric oxide synthase; ODC, ornithine decarboxylase; OAT, ornithine aminotransferase; P5C, ??Δ^1-pyrroline-5-carboxylate; P5CRd, P5C reductase; P5CDH, P5C dehydrogenase; αKG, α-ketoglutarate; Glu, glutamate. Not all reactants or cofactors are shown. Dashed lines indicate transport. Modified from Figure 5 of Ref. (70) and reprinted by permission of Academic Press.

Transcriptional Regulation

Despite the fact that many questions remain regarding the molecular bases for transcriptional regulation of the urea cycle enzyme genes, research in this area has been slow over the past several years. Consequently, relatively little new information on this topic has emerged since it was last reviewed in 1995 (109). For example, DNA regulatory elements involved in hormonal regulation of ornithine transcarbamylase, ASS, and ASL gene expression in liver remain to be identified. Research on regulation of CPS-I transcription has continued. Prior to 1995 functional analysis of the CPS-I promoter had been confined to the region within a few hundred bp of the transcription start site (reviewed in 109). In 1995 a far upstream enhancer required for liver-specific expression and hormonal induction of the rat CPS-I gene was reported to be located at either −6.3 kb (116) or −10 kb (40). Our group has independently localized the region responsive to glucocorticoid and cyclic AMP to −6.3 kb (D. Kepka-Lenhart & S. Morris, unpublished results), corresponding to a DNase I hypersensitive site in rat liver chromatin (H. Huang & S. Morris, unpublished results). Taken together, the results are consistent with the interpretation that the reported location of the enhancer at −10 kb resulted from incorrect orientation of a HindIII fragment in the CPS-I promoter.

The −6.3 kb enhancer region is necessary and largely sufficient for liver-specific, developmental and hormonal regulation of CPS-I expression (23, 24). Detailed analyses have revealed that this enhancer region is complex, containing DNA elements that bind the transcription factors CCAAT/enhancer binding protein (C/EBP), hepatocyte nuclear factor 3 (HNF3), the glucocorticoid receptor, and AP-1 within a 714-bp region (22, 81). Both C/EBP α and β have been implicated in regulating hepatic CPS-I expression (55, 56). The AP-1 element appears to be dispensable for the normal tissue-specific, developmental and hormonal pattern of CPS-I expression, as a 496-bp fragment lacking this element confers the normal pattern of CPS-I expression when fused to a minimal CPS-I promoter (23). A cAMP response element (CRE) is present within the CPS-I enhancer region (22) but the transcription factor(s) that interact with this element have not been identified. Glucorticoid response is conferred by a glucocorticoid response unit consisting of a glucocorticoid response element, HNF3 and C/EBP elements (22). This is consistent with our earlier observation that glucocorticoid induction of CPS-I mRNA in cultured rat hepatocytes exhibited the characteristics of an indirect response to glucocorticoids (84); that is, the lag in response probably reflected the glucocorticoid-dependent induction of C/EBP and/or HNF3.

ARGININE SYNTHESIS

Intestinal-Renal Axis

Arginine is not an essential amino acid for healthy adult humans and rodents because it is synthesized in a collaboration between epithelial cells of the small intestine and the proximal tubular cells of the kidney (reviewed in 72, 95, 97, 128).

This pathway is known as the intestinal-renal axis. Briefly, citrulline is generated in the small intestine as one of the products of glutamine metabolism and released into the blood. Little or no citrulline is extracted from the blood as it passes through the liver, but citrulline is efficiently taken up by the proximal renal tubules, where it is converted to arginine which is released into the blood. Arginine is classified as a conditionally essential or semi-essential amino acid owing to conditions in which endogenous synthesis is inadequate to meet the requirements for arginine (4). Such conditions may include reduced function of the small bowel or kidney as a consequence of disease, trauma, or surgery (118), as well as conditions involving heightened protein and amino acid catabolism, such as inflammation, sepsis, or during recovery from severe trauma.

The intestinal-renal axis of arginine synthesis becomes established postnatally. The small intestine plays the predominant role in arginine synthesis in the perinatal period but gradually shifts to citrulline production and arginine degradation (11, 32, 35, 72, 126, 129). Glucocorticoids appear to play a major role in this transition around the time of weaning (39, 127).

Citrulline-NO Cycle

Interest in the metabolic fate of citrulline increased greatly following the discovery of the mammalian nitric oxide synthase (NOS) enzymes and recognition of the fact that any mechanism affecting availability of arginine represented a potential control point for regulating NO synthesis (76). The ability of many nonhepatic cell types to convert citrulline to arginine had already been known for many years (e.g., 36); indeed, virtually all cells tested have detectable levels of ASS and ASL activity (50, 96). However, the physiologic significance of this capability was unclear. It is now appreciated that many NO-producing cells recycle citrulline, one of the products of the NOS-catalyzed reaction, to arginine in a pathway termed the arginine-citrulline cycle or the citrulline-NO cycle (Figure 3). We use the latter terminology by analogy with the ornithine-urea cycle. John Vane's group demonstrated conversion of citrulline to arginine in NO-producing endothelial cells (46), and our group was the first to demonstrate that this recycling pathway was co-induced with iNOS (89). We and others have demonstrated co-induction of iNOS and the arginine biosynthetic pathway for a wide range of cell types in humans and other mammals (reviewed in 69, 73, 74). In cultured cells this co-induction appears to be restricted to ASS, as ASL expression is not induced. In the whole animal, however, both ASS and ASL are induced in parallel with iNOS (83). The basis for this difference is unknown. The induction only of ASS in cultured cells is consistent with the notion that ASS represents the rate-limiting step in the conversion of citrulline to arginine; however, the validity of this assumption has not been tested for most of the cells used in these studies. Studies of neural tissue revealed that NOS enzymes and ASS or ASL are not expressed within the same cells (2, 14, 101, 124), raising the possibility of an intercellular citrulline-NO cycle.

Inhibition of transamination by (aminooxy)acetate abolished the ability of citrulline to substitute for arginine in supporting NO synthesis in astrocytes (101),

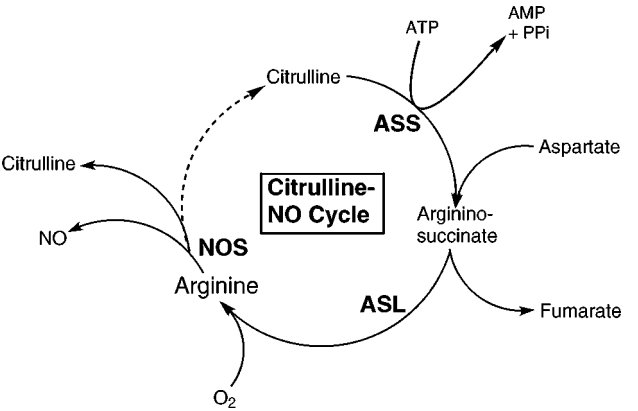

Figure 3 The citrulline-NO cycle. ASS, argininosuccinate synthetase; ASL, argininosuccinate lyase; NOS, nitric oxide synthase. The dashed line indicates that recycling of citrulline is variable and not quantitative. Modified from Figure 3 of Ref. (70) and reprinted by permission of Academic Press.

consistent with the notion that aspartate, the source of the nitrogen atom in the recycling pathway, is continuously generated by transamination. Although the implicit assumption that aspartate normally is not limiting is likely correct, there is little experimental data relevant to this assumption. In vascular smooth muscle cells, overexpression of ASS resulted in increased capacity for NO production (130), indicating that ASS activity rather than aspartate availability is limiting for arginine synthesis in these cells. Varying extracellular aspartate levels did not alter L-citrulline-dependent NO production in the chicken HD11 line (108), but this result may not be particularly relevant to most NO-producing cells because L-citrulline could support only very low levels of NO synthesis in the HD11 cells.

In contrast to the tight channeling and resultant high efficiency of the urea cycle, recycling of arginine to citrulline in nonhepatic cells is inefficient. This is clearly apparent from the fact that NO production by cultured cells is accompanied by accumulation of citrulline in the medium. Whether this inefficiency reflects a lack of channeling of citrulline from NOS to ASS or simply limiting levels of ASS activity, compared with NOS activity, or some combination of both, is unknown. Little information exists regarding the precise efficiency of citrulline-to-arginine recycling in different cells, which can be estimated by comparing the molar ratio of citrulline accumulated to stable end-products of NO (nitrate + nitrite + nitrosothiols) accumulated. For example, this ratio in the human AKN-1 cell line stimulated to express iNOS is 0.47 (65). Variable efficiency of the citrulline-NO cycle in different cell types has been inferred from differences in basal levels of ASS and ASL mRNAs, as well as differences in fold induction of these mRNAs among different cell types, but this has not been systematically investigated. However,

the fact that overexpression of ASS enhanced induced NO production in vascular smooth muscle cells (130) is consistent with this notion. A further implication of this study was that the rate of arginine uptake by stimulated vascular smooth muscle cells was insufficient to support maximal rates of NO synthesis. The ability of citrulline to replace arginine in supporting cellular NO synthesis has been used as a functional test for the existence of the recycling pathway (e.g., 5, 38, 88–90, 101, 131, 132) but is not necessarily indicative of the efficiency of this pathway because of potential differences in the availability of extracellular and intracellular citrulline pools for recycling. According to this test, it appears that L-citrulline has little or no ability to support NO synthesis in some iNOS-expressing cells (44, 108).

The contribution of the recycling pathway to induced NO synthesis is difficult to estimate, especially for cells in vivo, but it is likely that extracellular arginine provides the majority of the substrate for induced NO synthesis in many cell types. For example, induced NO synthesis is greatly reduced when cellular arginine uptake is inhibited (103, 106) or genetically ablated (86).

It is likely that induction of ASS and ASL expression in nonhepatic cells by cytokines and other inflammatory stimuli is primarily at the level of transcription. However, the transcription factors and DNA regulatory elements involved in induction of either of these genes by inflammatory stimuli have not been identified.

ARGININE CATABOLISM

One of the most exciting developments of the past several years has been the discovery of widespread and highly regulated expression of arginase activity in nonhepatic cells. The consequences of this expression have not been fully elucidated, but possible metabolic roles of the arginases include regulation of arginine availability for NO synthesis (reviewed in 12, 69, 70, 73) and production of ornithine for synthesis of polyamines, proline, and glutamate (reviewed in 74, 128) (see Figure 2). Recent results also indicate that elevated arginase activity may activate the GCN2 response in mammalian cells, probably by reducing intracellular arginine concentration (R. Ratan, personal communication). Thus, interest in arginase beyond a role in the urea cycle is growing significantly.

As noted above, arginase exists as two distinct isozymes. Unlike the primarily liver-specific pattern of arginase I expression, arginase II expression in healthy adults is nearly ubiquitous, albeit at relatively low levels (42, 75, 117). The elevation of plasma arginine levels in adult, nonstressed arginase II knockout mice (102) indicates that this enzyme contributes significantly to maintaining arginine homeostasis, consistent with tracer studies indicating that arginine degradation plays a major role in this process (17, 18). Although it has been speculated that arginase II expression in kidney might play a role in renal function, initial analyses have not revealed any apparent changes in urinary content or volume in the arginase II knockout mice (O. Shi, S. Morris, & W. O'Brien, unpublished results).

Recent studies of nonhepatic arginase expression have focused primarily on macrophages. Arginase expression in primary macrophages and macrophage cell

lines is regulated by a wide range of individual pro- and antiinflammatory cytokines and other agents—as well as by combinations thereof—and is highly complex (see, e.g., Table II in Ref. 74). This includes responses to bacterial lipopolysaccharide (LPS), IL-4, IL-10, IL-13, TGF, glucocorticoids, catecholamines, and cAMP analogues, as well as to hypoxia and trauma (1, 9, 10, 13, 20, 26, 27, 42, 43, 47, 60, 63, 64, 67, 77, 79, 80, 85, 91, 92, 99, 105, 119). cAMP strongly synergizes with LPS and cytokines in induction of the arginases. By analogy with the inducible and constitutive NOS isozymes, arginase I expression has sometimes been characterized as inducible and arginase II expression as constitutive, but this is an oversimplification. Although early studies did not identify the arginase isoform(s) that were induced, it is now known that, depending on the stimulus, cell type and animal species, increased arginase activity can reflect increased expression of arginase I, arginase II, or both isozymes. In all cases studied to date increased arginase activities and expression reflect increases in levels of the corresponding mRNAs, most likely owing to increased transcription of the arginase genes.

Consequences of high arginase activity in macrophages can include reductions in NO production (19, 41, 98, 110), potentially limiting host defenses against NO-sensitive pathogens. Based merely on the K_m's of the enzymes for arginine (2-20 mM for arginase, 2-20 μM for the NOS enzymes), it might not be expected that arginase would have a significant impact on cellular NO production. However, the V_{max} of arginase at physiologic pH is more than 1000 times that of the NOS enzymes, resulting in similar rates of arginine usage at physiologic arginine concentrations (128). It should be borne in mind that not all levels of arginase expression in macrophages result in inhibition of NO production (53) and that some stimuli that induce arginase expression may independently inhibit iNOS expression. In addition, N^G-hydroxy-arginine, an intermediate in NO synthesis, can accumulate to sufficient levels to inhibit arginase activity (15). These considerations make it difficult to predict with precision the consequences of co-expressing arginases and NOS enzymes.

It should be emphasized that arginase also can modulate NO synthesis in noninflammatory conditions. The best examples of this are the inhibitory effects of arginase on NO synthesis in smooth muscle, as revealed by the use of newly developed arginase inhibitors. Thus, arginase inhibitors enhance relaxation of smooth muscle in sphincter muscle (3) and in penile tissue (28, 54). The latter results reveal a new physiologic role for arginase in erectile function and indicate that it may be a useful target for development of drugs to treat erectile dysfunction. As these effects of arginase presumably reflect reduced availability of substrate for cNOS, nutritional supplementation with arginine may improve erectile function, particularly in aging individuals (68, 133).

It is important to recognize that the impact of changes in arginase expression is not limited to effects on NO synthesis. We and others have shown recently that arginase activity can be a limiting factor for synthesis of polyamines and proliferation in some cells (53, 61, 62, 104, 123). These observations have significant implications for tumorigenesis, atherogenesis, and wound healing.

Elucidation of the transcriptional mechanisms involved in these complex patterns of arginase expression in macrophages and other nonhepatic cell types is just beginning. The 5′ flanking region of the murine arginase I gene contains elements involved in transcriptional responses to IL-4, cAMP, TGFβ, dexamethasone, and LPS (M. Gray & S. M. Morris, unpublished results). The IL-4 response is conferred by a STAT6 element located approximately 3.0 kb upstream of the transcription start site; identification of other elements is currently in progress. Elements involved in transcriptional responses to LPS and cAMP are located in the 5′ flanking region of the murine arginase II gene (M. Gray & S. M. Morris, unpublished results). Among these are an Sp1 site at approximately −50 bp that is required for the response to LPS but not to cAMP. Unlike many LPS-inducible genes, the LPS-responsive region of the arginase II promoter contains no NFκB elements.

PERSPECTIVES AND FUTURE DIRECTIONS

Although we have clearly learned a great deal about the function and regulation of the urea cycle since it was first described 70 years ago (58), gaps not only remain in our understanding of this pathway but new information on the constituent enzymes of this pathway continues to emerge, particularly as regards their regulation and metabolic roles in nonhepatic cells. As examples of gaps in our knowledge, two striking yet incompletely understood features of the urea cycle enzymes are their high level expression in adult liver and the remarkable coordination of changes in levels of the individual enzymes in vivo. Although transcription is a major component of the changes in expression of these enzymes, analyses of the promoter regions of their genes to date have failed to reveal a common basis for their high-level expression in liver or for their coordinate responses to changes in dietary protein intake. The element that comes closest to being a common regulatory element is the C/EBP binding site, which is present in at least one copy in three of the five urea cycle enzyme genes (109). As the efficient channeling of reactants between successive enzymes of this pathway implies some close physical interaction between these enzymes in a metabolon (121), it is tempting to speculate that this association may play some role in coordinating their expression at the protein level; for example, enzymes that are in excess of the amount required for the metabolon may be rapidly degraded.

The paucity of quantitative information regarding fluxes through many of the arginine metabolic pathways indicated in Figure 1 remains a major obstacle to understanding the physiologic or pathophysiologic roles of the arginine metabolic enzymes in individual cell types and organs. When evaluating the results of metabolic studies using cultured cells, it should be borne in mind that arginine levels in standard tissue culture media are supraphysiologic. For example, arginine concentrations in DMEM and RPMI media are approximately 0.4 and 1.2 mM, respectively, whereas plasma arginine concentrations in adult humans and mice are approximately 0.1 mM and in adult rats are approximately 0.25 mM.

With the exception of the urea cycle and NO synthesis, we know relatively little about how activities of these pathways vary in individuals with age, variations in diet, or in response to injury or disease. For example, it has been known for nearly 40 years that changes in levels of individual amino acids such as arginine, citrulline, and leucine result in altered expression of arginine metabolic enzymes (reviewed in 72), but the basis for this remains unknown. Results of recent work on regulation of mammalian gene expression by individual amino acids (e.g., 37, 45, 51) may shed light on this phenomenon. Moreover, identification of which of the various enzymes involved in arginine metabolism are expressed in different organs and cell types is incomplete and thus remains an active area of investigation. For example, strong expression of type I arginase in nonhepatic cells in response to inflammatory stimuli and even the existence of arginine decarboxylase and agmatinase in mammalian cells was discovered less than 8 years ago, indicating that we still have much to learn in this area. However, the increasing application of DNA microarray analysis should allow us to begin addressing this problem. Even so, microarray data represents only a starting point because it does not provide information about protein levels or activities of the enzymes, nor does it identify which cell types in specific organs express the enzymes.

Many questions raised about the roles and regulation of the urea cycle enzymes in this forum in 1992 (72) have yet to be fully addressed. Moreover, a wealth of new information about expression of some of these enzymes has raised new questions. This is indeed a time of "new and exciting developments" in arginine metabolism (6). Thus, the future continues to hold great promise for exciting new advances in nutrition, physiology, biochemistry, immunology, and oncology for investigators who choose to enter into this rich area of investigation.

ACKNOWLEDGMENTS

The author expresses his gratitude to the past and present members of his laboratory and to his collaborators who have contributed to much of the work cited here. Work in the author's laboratory was supported in part by NIH grant RO1 GM57384.

LITERATURE CITED

1. Albina JE, Henry WL Jr, Mastrofrancesco B, Martin BA, Reichner JS. 1995. Macrophage activation by culture in an anoxic environment. *J. Immunol.* 155:4391–96
2. Arnt-Ramos LR, O'Brien WE, Vincent SR. 1992. Immunohistochemical localization of argininosuccinate synthetase in the rat brain in relation to nitric oxide synthase-containing neurons. *Neuroscience* 51:773–89
3. Baggio R, Emig FA, Christianson DW, Ash DE, Chakder S, Rattan S. 1999. Biochemical and functional profile of a newly developed potent and isozyme-selective arginase inhibitor. *J. Pharmacol. Exp. Ther.* 290:1409–16

4. Barbul A. 1986. Arginine: biochemistry, physiology, and therapeutic implications. *J. Parenter. Enter. Nutr.* 10:227–38
5. Baydoun AR, Bogle RG, Pearson JD, Mann GE. 1994. Discrimination between citrulline and arginine transport in activated macrophages: inefficient synthesis of NO from recycling of citrulline to arginine. *Br. J. Pharmacol.* 112:487–92
6. Beaumier L, Castillo L, Yu Y-M, Ajami AM, Young VR. 1996. Arginine: new and exciting developments for an "old" amino acid. *Biomed. Environ. Sci.* 9:296–315
7. Beliveau Carey G, Cheung CW, Cohen NS, Brusilow S, Raijman L. 1993. Regulation of urea and citrulline synthesis under physiological conditions. *Biochem. J.* 292:241–47
8. Deleted in proof
9. Bernard AC, Fitzpatrick EA, Maley ME, Gellin GL, Tsuei BJ, et al. 2000. Beta adrenoreceptor regulation of macrophage arginase activity. *Surgery* 127:412–18
10. Bernard AC, Mistry SK, Morris SM Jr., O'Brien WE, Tsuei BJ, et al. 2001. Alterations in arginine metabolic enzymes in trauma. *Shock* 15:215–19
11. Blachier F, M'Rabet-Touil H, Posho L, Darcy-Vrillon B, Duee P-H. 1993. Intestinal arginine metabolism during development. *Eur. J. Biochem.* 216:109–17
12. Boucher JL, Moali C, Tenu JP. 1999. Nitric oxide biosynthesis, nitric oxide synthase inhibitors and arginase competition for L-arginine utilization. *Cell. Mol. Life Sci.* 55:1015–28
13. Boutard V, Havouis R, Fougueray B, Philippe C, Moulinoux JP, Baud L. 1995. Transforming growth factor-β stimulates arginase activity in macrophages. *J. Immunol.* 155:2077–84
14. Braissant O, Gotoh T, Loup M, Mori M, Bachmann C. 1999. L-arginine uptake, the citrulline-NO cycle and arginase II in the rat brain: an in situ hybridization study. *Molec. Brain Res.* 70:231–41
15. Buga GM, Singh R, Pervin S, Rogers NE, Schmitz DA, et al. 1996. Arginase activity in endothelial cells: Inhibition by N^G-hydroxyarginine during high-output nitric oxide production. *Am. J. Physiol. Heart Circ. Physiol.* 271:H1988–98
16. Camacho JA, Obie C, Biery B, Goodman BK, Hu C-A, et al. 1999. Hyperornithinaemia-hyperammonaemia-homocitrullinemia syndrome is caused by mutations in a gene encoding a mitochondrial ornithine transporter. *Nat. Genet.* 22:151–58
17. Castillo L, Chapman TE, Sanchez M, Yu YM, Burke JF, et al. 1993. Plasma arginine and citrulline kinetics in adults given adequate and arginine-free diets. *Proc. Natl. Acad. Sci. USA* 90:7749–53
18. Castillo L, Sanchez M, Chapman TE, Ajami A, Burke JF, Young VR. 1994. The plasma flux and oxidation rate of ornithine adaptively decline with restricted arginine intake. *Proc. Natl. Acad. Sci. USA* 91:6393–97
19. Chang C-I, Liao JC, Kuo L. 1998. Arginase modulates nitric oxide production in activated macrophages. *Am. J. Physiol. Heart Circ. Physiol.* 43:H342–48
20. Chang C-I, Zoghi B, Liao JC, Kuo L. 2000. The involvement of tyrosine kinases, cyclic AMP/protein kinase A, and p38 mitogen-activated protein kinase in IL-13-mediated arginase I induction in macrophages: Its implications in IL-13-inhibited nitric oxide production. *J. Immunol.* 165:2134–41
21. Cheung C-W, Cohen NS, Raijman L. 1989. Channeling of urea cycle intermediates *in situ* in permeabilized hepatocytes. *J. Biol. Chem.* 264:4038–44
22. Christoffels VM, Grange T, Kaestner KH, Cole TJ, Darlington GJ, et al. 1998. Glucocorticoid receptor, C/EBP, HNF3, and protein kinase A coordinately activate the glucocorticoid response unit of the carbamoylphosphate synthetase I gene. *Mol. Cell. Biol.* 18:6305–15
23. Christoffels VM, Habets PEMH, Das AT, Clout DEW, van Roon MA, et al. 2000. A single regulatory module of the carbamylphosphate synthetase I gene

executes its hepatic program of expression. *J. Biol. Chem.* 275:40020–27
24. Christoffels VM, van den Hoff MJ, Lamers MC, van Roon MA, de Boer PA, et al. 1996. The upstream regulatory region of the carbamoyl-phosphate synthetase I gene controls its tissue-specific, developmental, and hormonal regulation in vivo. *J. Biol. Chem.* 271:31243–50
25. Cohen NS, Cheung C-W, Raijman L. 1987. Channeling of extramitochondrial ornithine to matrix ornithine transcarbamylase. *J. Biol. Chem.* 262:203–8
26. Corraliza IM, Modolell M, Ferber E, Soler G. 1997. Involvement of protein kinase A in the induction of arginase in murine bone marrow-derived macrophages. *Biochim. Biophys. Acta* 1334:123–28
27. Deleted in proof
27a. Corvi MM, Soltys CL, Berthiaume LG. 2001. Regulation of mitochondrial carbamoylphosphate synthetase 1 activity by active site fatty acylation. *J. Biol. Chem.* 276:45704–12
28. Cox JD, Kim NN, Traish AM, Christianson DW. 1999. Arginase-boronic acid complex highlights a physiological role in erectile function. *Nat. Struct. Biol.* 6:1043–47
29. Curthoys NP, Watford M. 1995. Regulation of glutaminase activity and glutamine metabolism. *Annu. Rev. Nutr.* 15:133–59
30. Cynober L, Boucher JL, Vasson M-P. 1995. Arginine metabolism in mammals. *Nutr. Biochem.* 6:402–13
31. Daghigh F, Fukuto JM, Ash DE. 1994. Inhibition of rat liver arginase by an intermediate in NO biosynthesis, N^G-hydroxy-L-arginine: implications for the regulation of nitric oxide biosynthesis by arginase. *Biochem. Biophys. Res. Commun.* 202:174–80
32. de Jonge WJ, Dingemanse MA, De Boer PA, Lamers WH, Moorman AFM. 1998. Arginine-metabolizing enzymes in the developing rat small intestine. *Pediatric Res.* 43:442–51
33. Dhahbi JM, Mote PL, Wingo J, Rowley BC, Cao SX, et al. 2001. Caloric restriction alters the feeding response of key metabolic enzyme genes. *Mech. Ageing Dev.* 122:1033–48
34. Dhahbi JM, Mote PL, Wingo J, Tillman JB, Walford RL, Spindler SR. 1999. Calories and aging alter gene expression for gluconeogenic, glycolytic, and nitrogen-metabolizing enzymes. *Am. J. Physiol. Endocrinol. Metab.* 277:E352–60
35. Dugan ME, Knabe DA, Wu G. 1995. The induction of citrulline synthesis from glutamine in enterocytes of weaned pigs is not due primarily to age or change in diet. *J. Nutr.* 125:2388–93
36. Eagle H. 1959. Amino acid metabolism in mammalian cell cultures. *Science* 130:432–37
37. Fafournoux P, Bruhat A, Jousse C. 2000. Amino acid regulation of gene expression. *Biochem. J.* 351:1–12
38. Flodstrom M, Morris SM Jr, Eizirik DL. 1996. Role of the citrulline-nitric oxide cycle in the functional response of adult human and rat pancreatic islets to cytokines. *Cytokine* 8:642–50
39. Flynn NE, Meininger CJ, Kelly K, Ing NH, Morris SM Jr, Wu G. 1999. Glucocorticoids mediate the enhanced expression of intestinal type II arginase and argininosuccinate lyase in postweaning pigs. *J. Nutr.* 129:799–803
40. Goping IS, Lamontagne S, Shore GC, Nguyen M. 1995. A gene-type-specific enhancer regulates the carbamyl phosphate synthetase I promoter by cooperating with the proximal GAG activating element. *Nucleic Acids Res.* 23:1717–21
41. Gotoh T, Mori M. 1999. Arginase II downregulates nitric oxide (NO) production and prevents NO-mediated apoptosis in murine macrophage-derived RAW 264.7 cells. *J. Cell Biol.* 144:427–34
42. Gotoh T, Sonoki T, Nagasaki A, Terada K, Takiguchi M, Mori M. 1996. Molecular cloning of cDNA for nonhepatic mitochondrial arginase (arginase II) and comparison of its induction with nitric oxide

synthase in a murine macrophage-like cell line. *FEBS Lett* 395:119–22

43. Hammermann R, Hey C, Schafer N, Racke K. 2000. Phosphodiesterase inhibitors and forskolin up-regulate arginase activity in rabbit alveolar macrophages. *Pulmonary Pharmacol. Therap.* 13:141–47
44. Hammermann R, Bliesener N, Mossner J, Klasen S, Wiesinger H, et al. 1998. Inability of rat alveolar macrophages to recycle L-citrulline to L-arginine despite induction of argininosuccinate synthetase mRNA and protein, and inhibition of nitric oxide synthesis by exogenous citrulline. *Naunyn-Schmiedebergs Arch. Pharmacol.* 358:601–7
45. Harding HP, Novoa II, Zhang Y, Zeng H, Wek R, et al. 2000. Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol. Cell* 6:1099–108
46. Hecker M, Sessa WC, Harris HJ, Anggard EE, Vane JR. 1990. The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor: cultured endothelial cells recycle L-citrulline to L-arginine. *Proc. Natl. Acad. Sci. USA* 87:8612–16
47. Hey C, Wessler I, Racke K. 1995. Nitric oxide synthase activity is inducible in rat, but not rabbit alveolar macrophages, with a concomitant reduction in arginase activity. *Naunyn-Schmiedebergs Arch. Pharmacol.* 351:651–59
48. Iijima M, Jalil A, Begum L, Yasuda T, Yamaguchi N, et al. 2001. Pathogenesis of adult-onset type II citrullinemia caused by deficiency of citrin, a mitochondrial solute carrier protein: tissue and subcellular localization of citrin. *Adv. Enzyme Regul.* 41:325–42
49. Indiveri C, Tonazzi A, Stipani I, Palmieri F. 1997. The purified and reconstituted ornithine/citrulline carrier from rat liver mitochondria: electrical nature and coupling of the exchange reaction with H^+ translocation. *Biochem. J.* 327:349–56

49a. Ignarro LJ, ed. 2000. *Nitric Oxide. Biology and Pathobiology*. San Diego, CA: Academic Press

50. Jackson MJ, Beaudet AL, O'Brien WE. 1986. Mammalian urea cycle enzymes. *Annu. Rev. Genet.* 20:431–64
51. Jefferson LS, Kimball SR. 2001. Amino acid regulation of gene expression. *J. Nutr.* 131:2460S–66S
52. Jenkinson CP, Grody WW, Cederbaum SD. 1996. Comparative properties of arginases. *Comp. Biochem. Physiol.* 114B:107–32
53. Kepka-Lenhart D, Mistry SK, Wu G, Morris SM Jr. 2000. Arginase I: a limiting factor for nitric oxide and polyamine synthesis by activated macrophages? *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 279:R2237–42
54. Kim NN, Cox JD, Baggio RF, Emig FA, Mistry SK, et al. 2001. Probing erectile function: S-(2-boronoethyl)-L-cysteine binds to arginase as a transition state analogue and enhances smooth muscle relaxation in human penile corpus cavernosum. *Biochemistry* 40:2678–88
55. Kimura T, Chowdhury S, Tanaka T, Shimizu A, Iwase K, et al. 2001. CCAAT/enhancer-binding protein beta is required for activation of genes for ornithine cycle enzymes by glucocorticoids and glucagon in primary-cultured hepatocytes. *FEBS Lett.* 494:105–11
56. Kimura T, Christoffels VM, Chowdhury S, Iwase K, Matsuzaki H, et al. 1998. Hypoglycemia-associated hyperammonemia caused by impaired expression of ornithine cycle enzyme genes in C/EBPα knockout mice. *J. Biol. Chem.* 273:27505–10
57. Kobayashi K, Sinasac DS, Iijima M, Boright AP, Begum L, et al. 1999. The gene mutated in adult-onset type II citrullinaemia encodes a putative mitochondrial carrier protein. *Nat. Genet.* 22:159–63
58. Krebs HA, Henseleit K. 1932. Untersuchungen uber die harnstoffbildung im tierkorper. *Hoppe-Seylers Z. Physiol. Chem.* 210:33–66

59. Latasa MU, Carretero MV, Garcia-Trevijano ER, Torres L, Mato JM, Avila MA. 2000. Identification of argininosuccinate lyase as a hypoxia-responsive gene in rat hepatocytes. *J. Hepatol.* 33:709–15
60. Deleted in proof
61. Li H, Meininger CJ, Hawker JR Jr, Haynes TE, Kepka-Lenhart D, et al. 2001. Regulatory role of arginase I and II in syntheses of nitric oxide, polyamines and proline in endothelial cells. *Am. J. Physiol. Endocrinol. Metab.* 280:E75–82
62. Li H, Meininger CJ, Hawker JR Jr, Kelly KA, Morris SM Jr, Wu G. 2002. Activities of arginase I and II are limiting for endothelial cell proliferation. *Am. J. Physiol.* 282:R64–69
63. Louis CA, Mody V, Henry WL Jr, Reichner JS, Albina JE. 1999. Regulation of arginase isoforms I and II by IL-4 in cultured murine peritoneal macrophages. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 276:R237–42
64. Louis CA, Reichner JS, Henry WL Jr., Mastrofrancesco B, Gotoh T, et al. 1998. Distinct arginase isoforms expressed in primary and transformed macrophages: regulation by oxygen tension. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 274:R775–82
65. Marzinzig M, Nussler AK, Stadler J, Marzinzig E, Bartlen W, et al. 1997. Improved methods to measure end products of nitric oxide in biological fluids: Nitrite, nitrate, and S-nitrosothiols. *Nitric Oxide* 1:177–89
66. Mistry SK, Burwell TJ, Chambers RM, Rudolph-Owens L, Spaltmann F, et al. 2002. Cloning of human agmatinase. An alternate path for polyamine synthesis induced in liver by hepatitis B virus. *Am. J. Physiol.* 282:G375–81
67. Deleted in proof
68. Moody JA, Vernet D, Laidlaw S, Rajfer J, Gonzalez-Cadavid NF. 1997. Effects of long-term oral administration of L-arginine on the rat erectile response. *J. Urol.* 158:942–47
69. Mori M, Gotoh T. 2000. Regulation of nitric oxide production by arginine metabolic enzymes. *Biochem. Biophys. Res. Commun.* 275:715–19
70. Mori M, Gotoh T. 2000. Relationship between arginase activity and nitric oxide production. See Ref. 49a, pp. 199–208
71. Morimoto BH, Brady JF, Atkinson DE. 1990. Effect of level of dietary protein on arginine-stimulated citrulline synthesis. Correlation with mitochondrial N-acetylglutamate concentrations. *Biochem. J.* 272:671–75
72. Morris SM Jr. 1992. Regulation of enzymes of urea and arginine synthesis. *Annu. Rev. Nutr.* 12:81–101
73. Morris SM Jr. 1999. Arginine synthesis, metabolism, and transport: Regulators of nitric oxide synthesis. In *Cellular and Molecular Biology of Nitric Oxide*, ed. JD Laskin, DL Laskin, pp. 57–85. New York: Marcel Dekker
74. Morris SM Jr. 2000. Regulation of arginine availability and its impact on NO synthesis. See Ref. 49a, pp. 187–97
75. Morris SM Jr, Bhamidipati D, Kepka-Lenhart D. 1997. Human type II arginase: sequence analysis and tissue-specific expression. *Gene* 193:157–61
76. Morris SM Jr, Billiar TR. 1994. New insights into the regulation of induced nitric oxide synthesis. *Am. J. Physiol. Endocrinol. Metab.* 266:E829–39
77. Morris SM Jr, Kepka-Lenhart D, Chen LC. 1998. Differential regulation of arginases and inducible nitric oxide synthase in murine macrophage cells. *Am. J. Physiol. Endocrinol. Metab.* 275:E740–47
78. Morris SM Jr, Moncman CL, Rand KD, Dizikes GJ, Cederbaum SD, O'Brien WE. 1987. Regulation of mRNA levels for five urea cycle enzymes in rat liver by diet, cyclic AMP, and glucocorticoids. *Arch. Biochem. Biophys.* 256:343–53
79. Munder M, Eichmann K, Modolell M. 1998. Alternative metabolic states in murine macrophages reflected by the

nitric oxide synthase/arginase balance: Competitive regulation by $CD4^+$ cells correlates with Th1/Th2 phenotype. *J. Immunol.* 160:5347–54

80. Munder M, Eichmann K, Moran JM, Centeno F, Soler G, Modollel M. 1999. Th1/Th2-regulated expression of arginase isoforms in murine macrophages and dendritic cells. *J. Immunol.* 163:3771–77
81. Musa DMAA, Kobayashi K, Yasuda I, Iijima M, Christoffels VM, et al. 1999. Involvement of a cis-acting element in the suppression of carbamoyl phosphate synthetase I gene expression in the liver of carnitine-deficient mice. *Mol. Genet. Metab.* 68:346–56
82. Deleted in proof
83. Nagasaki A, Gotoh T, Takeya M, Yu Y, Takiguchi M, et al. 1996. Coinduction of nitric oxide synthase, argininosuccinate synthetase, and argininosuccinate lyase in lipopolysaccharide-treated rats. RNA blot, immunoblot, and immunohistochemical analyses. *J. Biol. Chem.* 271:2658–62
84. Nebes VL, Morris SM Jr. 1988. Regulation of messenger ribonucleic acid levels for five urea cycle enzymes in cultured rat hepatocytes. Requirements for cyclic adenosine monophosphate, glucocorticoids, and ongoing protein synthesis. *Mol. Endocrinol.* 2:444–51
85. Deleted in proof
86. Nicholson B, Manner CK, Kleeman J, MacLeod CL. 2001. Sustained nitric oxide production in macrophages requires the arginine transporter CAT2. *J. Biol. Chem.* 276:15881–85
87. Nissim I, Yudkoff M, Brosnan JT. 1996. Regulation of [15N]urea synthesis from [5-15N]glutamine. Role of pH, hormones, and pyruvate. *J. Biol. Chem.* 271:31234–42
88. Norris KA, Schrimpf JE, Flynn JL, Morris SM Jr. 1995. Enhancement of macrophage microbicidal activity: Supplemental arginine and citrulline augment nitric oxide production in murine peritoneal macrophages and promote intracellular killing of *Trypanosoma cruzi*. *Infect. Immun.* 63:2793–96
89. Nussler AK, Billiar TR, Liu ZZ, Morris SM Jr. 1994. Coinduction of nitric oxide synthase and argininosuccinate synthetase in a murine macrophage cell line. Implications for regulation of nitric oxide production. *J. Biol. Chem.* 269:1257–61
90. Nussler AK, Liu ZZ, Hatakeyama K, Geller DA, Billiar TR, Morris SM Jr. 1996. A cohort of supporting metabolic enzymes is coinduced with nitric oxide synthase in human tumor cell lines. *Cancer Lett.* 103:79–84
91. Ochoa JB, Bernard AC, Mistry SK, Morris SM Jr., Figert PL, et al. 2000. Trauma increases extrahepatic arginase activity. *Surgery* 127:419–26
92. Ochoa JB, Bernard AC, O'Brien WE, Griffen MM, Maley ME, et al. 2001. Arginase I expression and activity in human mononuclear cells after injury. *Ann. Surg.* 233:393–99
93. O'Sullivan D, Brosnan JT, Brosnan ME. 1998. Hepatic zonation of the catabolism of arginine and ornithine in the perfused rat liver. *Biochem. J.* 330:627–32
94. Palmieri L, Pardo B, Lasorsa FM, del Arco A, Kobayashi K, et al. 2001. Citrin and aralar1 are Ca(2+)-stimulated aspartate/glutamate transporters in mitochondria. *EMBO J.* 20:5060–69
95. Rabier D, Kamoun P. 1995. Metabolism of citrulline in man. *Amino Acids* 9:299–316
96. Ratner S. 1973. Enzymes of arginine and urea synthesis. *Adv. Enzymol.* 39:1–90
97. Reyes AA, Karl IE, Klahr S. 1994. Role of arginine in health and in renal disease. *Am. J. Physiol. Renal Physiol.* 267:F331–46
98. Rutschman R, Lang R, Hesse M, Ihle JN, Wynn TA, Murray PJ. 2001. Cutting edge: Stat6-dependent substrate depletion regulates nitric oxide production. *J. Immunol.* 166:2173–77
99. Ryan JL, Yohe WB, Morrison DC. 1980.

Stimulation of peritoneal cell arginase by bacterial lipopolysaccharide. *Am. J. Pathol.* 99:451–62
100. Saheki T, Li MX, Kobayashi K. 2000. Antagonizing effect of AP-1 on glucocorticoid induction of urea cycle enzymes: a study of hyperammonemia in carnitine-deficient, juvenile visceral steatosis mice. *Mol. Genet. Metab.* 71:545–51
101. Schmidlin A, Wiesinger H. 1998. Argininosuccinate synthetase: localization in astrocytes and role in the production of glial nitric oxide. *Glia* 24:428–36
102. Shi O, Morris SM Jr, Zoghbi H, Porter CW, O'Brien WE. 2001. Generation of a mouse model for arginase II deficiency by targeted disruption of the arginase II gene. *Mol. Cell. Biol.* 21:811–13
103. Simmons WW, Closs EI, Cunningham JM, Smith TW, Kelly RA. 1996. Cytokines and insulin induce cationic transporter (CAT) expression in cardiac myocytes. *J. Biol. Chem.* 271:11694–702
104. Singh R, Pervin S, Karimi A, Cederbaum S, Chaudhuri G. 2000. Arginase activity in human breast cancer cell lines: N^{ω}-hydroxy-L-arginine selectively inhibits cell proliferation and induces apoptosis in MDA-MB-468 cells. *Cancer Res.* 60:3305–12
105. Sonoki T, Nagasaki A, Gotoh T, Takiguchi M, Takeya M, et al. 1997. Coinduction of nitric-oxide synthase and arginase I in cultured rat peritoneal macrophages and rat tissues *in vivo* by lipopolysaccharide. *J. Biol. Chem.* 272:3689–93
106. Stevens BR, Kakuda DK, Yu K, Waters M, Vo CB, Raizada MK. 1996. Induced nitric oxide synthesis is dependent on induced alternatively spliced CAT-2 encoding L-arginine transport in brain astrocytes. *J. Biol. Chem.* 271:24017–22
107. Stewart PM, Walser M. 1980. Short term regulation of ureagenesis. *J. Biol. Chem.* 255:5270–80
108. Su C-L, Austic RE. 1999. The recycling of L-citrulline to L-arginine in a chicken macrophage cell line. *Poultry Sci.* 78:353–55
109. Takiguchi M, Mori M. 1995. Transcriptional regulation of genes for ornithine cycle enzymes. *Biochem. J.* 312:649–59
110. Tenu J-P, Lepoivre M, Moali C, Brollo M, Mansuy D, Boucher J-L. 1999. Effects of the new arginase inhibitor N^{ω}-hydroxy-nor-L-arginine on NO synthase activity in murine macrophages. *Nitric Oxide* 3:427–38
111. Tillman JB, Dhahbi JM, Mote PL, Walford RL, Spindler SR. 1996. Dietary calorie restriction in mice induces carbamyl phosphate synthetase I gene transcription tissue specifically. *J. Biol. Chem.* 271:3500–6
112. Tomomura M, Imamura Y, Horiuchi M, Koizumi T, Nikaido H, et al. 1992. Abnormal expression of urea cycle enzyme genes in juvenile visceral steatosis (jvs) mice. *Biochim. Biophys. Acta* 1138:167–71
113. Tomomura M, Imamura Y, Tomomura A, Horiuchi M, Saheki T. 1994. Abnormal gene expression and regulation in the liver of jvs mice with systemic carnitine deficiency. *Biochim. Biophys. Acta* 1226:307–14
114. Tomomura M, Tomomura A, Dewan MA, Saheki T. 1996. Long-chain fatty acids suppress the induction of urea cycle enzyme genes by glucocorticoid action. *FEBS Lett.* 399:310–12
115. Valle D, Simell O. 2001. The hyperornithinemias. In *The Metabolic & Molecular Bases of Inherited Disease*, ed. CR Scriver, AL Beaudet, WS Sly, D Valle, B Childs, et al, pp. 1857–95. New York: McGraw-Hill
116. van den Hoff MJ, van de Zande LP, Dingemanse MA, Das AT, Labruyere W, et al. 1995. Isolation and characterization of the rat gene for carbamoylphosphate synthetase I. *Eur. J. Biochem.* 228:351–61
117. Vockley JG, Jenkinson CP, Shukula H, Kern RM, Grody WW, Cederbaum SC. 1996. Cloning and characterization of the

human type II arginase gene. *Genomics* 38:118–23
118. Wakabayashi Y, Yamada E, Yoshida T, Takahashi H. 1994. Arginine becomes an essential amino acid after massive resection of rat small intestine. *J. Biol. Chem.* 269:32667–71
119. Wang WW, Jenkinson CP, Griscavage JM, Kern RM, Arabolos NS, et al. 1995. Co-induction of arginase and nitric oxide synthase in murine macrophages activated by lipopolysaccharide. *Biochem. Biophys. Res. Commun.* 210:1009–16
120. Waterlow JC. 1999. The mysteries of nitrogen balance. *Nutr. Res. Rev.* 12:25–54
121. Watford M. 1989. Channeling in the urea cycle: a metabolon spanning two compartments. *Trends Biochem. Sci.* 14:313–14
122. Watford M. 1993. Hepatic glutaminase expression: relationship to kidney-type glutaminase and to the urea cycle. *FASEB J.* 7:1468–74
123. Wei LH, Wu G, Morris SM Jr., Ignarro LJ. 2001. Elevated arginase I expression in rat aortic smooth muscle cells increases cell proliferation. *Proc. Natl. Acad. Sci. USA* 98:9260–64
124. Wiesinger H. 2001. Arginine metabolism and the synthesis of nitric oxide in the nervous system. *Prog. Neurobiol.* 64:365–91
125. Wu G. 1995. Urea synthesis in enterocytes of developing pigs. *Biochem. J.* 312:717–23
126. Wu G, Knabe DA, Flynn NE. 1994. Synthesis of citrulline from glutamine in pig enterocytes. *Biochem. J.* 299:115–21
127. Wu G, Meininger CJ, Kelly K, Watford M, Morris SM Jr. 2000. A cortisol surge mediates the enhanced expression of pig intestinal pyrroline-5-carboxylate synthase during weaning. *J. Nutr.* 130:1914–19
128. Wu G, Morris SM Jr. 1998. Arginine metabolism: nitric oxide and beyond. *Biochem. J.* 336:1–17
129. Wu R-Y, Borbolla AG, Knabe DA. 1994. The uptake of glutamine and release of arginine, citrulline and proline by the small intestine of developing pigs. *J. Nutr.* 124:2437–44
130. Xie L, Gross SS. 1997. Argininosuccinate synthetase overexpression in vascular smooth muscle cells potentiates immunostimulant-induced NO production. *J. Biol. Chem.* 272:16624–30
131. Zhang WY, Gotoh T, Oyadomari S, Mori M. 2000. Coinduction of inducible nitric oxide synthase and arginine recycling enzymes in cytokine-stimulated PC12 cells and high output production of nitric oxide. *Brain Res. Mol. Brain Res.* 83:1–8
132. Zhang WY, Takiguchi M, Koshiyama Y, Gotoh T, Nagasaki A, et al. 1999. Expression of citrulline-nitric oxide cycle in lipopolysaccharide and cytokine-stimulated rat astroglioma C6 cells. *Brain Res.* 849:78–84
133. Zorgniotti AW, Lizza EF. 1994. Effect of large doses of the nitric oxide precursor, L-arginine, on erectile dysfunction. *Int. J. Impotence Res.* 6:33–36