JOURNAL OF BACTERIOLOGY, Apr. 1983, p. 344–350
0021-9193/83/040344-07$02.00/0

Vol. 154, No. 1

Proteins Induced by Aerobiosis in *Escherichia coli*

MARTIN W. SMITH AND FREDERICK C. NEIDHARDT*

Department of Microbiology and Immunology, The University of Michigan, Ann Arbor, Michigan 48109

Received 27 September 1982/Accepted 10 January 1983

The role of protein induction and repression in the adaptation of *Escherichia coli* to changes in the supply of oxygen and other electron acceptors is only poorly understood. We have studied the changes in cellular protein composition associated with this adaptation by measuring the levels of 170 individual polypeptides produced during aerobic or anaerobic growth of *E. coli*, with and without nitrate. Nineteen polypeptides had levels highest during aerobic growth. These proteins include the enzymes of the pyruvate dehydrogenase complex, several tricarboxylic acid cycle enzymes, superoxide dismutase, and tetrahydropteroyltriglutamate transmethylase. The other aerobiosis-induced proteins have not been identified. These polypeptides are major cellular proteins during aerobic growth and display several different patterns of regulation in response to medium composition. Induction ratios for oxygen ranged from 2.2 to 11.2, with one exceptional member, superoxide dismutase, increasing 71-fold with aeration. Most of the proteins were also induced by nitrate during anaerobic growth. The time course of induction after shifts in oxygen supply revealed similarities in response among proteins of related function or metabolic regulation class. These results are discussed in relation to previously reported information on the identified aerobiosis-induced proteins.

Escherichia coli is capable of coupling its oxidative metabolism with energy-consuming reactions by either respiratory or fermentative processes, depending on the availability of an acceptable respiratory electron acceptor. When oxygen, nitrate, or one of several other compounds is present, terminal respiration allows energy production and membrane energization. In the absence of such compounds, fermentative pathways maintain a favorable redox balance, energy comes principally from substrate-level reactions, and membrane energization is by ATP hydrolysis.

Although the transition between respiratory and fermentative growth is made readily and smoothly (21), it is accompanied by alterations in the rate, route, and efficiency of carbon source utilization, in the pathways of electron flow, and in certain biosynthetic steps.

The studies reported here concern the contribution of cellular protein composition to the growth of *E. coli* in the presence of oxygen and other electron acceptors. We report (i) the enumeration of proteins produced in increased amounts by *E. coli* in the presence of oxygen; (ii) measurement of their induction ratios for oxygen and nitrate; (iii) their molecular abundance; (iv) the kinetics of their synthesis after shifts in oxygen supply; and (v) their biochemical identification, where known.

MATERIALS AND METHODS

Bacterial strain, media, and growth and labeling conditions. *E. coli* K-12 strain W3110 (Thi$^-$) was used throughout this work. Cells were grown in MOPS minimal medium modified as previously described (21). Anaerobic cultures were grown in an anaerobic glove chamber designed to allow manipulation of a culture without disturbance of the anaerobic environment. The chamber, methods for shifting cultures between aerobic and anaerobic conditions, and radiolabeling techniques have been described in the companion paper (21).

Analytical techniques. The preparation of extracts, analysis by two-dimensional gel electrophoresis, and measurement of protein spots have been previously described (18, 21). A sample of partially purified *E. coli* succinate dehydrogenase was kindly provided by E. C. C. Lin. Methods for identification of polypeptide spots on gels by comigration with purified proteins have been described (3).

RESULTS

Steady-state levels of polypeptides produced during aerobic growth. Of 170 individual polypeptides sampled from two-dimensional gels, 19 had steady-state levels higher during aerobic growth than during anaerobic. These proteins are indicated in Fig. 1 with squares and are numbered according to their alpha-numeric designation in Table 1. Steady-state induction ratios for the proteins are given in Table 1 as the level

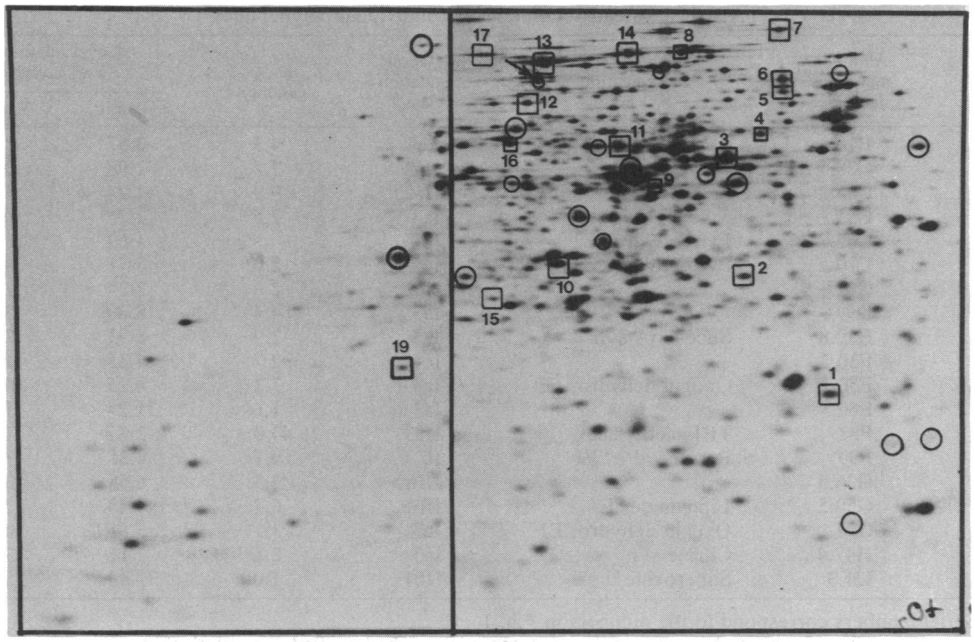

FIG. 1. Autoradiograms of two-dimensional gels of aerobically grown *E. coli* K-12 strain W3110 labeled with $^{35}SO_4$. This is a composite of two gels prepared with the same extract: right, pH 5 to 7 ampholine equilibrium gel; left, pH 3 to 10 non-equilibrium gel. The aerobiosis-induced polypeptides are marked by squares and are numbered as in Table 1. Conditions for electrophoresis and, for comparison, gels prepared from anaerobically grown cells are shown in Fig. 1 of the accompanying paper (21). The location of protein G74, pyruvate formate lyase, α-subunit, is indicated with an arrow.

during aerobic growth in glucose minimal medium or during anaerobic growth with nitrate as electron acceptor, both relative to the level during anaerobic growth in unsupplemented medium. Aerobic induction ratios for 18 of the proteins ranged from 2.2 to 11.2; protein I21.3 was exceptional with a ratio of 71.

Most of the proteins were also induced by the addition of nitrate to an anaerobic culture. Nitrate is generally a weaker inducer than air. It had little or no effect on the level of proteins F30.2, F50.3, F60.3, F88, and G97 and even repressed protein C55.

Induction ratios for nine control proteins sampled in the same experiments are given in Table 1 of the accompanying paper (21). They are proteins of known biochemical identity and metabolic activity and display a wide variety of regulatory patterns. The levels of these and most other proteins sampled in the survey were insensitive to both oxygen and nitrate. Other proteins were markedly repressed by oxygen (and responded variably to nitrate), and still others were induced by nitrate, but were detectable only during anaerobic growth. The latter set of proteins will be the subject of later reports.

Molecular abundance and metabolic regulation of the aerobiosis-induced polypeptides. Of the polypeptides sampled in the survey, the composite weight was at least 700 μg/mg of total protein (70%). The average weight fraction for an individual polypeptide was thus about 4 μg/mg. This value is somewhat higher than that for all proteins produced under this condition, since sampling favored the more abundant polypeptides. In fact, assuming that about 1,000 polypeptides are produced by *E. coli* under a given condition (an assumption based on the examination of hundreds of independent gels), this value is likely to be about five times the real average. Most of the polypeptides in Table 1 have weight fractions between 2 and 10 and thus are fairly major proteins. Polypeptide F88 was among the most abundant polypeptides, comprising almost 4.5% of the total cellular protein. Collectively, the 19 polypeptides comprised over 10% of the total protein weight in aerobically grown cells and 3% during anaerobic growth.

The levels of the aerobiosis-induced polypeptides varied widely during aerobic growth on media differing in principal carbon and energy source and in biosynthetic demand. Table 1 lists the metabolic regulation classes of the proteins (see reference 18 for a detailed description of the classification).

Many of the regulation classes are represented

TABLE 1. Steady-state levels of individual polypeptides

Serial no.[a]	Alpha-numeric designation[b]	Identification[c]	Metabolic regulation class[d]	α'_{+O_2} (μg/mg)[e]	Level relative to anaerobic	
					Aerobic	Nitrate
1	B18.4		Ia4	4.3	3.57	2.28
2	C28.5		IIa2	2.5	2.98	2.55
3	C45.2		IIa2	9.9	11.24	3.14
4	C55		Ia3	2.5	2.76	0.35
5	C62.7	Pyr dehydro, E2	Ic		4.69	3.94
6	C70	Pyr dehydro, E2	Ic	2.0	5.71	4.15
7	C137		IIa2	2.3	2.25	2.07
8	D99		Ia3	4.4	8.00	3.22
9	E39.8	Succ-CoA syn	Ia3	2.9	8.93	2.13
10	F30.2		Ic	2.7	4.35	1.61
11	F50.3	Oxoglu dehydro, E2	Ia3	3.3	6.25	0.79
12	F60.3		Ia3	1.6	11.24	1.78
13	F88	TPT methylase	IIa3	44.0	2.85	1.92
14	F99	Pyr dehydro, E1	Ic	9.7	4.31	3.79
15	G26.5		IIIa	2.5	6.58	3.63
16	G50.5	Lipoamide, E3	IIb1	6.7	4.48	2.21
17	G97	Oxoglu dehydro, E1	Ia3	3.1	5.81	0.88
18	H47.4	Citrate syn	Ia3	2.1	2.17	3.13
19	I21.3	Superoxide dis	IIb1	1.0	71.43	5.00

[a] Serial numbers correspond to the numbers in Fig. 1.

[b] Proteins are listed with alpha-numeric designations as described by Pederson et al. (18).

[c] Biochemical identification of the named proteins has been reported previously (3, 19). Abbreviations used: Pyr dehydro, E1 and E2, pyruvate dehydrogenase complex enzymes 1 and 2 (pyruvate dehydrogenase and dihydrolipoamide acetyltransferase, respectively); Succ-CoA syn, succinyl coenzyme A synthase; Oxoglu dehydro, E1 and E2, 2-oxoglutarate dehydrogenase and dihydrolipoamide succinyltransferase, respectively; TPT methylase, tetrahydropteroyltriglutamate methyltransferase; Lipoamide, E3, lipoamide dehydrogenase; Citrate syn, citrate synthase; Superoxide dis, superoxide dismutase, mangano-enzyme.

[d] See reference 18 for a complete description of the classification.

[e] These values were measured by determining the total radioactivity in the individual spot on a gel from cells labeled with [U-^{14}C]glucose. The methods for this determination and the value for protein elongation factor G (16.6 μg/mg), from which all others are calculated, are from Pedersen et al. (18).

among the 19 polypeptides. Ten proteins (those in classes Ia3, Ia4, IIb, and III) had highest levels during growth on oxidizable carbon sources (acetate and glycerol). These include four known tricarboxylic acid cycle enzymes (E39.8, F50.3, G97, and H47.4), superoxide dismutase (the manganese-containing enzyme), and lipoamide dehydrogenase (G50.5). The protein components unique to the pyruvate dehydrogenase complex, C62.7, C70, and F99, and protein F30.2 reached highest levels during growth on rich media. Polypeptides C62.7 and C70 are two forms of dihydrolipoyl acetyltransferase. They have the same isoelectric point but differ in apparent molecular weight by approximately 7,000. Although the basis for the doublet is not known, by biochemical, genetic, and regulatory criteria they have been found to be products of the same gene, *aceF* (unpublished data). The class IIa2 and IIa3 proteins, C28.5, C45.2, C137, and F88, reached maximum levels during aerobic growth on glucose minimal medium and their lowest levels during aerobic growth in rich media. All of the other polypeptides showed minimum levels during anaerobic growth.

Time course of polypeptide synthesis after shifts in oxygen supply. The relative rates of synthesis of the aerobiosis-induced polypeptides after abrupt shifts in oxygen supply are given in Fig. 2.

The polypeptides reported in Fig. 2A are the enzyme components of the pyruvate dehydrogenase complex. They are produced from three very closely linked genes, *aceE* (F99), *aceF* (C62.7 and C70), and *lpd* (G50.5), at least two of which (*aceEF*) are coexpressed.

Figure 2B gives data for six other polypeptides, including the tricarboxylic acid cycle enzymes. They are all members of the metabolic regulation class Ia3 or the closely related class Ia4. Two of these, F50.3 and G97, are products of cotranscribed genes. These two sets of polypeptides (Fig. 2A and B) displayed very similar patterns of response to shifts in oxygen supply.

Synthesis of the two polypeptides displayed in Fig. 2C, F88 (tetrahydropteroyltriglutamate transmethylase) and I21.3 (superoxide dismutase), was rapidly and almost completely shut off after the shift to anaerobiosis. For F88 this response was transient, recovering to a final

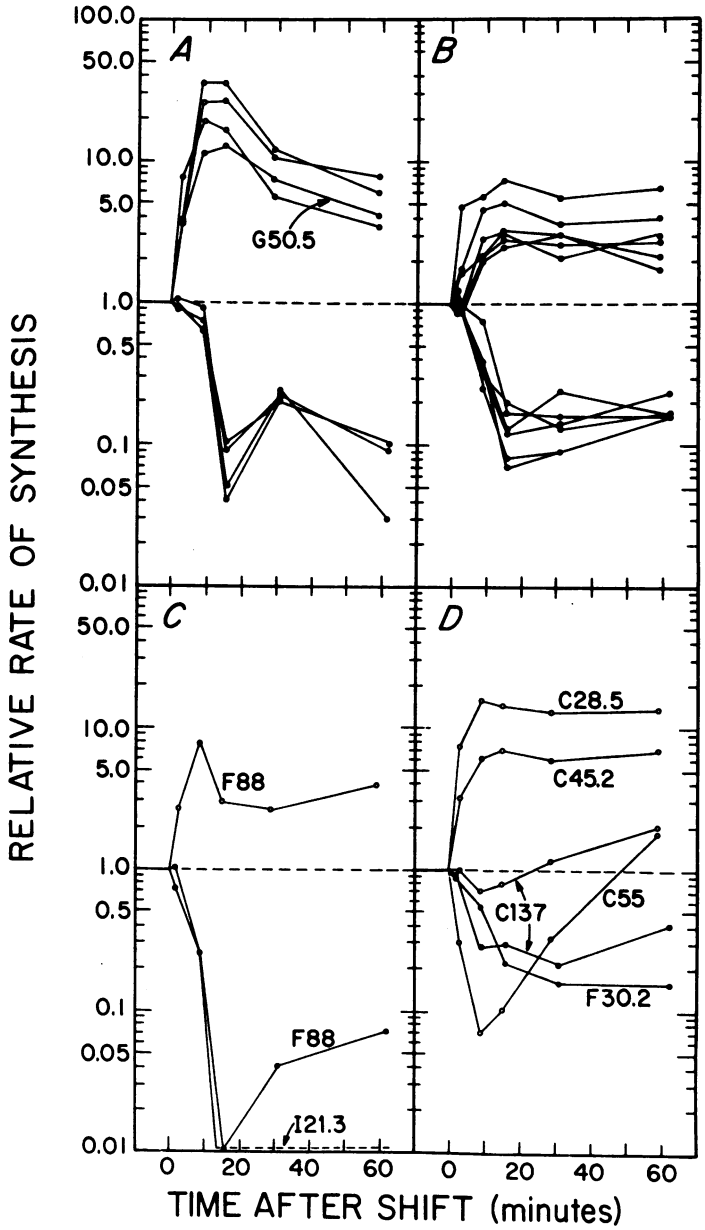

FIG. 2. Relative rates of synthesis of individual proteins at various times after shifts in oxygen supply. (●) Rates after a shift from aerobic to anaerobic; (○) rates after a shift from anaerobic to aerobic. All values are normalized to the measured preshift rate of synthesis. (A) Proteins C62.7, C70, F99, and G50.5; (B) proteins B18.4, D99, E39.8, F50.3, F60.3, and G97; (C) proteins F88 and I21.3 (● only); (D) proteins C28.5 (○ only), C45.2 (○ only), C55 (○ only), C137, and F30.2 (● only). Data for polypeptides G26.5 and H47.4 are not shown.

postshift synthesis rate of about one-third the aerobic rate (Table 1); for I21.3 the response was indefinite.

Figure 2D gives the responses of five other unidentified polypeptides. They are plotted here either because their response was unusual or because data for only one shift were obtained. The responses of polypeptides C55 and C137 to the shift to air are particularly noteworthy. They were characterized by a transient and, in the case of C55, dramatic decrease in rate of synthesis during the first 10 min, followed by gradual recovery during the rest of the time course.

The responses of selected control proteins to

the same shifts were given in Fig. 3D of the preceding report (21). These proteins generally gave little response to the shifts. Some, however, displayed transient fluctuations in rate of synthesis immediately after the shifts.

DISCUSSION

The physiological characteristics distinguishing fermenting cells of *E. coli* from those respiring actively are reasonably well understood. Differences in the rate and mechanisms of carbon, energy, and electron flow have been identified, and their roles in the adaptation between fermentative and respiratory metabolism are evident (8, 14, 23).

Changes in the activity of allosteric enzymes in key pathways of carbon and energy metabolism play a significant role in achieving this adaptation (20). The overall significance of changes in the amounts of enzymes and other proteins, however, has not been fully assessed. This and the preceding report mark the initiation of studies concerning the changes in cellular protein composition associated with the availability of oxygen and other respiratory electron acceptors to *E. coli*.

Depletion of oxygen from a culture growing in glucose minimal medium causes the induction of at least 18 polypeptides (21). This set of proteins is diverse with respect to molecular abundance and metabolic regulation during aerobic growth. Their transient responses to an abrupt shift in oxygen supply, however, suggest that these proteins may share sensitivity to a limited number of metabolic signals generated during a shift.

The present study revealed 19 polypeptides whose levels are increased by aerobic conditions. Half of these have been identified on our two-dimensional gels. These include components of two closely related pyridine nucleotide-linked dehydrogenases (pyruvate and 2-oxoglutarate dehydrogenase complexes) well known for their role in oxidative metabolism. These enzyme complexes each contain two unique subunits which are coexpressed from the *aceEF* and *sucAB* operons, respectively, but they share a third subunit, lipoamide dehydrogenase. Detailed genetic analysis of this interesting system has been made by Guest and his co-workers (9, 15).

Other studies have revealed that the pyruvate dehydrogenase complex is inducible by pyruvate during aerobic growth (4), repressed by anaerobiosis, and derepressed by nitrate during anaerobic growth (25). It is possible, though unlikely, that this anaerobic repression and its relief by nitrate are the result of fluctuations in pyruvate levels.

The ten identified polypeptides listed in Table 1 also include at least three tricarboxylic acid cycle enzymes (succinyl coenzyme-A synthase, 2-oxoglutarate dehydrogenase complex, and citrate synthase); protein F60.3 has tentatively been identified as succinate dehydrogenase by its comigration with the major species of a nearly pure preparation of the enzyme. Anaerobic repression has been reported for 2-oxoglutarate dehydrogenase (1), isocitrate dehydrogenase, malate dehydrogenase, citrate synthase, and aconitase (1, 13, 24). Many of the tricarboxylic acid cycle enzymes are also subject to catabolite repression (5, 20, 22). We have studied a set of 10 polypeptides, including the known tricarboxylic acid cycle enzymes, which share a very broad spectrum of regulatory behaviors (M. W. Smith, R. A. VanBogelen, and F. C. Neidhardt, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, K93, p. 142). These include (i) an inverse relationship between steady-state level and growth rate, (ii) induction by acetate and oxygen, (iii) repression by glucose, and (iv) an unusual response to cyclic AMP availability. These results suggest that a subset of the aerobiosis-induced polypeptides share sensitivity to a number of other regulatory signals.

The manganese-containing superoxide dismutase is induced by aerobiosis. It displays the highest induction ratio for oxygen among the polypeptides studied (Table 1) and, in fact, is virtually invisible on two-dimensional gels prepared from anaerobically grown cells. The enzyme apparently serves to protect the cell from superoxide ions generated by terminal respiration (11). Induction of superoxide dismutase by oxygen (which may be mediated through superoxide itself) has been reported previously (6, 12).

Finally, the *metE* product, vitamin B_{12}-independent tetrahydropteroyltriglutamate transmethylase, is induced by air. This enzyme catalyzes the last step in methionine synthesis in the absence of vitamin B_{12}. When cells are provided the vitamin, the B_{12}-independent enzyme is repressed (17) and an isozyme requiring vitamin B_{12} is used. The high percentage of cellular protein represented by the *metE* product has been attributed to its unusually low turnover number (7). In fact, under optimal conditions of assay, the B_{12}-dependent methyltransferase is almost 60 times as active as the B_{12}-independent enzyme. It is interesting that the vitamin B_{12}-dependent reaction is inhibited by oxygen (7) and that certain *metE* mutants do not require methionine for anaerobic growth (unpublished data). These results suggest the evolution of two pathways for methionine synthesis in *E. coli*, one aerobic and the other anaerobic.

Most of the polypeptides reported in this and the accompanying paper (21) responded to nitrate as they do to oxygen. Induction or repres-

sion by oxygen and nitrate may be the result of separate, unrelated metabolic signals. On the other hand, the establishment of electron transfer through an energy-yielding respiratory chain may be the key element to which these polypeptides are sensitive. The respiratory chains directed to oxygen and nitrate in *E. coli* have certain components in common (quinones and heme-containing electron carriers; 10), and it is conceivable that one of these components, when triggered by the presence or absence of a respiratory electron acceptor, can mediate a signal to the appropriate promoters. These possibilities can be tested by examining polypeptides known to respond to electron acceptor supply in cells blocked at various steps of electron transport by mutation or chemical inhibition.

The most interesting question, of course, is whether the polypeptides that are elevated by changes in electron acceptor supply share a regulatory mechanism. In fact, the goal of this work must now be the identification of (i) the metabolic signals and (ii) the regulatory elements (repressor, activator proteins) that are responsible for the adjustments we have observed.

There are several well-known instances of sets of genes that are coregulated though in different operons. In the case of the arginine biosynthetic pathway in *E. coli*, a set of nine genes in six operons are subject to a common repressor (2). Maas and Clark (16) have defined such a set as a regulon, i.e., a set of genes sharing a common regulatory element. How does one refer to a set of genes that share a behavioral response and may or may not share a molecular regulatory mechanism? We propose the term "stimulon" to refer to a set of genes (or a set of regulons) whose products are increased in reponse to a common environmental stimulus, irrespective of molecular mechanism. This definition is intentionally broad since it represents an inquiring "first look" at the set of proteins that share a regulatory behavior. The polypeptides reported in this and the accompanying paper (21) are considered representative of two stimulons: the aerobic and anaerobic stimulons.

The criteria for the definition of a regulon demand rigorous investigation on the genetic and molecular levels. To this end, we have recently isolated an *E. coli* strain which has abnormally high anaerobic levels of a subset of the 19 aerobically induced proteins. Should this defect prove to result from a single, identifiable mutation, this finding may suggest the existence of a regulon within the aerobic stimulon.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM-17892 from the National Institute of General Medical Sciences. M.W.S. was supported in part by Public Health Service Predoctoral Genetics Training Grant GM-07544 awarded to the Department of Human Genetics, The University of Michigan.

LITERATURE CITED

1. **Amarasingham, C. R., and B. D. Davis.** 1965. Regulation of α-ketoglutarate dehydrogenase formation in *Escherichia coli*. J. Biol. Chem. **240**:3664–3668.
2. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1–56.
3. **Bloch, P. L., T. A. Phillips, and F. C. Neidhardt.** 1980. Protein identifications on O'Farrell two-dimensional gels: locations of 81 *Escherichia coli* proteins. J. Bacteriol. **141**:1409–1420.
4. **Dietrich, J., and U. Henning.** 1970. Regulation of pyruvate dehydrogenase complex synthesis in *Escherichia coli* K-12. Eur. J. Biochem. **14**:258–269.
5. **Dills, S. S., and W. J. Dobrogosz.** 1977. Cyclic adenosine 3',5'-monophosphate regulation of membrane energetics in *Escherichia coli*. J. Bacteriol. **131**:854–865.
6. **Fee, J. A., A. C. Lees, P. L. Bloch, and F. C. Neidhardt.** 1978. The role of superoxide in the induction of superoxide dismutase and oxygen toxicity, p. 635–658. *In* W. S. Caughey (ed.), Symposium on the biochemical and clinical aspects of oxygen. Academic Press, Inc., New York.
7. **Flavin, M.** 1975. Methionine biosynthesis, p. 457–503. *In* D. M. Greenberg (ed.), Metabolism of sulfur compounds, vol. VII, Metabolic pathways, 3rd ed. Academic Press, Inc., New York.
8. **Gray, C. T., J. W. T. Wimpenny, D. E. Hughes, and M. R. Mossman.** 1967. Regulation of metabolism in facultative bacteria. I. Structural and functional changes in *Escherichia coli* associated with shifts between the aerobic and anaerobic states. Biochim. Biophys. Acta **117**:22–32.
9. **Guest, J. R., and P. E. Stephens.** 1980. Molecular cloning of the pyruvate dehydrogenase complex genes of *Escherichia coli*. J. Gen. Microbiol. **121**:277–292.
10. **Haddock, B. A., and C. W. Jones.** 1977. Bacterial respiration. Bacteriol. Rev. **41**:48–99.
11. **Hassan, H. M., and I. Fridovich.** 1977. Enzymatic defenses against the toxicity of oxygen and of streptonigrin in *Escherichia coli*. J. Bacteriol. **129**:1574–1583.
12. **Hassan, H. M., and I. Fridovich.** 1977. Regulation of synthesis of superoxide dismutase in *Escherichia coli*. J. Biol. Chem. **252**:7667–7672.
13. **Hespell, R. B.** 1976. Glycolytic and tricarboxylic acid cycle enzyme activities during intraperiplasmic growth of *Bdellovibrio bacteriovorus* on *Escherichia coli*. J. Bacteriol. **128**:677–680.
14. **Krebs, H. A.** 1972. The Pasteur effect and the relations between respiration and fermentation, p. 1–34. *In* P. N. Campbell and F. Dickens (ed.), Essays in biochemistry, vol. 8. Academic Press, Inc., New York.
15. **Langley, D., and J. R. Guest.** 1977. Biochemical genetics of the α-keto acid dehydrogenase complexes of *Escherichia coli*: isolation and biochemical properties of deletion mutants. J. Gen. Microbiol. **99**:263–276.
16. **Maas, W. K., and A. J. Clark.** 1964. Studies on the mechanisms of repression of arginine biosynthesis in *Escherichia coli*. II. Dominance of repressibility in diploids. J. Mol. Biol. **8**:365–370.
17. **Mulligan, J. T., W. Margolin, J. H. Krueger, and G. C. Walker.** 1982. Mutations affecting regulation of methionine biosynthetic genes isolated by use of *met-lac* fusions. J. Bacteriol. **151**:609–619.
18. **Pedersen, S., P. L. Bloch, S. Reeh, and F. C. Neidhardt.** 1978. Patterns of protein synthesis in *E. coli*: a catalog of the amount of 140 individual proteins at different growth rates. Cell **14**:179–190.
19. **Phillips, T. A., P. L. Bloch, and F. C. Neidhardt.** 1980. Protein identifications on O'Farrell two-dimensional gels: locations of 55 additional *Escherichia coli* proteins. J.

Bacteriol. **144**:1024–1033.
20. **Sanwal, B. D.** 1970. Allosteric control of amphibolic pathways in bacteria. Bacteriol. Rev. **34**:20–39.
21. **Smith, M. W., and F. C. Neidhardt.** 1983. Proteins induced by anaerobiosis in *Escherichia coli*. J. Bacteriol. **154**:336–343.
22. **Takahashi, Y.** 1975. Effect of glucose and cyclic adenosine 3′,5′-monophosphate on the synthesis of succinate dehydrogenase and isocitrate lyase in *Escherichia coli*. J. Biochem. **78**:1097–1100.
23. **Thomas, A. D., H. W. Doelle, A. W. Westwood, and G. L. Gordon.** 1972. Effect of oxygen on several enzymes involved in the aerobic and anaerobic utilization of glucose in *Escherichia coli*. J. Bacteriol. **112**:1099–1105.
24. **Wimpenny, J. W. T., and J. A. Cole.** 1967. The regulation of metabolism in facultative bacteria. III. The effect of nitrate. Biochim. Biophys. Acta **148**:233–242.
25. **Yamamoto, I., and M. Ishimoto.** 1975. Effect of nitrate reduction on the enzyme levels in carbon metabolism in *Escherichia coli*. J. Biochem. **78**:307–315.