The Leucine-Responsive Regulatory Protein, a Global Regulator of Metabolism in *Escherichia coli*

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INTRODUCTION

More than 10 years ago, both Fraser and Newman (47) and Quay and Oxender (121) suggested that leucine might act as a regulatory signal for *Escherichia coli* because it affected expression of several unrelated operons. Adding leucine to the growth medium of *E. coli* reduced expression of some operons and increased expression of others. In at least some of these cases, the effect of leucine is now known to be mediated by Lrp (leucine-responsive regulatory protein), a protein encoded by *lrp*. It has also become clear that not all Lrp-responsive operons are affected by leucine when cells are grown in a minimal medium.

lrp, located near min 20 on the *E. coli* chromosome, was first identified as a locus (*livR*) that affected the transport of branched-chain amino acids (6). Subsequently, mutations in *lrp* were identified by three groups of investigators studying different operons. The fact that mutations in *lrp* affected the expression of operons involved in amino acid biosynthesis (*ilvIH, serA*) (86, 119), amino acid degradation (*sdaA, tdh*) (86), and peptide transport (*oppABCDF*) (11) suggested that *lrp* might play some general role in amino acid metabolism, perhaps similar to the role played by *crp* in carbohydrate metabolism.

More recently, *lrp* was independently identified as a regulatory gene affecting the expression of pilin biosynthesis in a number of systems (16, 20) and the expression of *lysU*, a gene encoding one of two lysyl-tRNA synthetases (52, 69). Taken together, these results establish that *lrp* is a significant *E. coli* regulatory gene and invite speculation as to the physiological and adaptive significance of this gene.

There have been two systematic attempts to identify *E. coli* operons affected by Lrp and leucine: a two-dimensional electrophoretic analysis of polypeptides performed by Ernsting et al. (43), and an analysis of anonymous operons identified by Lin et al. after λ*placMu* mutagenesis (84). A compilation of genes and operons regulated by Lrp uncovered by genetic analyses and by these two systematic surveys is shown in Table 1. The table does not include 21 unidentified polypeptides whose expression is regulated by Lrp (43), nor does it include the group of unidentified genes identified by Lin et al. using transposon mutagenesis (84).

Altogether, there may be several dozen operons whose expression is regulated by Lrp. These operons are defined here as members of the Lrp regulon. For a substantial fraction of these operons, there is at least some evidence that they are regulated directly by Lrp, rather than indirectly.

When viewed as a whole, the most striking aspect of the Lrp regulon is the number of different patterns of regulation in response to Lrp and leucine and perhaps to other proteins (Table 2). The patterns summarized in Table 2 are best considered in pairs. For the first pair (patterns 1 and 4), Lrp activates or represses expression and leucine antagonizes the effect of Lrp. Thus, for example, Lrp activates transcription from the *ilvIH* promoter and leucine causes repression by interfering with the action of Lrp. On the other hand, Lrp represses expression from the *sdaA* promoter and leucine induces expression of this operon, presumably by interfering with the repressive action of Lrp.

For the second pair (patterns 2 and 5), Lrp again activates or represses expression but the action of Lrp is potentiated by leucine. Thus, for example, Lrp acts negatively on *livJ* and *livKHMGF* expression, and this negative effect requires the presence of leucine. On the other hand, Lrp is required for *fimB*- and *fimE*-promoted phase variation of type I pili, and the effect is potentiated by leucine (16).

For the third pair (patterns 3 and 6), Lrp either activates or represses but the Lrp-related regulation is relatively insensitive to leucine, at least during growth of cells in minimal medium. For example, the *pap* operon of *E. coli* requires Lrp for expression, but this effect of Lrp is independent of leucine. Some polypeptides identified by Ernsting et al. by two-dimensional gel electrophoresis, including OmpC, show the opposite pattern: Lrp represses, and the repression is insensitive to leucine (43). More than half of the Lrp-regulated polypeptides seen by electrophoretic analysis showed expression that was insensitive to leucine under the conditions used.

It is instructive to compare Lrp with Crp, another, more thoroughly studied, *E. coli* regulatory protein. Like Lrp, Crp activates transcription of some operons and represses transcription of others. However, in the case of Crp, both activation and repression require that Crp interact with cyclic AMP (cAMP) (2, 90). Lrp also interacts with a ligand, but the ligand has effects that cannot be explained solely by a single mode of action, such as reducing the DNA-binding ability of Lrp. The complex regulatory patterns summarized in Table 2 may ultimately be understood in terms of effects of leucine upon the specificity of DNA binding or in terms of interactions of Lrp and leucine with other proteins.

Several reviews of the Lrp regulon, which emphasize the identification of genes in the regulon (102) and the possibility that Lrp participates in the maintenance of chromosomal structure and organization (31), have recently been published. The present review is organized in three parts. The first part is concerned with the broad question of how Lrp and leucine interact to regulate gene expression. It is focused mainly on Lrp and its interaction with DNA, on the organization and regulation of the *lrp* operon, and on the way in which Lrp regulates expression of several operons. The second part contains a short description of each of the operons known to be regulated by Lrp. The third part, concerned with the effects of Lrp on metabolism, relates to the physiological role of Lrp.

TABLE 1. Loci regulated by Lrp

Locus	Product	Reference(s)
Amino acid biosynthesis		
ilvIH	AHAS	119
leuABCD	Enzymes involved in leucine biosynthesis	84
serA	D-3-Phosphoglycerate dehydrogenase	86, 127
glnALG	Glutamine synthetase (*glnA*) and genes that regulate *glnA* (*glnLG*)	43
gltBDF	Glutamate synthase (*gltBD*)	43, 44
glyA	Serine hydroxymethyltransferase	31
Amino acid degradation		
gcv	Glycine cleavage pathway	84
tdh, kbl	Threonine dehydrogenase; 2-amino-3-ketobutyrate CoA ligase	86, 127
sdaA	Serine deaminase	86
Transport		
livJ	Binding protein for isoleucine, valine, and leucine transport	62
livKHMGF	Binding protein for leucine transport (*livK*); membrane components for branched-chain amino acid transport (*livHMGF*)	62
oppABCDF	Binding protein and membrane components for oligopeptide transport	11, 64
ompC	Outer membrane porin C	43
ompF	Outer membrane porin F	43
micF	Antisense RNA; translational inhibitor of *ompF*	46
Pilin synthesis		
daa	F1845 pili	14, 156
fae	K88 pili	68
fan	K99 pili	22
fim	Type I pili	16
pap	P pili	22
sfa	S pili	157
Miscellaneous		
lrp	Leucine-responsive regulatory protein	84, 117
lysU	Lysyl-tRNA synthetase form II	52, 69, 85
osmY	OsmY, an 18-kDa periplasmic protein of unknown function	78
pnt	Pyridine nucleotide transhydrogenase	31
W protein	Required for efficient translation of some mRNAs	43

INVOLVEMENT OF Lrp AND LEUCINE IN REGULATING OPERON EXPRESSION

Properties of Lrp

Lrp has been purified to near homogeneity from a wild-type strain (166) and from a strain that produces a mutant Lrp that is insensitive to leucine (168). It has also been purified to apparent homogeneity from an overproducing strain of *E. coli* (44). Purified Lrp gives the same mobility shift pattern with DNA from the *ilvIH* promoter region as does Lrp in crude extracts (168). Lrp has a pI of about 9.3 and a monomeric molecular mass of 18.8 kDa, and at a concentration of 10 μM it exists as a dimer in solution (168). Some partial diploid strains containing both wild-type and mutant *lrp* alleles had

TABLE 2. Patterns of regulation of target genes by Lrp

Pattern no.	Effect of Lrp and leucine	Example
	Lrp activates	
1.	Leucine antagonizes the effect	*ilvIH*
2.	Leucine potentiates the effect	*fimB*- and *fimE*-promoted switching
3.	Leucine has little effect	*papBA*
	Lrp represses	
4.	Leucine antagonizes the effect	*sdaA*
5.	Leucine potentiates the effect	*livJ, livKHMGF*
6.	Leucine has little effect	*ompC*

properties different from either parent (62), supporting the idea that Lrp exists as a multimer in vivo.

E. coli cells grown in a glucose-based minimal medium contain about 3,000 molecules of Lrp per cell, as estimated by titration of Lrp in crude extracts with an antibody (168). The in vivo concentrations of Lrp and of Lrp-binding sites upstream of *ilvIH* are about 10^{-6} and 10^{-9} M, respectively (168). At these concentrations in vitro, all of the binding sites upstream of *ilvIH* would contain bound Lrp, even in the presence of leucine. Since expression of *ilvIH* in vivo is affected by leucine, the chemical activity of Lrp in cells may be low, either because most of it is bound nonspecifically to DNA or because Lrp binds more poorly to specific DNA sites in vivo than expected from the results of in vitro studies.

Lrp is not related to the large families of bacterial regulatory proteins such as the LysR family or the two-component family of bacterial regulatory proteins, nor does it show relatedness to other well-known regulatory proteins such as Crp or Fnr or to abundant DNA-binding proteins such as integration host factor or the histone-like regulatory proteins. It is related to AsnC, an *E. coli* protein that regulates expression of *asnA* (which encodes asparagine synthetase A) (37, 75). The amino acid sequences of Lrp and AsnC are 25% identical, and at least another 25% show close similarity (168).

AsnC has a region centered at amino acid 35 that is similar to regions of other proteins known to have helix-turn-helix (HTH) motifs (38, 75). HTH motifs consist of a short α-helix,

FIG. 2. Organization of Lrp-binding sites upstream of *ilvIH*. +1 represents the transcription start site, and the centers of binding sites are shown relative to this position.

FIG. 1. (A) Locations of mutations in Lrp. The position of every 10th amino acid is denoted by a dot. Redrawn from reference 118. (B) Amino acid sequence of Lrp (168). Mutations affecting DNA binding: D13Y, L34P, L40F, S41P, P44T, L46P, R48C, Y61H, L65P, and L70P. Mutations affecting activation: V76A, P90L, F113L, T119L, and S125P. Mutations affecting the response to leucine: L108F, D114E, M124R, L136R, Y147H, V148F, and V149A.

a turn that almost always includes a glycine, and a second short helix (the recognition helix) that is thought to form specific base contacts with DNA. When analyzed by either the method of Brennan and Matthews (24) or that of Dodd and Egan (38), Lrp shows a high probability of having the first helix and turn between residues 29 and 41 but only a poor match in the region of the recognition helix (117). Direct evidence for an HTH motif within Lrp was provided by an analysis of several dozen *lrp* mutations, isolated on the basis of their effects on expression of the *ilvIH* promoter (118). The majority of the mutations that prevented binding of Lrp to DNA are clustered within the putative HTH region mentioned above (Fig. 1). Each of three mutations that affected the first putative α-helix reduced the HTH similarity score (38). On the other hand, two other mutations that affected the putative recognition helix had little effect on the similarity score and for a third the similarity score was even markedly elevated (118). These last results suggest that the specific binding of Lrp to *ilvIH* DNA may require particular amino acids which are not normally present at those positions within the HTH regions of other DNA-binding proteins.

Further analysis of the above-mentioned *lrp* mutations identified a group that retained DNA binding but resulted in reduced operon expression (118). These mutations, which potentially affect the ability of Lrp to activate transcription from the *ilvIH* promoter, are located within the middle region of Lrp (Fig. 1). Mutations that affect interactions between Lrp dimers might also be included in this group. Alternatively, they might appear as mutations abolishing DNA binding, which was assessed by mobility shift assays with *ilvIH* DNA. Since binding to *ilvIH* DNA involves Lrp binding to multiple sites cooperatively, mutations affecting cooperativity may lead to failure to detect binding.

A third group of mutations allowed normal expression from the *ilvIH* promoter in cells grown in the absence of leucine but prevented leucine from repressing the *ilvIH* operon (118). These mutations which caused Lrp to be insensitive to leucine mapped to the C-terminal one-third of the molecule (Fig. 1).

It should be noted that in only a few cases have any of the above-mentioned mutations been tested for their effects on other Lrp-sensitive operons. Furthermore, besides transposon insertions, few mutations within *lrp* have been isolated on the basis of their effects on operons other than *ilvIH*.
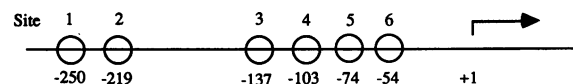
Interaction of Lrp with DNA

Consensus sequence. The *ilvIH* operon of *E. coli* encodes one of the enzymes involved in branched-chain amino acid biosynthesis. In vitro, purified Lrp bound with high affinity to at least six sites upstream of the *ilvIH* promoter as judged by methidiumpropyl-EDTA (MPE) footprinting (159). The organization of these sites is shown in Fig. 2.

Salmonella typhimurium LT2 also contains an *ilvIH* operon (144), but this operon is not functional because of a point mutation near the 5' end of the *ilvI* gene (129). MPE footprinting of binding sites upstream of the *S. typhimurium* *ilvIH* operon also identified six binding sites, three of them similar to corresponding sites in *E. coli* and three at different relative positions (161). A consensus sequence derived from a comparison of the 12 *ilvIH* binding sites from the two organisms is shown in Fig. 3 (161).

A computer analysis of 11 Lrp regulon-related sequences by Rex et al. (4 from *ilvIH*, 2 from *lysU*, and 1 each from *tdh*, *sdaA*, *oppA*, *oppBDCF*, *livJ*, and *livKHMGF*) suggested TTTATTCt NaAT as a potential consensus sequence (127). This consensus, shown in Fig. 3, is similar in part to that derived by comparison of *ilvIH* sequences. In their studies of the regulation of the *tdh* operon, Rex et al. defined by deletion analysis a 25-bp region just upstream of the *tdh* promoter that was required for induction of operon expression by exogenous leucine. A 12-bp sequence within that region differs from the consensus they defined by only 1 bp and is probably a site at which Lrp binds.

The consensus sequences shown in Fig. 3, derived from an analysis of a limited number of sequences, will almost certainly be modified as other sites are analyzed. To date, DNase I footprinting experiments have not proven very useful in defining binding sites because these footprints extend over many dozens of base pairs without defining regions that contact Lrp. Other kinds of footprinting studies, such as MPE footprinting, are required to define binding sites, and these have not yet been reported for operons other than *ilvIH*. A preliminary analysis of the *pap*, *sfa*, and *daa* operons, however, suggests that the consensus sequences shown in Fig. 3 may not adequately account for all Lrp-binding sites. van der Woude et al. pointed out that the control regions of these operons contain a TTTTAT sequence within regions that are not protected by Lrp but do not contain this sequence within a region that is thought to be important for Lrp binding (156). In some preliminary studies of *pap* DNA, regions protected by Lrp against methylation all contained the sequence GNN(N)TTTT (88a).

Clearly, more work will be required to define the requirements for Lrp binding. There may be a distinct consensus

Comparison of 12 sites upstream of *ilvIH*   A g a A T T T T A T t c T
Comparison of Lrp regulon sequences   T T T A T T C t N a A T

FIG. 3. Consensus sequences for Lrp binding to DNA derived from an MPE footprinting analysis of 12 sites upstream of the *ilvIH* operon and from a comparison of sequences within or near genes controlled by Lrp.

sequence, albeit one with complicated rules, as is the case for the consensus sequence for OxyR binding (152). However, the possibility must be entertained that Lrp recognizes not a consensus but some other feature of DNA such as bending or a pattern of repeating A's and T's.

Stoichiometry and cooperativity of binding. The stoichiometry of Lrp binding to a single Lrp-binding site in *ilvIH* (site 2 in Fig. 2) has been determined. One dimer of Lrp binds to one site (160a).

The binding of Lrp to at least some of the six sites upstream of the *ilvIH* operon is cooperative (159). Lrp bound most tightly to sites 1 and 2 (centers separated by 31 bp [Fig. 2]), with an apparent dissociation constant of about 5×10^{-9} M. Binding of Lrp to one of these sites, when measured by the method of Tsai et al. (154), was more than 100-fold tighter if the other site was already occupied by Lrp than if it was unoccupied. Mutations in site 2 reduced binding to both sites 1 and 2 more than 10-fold, whereas mutations in site 1 reduced binding only 2-fold but greatly reduced cooperativity. Binding to sites 3, 4, and 5 was highly cooperative (about 1,000-fold). Mutations in either site 3, site 4, or site 5 reduced binding to all three sites. A mutation in site 6 reduced binding to site 6 but did not affect either cooperative binding to sites 3, 4, and 5 or expression. Little or no cooperativity was observed between sites 1 + 2 and sites 3 + 4 + 5 (159).

Bending caused by binding. Naked DNA upstream of the *ilvIH* promoter is bent by about 110° (158a), with the center of bending mapping to position −115 as determined by gel electrophoresis (171). Similar experiments have demonstrated that upon binding Lrp, the DNA upstream of the *ilvIH* promoter is bent further. By using vectors described by Kim et al. to generate circularly permuted fragments (73), Lrp induced a bend of about 52° upon binding to a single site, and the angle of bending was increased to at least 135° when Lrp bound to two adjacent sites (160). Thus, as for Crp and integration host factor (153), an important function of Lrp may be to bend DNA.

DNase I footprints obtained with *ilvIH*, *lysU*, and *pap* DNA did not look like typical footprints in which a relatively short region of DNA was protected from cleavage. Rather, the footprints of these DNAs were all extended over a region of more than 100 bp (85, 107, 160). It seems unlikely that Lrp, which is a homodimer with a molecular weight of 38,000, binds tandemly over the entire protected region. It is more likely that Lrp causes DNA to be looped or to be wrapped around a core of Lrp. Consistent with the latter possibility is the distinct phasing of regions of protection and regions of hypersensitivity to cleavage with a periodicity of about 10 bp. Extensive DNase I footprints showing phased regions of protection have been observed in some other systems, including curved DNA in minicircles (40), DNA in nucleosomes (40), and DNA looped out through interaction between lambda *cI* repressors bound to sites artificially placed far apart (67).

Lrp binding can be affected by DNA methylation and vice versa. With few exceptions, adenine residues within GATC sequences in *E. coli* are methylated as a result of the action of the Dam methylase (91). In cases when an Lrp-binding site contains a GATC sequence, the nature of Lrp binding can be affected by whether the GATC sequence is methylated. Several operons involved in pilin biosynthesis contain GATC sequences (reviewed in more detail below). Nou et al. have shown that binding of Lrp to *pap* regulatory DNA is controlled by methylation of two such sites, called GATC[1028] and GATC[1130] (107). Whereas Lrp bound to the site containing GATC[1130] whether or not this sequence was methylated, the
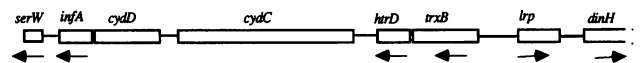

FIG. 4. Genes in the 19.8-min region of the *E. coli* chromosome. Arrows represent the direction of transcription. In the genomic restriction map of Rudd, *serW* is at 934.1 kb and *lrp* is at 940.8 kb (132).

DNase I footprint for Lrp binding to GATC[1130] was clearly different for the methylated and nonmethylated sites (107).

These results invite speculation whether there are other GATC sites that remain unmethylated because of an interaction with Lrp. Although 11 GATC sites in the origin of DNA replication in *E. coli* are sequestered from methylation after passage of the replication fork (27), Smith et al. showed that Lrp was not responsible for this delayed methylation (142). However, recent data indicate that Lrp is required for methylation protection of a GATC site located at 77 min on the *E. coli* chromosome (60b). Lrp appeared to act directly to inhibit methylation since Lrp specifically bound to a DNA fragment containing this GATC site. Notably, this site became fully methylated upon addition of leucine to the growth medium, suggesting that binding of Lrp to this site was inhibited by leucine. It is not yet known what gene(s) might be controlled by binding of Lrp to this GATC site.

Organization and Regulation of the *lrp* Operon

Organization of the *lrp* operon. The location of *lrp* relative to other genes in the min 20 region of the *E. coli* chromosome is shown in Fig. 4 (35, 52). The nucleotide sequences of *trxB* (133), *lrp* (11, 168), and the region between them (11, 162) have been determined. The start point for *lrp* transcription, determined by primer extension and deletion mapping (162), is located 267 nucleotides upstream from the translational start of *lrp*. Inspection of this sequence suggests that it is an untranslated leader region. It is not yet clear whether there are genes downstream of *lrp* that are transcribed from the *lrp* promoter. *dinH*, a gene that is regulated by LexA, is immediately downstream of *lrp* and is transcribed in the same direction (83). It is likely that *dinH* has a separate promoter because the expression of *dinH* was markedly increased after treatment of *E. coli* with mitomycin (83) and that induction was not dependent on *lrp* or its promoter (60a). The exact location of that promoter has not been established, but there is a putative LexA-binding site 35 bp downstream of the end of the *lrp* gene (83). Whether some basal expression of *dinH* occurs by readthrough transcription from the *lrp* promoter remains to be established.

Autogenous regulation. Studies with *lrp-lacZ* transcriptional fusions indicate that *lrp* is autogenously regulated. Expression from the reporter gene was about 3-fold higher in a strain lacking Lrp than in a parent strain and 5- to 10-fold lower in a strain that produced elevated levels of Lrp (84, 117, 162). Most of the autogenous regulation by Lrp could be accounted for by binding of Lrp to a site located immediately upstream of the *lrp* promoter. A mutation in that site significantly decreased repression caused by Lrp and reduced binding in vitro (162).

The fact that wild-type strains of *E. coli* have a relatively high content of Lrp (about 3,000 dimers per cell) (168) suggests that the autogenous regulation of *lrp* is not particularly sensitive, which in turn suggests that the dissociation constant for the Lrp/*lrp* DNA complex is relatively high. The latter dissociation constant was determined by Wang et al. (162) to be 4 times higher than for binding to *ilvIH* sites 1 and 2 (159) and 12 times higher than to a site(s) upstream from *gltBDF* (44).

Effect of growth conditions on *lrp* expression. As measured by using *lrp-lacZ* fusions, neither leucine (84, 117) nor any of the other amino acids, when added singly to glucose minimal medium (117), affected expression from the *lrp* promoter. However, in a rich medium such as LB (84) or in a defined rich medium (glucose, morpholinepropanesulfonic acid [MOPS], amino acids, purines, pyrimidines, and vitamins) (30b, 171a), expression from the *lrp* promoter was repressed 4- to 10-fold. This repression did not require Lrp, because it was observed in a strain having an inactive *lrp* gene (84). As pointed out by Lin et al., even though Lrp controls its own synthesis by autogenous regulation, some other mechanism must override this regulation during growth in rich media (84). The mechanism by which rich medium affects *lrp* expression is not known, but much of the effect was observed when the 20 amino acids and the four nitrogen bases used for RNA synthesis were added to the medium (30b).

The conclusion that expression from the *lrp* promoter is lower for cells grown in a rich medium than in a minimal medium is based on experiments in which transcriptional fusions to a *lacZ* reporter gene were used. Although it is reasonable that Lrp levels are proportionately reduced in cells grown in a rich medium, this has not been demonstrated directly by using an antibody-based assay for Lrp. In the remainder of this review, it is assumed that Lrp levels are 4- to 10-fold lower in cells grown in rich media than in minimal media.

Effects of Lrp on Operon Expression

Direct versus indirect effects. In at least some cases, the effect of Lrp on a target operon has been shown to be direct (binding of Lrp activates or represses transcription of the target operon) rather than indirect (Lrp affects expression of another regulatory gene which, in turn, affects expression of the target operon). For the *ilvIH* operon, the evidence for direct action is as follows. In vitro, purified Lrp bound with high affinity to at least six sites upstream of the *ilvIH* promoter and mutations in each of these sites except one prevented binding to that site and lowered in vivo expression of the operon (Fig. 2) (159). In addition, Lrp stimulated transcription from the *ilvIH* promoter in vitro in a system containing purified RNA polymerase and purified Lrp (167).

The results of binding studies involving gel retardation techniques suggest that a number of other operons are directly controlled by Lrp. These operons include *daa* (157), *fim* (15a), *gltBDF* (44), *lysU* (85), *micF* and *ompC* (46), *pap* (107), and *sfa* (157). Furthermore, mutations in the apparent consensus sequence (TTTATCCTGAAT) for Lrp in the upstream region of the *tdh* operon resulted in constitutive expression of a reporter gene (127), consistent with a direct effect of Lrp on this operon as well.

Can Lrp act from a distance? The fact that there are six Lrp-binding sites located within a several-hundred-base-pair region upstream of the *ilvIH* promoter raises the question whether activation of transcription by Lrp requires binding at or near the promoter or whether Lrp can act from a more distant site. In considering this question, it is instructive to refer to the analysis of bacterial promoters by Collado-Vides et al. (30) and Gralla (59). They suggested that *E. coli* and *S. typhimurium* promoters that were positively controlled could be divided into two groups, one of them being a large collection of sigma 70 promoters for which activation can be understood in terms of direct interaction between a neighboring activator and RNA polymerase (30, 59). The other group was made up of a much smaller number of sigma 54 promoters.

For this group, binding sites for activator proteins were located far (up to 270 bp) upstream of the promoter and could be moved even further away without destroying activator function (30, 59). Activation of these sigma 54 promoters was imagined to occur through a looping mechanism, bringing the activator in contact with RNA polymerase. Some recent in vitro transcription experiments with purified RNA polymerase strongly suggest that the *ilvIH* and *gltBDF* promoters are recognized by a sigma 70 polymerase (44a, 167). Furthermore, experiments performed by Sacco et al. and confirmed by us have shown that moving the block of six Lrp-binding sites hundreds of base pairs further upstream destroyed the ability of Lrp to activate *ilvIH* expression in vivo (134, 158a). Taken together, these results suggest that Lrp activates transcription by binding to one of the downstream sites and directly interacting with a neighboring RNA polymerase. Wang and Calvo (159) argued that Lrp bound to site 5 probably activated transcription and that cooperative binding to sites 3 and 4 helped to deliver Lrp to site 5. Binding of Lrp to sites 1 and 2, centered at -250 and -219, respectively, was postulated to increase the effectiveness of site 5 through formation of a large nucleoprotein complex.

Does Lrp interact with other proteins? As mentioned in the Introduction, one of the interesting features of the Lrp regulon is the number of different patterns of regulation that result from the interaction of Lrp, leucine, and perhaps other proteins. How is it that leucine can overcome the effect of Lrp in one case (for example, the activation of *ilvIH*), and potentiate the effect of Lrp in another case (for example, the repression of *liv* operons by Lrp)? Why is it that some members of the Lrp regulon are affected by leucine and others are not?

One possible explanation for these phenomena is that in some cases Lrp interacts with another protein. This may be the case for regulation of *pap*, an operon whose expression is affected by Lrp but not by leucine. PapI, a regulatory protein encoded by the *pap* operon, did not bind to *pap* DNA by itself but affected both the pattern of bands observed in gel mobility experiments and the DNase I footprint pattern that was induced by Lrp (107). Thus, it is possible that PapI interacts directly with Lrp and that this interaction is responsible for the observed leucine insensitivity of the *pap* operon.

Effects of Leucine on Lrp-Mediated Regulation

At least some effects of leucine in vivo are mediated by Lrp. Table 3 lists the effects of leucine on expression of *lacZ* operon fusions to a number of members of the Lrp regulon and compares the effect of leucine in *lrp*$^+$ and *lrp* backgrounds. In most cases examined, leucine either increased or decreased expression of a target gene in an *lrp*$^+$ strain but had little effect on that target gene in an *lrp* strain. Thus, for these operons, the effect of leucine on transcription appears to be mediated by Lrp. It can also be seen that for strains growing in minimal media, the ability of leucine to antagonize the effect of Lrp on transcription varied greatly, with the effect of exogenous leucine ranging from substantial antagonism of the effect of Lrp (*tdh*, *sdaA*) to nearly no effect (*gltBDF*, *papBA*, *gcv*, *ompC*) to potentiation of the effect of Lrp (*livJ*, *livK*).

In addition, studies with strains having mutations within *lrp* showed that at least some of the in vivo effects of leucine are directly mediated by Lrp. For wild-type strains of *E. coli*, leucine repressed in vivo expression of the *ilvIH* operon 5- to 10-fold (145) and reduced binding of Lrp to sites upstream of the *ilvIH* promoter in vitro (128). Recent in vivo footprinting studies by M. Sacco and her colleagues showed very much the same result: leucine reduced Lrp binding to at least some of the sites upstream of the *ilvIH* operon (133a). By contrast, in

TABLE 3. Effect of leucine on transcription of operon fusions in the Lrp regulon

Strain	Relevant genotype	β-Galactosidase activity (Miller units)		Expression ratios		Reference
		−Leu	+Leu	−Leu/+Leu	+Lrp/−Lrp	
CP67	gcv-lacZ	1,675	1,450	1.2		84
CP67/lrp::Tn10	gcv-lacZ lrp201::Tn10	75	75	1.0	22	
BE3479	gltBDF-lacZ	441	196	2.3		44
BE3779	BE3479 lrp-35::Tn10	9.6	9.6	1.0	46	
CV975	ilvIH-lacZ	31	7	4.4		119
CV1008	CV975 lrp-35::Tn10	1.0	1.0	1.0	31	
BE54	ilvIH-lacZ	54	9.9	5.5		44
BE55	BE54 lrp-35::Tn10	1.8	ND^a		31	
SH151	livJ-lacZ	1,200	30	40		62
SH154	SH151 lrp-35::Tn10	1,500	1,300		0.02^b	
SH152	livK-lacZ	350	30	12		62
SH155	SH152 lrp-35::Tn10	1,150	1,100	1.1	0.03^b	
CV1069	lrp-lacZ	283	443	0.64		30a
MEW45(pLRN1)	lrp-lacZ pACYC184 lrp	150	200	0.75		84
MEW45(pACYC184)	lrp-lacZ pACYC184	1,550	1,475	1.1	10.3	
MF67	micF-lacZ	250	ND			46
MF66	MF67 lrp-35::Tn10	500	ND		0.5	
MF5.4	ompC-lacZ	45	55	0.82		46
MF6.5	MF5.4 lrp-35::Tn10	83	81	1.0	0.5	
SS5136	oppA-lacZ	208	820	0.25		9
SS5136/lrp::Tn10	oppA-lacZ lrp::Tn10				0.125	141a^d
DL379	papBA-lacZ	1,239	1,391	0.89		22^c
DL850	DL379 lrp-20::Tn10	37	22	1.7	33	
MF50.1	sdaA-lacZ	70	493	0.14		30a
SPC1.1	MF50.1 lrp-35::Tn10	1,103	987	1.1	0.06	
MEW22	sdaA-lacZ	56	225	0.25		86
MEW36	MEW22 lrp-201::Tn10	425	530	0.80	0.13	
DRN-1	serA-lacZ	2,350	1,360	1.7		86
MEW38	DRN-1 lrp-201::Tn10	450	500	0.90	5.2	
BW3912 (λserA)	serA-lacZ	446	189	2.4		127
SP1359	BW3912 (λserA) lrp::Tn10	104	89	1.2	4.3	
BW3912(λpdr1)	tdh-lacZ	9.6	86.7	0.11		127
SP1300	BW3912(λpdr1) lrp::Tn10	194	120	1.6	0.049	

^{a} ND, not determined.
^{b} The +Lrp/−Lrp ratio was determined for values obtained in the presence of exogenous leucine.
^{c} β-Galactosidase assays were measured on extracts from cells grown in M9 glycerol medium.
^{d} Personal communication to Rex et al. (127).

strains having the lrp-1 mutation, the ilvIH operon was not repressed by exogenous leucine (119, 155). Moreover, Lrp-1 purified from such strains bound to ilvIH DNA in vitro but the binding was not reduced by leucine (168). As mentioned earlier, a mutational analysis identified a region of Lrp that seems to be particularly important for the leucine-responsive behavior of the protein (118).

The results summarized above support two important conclusions. First, leucine affects ilvIH expression directly, rather than indirectly by influencing some other operon that affects ilvIH expression. Second, leucine-mediated repression of ilvIH expression can be understood in simple terms: Lrp activates transcription from the ilvIH promoter by binding to specific sites, and leucine reduces such binding.

Leucine has also been shown to antagonize the binding of Lrp to the promoter regions of lysU (85), which is negatively regulated by Lrp, and gltBDF, which is positively regulated by Lrp (44).

Specificity of the leucine effect. For some of the operons known to be affected by leucine in vivo, alanine has been shown
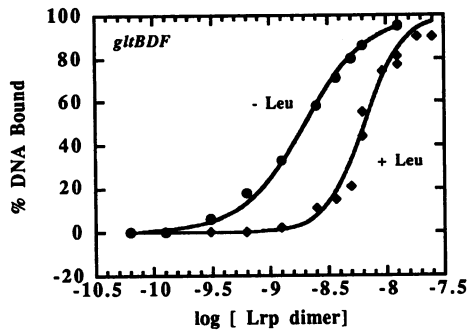
FIG. 5. Effect of leucine on Lrp binding to the *gltBDF* promoter. Data from gel mobility shift assays carried out in the absence or presence of saturating (30 mM) leucine were quantified by phosphor-imager scanning and plotted as percent DNA bound at various concentrations of Lrp. The data have been analyzed as described by Ernsting et al. (44). The apparent dissociation constant for Lrp binding to *gltBDF* DNA is 2.0 nM in the absence of leucine and 6.6 nM in the presence of 30 mM leucine. Reprinted from reference 44 with permission of the publisher.

to have a similar effect. This is true for *fan* (22, 34), *fim* (50), *ilvIH* (117), *lysU* (29), and *oppABCDF* (9). Platko (117) tested the effects of each of the naturally occurring amino acids except proline on *ilvIH* expression and found that only leucine and alanine had a detectable effect.

The effect of alanine on the patterns of proteins observed after two-dimensional electrophoresis was very similar to the effect of leucine, and no polypeptides that were regulated by alanine, but not by leucine, were identified (43).

In vitro, leucine, but not valine, isoleucine, or threonine, reduced the overall extent of binding of Lrp to *ilvIH* DNA (128). In contrast, Lin et al. reported that binding of Lrp to *lysU* DNA was reduced not only by leucine and alanine but also by isoleucine (85). Expression of the *lysU* operon in vivo, however, was not affected by isoleucine (85).

Leucine modulates, but does not abolish, the binding of Lrp to target operons. The effect of leucine on the affinity of Lrp in binding to the *gltBDF* promoter region has been examined quantitatively (44). Although leucine reduced binding, its effect was saturable, and at saturating concentrations of leucine, Lrp exhibited approximately a threefold reduction in its apparent affinity for the *gltBDF* promoter region (Fig. 5). These results strongly suggest that the binding of leucine to Lrp modulates but does not abolish the affinity of Lrp for the promoters of its target operons. In the cases of both the *gltBDF* and *ilvIH* operons, Lrp activates transcription both in the presence and in the absence of leucine. The data for negatively regulated operons shown in Table 3 also suggest that Lrp negatively regulates transcription of these operons both in the presence and in the absence of leucine.

The effects of leucine on positively regulated genes probably depend on the effective in vivo concentration of Lrp. Quantitative studies of the binding of Lrp to target DNA in the presence and absence of leucine suggest that the effect of leucine on positively regulated genes probably depends on the effective in vivo concentration of Lrp (44). At sufficiently high concentrations of Lrp, promoters will be complexed with activator regardless of the presence or absence of leucine, and all positively regulated genes and operons will be insensitive to leucine. Conversely, at sufficiently low concentrations of Lrp, all positively regulated genes and operons will be sensitive to leucine. Under any given growth condition, there will be a

hierarchy of positively regulated target genes, ranging from those with the lowest affinity for liganded Lrp, which are most sensitive to the effect of leucine, to those with the highest affinity for liganded Lrp, which are least sensitive to the effect of leucine. Also, for each target gene, there will be an Lrp concentration at which the effect of leucine on expression is maximal, i.e., a concentration of Lrp that balances the diminishing expression of a positively regulated gene as the Lrp concentration is decreased with the increasing leucine sensitivity as the Lrp concentration is decreased. From the in vitro studies of Lrp binding to *gltBDF* shown in Fig. 5, a maximal effect of leucine on expression should be seen at an Lrp dimer concentration of about 3 nM.

These considerations, together with the fact that *lrp* expression is repressed during growth in a rich medium, suggest a model for the regulation of genes in the Lrp regulon during shifts from minimal to rich media. The presence of leucine in rich medium is predicted to produce an immediate decrease in the effect of Lrp on positively regulated target genes, with a hierarchy of responsiveness of these target genes that depends primarily on the affinity of leucine-liganded Lrp for the target. Continued growth in a rich medium should lead to a decrease in the steady-state concentration of Lrp and thus to a continued decline in the transcription of positively regulated genes in the Lrp regulon. As noted above, transcription from the *lrp* promoter was 4- to 10-fold lower in cells grown in rich media than in minimal media. In Fig. 5, the predicted immediate effect of a rich medium containing leucine on transcription of *gltBDF* is a twofold decrease in expression (inferred from the change in DNA-binding effected by leucine at 5.5 nM Lrp dimer, which is estimated to be the effective in vivo concentration during growth in glucose minimal MOPS medium [44]). In these analyses it was assumed that the binding of Lrp to *gltBDF* DNA is both necessary and sufficient for transcriptional activation. Continued growth in rich medium would lead to further reductions in *gltBDF* expression as the concentration of Lrp in the cells decreased as a result of protein degradation and/or dilution during cell division. During steady-state growth in LB, the level of *gltBDF* expression was about 16-fold lower than during growth in glucose minimal MOPS medium (44), suggesting that a shift to LB resulted in a 3.5-fold decrease in the steady-state concentration of Lrp dimer.

This model for regulation of target genes by Lrp satisfactorily explains regulatory patterns 1 and 4 described in the Introduction. However, it does not explain how leucine can potentiate the repression of *liv* operons by Lrp or potentiate the activation of an operon by Lrp. One possible explanation is that Lrp binds to two different consensus sequences and that high concentrations of leucine decrease binding to sequences of one type and increase binding to the other. Another possibility is that there is only one type of Lrp-binding site but that the spacing of sites is different in operons representing different regulatory patterns. If such were the case, leucine might affect Lrp-Lrp interactions differently, depending upon the spacing. Further experiments are required to assess these possibilites.

Cooperativity associated with leucine effects. The quantitative gel shift measurements of Lrp binding to the *gltBDF* promoter region illustrate yet another feature that may be important in explaining preferential binding of Lrp upstream of certain operons when leucine is present. The curves used to fit the data shown in Fig. 5 were fitted by using the Hill equation. The apparent cooperativity, n, for this equation was greater in the presence of leucine than in its absence (44). The apparent cooperativity of Lrp binding to the low-affinity sites (3 + 4 + 5) upstream of *ilvIH* was also increased in the

presence of leucine. The origins of such enhanced cooperativity in the presence of leucine are not yet clear and may result either from dimerization of monomeric Lrp during binding to single sites or from increased interaction between Lrp dimers bound to adjacent sites when leucine is present. It may be possible to space Lrp-binding sites so that they are optimized for the interactions favored by leucine-saturated Lrp.

Genes That Are Evolutionarily Related to Lrp

Affinity-purified antibodies against Lrp reacted with Lrp-like proteins in a variety of members of the family Enterobacteriaceae, namely Serratia strain 8011, Enterobacter aerogenes, Klebsiella aerogenes, and S. typhimurium. Furthermore, proteins in crude extracts of these strains behaved like E. coli Lrp in gel retardation experiments that measured binding to DNA upstream of the ilvIH promoter (118a).

The lrp-like genes from S. typhimurium, K. aerogenes, Enterobacter aerogenes, and Serratia strain 8011 were cloned and shown to complement an lrp null mutation in an E. coli strain. The nucleotide sequence of each of these genes matches the E. coli sequence closely (about 87% identical), and the predicted amino acid sequences are even more closely related (99% identical) (118a). It is clear that there has been sufficient time for many of the positions to have been mutated, and the fact that with few exceptions only silent changes have survived suggests that most amino acid changes within Lrp are deleterious to enteric bacteria.

These results establish that an lrp-like gene exists in other enteric bacteria. There is also an lrp-like gene in Pseudomonas putida which shows 36% identity at the amino acid sequence level (89). This P. putida gene, called bkdR, lies immediately upstream of an operon involved with the catabolism of branched-chain amino acids (89). This latter operon, called here the bkdA1 operon, contains genes bkdA1, bkdA2, bkdB, and lpdV, which encode a branched-chain keto acid dehydrogenase multienzyme complex. bkdR and bkdA1 are separated by only a few hundred base pairs and are divergently transcribed. Strains having mutations that disrupted bkdR could not catabolize branched-chain amino acids, and this fact, plus the results of complementation studies, suggested that BkdR acts in trans to activate expression of the bkdA1 operon (89). As measured by gel mobility shift assays and DNase I footprinting, both purified BkdR and Lrp bound specifically to the region between bkdR and bkdA1 (142a).

The lrp-like gene from P. putida, when transferred to E. coli, codes for a protein that binds to anti-Lrp antibodies and that functions, albeit poorly, as an activator of the E. coli ilvIH promoter (118a). Furthermore, the E. coli lrp gene introduced into P. putida complements the bkdR gene (89).

These results demonstrate that at least one gram-negative organism only distantly related to E. coli has a gene that is related through evolution to lrp. It remains to be established whether bkdR has evolved to have a more specialized role limited to branched-chain amino acid catabolism or whether, like Lrp, it plays some more general role in coordinating metabolism. It is also possible that in addition to bkdR, there are other lrp-like genes in P. putida.

Comparison of Lrp with Other Regulatory Proteins

Typically, regulatory proteins alter the level of transcription of a target gene, and the regulation is modulated either by binding of a ligand to or by covalent modification of the regulatory protein. In contrast to the relatively small effects of leucine on the affinity of Lrp for its target DNA, many reg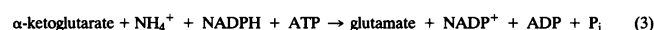ulatory proteins exhibit very large changes in specific binding affinity upon binding ligand or upon modification. Crp does not bind specifically to DNA in the absence of cAMP (2), whereas the affinity of the Lac repressor for the lac operator decreases more than 10,000-fold on binding inducer (93). Some other regulatory proteins retain significant affinity for their target DNA in both liganded and unliganded forms, as does Lrp, but transcription of the target gene may be altered significantly by binding ligand. Thus, the MerR protein represses merT when it is unliganded and activates transcription of merT when mercury is bound (109), and similar effects of arabinose binding are observed in the regulation of araBAD by AraC (88). In contrast to these "switch-like" regulatory proteins that turn transcription of the target gene off or on in response to the concentration of the regulatory ligand, Lrp modulates rather than abolishes transcription of target genes in response to leucine. In all the cases that have been studied, the effect of leucine is merely to modulate the binding of Lrp to the target gene and not to alter the effect of Lrp on transcription. If Lrp is a repressor in the absence of leucine, it remains a repressor in the presence of leucine.

OPERONS REGULATED BY Lrp

Amino Acid Biosynthesis

Five operons involved in amino acid biosynthesis are regulated by Lrp: the glnALG operon, which includes the coding sequence for glutamine synthetase; the gltBDF operon, which encodes glutamate synthase; the ilvIH operon, which encodes acetohydroxyacid synthase III (an isozyme involved in branched-chain amino acid biosynthesis); the leuABCD operon, which encodes enzymes that catalyze the biosynthesis of leucine; and serA, which encodes phosphoglycerate dehydrogenase (the enzyme catalyzing the first step in serine biosynthesis). Each of these operons is positively regulated by Lrp, although as described below, the effects on glnALG are indirect.

Regulation of gltBDF. Glutamate synthase catalyzes the first reaction shown below:

$$\alpha\text{-ketoglutarate} + \text{glutamine} + \text{NADPH} \rightarrow \text{NADP}^+ + 2\,\text{glutamate} \tag{1}$$

$$\text{NH}_4^+ + \text{glutamate} + \text{ATP} \rightarrow \text{glutamine} + \text{ADP} + P_i \tag{2}$$

$$\alpha\text{-ketoglutarate} + \text{NH}_4^+ + \text{NADPH} + \text{ATP} \rightarrow \text{glutamate} + \text{NADP}^+ + \text{ADP} + P_i \tag{3}$$

This reaction (equation 1) and the reaction catalyzed by glutamine synthetase (equation 2) are required for the assimilation of a low concentration of ammonia from exogenous media. The net result of the activities of these two enzymes (equation 3 above) is to convert 1 mol of α-ketoglutarate to glutamate. The same reaction can be catalyzed by glutamate dehydrogenase (equation 4):

$$\alpha\text{-ketoglutarate} + \text{NH}_4^+ + \text{NADPH} \rightarrow \text{glutamate} + \text{NADP}^+ \tag{4}$$
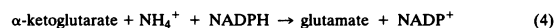
however, strains lacking glutamate dehydrogenase show no detectable phenotype, whereas gltBDF strains lacking glutamate synthase grow very slowly in media containing low levels of ammonia (1 mM) or containing nitrogen sources which generate ammonia slowly such as arginine or ornithine (126). The different phenotypes displayed by gltBDF strains and gdh strains may result from the fact that the K_m for ammonia is much lower for glutamate synthase than for glutamate dehydrogenase.

Glutamate synthase expression is not regulated by the level of ammonia in the medium, whereas glutamine synthetase expression is regulated predominantly in response to ammonia

levels. Glutamate synthase activity is highest in cells grown in minimal medium containing ammonia and is decreased in the presence of glutamate (13, 23) or in broth medium (23). The recent studies of Castano et al. have demonstrated that expression of GltF is required for glutamate-mediated repression of the *glt* operon (28).

The *gltBDF* operon was first identified as a member of the Lrp regulon by two-dimensional gel electrophoresis. When the total proteins of isogenic *lrp*$^+$ and *lrp* strains were analyzed, one of the polypeptides found to be expressed at reduced levels in an *lrp*::Tn*10* strain showed the migration properties of GltD, the small subunit of glutamate synthase (43). This tentative identification was confirmed by comigration with purified glutamate synthase and by enzyme assays of glutamate synthase. The levels of glutamate synthase activity were maximal in *lrp*$^+$ cells grown in glucose minimal MOPS medium supplemented with isoleucine and valine and were reduced about twofold when leucine was added to the medium. Glutamate synthase activity was undetectable in an isogenic *lrp* strain. Because the effect of Lrp on expression of glutamate synthase was so much greater than the effect of leucine, glutamate synthase was classified as a protein regulated by Lrp in a leucine-insensitive manner.

Studies with transcriptional fusions to *lacZ* demonstrated that the regulation of *gltBDF* by Lrp was at the level of transcription. Reporter gene expression was 44-fold higher in an *lrp*$^+$ strain than in a strain containing *lrp*::Tn*10*. In contrast, addition of leucine to the medium decreased expression of β-galactosidase 2.2-fold in the *lrp*$^+$ strain and had no effect on expression in the *lrp* strain. Thus, the effect of leucine on transcription requires a functional Lrp protein. Lrp was found to bind cooperatively to one or more sites upstream of the *gltBDF* promoter in mobility shift assays, and leucine reduced but did not abolish the affinity of Lrp for its target DNA (44). The exact position and number of these sites have not been determined, and mutational analysis has not yet established the functional significance of Lrp binding for the regulation of *gltBDF* transcription.

Regulation of *glnALG*. The *glnALG* operon encodes glutamine synthase (*glnA*) and two proteins involved in transcriptional regulation of *glnALG* expression, GlnL (NR$_{II}$) and GlnG (NR$_I$). The expression of this operon is regulated by the availability of ammonia, as sensed by the ratio of glutamine to α-ketoglutarate in the cell (126). The major promoter for *glnALG* transcription, *glnAp2*, is recognized by the σ^{54} subunit of RNA polymerase, and transcription from this promoter requires phosphorylated NR$_I$. The phosphorylation state of NR$_I$ is in turn regulated by NR$_{II}$ (which has both kinase and phosphatase activities) in response to the ratio of glutamine to α-ketoglutarate in the cell. The ratio of glutamine to α-keto-glutarate also controls adenylation of glutamine synthetase, a modification that results in greatly decreased activity of glutamine synthetase under physiological conditions. Adenylation is favored when the ratio of glutamine to α-ketoglutarate is high, indicating the adequate availability of nitrogen.

A two-dimensional electrophoretic analysis of cells grown in glucose minimal MOPS medium indicated that the expression of glutamine synthetase was reduced in *lrp* strains compared with *lrp*$^+$ strains and that Lrp also increased the ratio of the catalytically active unadenylated form of the enzyme to the much less active adenylated form (43). These findings were confirmed by assays of the total levels of glutamine synthetase activity in isogenic *lrp*$^+$ and *lrp* strains and of the ratio of adenylated to unadenylated forms of the enzyme. The *lrp* strain had about fourfold-lower total expression of glutamine synthetase during exponential growth in glucose minimal MOPS

medium lacking ammonia and with glutamine provided as the sole source of nitrogen, and 70% of the glutamine synthetase was in the adenylated inactive form, compared with 20% of the adenylated enzyme in the *lrp*$^+$ strain. Thus, the effective concentration of active glutamine synthetase in an *lrp* strain grown under nitrogen-limiting conditions is about 9% of the concentration in an *lrp*$^+$ strain. *lrp* strains are functionally Ntr$^-$, meaning that they do not show elevated levels of glutamine synthetase when grown under nitrogen-limiting conditions and that they are unable to induce other operons whose expression is regulated by GlnL and GlnG, such as those required for growth on arginine as the sole nitrogen source. *lrp* strains grow very slowly on media containing glutamine as the sole nitrogen source or on low levels of ammonia. They are also unable to use arginine or proline as the sole nitrogen source.

Mutants lacking glutamate synthase activity are also functionally Ntr$^-$, probably because glutamine cannot be converted to glutamate in these strains, and the intracellular ratio of glutamine to α-ketoglutarate is consequently high (23, 112, 114). Since very low levels of glutamate synthase are expressed in *lrp* strains, the possibility was examined that the effect of Lrp on glutamine synthetase expression was indirect and was secondary to its effect on glutamate synthase expression (43). The effect of Lrp on glutamine synthetase expression was shown to require a functional NR$_{II}$ protein. Thus, the effect of Lrp on glutamine synthetase expression requires a functional signal transduction pathway linking the cellular glutamine/α-ketoglutarate ratio to the transcription and covalent modification of glutamine synthetase. The studies by Castano et al. (28) have shown that regulation of glutamine synthetase expression by glutamate synthase also requires high expression of both GltD and GltF and that GltF exhibits sequence similarity to histidine kinases. They suggested that GltF may regulate glutamine synthetase expression by phosphorylating either NR$_I$ or NR$_{II}$.

In summary, it is probable, although not yet proven, that the effect of Lrp on glutamine synthetase levels is entirely due to the effect of Lrp on *gltBDF* transcription.

Regulation of *ilvIH*. The first step common to the biosynthesis of branched-chain amino acids is catalyzed by acetohydroxy acid synthase (AHAS). *ilvIH* encodes AHAS III, one of two AHAS isozymes that are present in *E. coli* K-12 (32). *ilvI* and *ilvH* together form an operon and code for polypeptides with molecular weights of 61,000 and 17,000, respectively (143). The *ilvI* polypeptide is absolutely required for AHAS III activity, whereas the *ilvH* polypeptide enhances AHAS III activity and also confers upon it sensitivity to inhibition by valine (139).

The expression of the *ilvIH* operon is repressed by leucine (33) at the level of transcription (145). Haughn et al. showed that sequences several hundred bases upstream from the *ilvIH* promoter were required for optimal expression from that promoter (63), and Ricca et al. identified in crude extracts of *E. coli* a protein that bound to those sequences (128). That protein was originally called IHB (*ilvIH*-binding protein), but the name was changed to Lrp (119) when it was recognized that Lrp affected a number of unrelated operons (11, 86, 119). Expression from the *ilvIH* promoter was reduced more than 30-fold in a strain lacking a functional Lrp (119). Evidence that Lrp directly activates expression from the *ilvIH* promoter and that leucine reduces the extent of activation was summarized above.

Some recent studies suggest that *ilvIH* expression is repressed by H-NS. *ilvIH* expression was higher in a strain containing a mutation in *hns* and lower in a strain having multiple copies of *hns*$^+$ on a plasmid. The effect of the *hns*

mutation was small in log-phase cells grown in a minimal medium, but more pronounced effects were observed in stationary-phase cells or in cells grown in minimal medium supplemented with leucine, especially at high osmolarity (82). The negative effect of H-NS may provide a greater range of *ilvIH* expression by reducing the basal level of expression when Lrp activity is low.

Regulation of *leuABCD*. Leucine is synthesized in most microorganisms by a pathway that is initiated by the condensation of α-ketoisovalerate with acetyl coenzyme A (acetyl-CoA). In both *E. coli* and *S. typhimurium*, the four genes responsible for leucine biosynthesis are organized as an operon and controlled by a transcription attenuation mechanism (53, 165). Some recent results from Lin et al. suggest that the *leuABCD* operon of *E. coli* may also be positively regulated by Lrp (84). Among the strains having λ p*lac*Mu insertions that they isolated were some that required leucine for growth and, from mapping experiments, probably had insertions in the *leu* operon. In media containing leucine, four of these isolates showed 10-fold-higher expression of reporter gene expression in an *lrp*⁺ strain than in an *lrp* strain. It is not yet clear whether the effect of Lrp on *leu* operon expression is direct or indirect. An indirect effect is possible since strains lacking Lrp have elevated capacity to transport leucine as a result of derepression of the high-affinity branched-chain amino acid transport system. That elevated capacity may lead to elevated intracellular concentrations of leucine and, as a result, to reduced *leu* operon expression.
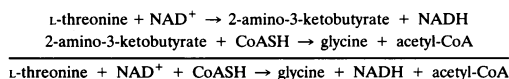
Regulation of *serA*. *serA* maps at min 63 (108) and encodes 3-phosphoglycerate dehydrogenase, an enzyme that catalyzes the first committed step in the biosynthesis of serine from glucose. The expression of *serA* was decreased during growth in media containing exogenous leucine but was not affected by exogenous serine (95). Lin et al. (86) subsequently demonstrated that expression of β-galactosidase in a *serA-lacZ* fusion strain was regulated by both Lrp and leucine (Table 3). These observations were confirmed by Rex et al. (127) (Table 3), who used a protein fusion of *lacZ* to nucleotide 545 of the *serA* coding region. In both cases, Lrp stimulated expression of β-galactosidase and the effect of Lrp was antagonized by leucine. Further studies on the regulation of *serA* by Lrp have not been reported, and, in particular, direct regulation of *serA* transcription by Lrp has not been demonstrated.

Amino Acid Degradation

Three operons involved in amino acid catabolism are regulated by Lrp. These are the *sdaA* operon coding for L-serine deaminase, the *tdh* operon encoding threonine dehydrogenase and 2-amino-3-ketobutyrate lyase, and the *gcv* operon encoding the proteins required for formation of one-carbon units from glycine.
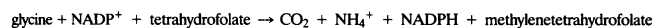
Regulation of *sdaA*. L-Serine deaminase, which catalyzes the catabolism of serine to pyruvate and ammonia, is induced by leucine (115). The effect of leucine on L-serine deaminase was shown to be mediated at the level of transcription and required a functional Lrp (86) (Table 3). The *sdaA* gene has been cloned and sequenced (150). To date, there is no published evidence on whether the regulation of *sdaA* by Lrp is direct.

Regulation of *tdh*. The enzymes of the *tdh* operon, threonine dehydrogenase and 2-amino-3-ketobutyrate lyase, catalyze the following reactions:

$$\text{L-threonine} + \text{NAD}^+ \rightarrow \text{2-amino-3-ketobutyrate} + \text{NADH}$$
$$\text{2-amino-3-ketobutyrate} + \text{CoASH} \rightarrow \text{glycine} + \text{acetyl-CoA}$$

$$\text{L-threonine} + \text{NAD}^+ + \text{CoASH} \rightarrow \text{glycine} + \text{NADH} + \text{acetyl-CoA}$$

Synthesis of glycine from threonine normally does not occur in media containing glucose. In such media, 3-phosphoglycerate serves as the precursor of serine, and serine is converted to glycine in a reaction catalyzed by serine hydroxymethyltransferase (the *glyA* gene product). However, early studies of metabolism in *E. coli* by Roberts et al. established that glycine served as the precursor for serine when either fructose or acetate was the carbon source (130). Several investigations provided evidence that glycine could be formed from threonine under certain circumstances (47, 158). Threonine dehydrogenase was subsequently shown to be induced by leucine (103), and a strong linkage was established between induction of the enzyme and the ability to synthesize glycine from exogenous threonine (103). The metabolic pathway required for conversion of threonine to glycine and serine was further studied by Ravnikar and Somerville (124, 125). The effect of leucine on threonine dehydrogenase activity was shown to require functional Lrp (86), while the investigations of Aronson et al. (10) have established that leucine affects transcription of the *tdh* operon.

Regulation of *gcv*. The catabolism of glycine proceeds by the reaction shown below.

$$\text{glycine} + \text{NADP}^+ + \text{tetrahydrofolate} \rightarrow \text{CO}_2 + \text{NH}_4^+ + \text{NADPH} + \text{methylenetetrahydrofolate}$$

The reactions required for the cleavage of glycine involve four enzymes referred to as T, H, P, and L. The L protein, lipoamide dehydrogenase (encoded by the *lpd* operon), is also part of α-keto acid dehydrogenase complexes in *E. coli*. The T, H, and P proteins are encoded by the *gcv* operon and are specific for glycine cleavage. This operon has recently been mapped to the 62-min region of the *E. coli* chromosome, and has been cloned and sequenced (110). The *gcv* operon is negatively regulated by PurR (169) and positively regulated by Lrp (84). The regulation of the *gcv* operon by Lrp has not been extensively studied, although it has been noted that the expression of a *gcv-lacZ* operon fusion was almost insensitive to leucine (84) (Table 3) but highly sensitive to the presence or absence of Lrp. It is not yet known whether the regulation of *gcv* by Lrp is direct or indirect. Wilson et al. identified GcvA as an unlinked, *trans*-acting positive regulator of *gcv* expression (170). It will be interesting to determine whether positive regulation of the *gcv* operon by Lrp requires a functional GcvA protein and whether *gcvA* is regulated by Lrp.

Whereas the *sdaA* and *tdh* operons are negatively regulated by Lrp, the *gcv* operon is positively regulated by Lrp, like the amino acid biosynthetic operons described above. The glycine cleavage pathway may allow the need for one-carbon units to be coordinated with the need for glycine (146). The positive regulation of glycine cleavage by Lrp may be rationalized because this coordination is most important when cells are actively synthesizing purines, pyrimidines, and amino acids. Thus, the glycine cleavage enzymes may provide one-carbon units for biosynthetic reactions, as well as a pathway for catabolism of glycine.

Transport

Six operons involved in nutrient transport are known to be regulated by Lrp. Two operons involved in high-affinity transport of branched-chain amino acids, the *livJ* and *livKHMGF* operons, are negatively regulated by Lrp, and that negative regulation is potentiated by leucine. This pattern of regulation leads to high-level expression of the operon during growth in minimal medium and its repression when leucine is present in the medium. Thus, although the high-affinity branched-chain amino acid transport proteins are negatively regulated by Lrp,

the pattern is similar to those of positively regulated operons that are expressed in minimal medium and down-regulated when leucine is present. The oligopeptide permease operon encoded by *oppABCDF* is also negatively regulated by Lrp, but in this case leucine abolishes repression. Lrp also increases the expression of *ompF* and decreases the expression of *ompC*. As will be discussed below, preliminary evidence suggests that the effect of Lrp on *ompF* expression requires a functional *micF* gene and that expression of the *micF* operon is also controlled by Lrp.

A minor serine-specific transport system in which serine uptake is coupled to proton uptake has also been shown to be induced by leucine (61, 72). Whether this leucine effect is mediated by Lrp has not been reported.

Regulation of *livJ* and *livKHMGF*. Branched-chain amino acids are transported into *E. coli* by a low-affinity system, LIV-II, and by two high-affinity systems, LIV-I and LS (98, 122). The LIV-1 transport system transports leucine, isoleucine, and valine, whereas the LS transport system transports only leucine. The last two systems share a common set of membrane components and are distinguished by the specificity of their periplasmic binding proteins (77). The LIV-1-binding protein binds L-leucine, L-isoleucine, and L-valine with approximately equal affinity and is coded for by the *livJ* gene. By contrast, the LS-binding protein binds D- and L-leucine but neither isoleucine nor valine and is coded for by *livK*, the first gene in an operon that also contains genes *livHMGF* coding for the common membrane components. *livJ* and *livKHMGF* are closely linked near min 76 on the *E. coli* chromosome within a region referred to as the LIV-I locus.

The LIV-I locus is regulated by leucine, as determined by assay of transport and leucine-binding activity of periplasmic shock fluids (5, 123). Mutants that were constitutively derepressed in transport of leucine through high-affinity systems were isolated and shown to have mutations in a locus, *livR*, that was located near min 20 (6). Haney et al. showed that *livR1* was an allele of *lrp* (62). Although *livR1* was the first allele of *lrp* isolated, *lrp* was chosen as the locus designation because it seemed to better convey the notion that regulation was not limited to a particular operon.

Strains lacking Lrp had a high, constitutive ability to transport leucine, and expression from the *livJ* and *livKHMGF* promoters was similarly high and constitutive (62). These results suggest that Lrp acts negatively on expression from these promoters. Since in a wild-type strain, leucine is required for repression of leucine transport and it reduces expression from the *livJ* and *livKHMGF* promoters, the implication is that leucine is required for Lrp-mediated repression. Indeed, in strains containing *lrp-1*, an allele that makes *ilvIH* expression insensitive to leucine (119), leucine transport and expression from *livJ* and *livKHMGF* promoters were no longer repressible by leucine (62). It is not yet clear whether the effects of Lrp upon *liv* operon expression are direct or indirect.

Regulation of *oppABCDF*. Enteric bacteria have a number of different permeases that allow active transport of peptides across the inner membrane. The focus here is on *oppABCDF*, an operon linked to *trp* that encodes a peptide transport system with rather broad specificity (12, 64). In *E. coli* K-12, exogenous leucine increased the transport velocity and accumulation level of peptides, and those increases required an intact *oppABCDF* operon (8). Consistent with these results is the finding that leucine increased the rate of synthesis of OppA, the peptide-binding protein located in the periplasm (8). The effect of leucine was at the level of transcription, as judged by measuring reporter gene expression in strains containing *opp-lacZ* fusions (9). Exogenous alanine and growth under anaer-

obic conditions also served to elevate expression from the *opp* operon promoter. Any two of the three inducing signals together gave the same level of induction as a single signal (9).

Austin et al. (11) isolated mutations in *oppI* that led to constitutive expression of the oligopeptide permease operon. They determined the map location of *oppI* to be near min 20, cloned *oppI*, and determined its nucleotide sequence (11). *oppI* was subsequently shown to be identical to *lrp* (119).

In *S. typhimurium*, there are at least two operons that encode peptide permeases, *oppABCDF* (located near *trp*) (79) and *tppB* (located at min 34) (55). Curiously, the *oppABCDF* operon of this strain was apparently expressed constitutively, whereas the expression of *tppB* was induced by exogenous leucine and anaerobiosis (64, 70). Particularly puzzling were the opposite results of ostensibly the same experiments performed by two different groups employing F'-bearing *opp-lacZ* fusions from *E. coli* that were transferred to *S. typhimurium*. Reporter gene expression was reported by Hiles et al. (64) to be constitutive but by Andrews and Short (9) to be induced by leucine. The basis for this discrepancy is not clear.

Regulation of *ompC*, *micF*, and *ompF*. A two-dimensional electrophoretic analysis of protein expression in isogenic *lrp*$^+$ and *lrp*::Tn*10* strains first identified OmpC and OmpF as members of the Lrp regulon (43). Expression of OmpC was increased about twofold and expression of OmpF was decreased about twofold in an *lrp* strain compared with the wild-type strain. These proteins were characterized as leucine insensitive. More-recent studies (46) established that Lrp decreased the expression of an *ompC-lacZ* transcriptional fusion (Table 3) and thus acts at the level of transcription on this gene. Leucine weakly antagonized the effect of Lrp on *ompC* expression, and, as in other cases, the effect of leucine required a functional Lrp protein. Mobility shift assays with DNA containing the *ompC-micF* intergenic region indicated that Lrp bound specifically to this region, forming at least two complexes. In contrast, the expression of β-galactosidase from an *ompF-lacZ* transcriptional fusion was not decreased in an *lrp* background, as would be expected from the effect of an *lrp* mutation on expression of OmpF. Rather, expression was slightly increased. This surprising observation suggested that regulation of OmpF by Lrp might be indirect. Since *ompF* translation is regulated by an antisense RNA encoded by the *micF* gene (4) and since the *micF* and *ompC* genes are divergently transcribed, these observations suggested that Lrp might be regulating the transcription of both *micF* and *ompC*. A preliminary indication that this hypothesis is correct is the effect of Lrp on a *micF-lacZ* transcriptional fusion, shown in Table 3 (46). The transcription of *micF*::*lacZ* was increased approximately twofold in an *lrp* strain compared with the isogenic wild-type strain.

Formation of Pili

Pili, also known as fimbriae, are composed of about 1,000 pilin protein subunits. They project from the surface of enteric bacteria, helping them to bind to eukaryotic cells and thus to colonize habitats such as the intestine or urinary tract. Pili produced by different strains of *E. coli* that have been studied intensively include P (106), K99 (49), S (138), F1845 (15), K88 (96), and type I (25) pili. About a dozen genes are required for formation of a single pilus type, including a gene that codes for the major pilin subunit, genes that encode minor pilin subunits that determine host cell specificity, genes that are required for pilin assembly, and genes that regulate expression of the aforementioned genes (87).

Environmental conditions such as temperature, media com-

position, and pH affect the expression of genes involved in pilus formation. In addition, some *E. coli* strains exhibit pilus phase variation, meaning that cells either produce pili of a particular type (phase ON) or do not (phase OFF). Progeny of cells of one type breed true but occasionally give rise to cells having the opposite phase phenotype.

Lrp plays an important role in the expression of some pilus genes and in pilus phase variation. The summaries below are focused on this role of Lrp, and readers are referred to a review by van der Woude et al. (156) and to some of the references cited below for a broader view.

Regulation of *pap* genes. The organization of *pap* (pyelonephritis-associated pili), the gene cluster required for the synthesis of P pili, is shown in Fig. 6A. *papA* encodes the major pilin subunit, and *papI* and *papB* are regulatory genes that control expression of the major promoter that is located just upstream of *papB*. *E. coli* strains carrying the *pap* gene cluster exhibit phase variation (156). Remarkably, the mechanism underlying this phase variation is not genetic (i.e., it is not due to mutation or rearrangement of DNA) (19). Rather, phase variation is regulated by the methylation state of two GATC sites, denoted $GATC^{1028}$ and $GATC^{1130}$ (Fig. 6A), that are located about 100 bp apart between *papI* and *papB* (18, 21). The phase ON state of phase variation was correlated with $GATC^{1028}$ being unmethylated and $GATC^{1130}$ being methylated, whereas for the phase OFF state the opposite was the case. In cells that either lacked the Dam methylase (enzyme that methylates the A within GATC) or had elevated levels of this enzyme, the switch was locked in the OFF position (18).

Except for a short time after DNA replication, GATC sites are expected to be methylated on both strands. Braaten et al. reasoned that GATC sites that remained unmethylated probably contained a protein that bound specifically to those sites, and they searched for a gene that coded for such a "methylation blocking factor." This search uncovered the locus *mbf* (20), and *mbf* was later shown to be identical to Lrp (22).

In strains lacking Lrp, expression from the *papBA* promoter was reduced about 40-fold and cells were locked in the phase OFF state (22). Leucine had no detectable effect on expression from the *papBA* promoter (22). Therefore, *papBA* belongs to the class of operons that are activated by Lrp and whose expression is unaffected by leucine.

Papl, the protein encoded by *papI*, is also required for expression from the *papBA* promoter. The binding of Papl and Lrp to *pap* DNA was investigated by Nou et al. by using DNase I footprinting (107). Papl did not bind to either methylated or unmethylated *pap* DNA. Lrp, on the other hand, caused the pattern of DNA cleavage to be perturbed over a region of about 120 bp, including about 45 bp to the right of $GATC^{1130}$ and 75 bp to the left (Fig. 6A). The footprint pattern was similar whether fully methylated DNA or unmethylated DNA templates were used, although distinct differences could be seen near $GATC^{1130}$. Significantly, the Lrp-related footprint was extended about 60 bp when Papl and Lrp were added together, and the extension was centered over $GATC^{1028}$. This was the case for unmethylated DNA but not for methylated DNA. These results, and the results of gel retardation experiments (107), suggest that either Papl directly interacts with Lrp (perhaps dependent upon DNA) or binding of Lrp perturbs the structure of nearby DNA so that Papl is able to bind to that DNA.

These studies direct attention to the methylation state of $GATC^{1028}$ as being the determinant of *papBA* promoter activity. Binding of Lrp and Papl in the region of $GATC^{1028}$ is postulated as being essential for *papBA* promoter activity, and that binding, in turn, is dependent on $GATC^{1028}$ being un
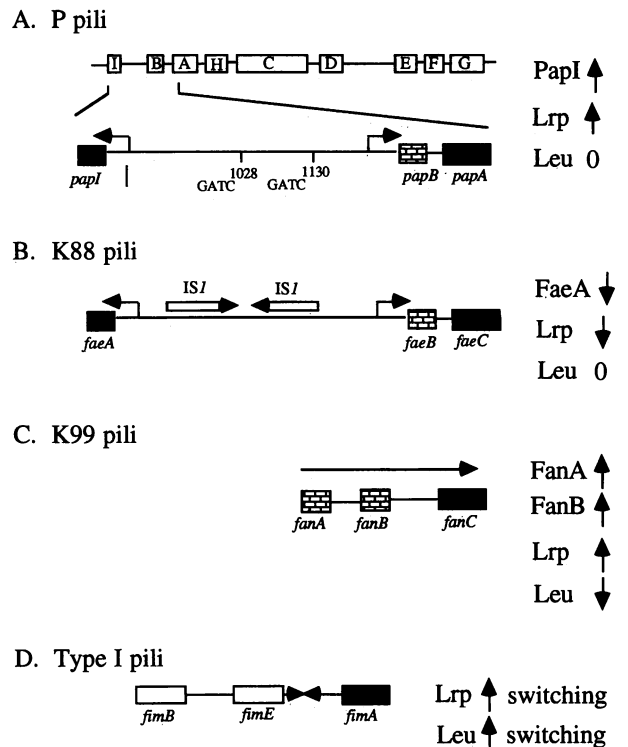

FIG. 6. Organization of the gene clusters involved in pilin biosynthesis. (A) P pili, encoded by *pap* genes. PapA is the major pilin subunit that forms the shaft of the pilus; PapH resembles a pilin and may terminate pilus growth; PapC is located in the outer membrane and forms the assembly platform for pilus growth; PapD is a chaperone that helps assembly of the pilus; and PapE, PapF, and PapG form the tip of the pilus. PapG determines the digalactoside-binding specificity of the pilus. Papl and PapB are regulatory proteins. Horizontal arrows represent promoters with the direction of transcription indicated. Redrawn from references 87 and 156. (B) K88 pili, encoded by *fae* genes. Only a part of the gene cluster is shown for this and other parts of the figure. IS*1* is an insertion element. Redrawn from reference 68. (C) K99 pili, encoded by *fan* genes. The arrow represents the direction of transcription of *fanA*, *fanB*, and *fanC*. There may be separate promoters for *fanA* and *fanB*. Redrawn from reference 131. (D) Type I pili, encoded by *fim* genes. The arrowheads represent a 314-bp invertible DNA segment. Modified from reference 94. Hatched rectangles identify genes that are related to *papA*, encoding the major pilin subunit. Brick-patterned rectangles and shaded rectangles identify genes that are related to *papB* and *papI*, respectively. Upward-pointing and downward-pointing arrows represent, respectively, stimulation and inhibition of rightward transcription by the indicated protein. For panel D, the stimulation is limited to the switching of the invertible element.

methylated (156). Other studies by Braaten et al. (21) support this conclusion. Cells containing the mutation $GATC^{1028} \rightarrow GCTC^{1028}$ were locked in the phase ON state. The $GCTC^{1028}$ mutation clearly prevented methylation, but the phase ON phenotype suggested that the mutation did not affect the interaction of Lrp and Papl with the $GATC^{1028}$ region. In vitro footprinting studies supported that notion (21). An important additional finding was that cells containing a $GATC^{1130} \rightarrow GCTC^{1130}$ mutation were stuck in the OFF position, suggesting that methylation at $GATC^{1130}$ is required for *papBA* promoter activity. This idea is given additional credence by the finding that the $GCTC^{1028}$ mutant, which was locked in the ON state in *dam*⁺ strains, was OFF in a *dam* strain (21).

In concluding this section, we want to emphasize one idea that emerges from these studies. The effect of Lrp in this system is dependent on another protein, PapI. This interaction, whether direct or not, may underlie some of the observed regulatory patterns that were discussed above.

Regulation of daa and sfa genes. A number of studies indicate that the *daa* genes, which encode F1845 pili, and the *sfa* genes, which encode S pili, are regulated in a manner similar to that described above for *pap*. The organization of the *daa* and *sfa* gene clusters is similar to that shown for *pap* in Fig. 6A. Furthermore, the region separating each pair contains an inverted repeat with embedded GATC sites that are separated by about 100 bp (14, 156). PapI, DaaF, and SfaC show significant amino acid sequence similarity (14, 57, 156), as do PapB, DaaA, and SfaB (14, 156). Moreover, F1845 and S pili, like P pili, show phase variation (137, 157), and that phase variation requires the Dam methylase and Lrp (157). In cells lacking Lrp, expression from the major *daa* promoter was reduced at least 60-fold (14, 157), and the same was true for the major *sfa* promoter (157). In addition, the pattern of methylation at the two GATC sites (denoted distal and proximal, relative to the major promoter) was the same for the three systems: phase ON, distal GATC unmethylated and proximal GATC methylated; phase OFF, distal GATC methylated and proximal GATC unmethylated (157). Finally, the results of DNase I footprinting performed with *daa* and *sfa* DNA and Lrp and Pap proteins (157) was generally consistent with the results of earlier studies with *pap* DNA (107).

Regulation of fae genes. The *fae* genes, which encode K88 pili (found on *E. coli* strains that cause diarrhea in pigs), are organized similarly to *pap* genes. *faeA* and *faeB* are transcribed divergently, and their putative protein products show considerable amino acid sequence similarity to PapI and PapB, respectively (68). In addition, the region separating *faeA* and *faeB*, like the corresponding *papI-papB* region, contains an inverted repeat with embedded GATC sites that are separated by about 100 bp (68, 156). These similarities, however, belie major differences (Fig. 6B). For one thing, two IS*1* elements are located between *faeA* and *faeB*, and this was observed in three independent *E. coli* isolates that had K88 pili (68). Furthermore, phase variation has not been observed for K88 pili. But most interesting, the basic mechanisms underlying the regulation of the *fae* and *pap* operons differ appreciably. For the *pap* operons, *papB* stimulates *papI* transcription (58) and PapI and Lrp both act positively to stimulate transcription from the major promoter (107). *faeB*, unlike *papB*, had no effect upon *fae* operon expression or K88 production (68). Moreover, Lrp and FaeA had negative effects, as evidenced by the fact that K88 pili production at 28°C was elevated six- to eightfold in mutants lacking either FaeA or Lrp or both. In addition, overproduction of FaeA nearly abolished K88 pili production, and replacing *faeA* (relatively weakly expressed) with PapI also caused K88 pili production to be severely reduced (68).

Although it seems clear in this case that FaeA and Lrp are acting negatively, the mechanism by which they act seems less clear. Results with *lacZ* transcriptional fusions showed only 2.4- and 1.5-fold increases in reporter gene expression in *faeA* and *lrp* mutants, respectively, suggesting that the effects of FaeA and Lrp may not be mainly on transcription (68).

Regulation of fan genes. K99 pili, which allow bacteria to attach to epithelial cells in the small intestines of lambs, calves, and piglets, are encoded by *fan* genes (97). The regulation of *fan* genes is clearly different from that of any of the other pilus genes described here. The gene that encodes the major pilin subunit, *fanC*, is preceded by two short genes, *fanA* and *fanB* (Fig. 6C).

The amino acid sequences of FanA and FanB are clearly related to each other and to PapB (131). Thus far, there has been no report of a *fan* gene that is homologous to *papI* or evidence that K99 pili undergo phase variation. Frameshift mutations in *fanA* or *fanB* reduced the extent of K99 pilus production 8- and 16-fold, respectively, suggesting that each of these loci has some important function. However, these mutations did not have much effect upon the expression of *fanC*, *fanD*, *fanE*, *fanF*, *fanG*, and *fanH*, as measured by analysis of the corresponding proteins in minicells (131), nor did they have much effect on transcription from the *fanA* promoter, as measured by using a *lacZ* transcriptional fusion (157). These results suggest that FanA and FanB may be involved in some step of pilus production other than controlling gene expression.

Strains lacking Lrp produced few K99 pili and had 70-fold-lower expression from the *fanA* promoter, as measured with a *fanABC-lacZ* transcriptional fusion (22). Thus, Lrp activates transcription from *fan* genes, as it does for *pap*, *daa*, and *sfa* genes. However, *fan* gene expression was markedly affected by leucine, whereas that was not the case for the others. Exogenous leucine or alanine at 100 μg/ml reduced expression from the *fanA* promoter about 10-fold (22).

Regulation of fim genes. Most *E. coli* strains contain type I pili, encoded by *fim* genes (also called *pil* genes) (111). It was recognized some time ago that type I pili undergo ON-OFF phase variation that is under transcriptional control (41). The basis for phase variation is inversion of a 314-bp DNA segment that lies upstream from *fimA*, the gene that encodes the major pilin subunit (1) (Fig. 6D). One of the orientations of the segment provides a promoter for *fimA* and downstream genes, and that orientation is characteristic of phase ON cells. Type I pilus phase variation is similar in some ways to phase variation of flagellar antigens in *Salmonella* species (174), but there are also important differences. One important difference between the two is that the invertible element in the *fim* system does not encode a protein required for the inversion event (1). Instead, two genes located immediately upstream of the invertible region, *fimE* and *fimB*, are directly involved in the inversion process (Fig. 6D) (74). FimE and FimB, which share 48% amino acid identity (74), are related to the integrase family of site-specific DNA recombinases (39, 42). Whereas FimE promotes ON to OFF inversion only, FimB stimulates switching in both directions (94). Until recently, strains believed to be wild type underwent slow (10^{-3}) and apparently random phase variation. At least some of these strains are now known to be *fimE* mutants (17). In true *fimB*⁺ *fimE*⁺ wild-type strains, very rapid ON-to-OFF switching promoted by *fimE* (0.3 per cell per generation) overrides the activity of *fimB*, ensuring that the OFF phase predominates.

Mutations in *himA* or *himD*, which code for subunits of integration host factor, markedly reduced the inversion frequency (39, 42), whereas mutations in *osmZ* (also called *pilG*; codes for H-NS) had the opposite effect (39, 48). More recently, Blomfield et al. (16) reported that Lrp had a marked effect on the inversion process. They showed that in the absence of Lrp, both the ON-to-OFF rate and the OFF-to-ON rate were reduced more than 100-fold. The lack of Lrp had little effect on expression of *fimE-lacZ* or *fimB-lacZ* fusions, suggesting that Lrp does not affect inversion by influencing the relative amounts of FimE or FimB.

Gally et al. investigated the effects of temperature and medium composition on switching (50). Both *fimB*- and *fimE*-promoted switching were stimulated by addition of branched-chain amino acids plus alanine to minimal medium, and this stimulation required Lrp. The rate of *fimE*-promoted switching was the same in both the supplemented minimal medium and

a defined rich medium, whereas the rate of *fimB*-promoted switching was eightfold lower in the latter medium. This difference was observed only in an *lrp*+ strain, suggesting that Lrp is involved in the differential control of FimB and FimE activity. The low rate of *fimB*-promoted switching in a rich medium might be due to reduced transcription of *lrp* that is known to occur during growth in rich medium (84).

These results are consistent with the idea that environmental cues determine the proportion of *E. coli* cells that produce type I pili.

Miscellaneous Operons

In addition to proteins involved in amino acid biosynthesis, amino acid catabolism, nutrient transport, and adherence to host tissues, three other proteins are known to be regulated by Lrp. The first of these is the Lrp protein itself, which is autogenously regulated at the level of transcription. The other two proteins are lysyl-tRNA synthetase form II encoded by the *lysU* gene and W protein (whose gene has not yet been identified). In addition, a recent review by D'Ari et al. (31) cites unpublished information suggesting that *glyA* (encoding serine hydroxymethyltransferase) is negatively regulated by Lrp and that *pnt* (encoding pyridine nucleotide transhydrogenase) and CP8 (a gene involved in utilization of xylose, ribose, arabinose, and rhamnose) are positively regulated by Lrp.

Regulation of *lysU*. In contrast to the other aminoacyl-tRNA synthetases, which are encoded by single genes, there are two unlinked genes encoding lysyl-tRNA synthetase isozymes, *lysU* and *lysS*. *lysU* maps at 92 min on the *E. coli* chromosome, and *lysS* maps at 62.1 min (81). The *lysU* gene is a member of the heat shock regulon (100). In addition, it was induced by exogenous leucine or alanine or during growth with D-fructose as a carbon source (66). *lysU* expression in *metK* strains was elevated (65), but the elevation is now known to have resulted from rapidly accumulated suppressor mutations in *lrp* (86). In fact, it was this realization that suggested that *lysU* might be a member of the Lrp regulon (86).

Recent work has confirmed this hypothesis. LysU was constitutive in an *lrp*::Tn*10* strain, and Lrp bound to the promoter region of the *lysU* gene as judged by both mobility shift assays and footprinting studies (52, 85, 116a). Transcription of *lysU* was initiated from a σ^{70} promoter located 90 to 120 bp upstream of the start codon of *lysU* (71, 81). Leveque et al. (80) identified transcription start sites at positions 88 and 80 nucleotides upstream of the start of translation. In the discussion that follows we assign the +1 position to the farther-upstream transcription start site identified by Leveque et al. and number all other sites with reference to this position. Lin et al. (85) performed DNase I footprinting studies with a fragment extending from positions −70 to +37 of the *lysU* gene. Lrp protected the region from −70 to +18 bp. Footprinting studies by Plateau and his colleagues (116a) showed protection of a region of the *lysU* gene spanning residues −127 to −16. Selection for mutants with constitutive expression of *lysU* at 30°C led to the discovery of mutations throughout this region, including a cluster of mutations at −46 to −43. Kawakami et al. independently obtained evidence for a negative regulatory element situated near position −44 of the *lysU* gene that was inactivated by IS*2* insertion (71). Lrp negatively regulates the expression of *lysU* and may bind to this negative regulatory element. Kawakami et al. showed that the disruption of this element suppressed cold-sensitive lethality of a null *lysS* mutant (71). This finding is consistent with the possibility that the negative element participates in thermal regulation of *lysU*. However, the recent studies of Ito et al. on the regulation

of *lysU* by Lrp showed that elevated expression of a *lysU-lacZ* translational fusion after a temperature shift was seen in both *lrp*+ and *lrp* strains (69), so Lrp does not appear to be involved in induction of *lysU* by heat shock. These workers found that induction of *lysU* expression by leucine or by elevated temperature was not seen in translational fusions that eliminated the first four codons of the *lysU* coding sequence, and they postulated positive translational regulation of *lysU*.

Regulation of *osmY*. Very recent studies by Lange et al. have provided evidence that Lrp regulates the expression of *osmY* during entry into stationary phase (78). OsmY is an 18.2-kDa periplasmic protein of unknown function that is induced both at high osmotic pressures (172) and during entrance into stationary phase (164). The induction that occurs during the entrance to stationary phase is at the level of transcription and requires sigma factor σ^S, encoded by *rpoS* (*katF*). This induction is also about fivefold higher in a rich medium than in a minimal medium (164). By contrast, exponential growth in either type of medium led to similarly low expression of *osmY*. The level of *osmY-lacZ* expression was repressed by Lrp both during exponential growth and after entrance into stationary phase. Repression by Lrp was greater in cells grown in minimal medium than in LB medium (78), consistent with the higher concentration of Lrp present in cells during exponential growth in glucose minimal medium compared with LB (44, 84). In an *rpoS* background, a small induction of *osmY-lacZ* was still seen on entry into stationary phase during growth in M9 glucose medium, and this induction required a functional Lrp. Thus, in the *rpoS* background, repression of *osmY-lacZ* expression by Lrp appears to be relieved during entry into stationary phase, although in a wild-type background Lrp clearly continues to repress *osmY-lacZ* expression in stationary phase. Lrp does not appear to be involved in the induction of *osmY-lacZ* seen at high osmotic pressures (78).

Regulation of *pnt*. In a recent review (31), unpublished data by Ambartsoumian and Newman was cited, indicating that the membrane-bound pyridine nucleotide transhydrogenase encoded by the *pntAB* operon was positively regulated by Lrp. Gerolimatos and Hanson (54) first established that the amount of pyridine nucleotide transhydrogenase was repressed when cells were grown in a rich medium or in a minimal medium containing leucine and partially repressed by exogenous alanine or methionine. The enzyme was not repressed by the other 17 amino acids added individually to the medium. They showed that repression of pyridine nucleotide transhydrogenase by leucine, alanine, or methionine was abolished in a strain containing a *livR* mutation which, as discussed above, is an allele of *lrp* encoding a leucine-insensitive but active Lrp protein. However, pyridine nucleotide transhydrogenase expression in the *livR* strain was still repressed in cells grown in a minimal medium containing casein hydrolysate. This latter result can now be understood in terms of Casamino Acids leading to lower concentrations of a leucine-insensitive Lrp, as discussed above.

Pyridine nucleotide transhydrogenase is one of three routes for providing NADPH in *E. coli*. Conceivably, the regulation of *pntAB* by Lrp reflects a special role that pyridine nucleotide transhydrogenase plays in supplying NADPH for amino acid uptake and metabolism. NADPH is required for biosynthesis of branched-chain and aromatic amino acids, proline, threonine, methionine, and lysine. In addition, it is important for assimilation of ammonia by glutamate dehydrogenase or by the combined action of glutamate synthase and glutamine synthetase.

Regulation of W protein. W, a protein of unknown function, stimulates translation in vitro. It stimulated the rate of trans-

TABLE 4. Generation times of the isogenic strains W3110 (*lrp+*), BE1 (*lrp-201*::Tn*10*), BE2 (*lrp-35*::Tn*10*), and BE3 (*lrp-1*) and the strains RG (*lrp+ metK+*) and RG62 (*lrp metK*) during exponential growth at 37°C in different media

Additions to MOPS minimal medium[a]	Generation time (min) of:					
	W3110	BE1	BE2	BE3	RG	RG62
0.4% Acetate	202	278	340	ND[b]	ND	ND
0.4% Glucose	57	80	99	58	57	65
0.4% Glucose + Ile + Val	55	74	84	55	ND	ND
0.4% Glucose + Ile + Val + Leu[c]	64	72	80	58	ND	ND
0.4% Glucose + 19 amino acids (−Met)	34	34	34	38	27	59

[a] The composition of MOPS and the concentrations of amino acid supplements are described in ref (99). All media contained thiamine.
[b] ND, not determined.
[c] Leucine was added to a final concentration of 10 mM.

lation of the hexapeptide fMet-Ala-Ser-Asn-Phe-Ser in the presence of all known translation factors, predominantly by preventing translation termination (51). Ganoza et al. (51) also showed that W protein stimulated ejection of noncognate tRNAs from ribosomes.

W is one of the proteins identified by two-dimensional electrophoresis of extracts from *lrp+* and *lrp* strains (43). This protein is positively regulated by Lrp, and expression is insensitive to the presence of leucine in the medium. Such regulation would be consistent with a role for W protein in preventing mistranslation in environments where the cellular pools of amino acids may be low and variable. Because the gene encoding W protein has not yet been identified, further studies on regulation of W by Lrp have not been performed. In the absence of any confirming evidence that the synthesis of this protein is regulated by Lrp, its identification as a member of the Lrp regulon must be considered preliminary.

PHYSIOLOGICAL ROLE OF Lrp

Growth Phenotype of *lrp* Mutations

Table 4 provides information on the generation times of isogenic *lrp+* and *lrp* strains in different media (43, 92). The growth rates of isogenic strains BE1, BE2, and W3110 are compared. Strain BE1 contains an *lrp*::Tn*10* mutation (transduced into strain W3110 from strain MEW26) (86), whereas strain BE2 contains a similar mutation, *lrp-35*::Tn*10*, derived from strain CV1008 (119). Blomfield et al. demonstrated that the transposon in the *lrp-35* allele was inserted into the 5' region of the *lrp* gene at position 196 relative to the start of translation (16). The position of the insertion in strain BE1 is not known. Although these strains have an isogenic background and both are prototrophic in glucose minimal medium, their generation times were not identical. However, although each strain grew more slowly than strain W3110 in minimal media, they all grew at the same rate in glucose MOPS medium supplemented with 19 amino acids (no Met). Thus, the phenotype of *lrp* strains is least evident in a rich medium, consistent with the fact that *lrp* positively regulates several genes involved in amino acid biosynthesis and negatively regulates other genes involved in amino acid catabolism.

The addition of isoleucine and valine to a glucose MOPS minimal medium had very little effect on the growth rate of strain W3110 but greatly decreased the generation time of the *lrp*::Tn*10* strains. The partial isoleucine and valine auxotrophy of *lrp* strains in this medium is most probably related to insufficient AHAS activity. This insufficiency is probably due to two separate phenomena: a 30-fold-reduced rate of transcription of *ilvIH* (119) and catabolite repression of *ilvBN* (151)

during growth on glucose. *ilvIH* and *ilvBN* code for two isozymes of AHAS that are present in the K-12 strain of *E. coli*.

Leucine is known to be toxic to wild-type strains of *E. coli* (56, 120), and this may explain the fact that leucine increased the generation time of strain W3110. However, leucine decreased the generation times of strains BE1 and BE2. Lin et al. argued that an *lrp* mutation creates a partial leucine auxotrophy and that this accounts for the stimulation of growth of *lrp* strains by leucine (86). Strains BE1 and BE2 grew more slowly than strain W3110, even in the presence of leucine, isoleucine, and valine, and so the absence of Lrp must create some limitation other than for these amino acids.

Strain BE3 contains the *lrp-1* allele, which encodes a leucine-insensitive Lrp. During growth on glucose MOPS minimal medium, this strain showed the same growth rate as strain W3110. As expected, isoleucine and valine had little effect on the generation time of strain BE3, since Lrp-1 is functional as a positive effector of *ilvIH*. Leucine did not inhibit the growth of strain BE3 in the presence of exogenous isoleucine and valine, suggesting that the toxic effect of leucine on growth of *lrp+* strains is mediated by the effect of leucine on Lrp. Strain BE3 grew more slowly than did *lrp+* strains on glucose MOPS medium supplemented with 19 amino acids (lacking Met). This strain may be unable to repress the biosynthesis of amino acids whose synthesis is normally positively regulated in a leucine-sensitive fashion, resulting in a diversion of energy and metabolites away from DNA replication. Strain BE3 may also be unable to induce pathways of amino acid catabolism important for optimal growth in a rich medium. Many of these pathways are normally repressed by Lrp in a leucine-sensitive fashion and would remain constitutively repressed in strain BE3.

Interaction between Lrp and Nitrogen Metabolism

Deficiencies in glutamate synthase expression are associated with the inability to induce nitrogen-regulated genes in response to nitrogen limitation. Since Lrp controls glutamate synthase expression, it can control the synthesis of the other nitrogen-regulated genes indirectly. When levels of Lrp are low, glutamate synthase expression will also be low, and the levels of nitrogen-regulated genes will remain low even under nitrogen-limiting conditions.

Operons and proteins regulated by nitrogen availability. It has long been known that in strains of *E. coli* and *S. typhimurium*, the synthesis of a number of proteins is induced under nitrogen-limiting conditions such as growth on glutamine, arginine, ornithine, and putrescine (76, 140, 173). These proteins include, in addition to glutamine synthetase, enzymes required for the conversion of arginine and ornithine to putrescine (and eventually succinate) (140, 141, 173) and for the degradation of cytosine deaminase (7) and *S. typhimurium*

proteins involved in the transport of histidine, glutamine, lysine-arginine-ornithine, and glutamate-aspartate (76, 148).

Some control elements involved in expression of nitrogen-regulated operons. Mutations leading to constitutive production of glutamine synthetase, and mapping in or near *glnALG*, also lead to constitutive production of other nitrogen-regulated operons (76, 114). We now know that mutations in *glnL* lead to constitutive expression of glutamine synthetase by preventing the dephosphorylation of phosphorylated NR$_I$ (26, 45). *glnL* mutations also lead to constitutive expression of other operons regulated by NR$_I$ (7, 140).

Mutations in *glnG* that lead to the absence of NR$_I$ lead to a failure to induce glutamine synthetase expression under nitrogen-limiting conditions, regardless of the presence or absence of GlnL (113). Such mutations also result in the failure to induce other nitrogen-regulated operons (140, 173). Thus, the expression of nitrogen-regulated operons appears to be controlled by GlnL (NR$_{II}$) and GlnG (NR$_I$).

The activities of NR$_I$ and NR$_{II}$ are regulated in response to the ratio of glutamine to α-ketoglutarate in the cell (126). When ammonia is abundant and the cellular ratio of glutamine to α-ketoglutarate is high, NR$_I$ is converted to its inactive, dephosphorylated form. Conversely, when ammonia is scarce and the ratio of glutamine to α-ketoglutarate is low, NR$_I$ is phosphorylated and transcription of nitrogen-regulated genes is induced.

Strains that lack glutamate synthase activity or that have greatly reduced levels of activity are unable to induce glutamine synthetase and other nitrogen-regulated proteins in response to nitrogen limitation (23, 112, 114). It is not yet known whether such strains have altered ratios of glutamine to α-ketoglutarate as a result of glutamate synthase deficiency or whether they have abnormalities in the signal transduction pathway linking this ratio to the activities of NR$_I$ and NR$_{II}$. The recent studies of Castano et al. (28) suggest that the abnormalities in nitrogen regulation seen in *gltBDF* strains lie in the signal transduction pathway. Expression of GltD and GltF, but not GltB, was shown to be necessary for normal induction of histidase (the *hut* operon product) in response to nitrogen limitation. Since both GltB and GltD are required for glutamate synthase activity, these results suggest that glutamate synthase activity is not required for control of nitrogen-regulated genes.

Lrp, by regulating the expression and activity of glutamate synthase, can override induction of nitrogen-regulated operons by ammonia limitation. *gltBD* is positively regulated by Lrp, and the levels of GltBD are reduced about 40-fold in *lrp* strains (43, 44). The expression of *gltF* has not yet been examined. Strains carrying a transposon insertion in *lrp* were unable to grow in a medium containing arginine or ornithine as the sole nitrogen source, which is characteristic of Ntr$^-$ strains unable to respond to nitrogen limitation. *lrp* strains also contained reduced levels of glutamine synthetase, and the enzyme was largely in the inactive adenylated form during growth on a nitrogen-limiting medium containing glutamine.

Glutamate synthase is not regulated by the nitrogen regulon in response to ammonia limitation (reviewed in reference 126). The evidence that glutamate synthase is directly regulated by Lrp (44) provides insight into the strategy for regulation of nitrogen metabolism in response to Lrp. When levels of Lrp and hence of glutamate synthase are high, genes of the nitrogen regulon are regulated by NR$_I$ in response to the availability of ammonia, which is thought to be sensed by the ratio of glutamine to α-ketoglutarate in the cell. When levels of Lrp are low (for instance during growth in rich media), levels of glutamate synthase are also low and ammonia limitation will

no longer be coupled to induction of nitrogen-regulated operons. During growth on complex media, particularly those containing peptides and amino acids, many factors may lead to fluctuations in the glutamine and α-ketoglutarate levels in the cell, including growth on media containing a source of glutamate and growth on media of high osmotic strength. Thus, it may be important to provide an alternate mechanism of sensing nitrogen availability under these conditions. Under these growth conditions, the need for ammonia assimilation is greatly reduced, since amino acids and nitrogen bases are the predominant nitrogenous compounds in bacterial cells.

Is Leucine an Lrp Antagonist at Physiologically Significant Concentrations?

Quantitative measurements of the effect of leucine on binding of Lrp to the *gltBDF* and *ilvIH* promoter regions, as assessed by mobility shift assays, indicated that relatively high levels of leucine were required to alter binding. Concentrations of leucine greater than 10 mM were required for a maximal effect, and the effect of leucine was half maximal at about 3 mM (44). Although the effect of leucine on binding of Lrp to the *gltBDF* promoter region appeared to be specific (similar concentrations of glutamate had no effect), one wonders whether such high levels of leucine occur under physiological conditions. Quay et al. (120) measured the concentrations of leucine in a wild-type strain of *E. coli* K-12. During growth in glucose minimal MOPS medium, the wild-type strain had an intracellular leucine concentration of 1.7 mM, and on addition of 0.4 mM exogenous leucine to the medium, the leucine concentration rose transiently to 11.7 mM and then dropped to 5.3 mM during steady-state growth in the leucine-containing medium. Comparison of these concentrations of leucine with plots of the effect of leucine on mobility shift assays of Lrp binding to *gltBDF* (44) suggest that intracellular leucine should have very little effect on binding during growth in glucose MOPS minimal medium and a nearly maximal effect on binding shortly after addition of leucine to the growth medium. Thus, leucine will be particularly effective as an antagonist shortly after a shift from a minimal medium to a medium containing leucine, when the cell has not yet had time to down-regulate high-affinity branched-chain amino acid transport in response to leucine. Quay et al. (120) also measured the effect of leucine on an isogenic *livR* strain that had been characterized by its inability to down-regulate branched-chain amino acid transport in response to leucine. The *livR* mutation was subsequently shown to be a mutation in *lrp* (62) that resulted in a leucine-insensitive Lrp protein. In the *livR* strain, the concentration of leucine during growth on glucose MOPS minimal medium was only 0.6 mM, but addition of leucine to the medium resulted in a much higher and more prolonged rise in the intracellular leucine concentration (to 15.9 mM). Steady-state growth in glucose MOPS minimal medium containing 0.4 mM leucine resulted in an intracellular leucine concentration of 1.0 mM. These effects can be rationalized by assuming that although the *livR* strain was unable to down-regulate the high-affinity branched-chain amino acid transport system in response to the addition of exogenous leucine, and thus the transient effect of adding leucine to the growth medium was more profound, this strain did retain the ability to control intracellular levels of leucine by other means.

In summary, in vivo leucine concentrations do reach and exceed those needed to show in vitro effects on Lrp-DNA interactions.
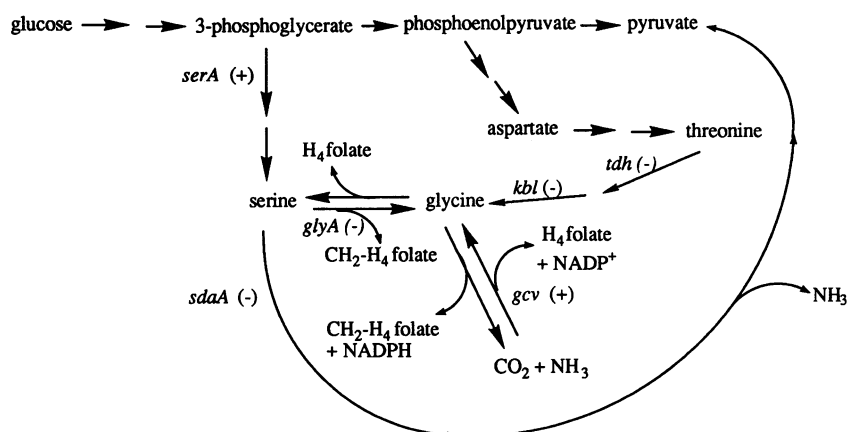
FIG. 7. Regulation of the genes involved in one-carbon metabolism by Lrp. (+) and (−) indicate that Lrp activates or represses, respectively, the operon in question.

Lrp Regulates Expression of Many Enzymes That Control the Generation of One-Carbon Units

A characteristic difference between growth of prototrophic bacteria in rich and minimal media is the demand for one-carbon units and glycine for biosynthesis of nucleotides and amino acids. De novo biosynthesis of purines requires one molecule of glycine and two one-carbon units, supplied directly as formyl tetrahydrofolate. The biosynthesis of histidine also requires one-carbon units and glycine, since histidine is derived from ATP. De novo biosynthesis of thymidylate requires a one-carbon unit, as does the synthesis of methionine. Furthermore, all protein translation is initiated with fMet, and thus a one-carbon unit is required for each polypeptide translated. Finally, one-carbon units must be supplied to methylate DNA, RNA, and a variety of small metabolites.

In cells of *E. coli* grown on glucose minimal medium, both one-carbon units and glycine are normally derived from serine, which is synthesized from 3-phosphoglycerate derived by glycolysis. It has been estimated that during growth in glucose minimal medium, 15% of the carbon from glucose is channeled into the formation of serine and glycine and the compounds derived from them (116). In contrast, growth in a rich medium, containing amino acids and nucleotides, greatly reduces the requirement for one-carbon units. During growth in a rich medium, assuming an adequate supply of glycine and serine, only the formation of thymidylate and fMet and the methylation of nucleic acids require one-carbon units. Thus, there is reason to suspect that one-carbon metabolism will be regulated in response to the nutrient content of the medium.

Figure 7 shows the reactions that generate or consume serine, glycine, and one-carbon units and identifies the enzymes that are positively or negatively regulated by Lrp.

Growth of cells in glucose minimal medium, where leucine is absent and the level of Lrp is relatively high, leads to expression of positively regulated target genes and repression of negatively regulated genes. Under these conditions, maximal diversion of glucose into serine biosynthesis occurs and serine is converted to glycine and one-carbon units, while deamination of serine to form pyruvate is minimized. Indeed, wild-type strains of *E. coli* cannot use serine as a source of nitrogen during growth in glucose MOPS minimal medium, but mutations in Lrp allow them to do so (86). Although there is a cryptic pathway permitting the synthesis of glycine from threonine, this pathway is not active in wild-type cells during growth

on glucose, and labeling experiments indicate that glycine is derived from serine (130). Thus, although the activity of serine hydroxymethyltransferase is said to be negatively regulated by Lrp (31), sufficient activity remains to supply the cell with glycine and one-carbon units derived from serine. Furthermore, the glycine cleavage enzymes are expressed and can be used to balance the demand for glycine and one-carbon units to meet the needs of biosynthesis.

In contrast, during growth in a rich medium, less glucose is diverted into serine biosynthesis, in keeping with the reduced demand for serine, and instead, excess serine is deaminated to form pyruvate. The rate of serine catabolism during growth in a rich medium is extremely high, and for this reason, the glucose-rich MOPS medium developed by Wanner and Neidhardt to ensure optimal growth to an A_{420} of 10 (163) contains 10 mM serine and 22 mM glucose, while the concentration of all other amino acids is 0.8 mM or less. Exogenous threonine, and perhaps aspartate and asparagine, may be catabolized to form glycine and acetyl-CoA, and serine hydroxymethyltransferase and serine deaminase will catalyze the conversion of glycine to pyruvate.

Although much remains to be learned about the role of Lrp in the regulation of one-carbon metabolism, the preliminary evidence suggests that this protein plays a major role in ensuring an adequate flux of carbon from glucose into serine biosynthesis during growth in glucose minimal medium and in ensuring that the catabolic pathway for the conversion of serine to pyruvate is activated during growth in a defined glucose-rich medium (163). Clearly, other major factors affect the regulation of one-carbon metabolism in response to the nutrient content of the medium. These include the effects of purines, many of them mediated by the PurR repressor, on enzymes involved in the generation of one-carbon units and glycine (36, 147, 169).

Lrp may also be involved in the alterations in one-carbon metabolism associated with growth at temperatures above 37°C. Catabolism of serine to acetate was increased in a wild-type strain following a shift from 28 to 44°C (93). Some data showing the effect of a shift in temperature from 28 to 42°C are given in Fig. 8. In an *lrp*⁺ strain grown in the presence of rifampin, the rate of serine catabolism increased somewhat, presumably because of a simple effect of temperature on the rate of reaction. In the absence of rifampin, there was a marked increase in the rate of product formation, suggesting
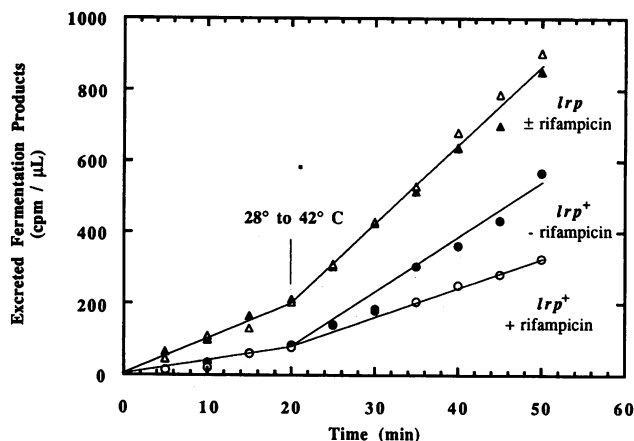
FIG. 8. Effect of temperature on the rate of serine catabolism. The excretion of volatile products derived from [3-^{3}H]serine was measured during steady-state growth in glucose MOPS minimal medium at 28°C and after a shift to 42°C. The procedures used have been described in reference 93. The major products derived from tritiated serine were acetate and water. Serine catabolism was compared in strain BE1 (lrp::Tn10) and its isogenic parent strain W3110, in the presence or absence of 200 μg of rifampin per ml. Where used, rifampin was added 10 min before the temperature shift. All values for excreted fermentation products were normalized for the ratio of A_{420} of the culture at 0 time to A_{420} at the time indicated (44a).

that increased serine catabolism required transcription (presumably of sdaA). By comparison, in an lrp strain, the rate of serine catabolism at 28°C was higher than in an lrp strain and the rate after a shift to 42°C was not affected by rifampin. Similar results were obtained for an lrp$^+$ strain grown in a medium containing leucine. These results are most simply interpreted as indicating that induction of sdaA expression following a temperature shift from 28 to 44°C results from the relief of repression of sdaA by Lrp. Under conditions where sdaA was not repressed by Lrp, for instance in the presence of leucine or in an lrp strain, very little effect of a temperature shift to 44°C on sdaA expression was seen. Increased L-serine deaminase activity during growth at 42 compared with 37°C was also noted by Newman et al. (101).

Elevated serine catabolism during growth at temperatures above 37°C, and possibly decreased serine biosynthesis at these temperatures, might contribute to decreased serine concentrations in heat-shocked cells and to resultant deficiencies in one-carbon units and glycine for nucleotide, nucleic acid, and protein biosynthesis. Indeed, Ambartsoumian et al. have shown that an lrp strain is auxotrophic for serine when grown at 42°C, although it is prototrophic at 37°C (3).

Mutations in lrp Suppress the Phenotype of metK Mutations

The metK gene encodes adenosylmethionine synthetase. The complete absence of adenosylmethionine would be expected to be lethal, but metK strains have low residual adenosylmethionine synthetase activity, and the level of residual activity is increased during growth in rich media or in the presence of leucine or methionine (60). These results suggest that there may be other isozymes of adenosylmethionine synthetase in K-12 strains of E. coli.

Strains containing a metK mutation rapidly accumulate suppressor mutations in lrp (86). Why does a mutation in lrp suppress the phenotype of a primary metK mutation? One possibility is that there is a second adenosylmethionine syn-

thetase isozyme that is normally cryptic or expressed at very low levels during growth on glucose minimal medium and that the expression of this isozyme is normally repressed by Lrp. Indeed, the studies of Satishchandran et al. identified an E. coli metX gene which encodes a second adenosylmethionine synthetase isozyme that was normally repressed in glucose MOPS minimal medium and induced during growth on rich medium (135, 136). A mutation in lrp might lead to constitutive expression of this second isozyme and thus provide a source of adenosylmethionine in metK strains. Measurements of adenosylmethionine synthetase activity in strains having a kanamycin insert in the chromosomal metK gene indicated that activity was substantially reduced (0.5%) in these strains compared with that in their wild-type parents during growth in glucose minimal medium but that the activity levels of metK$^+$ and metK strains were similar during growth in LB (135). These results support the idea that there is a gene coding for a second adenosylmethionine synthetase isozyme, and it will be interesting to see if Lrp represses the expression of this second gene.

Lin et al. (86) showed that metK strains were quite unstable and that within 30 generations, 19% of the cells had accumulated secondary mutations in lrp. These secondary mutations increased the growth rate of metK strains and thus were favored in continuous culture. They postulated that most laboratory metK strains are actually metK lrp double mutants.

It is important to distinguish the properties of metK lrp strains that are due to the metK mutation and those that are due to secondary mutations in lrp. The phenotype of one such double mutant strain, RG62, has been extensively characterized (60, 92, 93). This strain was prototrophic at 37°C but required serine and isoleucine for exponential growth at 44°C (92). Serine catabolism was constitutively elevated in this strain and was not further increased by addition of leucine to the medium or by heat shock (93). Strain RG62 was derepressed for methionine biosynthesis, and in contrast to normal strains, in which methionine limits the rate of growth at 44°C, its growth at this temperature was not stimulated by methionine (92). LysU (lysyl-tRNA synthetase form II), which is normally expressed at high levels only in heat-shocked cells, was constitutively expressed in strain RG62 (65). The constitutive expression of lysU (85) and the elevated rate of serine catabolism (85, 93) seen in strain RG62 (metK lrp) are properties of lrp strains. The phenotype of strain RG62 also included the failure to induce two other heat shock proteins, C14.7 and G13.5, during a heat shock in glucose minimal medium (92). The failure to induce C14.7 and G13.5 after a heat shock was not seen in an lrp strain (43), and induction of these proteins was restored by transformation of strain RG62 with a plasmid containing the metK gene. The transformed strain was also normally repressed for metE expression when grown in the presence of methionine, whereas in strain RG62 metE expression was not repressed. Thus, derepression of methionine biosynthesis appears to be associated with the metK mutation.

OVERVIEW: FEAST VERSUS FAMINE

The information presented in this review suggests the following working hypothesis. Lrp functions to regulate metabolic pathways in E. coli in response to the availability of amino acids and nitrogen bases in the external environment. Lrp positively regulates genes that function during famine and negatively regulates genes that function during a feast. During growth in minimal media, the level of Lrp in cells is high and intracellular concentrations of leucine are relatively low. Under these conditions, genes that are positively regulated by Lrp

TABLE 5. Genes and proteins in the Lrp regulon

Positively regulated by Lrp	Negatively regulated by Lrp
ilvIH (AHAS III)	*sdaA* (L-serine deaminase)
serA (3-phosphoglycerate dehydrogenase)	*tdh* (threonine dehydrogenase)
gcv (glycine cleavage system)	*lysU* (lysyl-tRNA synthetase II)
gltBDF (glutamate synthase)	*lrp* (Lrp)
glnALG (glutamine synthetase)	*micF* (antisense RNA)
ompF (outer membrane porin F)	*ompC* (outer membrane porin C)
papBA (P pili)	*livJ* (leucine-binding protein)[a]
fanABC (K99 pili)	*livKHMGF* (LIV-I transport)[a]
daaAB (F1845 pili)	*glyA* (serine hydroxymethyltransferase)
sfaBA (S pili)	*oppABCDF* (oligopeptide permease)
fimB- and *fimE*-promoted switching of type I pili[b]	
pnt (pyridine nucleotide transhydrogenase)	
CP8 (sugar utilization)	
leuABCD (leucine biosynthesis)	

[a] These operons are negatively regulated by Lrp, but repression is markedly increased in the presence of leucine. Thus, they are expressed during growth in glucose minimal medium but repressed during growth in a medium containing leucine.

[b] FimB- and FimE-promoted switching is stimulated by alanine, leucine, and isoleucine/valine, and this stimulation requires functional Lrp.

are expressed at maximal levels and genes that are negatively regulated by Lrp are maximally repressed. Table 5 summarizes the positively and negatively regulated genes and proteins in the Lrp regulon. Among the positively regulated cases are genes involved in amino acid biosynthesis (*serA*, *ilvIH*, and *leu*), genes involved in the generation of one carbon units (*gcv*), and genes involved in the assimilation of ammonia (*gltBDF* and *glnA*). Pyridine nucleotide transhydrogenase expression is also positively regulated by Lrp, in keeping with the demand for NADPH for the biosynthesis of amino acids and assimilation of ammonia during growth in minimal medium. The positive regulation of these genes by Lrp leads to maximal induction during growth in glucose minimal medium, to decreased expression in media containing leucine or alanine, and to repression in rich media. After a shift from minimal to rich medium, there will be an immediate drop in the expression of leucine-sensitive target genes in response to the leucine content of rich medium and then a more gradual drop in expression as the 3,000 molecules of Lrp per cell grown in glucose minimal medium (166) are reduced by degradation or cell division (44).

In general, formation of pili that allow cells to colonize specialized environments requires Lrp. Pili may be expressed at higher levels during growth in a nutrient-deficient medium in which the concentration of Lrp is high, provided that other factors do not dominate regulation of expression. An abundance of pili may help bacteria adhere to epithelial cells and thus maintain their presence in the specialized environment. Under conditions of nutrient excess, on the other hand, relatively rapidly growing cells should not have difficulty maintaining their presence, and low levels of pili may allow some cells to move to new hosts.

Among the negatively regulated genes are those involved in the catabolism of serine (*sdaA*) and threonine (*tdh*) and those involved in the transport of oligopeptides (*oppABCDF*). The regulation of the outer membrane porins OmpF and OmpC by Lrp is also consistent with the hypothesis that Lrp mediates adjustments to the nutritional composition of the medium. During growth in minimal media, expression of the OmpF porin is increased by Lrp and expression of the OmpC porin is decreased (43). The OmpF porin has a larger pore size than OmpC, permitting a more rapid entry of nutrients at low concentration in the external medium (104); reviewed in reference 105), and is thus well adapted for growth of cells under conditions where the nutrient content of the medium is

low and diffusion of nutrients into cells may limit the rate of growth. Both OmpC and OmpF prefer neutral molecules and cations over anions, and particularly exlude lipophilic anions. It has been hypothesized that the OmpC porin diameter is sufficiently small to exclude bile salts and that the expression of this porin, rather than OmpF, may facilitate the survival of *E. coli* in the gut (105).

OmpR and EnvZ are known to play an important role in the modulation of *ompF* and *ompC* transcription in response to the osmolarity of the medium (149). Recent studies have established that Lrp exerts its effect on *ompF* and *ompC* expression independently of OmpR and EnvZ and that OmpF and OmpC expression is normally regulated by osmolarity in an *lrp* strain (46).

Within the group of genes that are positively regulated by Lrp, there is a broad range of sensitivity to leucine. The regulatory model proposed by Ernsting et al. (44) suggests that leucine sensitivity is determined by the intrinsic affinity of the target gene for Lrp, with leucine-insensitive genes exhibiting a higher affinity for Lrp than leucine-sensitive genes do. The differing affinities of the target genes for Lrp create a hierarchy of response to changes in Lrp and/or leucine concentration. Those positively regulated genes with the lowest affinity for Lrp will be the first to be repressed as the leucine concentration rises or the Lrp concentration drops.

As discussed above, there is evidence that the concentration of Lrp is itself regulated, in part, by autogenous regulation of *lrp* transcription. Although we still know little about the factors that regulate Lrp expression, it is likely that Lrp levels are higher in glucose minimal medium than in media containing nitrogen bases and amino acids. Thus, growth in a rich medium, especially one containing leucine, may result in increased expression of genes that are negatively regulated by Lrp and in decreased expression of genes that are positively regulated by Lrp.

A few genes show regulation that cannot be fit simply into the feast-versus-famine pattern shown in Table 5. These cases have been marked by asterisks. For the proteins involved in high-affinity branched-chain amino acid transport, regulation by Lrp is negative and is stimulated in the presence of leucine (43, 62). Thus, one might expect the branched-chain amino acid transport system to be maximally expressed in minimal medium, maximally repressed during growth in minimal medium containing leucine, and expressed at an intermediate level during growth in a rich medium in which the Lrp

concentration is reduced. The only positive regulation by Lrp that is known to be stimulated by leucine is the regulation of *fimE*- and *fimB*-promoted switching (50).

Despite some caveats, the overall view of Lrp as providing coordination of metabolism during shifts between feast and famine affords a reasonable working hypothesis. Lrp modulates transcription of some target genes in response to leucine, and the level of expression of target genes appears to be sensitive to the intracellular concentration of Lrp. Understanding how *lrp* transcription is itself regulated by nutrients and other factors will be the next step in understanding the physiological significance of the Lrp regulon.

ACKNOWLEDGMENTS

REFERENCES

1. **Abraham, J. M., C. S. Freitag, J. R. Clements, and B. I. Eisenstein.** 1985. An invertible element of DNA controls phase variation of type 1 fimbriae of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82:**5724–5727.
2. **Ahdya, S., and S. Garges.** 1990. Positive control. J. Biol. Chem. **265:**10797–10800.
3. **Ambartsoumian, G., R. D'Ari, R. T. Lin, and E. B. Newman.** Altered amino acid metabolism in *lrp* mutants of *E. coli* K-12 and their derivatives. J. Gen. Microbiol., in press.
4. **Andersen, J., and N. Delihas.** 1990. *micF* RNA binds to the 5' end of *ompF* mRNA and to a protein from *Escherichia coli*. Biochemistry **29:**9249–9256.
5. **Anderson, J. J., and D. L. Oxender.** 1977. *Escherichia coli* transport mutants lacking binding protein and other components of the branched-chain amino acid transport systems. J. Bacteriol. **130:**384–392.
6. **Anderson, J. J., S. C. Quay, and D. L. Oxender.** 1976. Mapping of two loci affecting the regulation of branched-chain amino acid transport in *Escherichia coli* K-12. J. Bacteriol. **126:**80–90.
7. **Anderson, L., M. Kilstrup, and J. Neuhard.** 1989. Pyrimidine, purine and nitrogen control of cytosine deaminase synthesis in *Escherichia coli* K-12. Involvement of the *glnLG* and *purR* genes in the regulation of *codA* expression. Arch. Microbiol. **152:**115–118.
8. **Andrews, J. C., T. C. Blevins, and S. A. Short.** 1986. Regulation of peptide transport in *Escherichia coli*: induction of the *trp*-linked operon encoding the oligopeptide permease. J. Bacteriol. **165:**428–433.
9. **Andrews, J. C., and S. A. Short.** 1986. *opp-lac* operon fusions and transcriptional regulation of the *Escherichia coli trp*-linked oligopeptide permease. J. Bacteriol. **165:**434–432.
10. **Aronson, B. D., M. Levinthal, and R. L. Somerville.** 1989. Activation of a cryptic pathway for threonine metabolism via specific IS3-mediated alteration of promoter structure in *Escherichia coli*. J. Bacteriol. **171:**5503–5511.
11. **Austin, E. A., J. C. Andrews, and S. A. Short.** 1989. Selection, characterization, and cloning of *oppI*, a regulator of the *E. coli* oligopeptide permease operon. Abstr. Mol. Genet. Bacteriophage, p. 153. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Barak, Z., and C. Gilvarg.** 1974. Triornithine resistant strains of *Escherichia coli*. Isolation, definition, and genetic studies. J. Biol. Chem. **249:**143–148.
13. **Bender, R. A., A. Macaluso, and B. Magasanik.** 1976. Glutamate dehydrogenase: genetic mapping and isolation of regulatory mutants of *Klebsiella aerogenes*. J. Bacteriol. **127:**141–148.
14. **Bilge, S. S., J. M. Apostol, Jr., K. J. Fullner, and S. L. Moseley.** 1993. Transcriptional organization of the F1845 fimbrial adhesin determinant of *Escherichia coli*. Mol. Microbiol. **7:**993–1006.

15. **Bilge, S. S., C. R. Clausen, W. Lau, and L. L. Moseley.** 1989. Molecular characterization of a fimbrial adhesin, F1845, mediating diffuse adherence of diarrhea-associated *Escherichia coli* to HEp-2 cells. J. Bacteriol. **171:**4281–4289.
15a.**Blomfield, I. C.** Personal communication.
16. **Blomfield, I. C., P. J. Calie, K. J. Eberhardt, M. S. McClain, and B. I. Eisenstein.** 1993. Lrp stimulates phase variation of type 1 fimbriation in *Escherichia coli* K-12. J. Bacteriol. **175:**27–36.
17. **Blomfield, I. C., M. S. McClain, J. A. Princ, P. J. Calie, and B. I. Eisenstein.** 1991. Type 1 fimbriation and *fimE* mutants of *Escherichia coli* K-12. J. Bacteriol. **173:**5298–5307.
18. **Blyn, L. B., B. A. Braaten, and D. A. Low.** 1990. Regulation of *pap* pilin phase variation by a mechanism involving differential Dam methylation states. EMBO J. **9:**4045–4054.
19. **Blyn, L. B., B. A. Braaten, C. A. White-Ziegler, D. A. Rolfson, and D. A. Low.** 1989. Phase-variation of pyelonephritis-associated pili in *Escherichia coli*: evidence for transcriptional regulation. EMBO J. **8:**613–620.
20. **Braaten, B. A., L. B. Blyn, B. S. Skinner, and D. A. Low.** 1991. Evidence for a methylation-blocking factor (*mbf*) locus involved in *pap* pilus expression and phase variation in *Escherichia coli*. J. Bacteriol. **173:**1789–1800.
21. **Braaten, B. A., X. Nou, L. S. Kaltenbach, and D. A. Low.** 1994. Methylation patterns in *pap* regulatory DNA control pyelonephritis-associated pili phase variation in *Escherichia coli*. Cell **76:**577–588.
22. **Braaten, B. A., J. V. Platko, M. van Woude, F. K. de Graaf, J. M. Calvo, and D. A. Low.** 1992. Leucine responsive regulatory protein (Lrp) controls the expression of both the Pap and Fan pili operons in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **89:**4250–4254.
23. **Brenchley, J. A., C. A. Baker, and L. G. Patil.** 1975. Regulation of the ammonia assimilatory enzymes in *Salmonella typhimurium*. J. Bacteriol. **124:**182–189.
24. **Brennan, R. G., and B. W. Matthews.** 1989. The helix-turn-helix DNA binding motif. J. Biol. Chem. **264:**1903–1906.
25. **Brinton, C. C., Jr., P. Gemski, S. Falkow, and L. S. Baron.** 1961. Location of the piliation factor on the chromosome of *Escherichia coli*. Biochem. Biophys. Res. Commun. **5:**293–298.
26. **Bueno, R., G. Pahel, and B. Magasanik.** 1985. Role of *glnB* and *glnD* gene products in regulation of the *glnALG* operon of *Escherichia coli*. J. Bacteriol. **164:**816–822.
27. **Campbell, J. L., and N. Kleckner.** 1990. *E. coli* and the *dnaA* gene promoter are sequestered from *dam* methyltransferase following passage of the chromosomal replication fork. Cell **62:**967–979.
28. **Castano, I., N. Flores, F. Valle, A. A. Covarrubias, and F. Bolivar.** 1992. *gltF*, a member of the *gltBDF* operon of *Escherichia coli*, is involved in nitrogen-regulated gene expression. Mol. Microbiol. **6:**2733–2741.
29. **Clark, R. L., and F. C. Neidhardt.** 1990. Roles of the two lysyl-tRNA synthetases of *Escherichia coli*: analysis of nucleotide sequences and mutant behavior. J. Bacteriol. **172:**3237–3243.
30. **Collado-Vides, J., B. Magasanik, and J. D. Gralla.** 1991. Control site location and transcriptional regulation in *Escherichia coli*. Microbiol. Rev. **55:**371–394.
30a.**Collins, S., and R. G. Matthews.** Unpublished data.
30b.**Collins, S., C. Smith, and R. G. Matthews.** Unpublished data.
31. **D'Ari, R., R. T. Lin, and E. B. Newman.** 1993. The leucine-responsive regulatory protein: more than a regulator? Trends Biochem. Sci. **18:**260–263.
32. **DeFelice, M., C. T. Lago, C. H. Squires, and J. M. Calvo.** 1982. Acetohydroxy acid synthase isoenzymes of *Escherichia coli* K12 and *Salmonella typhimurium*. Ann. Microbiol. Inst. Pasteur. **133A:**251–256.
33. **DeFelice, M., and M. Levinthal.** 1977. The acetohydroxy acid synthase III isoenzyme of *Escherichia coli* K-12: regulation of synthesis by leucine. Biochem. Biophys. Res. Commun. **79:**82–87.
34. **de Graaf, F. K., P. Klaasen-Boor, and J. E. van Hees.** 1980. Biosynthesis of the K99 surface antigen is repressed by alanine. Infect. Immun. **30:**125–128.
35. **Delaney, J. M., and C. Georgopoulos.** 1992. Physical map locations of the *trxB*, *htrD*, *cydC*, and *cydD* genes of *Escherichia coli*. J. Bacteriol. **174:**3824–3825.

36. Dev, L. K., and R. J. Harvey. 1984. Regulation of synthesis of serine hydroxymethyltransferase in chemostat cultures of *Escherichia coli*. J. Biol. Chem. 259:8394–8401.

37. deWind, N., M. deJong, M. Meijer, and A. R. Stuitje. 1985. Site-directed mutagenesis of the *Escherichia coli* chromosome near *oriC*: identification and characterization of *asnC*, a regulatory element in *E. coli* asparagine metabolism. Nucleic Acids Res. 13:8797–8811.

38. Dodd, L. B., and J. B. Egan. 1987. Systematic method for the detection of potential lambda cro-like DNA-binding regions in proteins. J. Mol. Biol. 194:557–564.

39. Dorman, C. J., and C. F. Higgins. 1987. Fimbrial phase variation in *Escherichia coli*: dependence on integration host factor and homologies with other site-specific recombinases. J. Bacteriol. 169:3840–3843.

40. Drew, H. R., and A. A. Travers. 1985. DNA bending and its relation to nucleosome positioning. J. Mol. Biol. 186:773–790.

41. Eisenstein, B. I. 1981. Phase variation of type I fimbriae in *Escherichia coli* is under transcriptional control. Science 214:337–339.

42. Eisenstein, B. L., D. S. Sweet, V. Vaughn, and D. L. Friedman. 1987. Integration host factor is required for the DNA inversion that controls phase variation in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 84:6506–6510.

43. Ernsting, B. R., M. R. Atkinson, A. J. Ninfa, and R. G. Matthews. 1992. Characterization of the regulon controlled by the leucine-responsive regulatory protein in *Escherichia coli*. J. Bacteriol. 174:1109–1118.

44. Ernsting, B. R., J. W. Denninger, R. M. Blumenthal, and R. G. Matthews. 1993. Regulation of the *gltBDF* operon of *Escherichia coli*: how is a leucine-insensitive operon regulated by the leucine-responsive regulatory protein? J. Bacteriol. 175:7160–7169.

44a. Ernsting, B. R., and R. G. Matthews. Unpublished data.

45. Feng, J., M. R. Atkinson, W. McCleary, J. B. Stock, B. L. Wanner, and A. J. Ninfa. 1992. Role of phosphorylated metabolic intermediates in the regulation of glutamine synthetase synthesis in *Escherichia coli*. J. Bacteriol. 174:6061–6070.

46. Ferrario, M., B. R. Ernsting, R. M. Blumenthal, and R. G. Matthews. The leucine-responsive regulatory protein of *Escherichia coli* negatively regulates transcription of *ompC* and *micF* and positively regulates translation of *ompF*. Submitted for publication.

47. Fraser, J., and E. B. Newman. 1975. Derivation of glycine from threonine in *Escherichia coli* K-12 mutants. J. Bacteriol. 122:810–817.

48. Freitag, C. S., J. M. Abraham, J. R. Clements, and B. I. Eisenstein. 1985. Genetic analysis of the phase variation control of expression of type 1 fimbriae in *Escherichia coli*. J. Bacteriol. 162:668–675.

49. Gaastra, W., and F. K. deGraaf. 1982. Host-specific fimbrial adhesins of noninvasive enterotoxigenic *Escherichia coli* strains. Microbiol. Rev. 46:129–161.

50. Gally, D. L., J. A. Bogan, B. I. Eisenstein, and I. C. Blomfield. 1993. Environmental regulation of the *fim* switch controlling type 1 fimbrial phase variation in *Escherichia coli* K-12: effects of temperature and media. J. Bacteriol. 175:6186–6193.

51. Ganoza, M. C., C. Cunningham, and R. M. Green. 1985. Isolation and point of action of a factor from *Escherichia coli* required to reconstruct translation. Proc. Natl. Acad. Sci. USA 82:1648–1652.

52. Gazeau, M., F. Delort, P. Dessen, S. Blanquet, and P. Plateau. 1992. *Escherichia coli* leucine-responsive regulatory protein Lrp controls lysyl-tRNA synthetase expression. FEBS Lett. 300:254–258.

53. Gemmill, R. M., S. R. Wessler, E. B. Keller, and J. M. Calvo. 1979. The leucine operon of *Salmonella typhimurium* is controlled by an attenuation mechanism. Proc. Natl. Acad. Sci. USA 76:4941–4945.

54. Gerolimatos, B., and R. L. Hanson. 1978. Repression of *Escherichia coli* pyridine nucleotide transhydrogenase by leucine. J. Bacteriol. 134:394–400.

55. Gibson, M. M., M. Price, and C. F. Higgins. 1984. Genetic characterization and molecular cloning of the tripeptide permease (*tpp*) genes of *Salmonella typhimurium*. J. Bacteriol. 160:122–130.

56. Gollop, N., H. Tavori, and Z. Barak. 1982. Acetohydroxy acid synthase is a target for leucine-containing peptide toxicity in *Escherichia coli*. J. Bacteriol. 149:387–390.

57. Goransson, M., K. Forsman, and B. E. Uhlin. 1988. Functional and structural homology among regulatory cistrons of poli-adhesin determinants in *Escherichia coli*. Mol. Gen. Genet. 212:412–417.

58. Goransson, M., K. Forsman, P. Nilsson, and B. E. Uhlin. 1989. Upstream activating sequences that are shared by two divergently transcribed operons mediate cAMP-CRP regulation of pilus-adhesin in *Escherichia coli*. Mol. Microbiol. 3:1557–1565.

59. Gralla, J. D. 1991. Transcriptional control-lessons from an *E. coli* promoter data base. Cell 66:415–418.

60. Greene, R. C., J. S. V. Hunter, and E. H. Coch. 1973. Properties of *metK* mutants of *Escherichia coli* K-12. J. Bacteriol. 115:57–67.

60a. Gregg-Jolly, L. Personal communication.

60b. Hale, W. B., M. van der Woude, and D. A. Low. 1994. Analysis of nonmethylated GATC sites in the *Escherichia coli* chromosome and identification of sites that are differentially methylated in response to environmental stimuli. J. Bacteriol. 176:3438–3441.

61. Hama, H., T. Shimamoto, M. Tsuda, and T. Tsuchiya. 1988. Characterization of a novel L-serine transport system in *Escherichia coli*. J. Bacteriol. 170:2236–2239.

62. Haney, S. A., J. V. Platko, D. L. Oxender, and J. M. Calvo. 1992. Lrp, a leucine-responsive protein, regulates branched-chain amino acid transport genes in *Escherichia coli*. J. Bacteriol. 174:108–115.

63. Haughn, G. W., C. H. Squires, M. DeFelice, C. T. Lago, and J. M. Calvo. 1985. Unusual organization of the *ilvIH* promoter of *Escherichia coli*. J. Bacteriol. 163:186–198.

64. Hiles, I. D., M. P. Gallagher, D. J. Jamieson, and C. F. Higgins. 1987. Molecular characterization of the oligopeptide permease of *Salmonella typhimurium*. J. Mol. Biol. 195:125–142.

65. Hirshfield, I. N., P. L. Bloch, R. A. VanBogelen, and F. C. Neidhardt. 1981. Multiple forms of lysyl-transfer ribonucleic acid synthetase in *Escherichia coli*. J. Bacteriol. 146:345–351.

66. Hirshfield, I. N., F.-M. Yeh, and L. E. Sawyer. 1975. Metabolites influence control of lysine transfer ribonucleic acid synthetase formation in *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA 72:1364–1367.

67. Hochschild, A., and M. Ptashne. 1986. Cooperative binding of λ repressors to sites separated by integral turns of the DNA helix. Cell 44:681–687.

68. Huisman, T. T., D. Bakker, P. Klaasen, and F. K. de Graaf. 1994. Leucine-responsive regulatory protein, IS1 insertions, and the negative regulator FaeA control the expression of the *fae* (K88) operon in *Escherichia coli*. Mol. Microbiol. 11:525–536.

69. Ito, K., K. Kawakami, and Y. Nakamura. 1993. Multiple control of *Escherichia coli* lysyl-tRNA synthetase expression involves a transcriptional repressor and a translational enhancer element. Proc. Natl. Acad. Sci. USA 90:302–306.

70. Jamieson, D. J., and C. F. Higgins. 1984. Anaerobic and leucine-dependent expression of a peptide transport gene in *Salmonella typhimurium*. J. Bacteriol. 160:131–136.

71. Kawakami, K., K. Ito, and Y. Nakamura. 1992. Differential regulation of two genes encoding lysyl-tRNA synthetases in *Escherichia coli*: *lysU*-constitutive mutants compensate for a *lysS* null mutation. Mol. Microbiol. 6:1739–1745.

72. Kayahara, T., P. Thelen, W. Ogawa, K. Inaba, M. Tsuda, E. B. Goldberg, and T. Tsuchiya. 1992. Properties of recombinant cells capable of growing on serine without NhaB Na$^+$/H$^+$ antiporter in *Escherichia coli*. J. Bacteriol. 174:7482–7485.

73. Kim, J., C. Zwieb, C. Wu, and S. Adhya. 1989. Bending of DNA by gene-regulatory proteins: construction and use of a DNA bending vector. Gene 85:15–24.

74. Klem, P. 1986. Two regulatory *fim* genes, *fimB* and *fimE*, control the phase variation of type I fimbrae in *Escherichia coli*. EMBO J. 5:1389–1393.

75. Kolling, R., and H. Lother. 1985. AsnC: an autogenously regulated activator of asparagine synthetase A transcription in *Escherichia coli*. J. Bacteriol. 164:310–315.

76. Kustu, S. G., N. C. McFarland, S. P. Hui, B. Esmon, and G. Ferro-Luzzi Ames. 1979. Nitrogen control in *Salmonella typhi-*

murium: coregulation of synthesis of glutamine synthetase and amino acid transport systems. J. Bacteriol. **138**:218–234.

77. **Landick, R., and D. L. Oxender.** 1985. The complete nucleotide sequences of the *Escherichia coli* LIV-BP and LS-BP genes. J. Biol. Chem. **260**:8257–8261.

78. **Lange, R., M. Barth, J. Bohringer, and R. Hengge-Aronis.** 1993. Complex transcriptional control of the σ^S-dependent stationary phase-induced and osmotically regulated *osmY* (*csi-5*) gene suggests novel roles for Lrp, IHF and cAMP-CRP in the stationary phase response of *Escherichia coli*. J. Bacteriol. **175**:7910–7917.

79. **Lenny, A. B., and P. Margolin.** 1980. Locations of the *opp* and *supX* genes of *Salmonella typhimurium* and *Escherichia coli*. J. Bacteriol. **143**:747–752.

80. **Leveque, F., M. Gazeau, M. Fromant, S. Blanquet, and P. Plateau.** 1991. Control of *Escherichia coli* lysyl-tRNA synthetase expression by anaerobiosis. J. Bacteriol. **173**:7903–7910.

81. **Leveque, F., P. Plateau, P. Dessen, and S. Blanquet.** 1990. Homology of *lysS* and *lysU*, the two *Escherichia coli* genes encoding distinct lysyl-tRNA synthetase species. Nucleic Acids Res. **18**:305–312.

82. **Levinthal, M., P. Lejeune, and A. Danchin.** 1994. The H-NS protein modulates the activation of the *ilvIH* operon of *Escherichia coli* K-12 by Lrp, the leucine regulatory protein. Molec. Gen. Genet. **242**:736–743.

83. **Lewis, L. K., M. E. Jenkins, and D. W. Mount.** 1992. Isolation of DNA damage-inducible promoters in *Escherichia coli*: regulation of *polB* (*dinA*), *dinG*, and *dinH* by LexA repressor. J. Bacteriol. **174**:3377–3385.

84. **Lin, R., R. D'Ari, and E. B. Newman.** 1992. *placMu* insertions in genes of the leucine regulon: extension of the regulon to genes not regulated by leucine. J. Bacteriol. **174**:1948–1955.

85. **Lin, R., B. Ernsting, I. N. Hirshfield, R. G. Matthews, F. C. Neidhardt, R. L. Clark, and E. B. Newman.** 1992. The *lrp* gene product regulates expression of *lysU* in *Escherichia coli* K-12. J. Bacteriol. **174**:2779–2784.

86. **Lin, R. T., R. D'Ari, and E. B. Newman.** 1990. The leucine regulon of *E. coli* K-12: a mutation in *rblA* alters expression of L-leucine-dependent metabolic operons. J. Bacteriol. **172**:4529–4535.

87. **Lindberg, F., B. Land, L. Johansson, and S. Normark.** 1987. Localization of the receptor-binding protein adhesin at the tip of the bacterial pilus. Nature (London) **328**:84–87.

88. **Lobell, R. B., and R. F. Schleif.** 1990. DNA looping and unlooping by AraC protein. Science **250**:528–532.

88a.**Low, D.** Personal communication.

89. **Madhusudhan, K. T., D. Lorenz, and J. R. Sokatch.** 1993. The *bkdR* gene of *Pseudomonas putida* is required for expression of the *bkd* operon and encodes a protein related to Lrp of *Escherichia coli*. J. Bacteriol. **175**:3934–3940.

90. **Mallick, U., and P. Herrlich.** 1979. Regulation of synthesis of a major outer membrane protein: cyclic AMP represses *Escherichia coli* protein III synthesis. Proc. Natl. Acad. Sci. USA **76**:5520–5523.

91. **Marinus, M. G., and N. R. Morris.** 1973. Isolation of deoxyribonucleic acid methylase mutants of *Escherichia coli* K-12. J. Bacteriol. **114**:1143–1150.

92. **Matthews, R. G., and F. C. Neidhardt.** 1988. Abnormal induction of heat shock proteins in an *Escherichia coli* mutant deficient in adenosylmethionine synthetase activity. J. Bacteriol. **170**:1582–1588.

93. **Matthews, R. G., and F. C. Neidhardt.** 1989. Elevated serine catabolism is associated with the heat shock response in *Escherichia coli*. J. Bacteriol. **171**:2619–2625.

94. **McClain, M. S., I. C. Blomfield, and B. I. Eisenstein.** 1991. Roles of *fimB* and *fimE* in site-specific DNA inversion associated with phase variation of type 1 fimbriae in *Escherichia coli*. J. Bacteriol. **173**:5308–5314.

95. **McKitrick, J. C., and L. I. Pizer.** 1980. Regulation of phosphoglycerate dehydrogenase levels and effect on serine synthesis in *Escherichia coli* K-12. J. Bacteriol. **141**:235–245.

96. **Mooi, F. R., M. van Buuren, G. Koopman, E. Roosendaal, and F. K. 1. deGraaf.** 1984. K88ab gene of *Escherichia coli* encodes a fimbria-like protein distinct from the K88ab fimbrial adhesin. J. Bacteriol. **159**:482–487.

97. **Moon, H. W.** 1990. Colonization factor antigens of enterotoxigenic *Escherichia coli* in animals. Curr. Top. Microbiol. Immunol. **151**:147–165.

98. **Nazos, P. M., T. Z. Su, R. Landick, and D. A. Oxender.** 1984. Branched-chain amino acid transport in *Escherichia coli*, p. 24–28. *In* L. Leive and D. Schlessinger (ed.), Microbiology— 1984. American Society for Microbiology, Washington, D.C.

99. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119**:736–747.

100. **Neidhardt, F. C., R. A. VanBogelen, and E. T. Lau.** 1983. Molecular cloning and expression of a gene that controls the high-temperature regulon of *Escherichia coli*. J. Bacteriol. **153**:597–603.

101. **Newman, E. B., D. Ahmad, and C. Walker.** 1982. L-Serine deaminase activity is induced by exposure of *Escherichia coli* K-12 to DNA-damaging agents. J. Bacteriol. **152**:702–705.

102. **Newman, E. B., R. D'Ari, and R. T. Lin.** 1992. The leucine-Lrp regulon in *E. coli*: a global response in search of a raison d'etre. J. Bacteriol. **68**:617–619.

103. **Newman, E. B., V. Kapoor, and R. Potter.** 1976. Role of L-threonine dehydrogenase in the catabolism of threonine and synthesis of glycine by *Escherichia coli*. J. Bacteriol. **126**:1245–1249.

104. **Nikaido, H., and E. Y. Rosenberg.** 1983. Porin channels in *Escherichia coli*: studies with liposomes reconstituted from purified proteins. J. Bacteriol. **153**:241–252.

105. **Nikaido, H., and M. Vaara.** 1987. Outer membrane, p. 7–22. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.

106. **Normark, S., M. Baga, M. Goransson, F. P. Lindberg, B. Lund, M. Norgren, and B. E. Uhlin.** 1986. Genetics and biogenesis of *Escherichia coli* adhesins, p. 113–143. *In* D. Mirelman (ed.), Microbial lectins and agglutinins: properties and biological activity. Wiley Interscience, New York.

107. **Nou, X., B. Skinner, B. Braaten, L. Blyn, D. Hirsch, and D. Low.** 1993. Regulation of pyelonephritis-associated pili phase-variation in *Escherichia coli*: binding of the PapI and the Lrp regulatory proteins is controlled by DNA methylation. Mol. Microbiol. **7**:545–553.

108. **O'Day, K., J. Lopilato, and A. Wright.** 1991. Physical locations of *bglA* and *serA* on the *Escherichia coli* K-12 chromosome. J. Bacteriol. **173**:1571.

109. **O'Halloran, T. V., B. Frantz, M. K. Shin, D. M. Ralston, and J. G. Wright.** 1989. The MerR heavy metal receptor mediates positive activation in a topologically novel transcription complex. Cell **56**:119–129.

110. **Okamura-Ikeda, K., Y. Ohmura, K. Jufiwara, and Y. Motokawa.** 1993. Cloning and nucleotide sequence of the *gcv* operon encoding the *Escherichia coli* glycine cleavage system. Eur. J. Biochem. **216**:539–548.

111. **Orskov, I., F. Orskov, A. Birch-Andersen, P. Klemm, and C. Svanborg-Eden.** 1982. Semin. Infect. Dis. **4**:97–103.

112. **Pahel, G., D. M. Rothstein, and B. Magasanik.** 1982. Complex *glnA-glnL-glnG* operon of *Escherichia coli*. J. Bacteriol. **150**:202–213.

113. **Pahel, G., and B. Tyler.** 1979. A new *glnA*-linked regulatory gene for glutamine synthetase in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **76**:4544–4548.

114. **Pahel, G., A. D. Zelenetz, and B. M. Tyler.** 1978. *gltBD* gene and regulation of nitrogen metabolism by glutamine synthetase in *Escherichia coli*. J. Bacteriol. **133**:139–148.

115. **Pardee, A. B., and L. S. Prestidge.** 1955. Induced formation of serine and threonine deaminases by *Escherichia coli*. J. Bacteriol. **70**:667–674.

116. **Pizer, L. I., and M. L. Potochny.** 1964. Nutritional and regulatory aspects of serine metabolism in *Escherichia coli*. J. Bacteriol. **88**:611–619.

116a.**Plateau, P.** Personal communication.

117. **Platko, J. V.** 1991. Genetic analysis of Lrp, a leucine-responsive regulatory protein of *Escherichia coli*. Ph. D. thesis. Cornell University, Ithaca, N.Y.

118. Platko, J. V., and J. M. Calvo. 1993. Mutations affecting the ability of *Escherichia coli* Lrp to bind DNA, activate transcription, or respond to leucine. J. Bacteriol. **175**:1110–1117.

118a.Platko, J. V., B. Tyler, D. Friedberg, and J. M. Calvo. Unpublished data.

119. Platko, J. V., D. A. Willins, and J. M. Calvo. 1990. The *ilvIH* operon of *Escherichia coli* is positively regulated. J. Bacteriol. **172**:4563–4570.

120. Quay, S. C., T. E. Dick, and D. L. Oxender. 1977. Role of transport systems in amino acid metabolism: leucine toxicity and the branched-chain amino acid transport systems. J. Bacteriol. **129**:1257–1265.

121. Quay, S. C., and D. L. Oxender. 1980. Regulation of membrane transport. p. 413–436. Plenum Press, New York.

122. Rahmanian, M., D. R. Claus, and D. L. Oxender. 1973. Multiplicity of leucine transport systems in *Escherichia coli*. J. Bacteriol. **116**:1258–1266.

123. Rahmanian, M., and D. L. Oxender. 1972. Derepressed leucine transport activity in *Escherichia coli*. J. Supramol. Struct. **1**:55–59.

124. Ravnikar, P. D., and R. Somerville. 1987. Genetic characterization of a highly effecient alternate pathway of serine biosynthesis in *Escherichia coli*. J. Bacteriol. **169**:2611–2617.

125. Ravnikar, P. D., and R. Somerville. 1987. Structural and functional analysis of a cloned segment of *Escherichia coli* DNA that specifies proteins of a C_4 pathway of serine biosynthesis. J. Bacteriol. **169**:4716–4721.

126. Reitzer, L. J., and B. Magasanik. 1987. Ammonia assimilation and the biosynthesis of glutamine, glutamate, aspartate, asparagine, L-alanine, and D-alanine, p. 302–320. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.

127. Rex, J. H., B. D. Aronson, and R. L. Somerville. 1991. The *tdh* and *serA* operons of *E. coli*: mutational analysis of the regulatory elements of leucine-responsive genes. J. Bacteriol. **173**:5944–5953.

128. Ricca, E., D. A. Aker, and J. M. Calvo. 1989. A protein that binds to the regulatory region of the *ilvIH* operon of *Escherichia coli*. J. Bacteriol. **171**:1658–1664.

129. Ricca, E., C. T. Lago, M. Sacco, and M. De Felice. 1991. Absence of acetohydroxy acid synthase III in *Salmonella typhimurium* is due to early termination of translation within the *ilvI* gene. Mol. Microbiol. **5**:1741–1744.

130. Roberts, R. B., P. H. Abelson, D. B. Cowie, E. T. Bolton, and R. J. Britten. 1955. Studies of biosynthesis of *Escherichia coli*. Carnegie Inst. Washington Publ. **612**:283–285.

131. Roosendaal, E., M. Boots, and F. K. de Graaf. 1987. Two novel genes, *fanA* and *fanB*, involved in the biogenesis of K99 fimbriae. Nucleic Acids Res. **15**:5973–5984.

132. Rudd, K. E. 1992. Alignment of *E. coli* DNA sequences to a revised, integrated genomic restriction map, p. 2.13–2.31. *In* J. H. Miller (ed.), A short course in bacterial genetics, vol. 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

133. Russel, M., and P. Model. 1988. Sequence of thioredoxin reductase from *Escherichia coli*. J. Biol. Chem. **263**:9015–9019.

133a.Sacco, M. Personal communication.

134. Sacco, M., E. Ricca, R. Marasco, R. Paradiso, and M. DeFelice. 1993. A stereospecific alignment between the promoter and the cis-acting sequence is required for Lrp-dependent activation of *ilvIH* transcription in *Escherichia coli*. FEMS Microbiol. Lett. **107**:331–336.

135. Satishchandran, C., J. C. Taylor, and G. D. Markham. 1990. Novel *Escherichia coli* K-12 mutants impaired in S-adenosylmethionine synthesis. J. Bacteriol. **172**:4489–4496.

136. Satishchandran, C., J. C. Taylor, and G. D. Markham. 1993. Isozymes of S-adenosylmethionine synthetase are encoded by tandemly duplicated genes in *Escherichia coli*. Mol. Microbiol. **9**:835–846.

137. Saukkonen, K. M. J., B. Nowicki, and M. Leinonen. 1988. Role of type 1 and S fimbriae in pathogenesis of *Escherichia coli* O18K1 bacteremia and meningitis in the infant rat. Infect. Immun. **56**:892–897.

138. Schmoll, T., J. Morschhauser, M. Ott, B. Ludwig, I. van Die, and J. Hacker. 1990. Complete genetic organization and functional aspects of the *Escherichia coli* S fimbrial adhesin determinant. Nucleotide sequence of the genes *sfaBCDEF*. Microb. Pathog. **9**:331–344.

139. Sella, C., O. Weinstock, Z. Barak, and D. M. Chipman. 1993. Subunit association in aceohydroxy acid synthase isozyme III. J. Bacteriol. **175**:5339–5343.

140. Shaibe, E., E. Metzer, and Y. S. Halpern. 1985. Control of utilization of L-arginine, L-ornithine, agmatine, and putrescine as nitrogen sources in *Escherichia coli* K-12. J. Bacteriol. **163**:938–942.

141. Shaibe, E., E. Metzler, and Y. S. Halpern. 1985. Metabolic pathway for the utilization of L-arginine, L-ornithine, agmatine, and putrescine as nitrogen sources in *Escherichia coli* K-12. J. Bacteriol. **163**:933–937.

141a.Short, S. A. Personal communication.

142. Smith, D. W., W. B. Stine, A. L. Svitil, A. Bakker, and J. W. Zyskind. 1992. *Escherichia coli* cells lacking methylation-blocking factor (leucine-responsive regulatory protein) have precise timing of initiation of DNA replication in the cell cycle. J. Bacteriol. **174**:3078–3082.

142a.Sokatch, J. R. Personal communication.

143. Squires, C. H., M. DeFelice, J. Devereux, and J. M. Calvo. 1983. Molecular structure of *ilvHI* and its evolutionary relationship to *ilvG* in *Escherichia coli* K12. Nucleic Acids Res. **11**:5299–5313.

144. Squires, C. H., M. DeFelice, C. T. Lago, and J. M. Calvo. 1983. The *ilvHI* locus of *Salmonella typhimurium*. J. Bacteriol. **154**:1054–1063.

145. Squires, C. H., M. DeFelice, S. R. Wessler, and J. M. Calvo. 1981. Physical characterization of the *ilvHI* operon of *Escherichia coli* K12. J. Bacteriol. **147**:797–804.

146. Stauffer, G. V. 1987. Biosynthesis of serine and glycine, p. 412–418. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.

147. Steiert, J. G., R. J. Rolfes, H. Zalkin, and G. V. Stauffer. 1990. Regulation of the *Escherichia coli glyA* gene by the *purR* gene product. J. Bacteriol. **172**:3799–3803.

148. Stern, M. J., C. F. Higgins, and G. Ferro-Luzzi Ames. 1984. Isolation and characterization of *lac* fusions to two nitrogen-regulated promoters. Mol. Gen. Genet. **195**:219–227.

149. Stock, J. B., A. J. Ninfa, and A. M. Stock. 1989. Protein phosphorylation and regulation of adaptive responses in bacteria. Microbiol. Rev. **53**:450–490.

150. Su, H., B. F. Lang, and E. B. Newman. 1989. L-Serine degradation in *Escherichia coli* K-12: cloning and sequencing of the *sdaA* gene. J. Bacteriol. **171**:5095–5102.

151. Sutton, A., and M. Freundlich. 1980. Regulation by cyclic AMP of the *ilvB*-encoded biosynthetic acetohydroxyacid synthase in *Escherichia coli* K-12. Mol. Gen. Genet. **178**:179–183.

152. Tartaglia, L. A., C. J. Gimeno, G. Storz, and B. N. Ames. 1992. Multidegenerate DNA recognition by the OxyR transcriptional regulator. J. Biol. Chem. **267**:2038–2045.

153. Travers, A. A. 1989. DNA conformation and protein binding. Annu. Rev. Biochem. **58**:427–452.

154. Tsai, S. Y., M. Tsai, and B. W. O'Malley. 1989. Cooperative binding of steroid hormone receptors contributes to transcriptional synergism at target enhancer elements. Cell **57**:443–448.

155. Ursini, M. V., P. Arcari, and M. DeFelice. 1981. Acetohydroxyacid synthase isoenzymes of *Escherichia coli* K-12: a *trans*-acting regulatory locus for *ilvIH* gene expression. Mol. Gen. Genet. **181**:491–496.

156. van der Woude, M. W., B. A. Braaten, and D. A. Low. 1992. Evidence for global regulatory control of pilus expression in *Escherichia coli* by Lrp and DNA methylation: model building based on analysis of *pap*. Mol. Microbiol. **6**:2429–2435.

157. van der Woude, M. W., and D. A. Low. 1994. Leucine responsive regulatory protein and deoxyadenosine methylase control the phase variation and expression of the *E. coli sfa* and *daa* pili operons. Mol. Microbiol. **11**:605–618.

158. Van Lenten, E. J., and S. Simmonds. 1965. Metabolic relations between l-threonine and glycine in *Escherichia coli*. J. Biol. Chem. 240:3361–3371.
158a.Wang, Q., and J. M. Calvo. Unpublished data.
159. Wang, Q., and J. M. Calvo. 1993. Lrp, a global regulatory protein of *E. coli*, binds cooperatively to multiple sites and activates transcription of *ilvIH*. J. Mol. Biol. 229:306–318.
160. Wang, Q., and J. M. Calvo. 1993. Lrp, a major regulatory protein in *Escherichia coli*, bends DNA and can organize the assembly of a higher-order nucleoprotein structure. EMBO J. 12:2495–2501.
160a.Wang, Q., M. Midkiff, and J. M. Calvo. Unpublished data.
161. Wang, Q., M. Sacco, E. Ricca, C. T. Lago, M. DeFelice, and J. M. Calvo. 1993. Organization of Lrp binding sites upstream of *ilvIH* in *Salmonella typhimurium*. Mol. Microbiol. 7:883–891.
162. Wang, Q., J. Wu, D. Friedberg, J. Platko, and J. M. Calvo. 1994. Regulation of the *Escherichia coli lrp* gene. J. Bacteriol. 176: 1831–1839.
163. Wanner, B., R. Kodaira, and F. C. Neidhardt. 1977. Physiological regulation of a decontrolled *lac* operon. J. Bacteriol. 130:212– 222.
164. Weichart, D., R. Lange, N. Henneberg, and R. Hengge-Aronis. 1993. Identification and characterization of stationary-phase inducible genes in *Escherichia coli*. Mol. Microbiol. 10:407–420.
165. Wessler, S. R., and J. M. Calvo. 1981. Control of *leu* operon expression in *Escherichia coli* by a transcription attenuation mechanism. J. Mol. Biol. 149:579–597.
166. Willins, D. A. 1991. Characterization of Lrp, a transcriptional

regulatory protein of *Escherichia coli*. Ph.D. thesis. Cornell University, Ithaca, N.Y.
167. Willins, D. A., and J. M. Calvo. 1992. In vitro transcription from the *Escherichia coli ilvIH* promoter. J. Bacteriol. 174:7648–7655.
168. Willins, D. A., C. W. Ryan, J. V. Platko, and J. M. Calvo. 1991. Characterization of Lrp, an *Escherichia coli* regulatory protein that mediates a global response to leucine. J. Biol. Chem. 266:10768–10774.
169. Wilson, R. L., L. T. Stauffer, and G. V. Stauffer. 1993. Roles of the GcvA and PurR proteins in negative regulation of the *Escherichia coli* glycine cleavage enzyme system. J. Bacteriol. 175:5129–5134.
170. Wilson, R. L., P. S. Steiert, and G. V. Stauffer. 1993. Positive regulation of the *Escherichia coli* glycine cleavage enzyme system. J. Bacteriol. 175:902–904.
171. Wu, H.-M., and D. M. Crothers. 1984. The locus of sequence-directed and protein-induced DNA bending. Nature (London) 308:509–513.
171a.Wu, J., and J. M. Calvo. Unpublished data.
172. Yim, H. H., and M. Villarejo. 1992. *osmY*, a new hyperosmotically inducible gene, encodes a periplasmic protein in *Escherichia coli*. J. Bacteriol. 174:3637–3644.
173. Zaboura, M., and Y. S. Halpern. 1978. Regulation of γ-aminobutyric acid acid degradation in *Escherichia coli* by nitrogen metabolism enzymes. J. Bacteriol. 133:447–451.
174. Zieg, J., and M. Simon. 1980. Analysis of the nucleotide sequence of an invertible controlling element. Proc. Natl. Acad. Sci. USA 77:4196–4200.