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REGULATION OF ENZYMES OF UREA AND ARGININE SYNTHESIS

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INTRODUCTION

Sixty years ago Krebs & Henseleit first described the ornithine-urea cycle (67), a paradigm for the elucidation of other metabolic cycles. The urea cycle is an essential metabolic pathway for disposal of the toxic metabolite ammonia in most terrestrial vertebrates, whereas in marine elasmobranchs (sharks, skates, and rays) the urea synthesized by this pathway is used for osmoregulation (3). The urea cycle is catalyzed by five enzymes—carbamyl phosphate synthetase I [CPS-I; carbamoyl-phosphate synthetase (ammonia), EC 6.3.4.16], ornithine transcarbamylase (OTC; carbamoylphosphate: L-ornithine carbamoyltransferase, EC 2.1.3.3), argininosuccinate synthetase

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[AS; L-citrulline: L-aspartate ligase (AMP-forming), EC 6.3.4.5], argininosuccinate lyase (AL; L-argininosuccinate arginine-lyase, EC 4.3.2.1), and arginase (L-arginine ureohydrolase, EC 3.5.3.1)—which effect the net conversion of two molecules of ammonia and one of bicarbonate into urea at the expense of four high energy phosphate bonds. Each enzyme is composed of a single type of polypeptide chain encoded by a single-copy nuclear gene (15, 59). Kinetic and structural features of these enzymes and clinical aspects of inherited defects in members of the urea cycle have been reviewed recently (5, 15, 59, 103).

The first two enzymes of the urea cycle are located within the mitochondrial matrix, and the remaining three enzymes are cytosolic. These enzymes appear to be associated with one another so that substrates and products move from one enzyme to another by channeling rather than by simple diffusion (145). Within the liver, the urea cycle enzymes are localized predominantly within periportal, rather than perivenous, hepatocytes (79). This spatial distribution, which can be modulated by diet and hormones (82), has functional significance for ammonia and glutamine metabolism (45, 79).

Although the urea cycle is generally thought of as a detoxification pathway for disposal of ammonium, the fact that one mole of bicarbonate also is disposed of for each two moles of ammonium has led to the notion that the urea cycle also is a mechanism for maintaining pH homeostasis via regulation of bicarbonate levels (4), a proposal that has not been accepted universally (42, 143). More recently, the urea cycle has been viewed also as a component of an intercellular glutamine cycle (45). Thus, even sixty years after its initial description the physiologic role of the urea cycle continues to be the subject of new ideas and controversies.

Although the complete urea cycle is expressed only in liver, some enzymes of this pathway are expressed at significant levels also in small intestine and kidney, thereby constituting an independent arginine biosynthetic pathway (Figure 1). Vertebrates require arginine for protein synthesis and for the synthesis of compounds such as creatine, polyamines, and the novel signalling molecule nitric oxide (47, 81). The relative contribution of endogenous arginine synthesis in meeting this requirement varies according to age, physiologic state, and species of animal (141). Young animals require dietary arginine for optimal growth whereas endogenous arginine synthesis largely meets the arginine requirement of most adult omnivores (16, 51, 120, 141). Both immature and adult carnivores require dietary arginine. An extreme example is the cat for whom an arginine-free diet may be fatal within hours (84). Although AS and AL are present also in brain (104), their physiologic role in this organ is unclear and thus is not considered here.

The hepatic capacity for urea synthesis is determined by regulating the abundance and catalytic efficiency of the enzyme catalyzing the rate-limiting

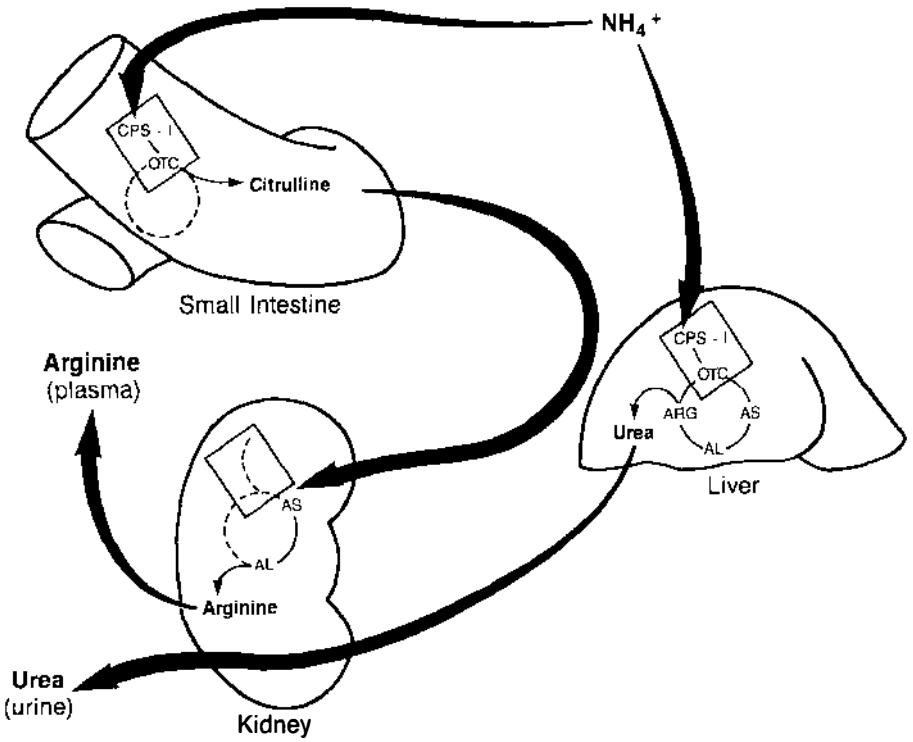

Figure 1 Interorgan relationships in urea and arginine synthesis. *Small rectangles* indicate the mitochondrial compartment. *Dotted lines* indicate missing parts of the urea cycle. Abbreviations: CPS-I, carbamyl phosphate synthetase-I; OTC, ornithine transcarbamylase; AS, argininosuccinate synthetase; AL, argininosuccinate lyase; ARG, arginase.

step. Based on enzyme activities measured under optimal conditions in the test tube, the potential ureagenic capacity of the liver is limited by AS abundance (103). However, as the urea cycle under normal physiologic conditions does not function at its full potential, rates of ureagenesis usually are controlled by substrate availability or catalytic efficiencies of the enzymes. Activities of the urea cycle enzymes are not known to be modulated by posttranslational modifications; regulation of catalytic efficiency is effected instead by varying concentrations of activators or inhibitors of the enzymes (15, 103). Thus, regulation of CPS-I by varying the concentration of its essential cofactor *N*-acetylglutamate is generally thought to be the principal mechanism for dynamically modulating ureagenic capacity in vivo (15, 30, 103). Recently, however, it has been proposed that arginase, which is at a branch point for utilizing arginine for ureagenesis or for other purposes such as protein or polyamine synthesis, may become rate-limiting under conditions

that alter its affinity for the cofactor Mn^{2+} (68). As regulation of rates of ureagenesis by varying substrate availability or catalytic efficiency of enzymes has been discussed elsewhere (79, 103), this review emphasizes the regulation of enzyme and mRNA abundance. Regulation of the urea cycle enzymes in fetal and perinatal periods also has been reviewed recently (79) and thus is not considered here.

UREA CYCLE ENZYMES IN LIVER

Responses to Diet

Since the beginning of this century it has been known that urea production in adult humans varies as a function of dietary protein intake (31). Changes in liver arginase activity according to dietary protein intake were reported in 1939 (71), representing one of the earliest examples of metabolic adaptation in mammals at the level of enzyme activity. Thirty years ago it was demonstrated that activities of all five urea cycle enzymes in rat liver varied as a function of dietary protein intake (115–117). Note, however, that metabolic regulation of urea cycle enzyme activities is species-specific, perhaps depending on the type of diet to which an animal is normally adapted. For example, in a strict carnivore such as the cat, adaptation of urea cycle enzyme activities in response to varying levels of dietary protein intake does not occur (107). Whether this lack of adaptation in cats involves reduced responses of the urea cycle enzymes to agents such as cAMP, insulin, and glucocorticoid is not known.

Activities of the urea cycle enzymes are highest in response to starvation and high-protein diets and are reduced to response to low-protein or protein-free diets (12, 14, 19, 22, 30, 76, 99, 114, 116). Variations in amino acid composition between different animal or vegetable dietary protein sources have little or no effect on the enzyme activities (22, 46, 117). However, feeding rats very high doses of some, but not all, individual amino acids can lead to increases in urea cycle enzyme activity (122). Because the latter study was conducted in vivo, the mechanism of these effects—such as possible selective effects on endocrine status—is unknown, and the authors noted that they had been unable to duplicate these results using cultured rat hepatocytes. Feeding rats ammonium acetate or ammonium citrate has not produced consistent effects on the urea cycle enzymes; when noted, effects were relatively small (54, 121, 122).

Diet-dependent changes in urea cycle enzyme activity are primarily the consequence of changes in enzyme mass (83, 114, 115, 122), which in turn largely reflect altered enzyme synthesis rates (119, 137). Abundances of urea

cycle enzyme mRNAs as a function of dietary protein intake generally correlate with the activities of these enzymes (83, 89, 112), thus indicating that dietary regulation of enzyme levels occurs primarily at a pretranslational step. Furthermore, this regulation is largely coordinate in degree for all five mRNAs (89). As these mRNAs are regulated also by agents such as cAMP, insulin, and glucocorticoids, it is likely that dietary responses are mediated primarily by hormones. These general assertions are probably accurate even though rigorous studies to document their validity for all five urea cycle enzymes have not been conducted, and some minor exceptions to these statements have been noted. For example, arginase activity may be regulated in vivo by varying the concentration of manganese (6, 108).

Disruption of nitrogen metabolism by conditions such as sepsis, trauma, uremia, or cancer also may result in altered urea synthesis and levels of urea cycle enzymes (12, 14, 17, 80, 101). However, as ureagenesis in these pathophysiologic states has not yet been studied extensively, information on regulatory signals and mechanisms is limited.

Hormonal Regulation

Treatment with glucagon or cAMP analogs results in increased activities and mRNA abundance for the urea cycle enzymes in rat liver, cultured hepatocytes, and hepatoma cell lines (13, 23, 35, 66, 72, 78, 94, 97, 109, 113, 124). To directly compare changes in enzyme and mRNA abundances is difficult owing to the large time differences in reaching new steady state values. The enzymes have half-lives of 3–9 days (40, 98, 119, 137, 142), whereas the mRNAs apparently have half-lives of several hours (97). Nevertheless, increases in enzyme activity apparently can be accounted for by increases in mRNA abundance (89, 97). In contrast to the other urea cycle enzymes, OTC is not responsive to cAMP analogs in cultured hepatocytes, in hepatoma cell lines, or in short-term treatment of rats (36, 89, 97). However, OTC is induced in rats treated with glucagon for several days (13, 124), suggesting that in vivo induction may represent an indirect response to glucagon or that response to cAMP is elicited only in the presence of some other agent, which has not been identified. Transcription rates of CPS-I, AS, and AL are rapidly stimulated by cAMP analogs (89, 97), which is indicative of a direct response to cAMP.

Activities of the urea cycle enzymes are reduced following adrenalectomy (19, 31, 77) and raised by glucocorticoid treatment of rats or of hepatoma cell lines (12, 19, 36, 40, 41, 77, 91, 94). As with cAMP, glucocorticoids also increase mRNA abundance in rats, cultured hepatocytes, and hepatoma lines, although precise correlations between enzyme activity and mRNA levels have not been determined in most instances (23, 26, 65, 97, 113). OTC is again

unusual in that its activity or mRNA exhibits little or no response to glucocorticoid (36, 89, 97, 113, 117). Transcription rates of CPS-I and AL are stimulated by glucocorticoid in cultured hepatocytes (94).

Responses to glucagon or cAMP analogs plus glucocorticoid are greater than to either agent alone (64, 66, 97). In cultured hepatocytes, the combination of cAMP and glucocorticoid was additive for AL mRNA and synergistic for CPS-I, AS, and arginase mRNAs, whereas OTC mRNA was unaffected (97). These results conflicted with those of other studies that found that glucocorticoid alone had little or no effect on enzyme activities in cultured hepatocytes but was permissive for the response to glucagon (35). However, these latter studies may not have observed increased enzyme activities owing to the lag in response to glucocorticoid (97) and the long half-lives of the enzymes. As enzyme activities and mRNA levels were not measured simultaneously in these studies, possibly the conflicting results represent differences in culture conditions among these laboratories or differences in glucocorticoid response at pre- and posttranslational steps. A combination of cAMP analog and glucocorticoid produced large increases in transcription rates of all urea cycle enzymes genes except OTC (94).

Urea excretion increases in diabetes, in part due to increased protein catabolism and increased food consumption. Activities of CPS-I, AS, AL, and arginase are elevated in diabetic rats, whereas OTC activity remains unchanged (6, 63, 125). The increased arginase activity reflects an increased concentration of its cofactor Mn^{2+} rather than any change in enzyme protein (6, 127). In cultured hepatocytes or hepatoma cell lines, insulin alone has little effect on urea cycle enzyme mRNAs but reduces the induction of CPS-I mRNA or synthesis rate by glucocorticoid, glucagon, or cAMP (63–66, 94) and also reduces the induction of arginase mRNA by cAMP (94); effects of cAMP and glucocorticoid on the urea cycle enzyme mRNAs are otherwise unchanged. Although these effects of insulin likely involve transcriptional control, experiments addressing this possibility have not been reported.

Inasmuch as thyroid status affects both synthesis and degradation of protein, thus influencing metabolism of nitrogenous metabolites, one might reasonably anticipate that thyroid hormone would prove to be a major regulator of urea cycle enzyme activity. However, relatively few studies of thyroid hormone action on this system have been performed, and the results obtained have not always been consistent (19, 31, 32, 38, 39, 74, 125). Because different diets were used in these studies, the extent to which nutritional factors influenced the variability in results is unknown. Overall, in vivo effects of thyroid hormone on urea cycle enzyme activities are modest. Although direct effects of thyroid hormone would best be elucidated by use of cultured hepatocytes or hepatoma cell lines, hepatocytes rapidly lose responsiveness to thyroid hormone in culture and thyroxine-responsive rat

hepatoma cell lines have not been described. The well-known effects of thyroid hormone on activities and mRNAs of the urea cycle enzymes in liver of the metamorphosing tadpole have been described previously (20, 85).

Effects of growth hormone on urea cycle enzymes are unclear. Treatment of normal rats with growth hormone resulted in decreased activities of AS, AL, and arginase but little or no change in CPS-I and OTC activities, which suggests that only the three cytosolic enzymes are responsive to growth hormone (77). A later study concluded that hypophysectomy decreased arginine synthetase and arginase activities whereas OTC activity remained unchanged (31); however, when enzyme activities are calculated as units per gram liver, the opposite conclusion is reached: OTC activity increases whereas activities of the other two enzymes decline slightly, if at all. Hypophysectomy of rats also increased activities of CPS-I and OTC in a more recent study, an effect that was reversed by administration of growth hormone; AS activity remained unchanged throughout (102). Differences in purity of the growth hormone preparations used in these studies could have contributed to the differing results. In any event, the activity changes reported are not great. Although hepatocytes have growth hormone receptors, we do not know whether any changes in urea cycle enzymes are the consequence of a direct action of growth hormone on these cells.

ARGININE BIOSYNTHETIC ENZYMES

Small Intestine

The small intestine is the principal source of circulating citrulline in adult mammals (146). Intestinal citrulline synthesis is catalyzed by CPS-I and OTC, which are localized in mucosal epithelial cells (43, 111). The regulation of intestinal CPS-I and OTC expression differs from that in liver. CPS-I activity in jejunum of adult mice was unaffected by altered dietary protein content (53), whereas OTC activity of rat small intestine decreased slightly when dietary protein content increased (147). As neither group assayed both enzymes, it is unclear whether these results reflect intrinsic differences in regulation of CPS-I and OTC in small intestine, species-specificity in dietary response, or differences in methodology. Moreover, assays of enzymes in intestinal homogenates are problematic owing to the presence of active proteases, and neither group apparently included protease inhibitors in the homogenization buffers.

Levels of CPS-I and OTC mRNAs in small intestine of adult rats were unaffected by treatment with glucagon or dexamethasone (113). However, levels of these mRNAs were only modestly affected in liver of the same animals, so it is unclear whether the apparent lack of an intestinal response reflects an intrinsic insensitivity to the hormones or reflects masking of a

hormone response owing to high basal expression. With regard to the latter possibility, CPS-I mRNA induction by cAMP or glucocorticoids is much higher in cultured rat hepatocytes than in rat liver owing to the much lower basal expression in the cultured hepatocytes; the final induced levels are very similar (97). Moreover, hormonal responsiveness may be a function of age, as a number of intestinal enzymes in rat lose glucocorticoid responsiveness during the third postnatal week (49).

Developmental expression of CPS-I and OTC has been examined by several groups. CPS-I activity in mouse jejunum was highest at birth, gradually declining to about a third of the highest value in adults (53). A similar profile was found for CPS-I mRNA in rat intestine (113). Reported developmental profiles for OTC activity are variable. OTC activity during development of mouse intestine was similar to that reported for CPS-I (27), whereas another group found little difference in intestinal OTC activity in mice from birth to 21 days, with slightly higher levels in the adult (73). OTC mRNA abundance in developing rat intestine paralleled that of CPS-I mRNA, declining between birth and maturity (113), and matched the OTC activity profile in developing mouse intestine (27). In contrast, OTC activity in small intestine of rat increased 5- to 6-fold from birth to adulthood (50). Developmental profiles of CPS-I and OTC mRNAs in small intestine differ significantly from those in liver (86, 113). As no group measured both mRNA abundance and enzyme activity, it is unclear whether this discrepancy reflects methodologic differences between laboratories, differences in expression between mouse and rat, or regulation at the translational or posttranslational level. As with the dietary studies, no group measured activities of both enzymes nor included protease inhibitors in the homogenization buffers, although Western blotting analysis showed intestinal OTC to be intact in one of these reports (27).

The roles of small intestine and kidney with regard to arginine biosynthesis change during development. Whereas levels of AS and AL are relatively high in liver and kidney but very low in small intestine of adults, leading to the relationship diagrammed in Figure 1, activities of these two enzymes are relatively high in small intestine but low in kidney for the first week or two after birth (53). This finding is consistent with the virtual absence of arginase activity in small intestine for two weeks after birth, followed by a rapid increase in activity to adult levels (50, 53, 73). Thus, the small intestine appears to be the principal arginine biosynthetic organ at and shortly after birth, whereas this function is divided between small intestine and kidney in adults.

Kidney

That kidney has a significant capacity for converting citrulline to arginine has been known for 50 years (7, 21). This conversion is carried out by AS and AL

(105), the third and fourth enzymes, respectively, of the urea cycle (Figure 1). These enzymes are localized in the proximal tubules of the kidney (70, 90). Physiologic studies have demonstrated that renal uptake of citrulline from the blood is closely matched by renal release of arginine into the circulation with little loss to the urine (48, 135, 146) and that the kidney is a major site of arginine biosynthesis in the rat (29). Renal production of arginine in the rat appears to be limited primarily by the availability of citrulline rather than by the capacity for conversion of citrulline to arginine (25). Although arginase activity is observed in kidney, it is much lower than in liver, it includes the activity of an isozyme distinct from liver arginase (126), and renal arginase activity is segregated from the major site of renal arginine biosynthesis (70).

Renal AS and AL activities and mRNA abundances increase as dietary protein intake increases, although the magnitude of this dietary response for these enzymes is less than in liver (87, 106). The effects of cAMP and glucocorticoid on abundance of these mRNAs differ for liver and kidney (87). Renal AS mRNA is induced by dibutyryl cAMP but not by dexamethasone, whereas this mRNA is induced by both agents in liver. The absence of a renal mRNA induction by dexamethasone is in apparent contrast to increased arginine synthetase (AS + AL) activity in kidney of rats receiving repeated injections of hydrocortisone (130). However, the duration of the treatment in the latter study raises the possibility that the increase was an indirect response to the hormone. Renal AL mRNA was unaffected by dibutyryl cAMP, dexamethasone, or a combination of these agents, whereas it was induced by dibutyryl cAMP in liver (87). Thus, the hormonal responses of these mRNAs in kidney differ qualitatively and quantitatively from their hormonal responses in liver of the same animals. These organ-specific hormonal responses may account, at least in part, for the organ-specific responses of mRNA abundance to dietary protein changes.

Messenger RNAs for renal AS and AL are measurable by 15 days of gestation in the mouse, corresponding to the time of appearance of immunoreactivity for these enzymes (90). These mRNAs increase coordinately during fetal and neonatal kidney development, whereas they exhibit clearly different developmental profiles in liver (86, 90). Developmental increases in AL activity and mRNA abundance are very similar in magnitude (53). Abundances of these mRNAs in kidney are unaffected by the hormonal changes that occur at parturition.

Cultured Cells

AS and AL are among the relatively few mammalian enzymes whose levels are regulated directly by metabolite concentration. Schimke reported nearly 30 years ago that activities of AS and AL in HeLa, KB, and L cells were repressed coordinately by arginine in the culture medium, whereas activities of these enzymes increased when arginine was replaced by citrulline in the

medium (118). Similarly, AS activity increased in cultured human lymphoblasts and in human RPMI 2650 cells (an epithelial tumor cell line) when arginine was replaced by citrulline in the culture medium, but AL activity was unchanged (57, 128). However, arginine deficiency causes no changes in activities of any of the urea cycle enzymes in cultured rat hepatocytes or in a rat hepatoma cell line (123). Thus, the mechanism for responding to variations in arginine concentration appears to be cell specific.

Changes in AS activity in cultured RPMI 2650 cells reflect changes in AS mRNA abundance (58). Measurements of AS mRNA precursor abundance, nuclear transcription rates, and expression of transfected AS minigenes indicate that the metabolite regulation is largely at the level of transcription (58). Because leucine starvation has effects similar to arginine starvation, the suggestion has been made that mammalian cells may have control mechanisms similar to those involved in general control of amino acid biosynthesis in *Saccharomyces cerevisiae* (58).

When cultured in the presence of canavanine, a toxic arginine analog, canavanine-resistant variants of human lymphoblast lines and RPMI 2650 cells, which express approximately 200-fold higher levels of AS, can be obtained (2, 128). AS activity in the canavanine-resistant cells is similar to that in normal liver. No change in AS gene copy number occurred in the canavanine-resistant cells, thus demonstrating that elevated AS expression was not caused by AS gene amplification (2, 129). Interestingly, AS expression in the canavanine-resistant cells was no longer subject to regulation by arginine (60, 128). Although the mechanism responsible for elevated AS expression in these cells remains unknown, the available data are consistent with a model that involves a positively acting factor (mechanism) that acts in *trans* (10, 11).

TRANSCRIPTIONAL CONTROL MECHANISMS

Although the extent to which changes in enzyme abundance reflect regulatory events at transcriptional, posttranscriptional, translational, or posttranslational steps has not been determined fully for the urea cycle enzymes in most instances, the evidence to date nonetheless indicates that altered enzyme abundance is primarily a consequence of altered mRNA abundance, which, in turn, is likely due to corresponding changes in transcription rates. This does not rule out the possibility of regulation at posttranscriptional steps under certain conditions, and translational control of CPS-I expression has been reported (138, 139). Although transcriptional control of urea cycle enzyme gene expression has been demonstrated in several laboratories, relatively little information is available regarding the types or organization of DNA regulatory elements present in the urea cycle enzyme genes. However, the presence

of certain hormonal response or tissue-specific expression elements can be inferred from the expression patterns of these genes. Although a variety of potential regulatory elements in the 5' flanking regions of some of the urea cycle enzyme genes have been identified by computer analysis, this review emphasizes DNA elements that have activity in cell transfection or in vitro transcription assays or that exhibit specific binding to nuclear proteins.

The promoter region of the rat CPS-I gene contains TATA and CAAT consensus elements at positions –21 and –82, respectively (69). CPS-I promoter activity in liver nuclear extracts is dependent on a C/EBP element at position –109 and an unidentified element(s) between –161 and –1200 (52). No consensus cAMP response elements (CREs) or glucocorticoid response elements (GREs) were readily apparent within 1 kb of the transcription start site (69). Functional analyses of CPS-I promoter elements by transfection assays have not been reported.

The promoters of the rat, mouse, and human OTC genes are atypical in that they appear to lack consensus TATA and CAAT elements at the usual positions (44, 133, 140). Approximately 1.3 kb of the 5' flanking region of the OTC gene is sufficient to direct expression of a transgene specifically within liver and intestine of mice (93). Liver-specific expression of the rat OTC gene, as judged by cell transfection assays, is conferred by 222 bp of the 5' flanking region, which apparently contains at least one negative and two positive regulatory elements (92), one of which binds a protein possibly related to the COUP transcription factor (136). In addition, a liver-specific enhancer element located at –11 kb contains binding sites for a factor related to C/EBP (92).

Isolation and characterization of the human AS promoter was complicated by the fact that the human genome contains one expressed AS gene and 14 pseudogenes (34). The human AS promoter contains a TATA element approximately at position –30, but no apparent CAAT element has been observed (33, 61). Studies of AS promoter function have been concerned with the mechanisms of repression by arginine and with over-expression in canavanine-resistant cells. AS minigene constructs containing both 5' flanking sequences and intragenic sequences are subject to arginine repression (9, 60). As this repression also occurs in a construct containing as little as 149 bp of 5' flanking sequence (9), intragenic DNA sequences may possibly be involved in the regulation by arginine. Intragenic sequences also appear to be involved in regulated AS expression in canavanine-resistant cells (11). AS DNA constructs that exhibit overexpression in canavanine-resistant cells have not been identified. Overexpression of the endogenous AS gene in the resistant cells may involve epigenetic changes in AS DNA or chromatin structure that are not easily duplicated with transfected DNA constructs.

The promoter region of the human AL gene contains no TATA element, but four potential Sp1 binding sites are located within 200 bp of the transcrip-

tion start site (1). No functional analysis of the AL promoter region has been reported.

Promoters of both rat and human liver arginase contain TATA and CAAT elements approximately at positions –25 and –60 to –70, respectively (100, 131). Potential GREs are found in the rat and human arginase promoter regions (100, 131), and a potential CRE has been noted for the human arginase 5' flanking region (131). A rat liver arginase construct containing nucleotides –90 to +286 is as efficient in in vitro transcription assays using rat liver nuclear extracts as is a construct containing nucleotides from –2.7 kb to +286 (132). A C/EBP-related factor footprints a region from –95 to –82 in the arginase promoter, thus suggesting a possible functional role for this element. However, because it was not reported whether the –90 to +286 construct retained this footprint, it is not clear whether this apparent C/EBP element is indeed functional. Deletion analysis indicates at least one positive DNA regulatory element is located in the region –90 to –51. This segment contains a region that can be footprinted by CTF/NF-1- and Sp1-related factors in a mutually exclusive fashion (132).

As the urea cycle enzyme genes generally exhibit coordinate expression in vivo, it is reasonable to believe that the 5' flanking regions of these genes share some DNA regulatory elements in common. Thus, at least three of these genes—CPS-I, OTC, and arginase—have DNA elements that are recognized by C/EBP or C/EBP-related factors (52, 92, 132). From their patterns of hormonal response, one can infer that, except for OTC, these genes likely contain functional CREs and GREs. However, the delayed response to dexamethasone and the requirement for ongoing protein synthesis in cultured hepatocytes suggest that these GREs require protein factors in addition to the glucocorticoid receptor in order to function (97). This hypothesis was proposed also for the phosphoenolpyruvate carboxykinase gene (95, 96), and evidence supporting this hypothesis has been reported (55). Based on their tissue-specific and developmental profiles, these genes are likely to have DNA elements that are recognized in common by one or more liver-specific transcription factors. Finally, at least four of the urea cycle enzyme genes—OTC, AS, AL, and arginase—share a common homologous sequence of unknown function (28, 100). As this element, termed the "urea cycle element" (UCE), is found also in the ornithine aminotransferase promoter (28), possibly this element may be involved in the coordinate expression of a larger group of genes, all involved in nitrogen metabolism. However, this hypothesis has yet to be tested, and the functional significance of the UCE in the urea cycle enzyme genes, if any, is unknown.

The presence of additional regulatory elements in at least two of the urea cycle enzyme genes—CPS-I and AS—is suggested by loss of expression of these, as well as of other liver-specific genes, in somatic cell hybrids

of hepatoma and fibroblast cells (18, 110). In the case of AS, this loss of expression, termed extinction, involves at least two genetic loci that act in *trans:* tissue-specific extinguisher-1 (TSE1), which maps to mouse chromosome 11 and human chromosome 17, and at least one other locus that has not been identified (134). A recent report states that TSE1 encodes a regulatory subunit of cAMP-dependent protein kinase (8, 62). The regulatory DNA elements of the AS gene that are involved in extinction have not yet been identified but presumably include elements recognized by either a CRE-binding protein (CREB) or some other factor that is phosphorylated by protein kinase A.

Regulatory mutants affecting urea cycle enzyme gene expression have been sought in hope that they may shed light on various control mechanisms. For example, researchers have identified mouse strains that carry mutations affecting the normal postnatal expression of the urea cycle enzymes as well as of other genes in liver (56, 88, 110). One group of mice carries radiation-induced deletions of a portion of chromosome 7 (88, 110). Whereas heterozygotes are phenotypically indistinguishable from wild-type normal mice, certain of these deletion mutants permit near-normal fetal development in the homozygous condition, but homozygotes die within a few hours of birth. Levels of the urea cycle enzymes, their mRNAs, and transcription rates are significantly below normal in liver of homozygous neonates, although the reductions in transcription rate are insufficient to fully account for the reduction in mRNA abundance (88, 110). The reductions in expression of the urea cycle enzymes in homozygotes are essentially coordinate in degree. Whether gene expression in liver of homozygotes is also abnormal prior to birth is not known. Expression of arginine biosynthetic enzymes in kidney and intestine of neonatal homozygotes is unaffected, suggesting that the mutants may have a defect in some regulatory pathway that is primarily affected in liver (88). The nature of the defect underlying the aberrant expression of the urea cycle enzymes and other liver-specific genes in the mutant mice has not been elucidated. The putative regulatory gene encompassed by the deletions must act in *trans,* as the deletions are unlinked to the structural genes whose expression is affected and the homozygotes appear to have defects at both transcriptional and posttranscriptional levels of control (88). The lack of a gene dosage effect suggests that the defect more likely represents the absence of a positive regulatory factor rather than increased expression of a negative regulatory factor.

Activities of the urea cycle enzymes are reduced also in liver of C3H-H-2°-jsv mice (56). In contrast to the previously described mutants, reductions in activities of the urea cycle enzymes in the jsv mice are not coordinate in degree nor are significant reductions observed until 25 days of age, subsequent to development of a fatty liver. Most likely the reductions in urea cycle

enzyme activities are secondary to other metabolic defects, as the ureagenic capacity of hepatocytes was markedly reduced in several animal models with fatty liver (75).

Finally, epigenetic events that affect expression of OTC, the only urea cycle enzyme gene located on the X chromosome, have been noted. Rat hepatoma cell lines that do not express OTC spontaneously give rise to OTC-expressing variants at a low frequency (24, 37). Although this frequency can be greatly increased by treatment with 5-azacytidine (24, 37), thus implying that the methylation state of the OTC gene influences its expression, a direct analysis of the methylation status of the OTC gene in expressing and nonexpressing cells has not been reported. Furthermore, in mice an increasing proportion of liver cells with a silent OTC gene on an inactive X chromosome region become OTC expressors with age (144), perhaps signifying important changes in gene expression patterns as females age. We do not know whether the changes in OTC expression in vivo involve DNA methylation.

CONCLUDING REMARKS

Genomic and cDNA clones for all the urea cycle enzymes have been isolated only within the past few years. Thus, the signals and mechanisms regulating the nutritional, hormonal, and developmental expression of these genes in liver and other organs are just beginning to be elucidated. For example, responses of these genes to agents such as cAMP, insulin, an glucocorticoid—or even to the complete absence of hormones—are being characterized and found to exhibit gene-specific patterns of expression (97). The finding of unique response patterns is intriguing because levels of these enzymes and their mRNAs in liver coordinately adapt to many physiologic changes in vivo (88, 89, 115, 116). What are the signals and mechanisms whereby coordinate expression of these genes is achieved in vivo?

The identification of *cis*-acting DNA elements and *trans*-acting regulatory factors undoubtedly will receive increasing attention, as transcription is very likely a major regulated step in expression of the genes encoding the urea and arginine biosynthetic enzymes. Integrating the results of such studies in a meaningful fashion will be challenging because at present the physiologic expression of a gene cannot be predicted merely by cataloging its individual regulatory elements. For example, in the case of genes that respond to both cAMP and glucocorticoids, it is unclear why some genes exhibit a synergistic response to a combination of these agents while others do not. In addition, a thorough understanding of the physiologic expression of these genes will require knowledge of how the regulatory factors themselves are regulated.

The cloned DNAs are powerful tools that can be used to transfect cultured cells or generate transgenic animals in order to address important questions

about the physiologic roles of these enzymes: What are the rate-limiting factors for ureagenesis under various physiologic and pathophysiologic conditions? Does the urea cycle play a role in regulating pH homeostasis (4)? Are the arginine biosynthetic enzymes rate-limiting for the production of nitric oxide in brain, macrophages, or other cell types (81)? How important is the spatial expression of the urea cycle enzymes in liver in regulating ammonia, urea, and glutamine metabolism (45)? Clinically, this information may be useful in the management of certain pathophysiologic conditions, and the cloned genes can be used also to treat inherited defects in these enzymes by gene therapy.

Recent years have seen greater appreciation of the complex inter- and intra-organ interplay in the metabolism of ammonia, urea, and arginine. Much is yet to be learned about the regulated metabolism of these essential compounds, as well as the interrelation between urea and arginine biosynthesis and other metabolic pathways. The means to explore challenging problems in these areas are at hand, and the future holds great promise for exciting advances in our understanding both of urea and arginine biosynthesis in particular and of metabolic regulation in general.

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