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Taxonomic Comparison of the Amino Termini of Microbial Cell Proteins

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A comparison was made of the distribution of amino terminal end groups in the cellular proteins of a number of microbes. Among the procaryotes, methionine is a highly variable but virtually ubiquitous major protein end group. This is consistent with its possible role as a general amino acid initiator of protein biosynthesis in the procaryotes. Generally, however, alanine is the most abundant of the major end groups, followed in decreasing order by serine, threonine, the acidic amino acids, and occasionally lysine. No other new major end-groups were found. Among 15 representatives of the *Enterobacteriaceae*, retention of the initiating methionine terminus of the cellular protein varies considerably at a tribal level and is randomized at a familial level. The profiles of the five remaining end groups, however, are strikingly uniform, and are, for example, close to but significantly different from those of the *Erwineae*. Among the taxonomically more heterogeneous *Bacillaceae*, end-group profiles vary more and are sometimes unrelated. End-group analysis is thus particularly useful as a molecular criterion of taxonomy in assessing familial homogeneity. Free NH_2 termini in eucaryote cell proteins are fewer, and they have increased acidic amino acid components and no methionine; they are otherwise similar to those of the procaryotes.

The cell proteins in *Escherichia coli* are biosynthesized beginning with *N*-formylmethionine at the amino terminus, the mature termini being formed later by a series of cleavages to give methionine and five or six other amino acids as the only significant general residues (1, 3, 7, 13, 16).

To examine phylogenetic similarities or divergences in the patterns of protein initiation and maturation at the site of the amino terminus, the overall profiles of the NH_2-terminal end groups were analyzed in representative microorganisms. Analyses were performed on whole cells and on non-particulate protein fractions. The results indicate that the distribution of NH_2-terminal amino acid residues is always nonrandom and is strongly conserved qualitatively, but with sufficient quantitative variation to provide a useful index of taxonomic divergence.

MATERIALS AND METHODS

Organisms were routinely grown in Nutrient Broth and were harvested during exponential or active growth at a cellular turbidity represented by an optical density of approximately 0.2 (660 nm). This is equivalent in *E. coli* to 50 μg of cell protein/ml. *Streptococcus faecium* was grown without shaking in Nutrient Broth with 0.25% tryptone, 0.2% yeast extract, 1.0% glucose, 0.4% phosphate buffer (*p*H 7), and thymine and adenine (2 mg/ml). The psychrophilic *Pseudomonas* B-16 was shaken at 15 C in Nutrient Broth with 3% NaCl. The growth media used for *Caulobacter*, *Crithidia*, and *Mycoplasma* have been previously described (5, 6, 10). The medium for *Saccharomyces carlsbergensis* (2) was supplemented with 0.7% hydrolyzed casein, and a modification of medium E used for *Chlorella* (4) was supplemented with 1% sucrose.

Micrococcus lysodeikticus (Worthington) and *Clostridium kluyveri* (Worthington) were obtained as lyophilized cells. Cells of *Desulfovibrio vulgaris* were supplied in the lyophilized state, and those of the methane fermenter isolated from impure *Methanobacillus omelianskii* had been extracted with 1:1 $CHCl_3$-CH_3OH. Cultures of *Rhodomicrobium vanniellii* and *Rhodospirillum rubrum* were grown photosynthetically in a medium of inorganic salts and 0.1% each sodium lactate, Nutrient Broth solids, and $NaHCO_3$.

To obtain the composite ribosomal and soluble protein fraction, 33% suspensions of washed cells in water were disrupted in the cold in a French press at 5,000 to 7,000 psi and then were centrifuged at 15,000 $\times$ *g* for 30 min. The supernatant liquids, as well as cultures and reconstituted lyophilized cells, were then acidified and washed with 10% trichloroacetic acid and acetone. Some cells were shaken overnight at 25 C

with 0.2 N ammonia (*p*H 10 to 11) to remove the D-alanine of teichoic acid, and the proteins were precipitated with trichloroacetic acid.

Proteins were solubilized in 8 M urea-bicarbonate solution and dinitrophenylated as previously described (9, 16). Dinitrophenyl (DNP)-amino acids were released by hydrolysis in concentrated HCl for 4 hr at 100 C; after recovery, a total of 0.3 to 0.8 μmole was assayed chromatographically, essentially as described by Waller and Harris (22). Corrections for hydrolytic and chromatographic loss were calculated from the recovery of each of the six most significant DNP-amino acids subjected together with undinitrophenylated *E. coli* cell protein to routine hydrolysis, extraction, and assay. The DNP-amino acids usually were acidified, reextracted into ether, dried, and reassayed in acetone to completely eliminate small residual chromatographic interferences from ε-DNP-lysine and DNP-methionine sulfoxide. Corrections for hydrolytic and chromatographic losses were calculated from the recovery of each of the six most significant DNP-amino acids subjected, together with undinitrophenylated *E. coli* protein, to routine hydrolysis, extraction, and assay. Recovery of end-group methionine was also followed with ^{35}S-methionine-labeled protein. DNP-methionine sulfoxide was kept inappreciable by the use of large amounts of cell preparations, 4-hr hydrolyses, and extraction into freshly opened ether supplied with preservative. Determinations lacking any of these precautions are referred to only qualitatively.

Almost all reported values are averaged from the assays of two or three different cellular batches. Agreement of values was generally within, and never significantly beyond, 1 part in 10. Whole-cell and combined ribosomal-soluble protein preparations with closely similar NH_2-terminal profiles were averaged as duplicates. Separated DNP-amino acids were frequently rechromatographed on silica gel in thin layer with a benzene-pyridine-acetic acid system (18), particularly when the recovery was qualitatively or quantitatively unusual. A chromogen arose occasionally after the ammonia incubation and moved on paper with glutamic and aspartic acids, but it was separated on silica gel and a correction for it was made. Total protein content was calculated from the optical absorption at 360 nm of ε-DNP-lysine in the DNP-protein hydrolysate after ether extraction. The levels of lysine in microbial cell proteins were taken from the values of Sueoka (20); in unreported proteins, the lysine level was considered to be 7%.

RESULTS

General survey of microorganisms. The NH_2-terminal amino acids in the procaryotic-cell proteins, listed in Table 1, comprise only the six or seven significant NH_2-terminal amino acids originally reported in *E. coli* (21, 22). End-group alanine and serine usually ranged from 48 to 56% and from 23 to 29%, respectively, of the total. The end-group alanine was, however, noticeably lower in two anaerobes, *M. omelianskii* and *C. kluyveri*. The levels of threonine and the acidic amino acids varied somewhat more. Glutamic and aspartic acids, which are reported together because of frequent overlapping, appeared in roughly similar amounts in all assays.

Of greatest significance is the presence of end-group methionine in the cell protein of almost all procaryotes, and its exclusion in the eucaryotes. Some of the organisms of Table 1, but relatively few, have as much NH_2-terminal methionine as does *E. coli* (21), and none contains appreciably more. The rate of NH_2-terminal methionine removal in *E. coli* after assembly of cell proteins has been shown to be generally heterogeneous, and much of it is slow (16). The continuous variation of NH_2-terminal methionine among the procaryotes would most likely be attributable to continuing differences in the efficiency of removal, reaching virtually 100% in *C. crescentis* and *R. rubrum*.

All of the gram-positive bacteria of Table 1 except *C. kluyveri* and *Corynebacterium* were given alkaline incubation before dinitrophenylation. Removal of NH_2-terminal alanine esterified to teichoic acid (19) is evident in Table 2 from the fall both in N-terminal alanine and in total N termini from unusually high levels to within the range of the gram-negative organisms of Table 1. Removal of this alanine is partial even at a neutral *p*H (Table 2), and appearas completed after NH_3 incubation overnight, since incubation for an additional 2 days had no further effect on *S. faecium* preparations. *S. aureus* is unique in its high complement of NH_2-terminal alanine (Table 1), even after the level of total NH_2 termini has been reduced to normal by NH_3 incubation.

Judging from initial analyses of the gram-positive bacteria without NH_3 incubation, teichoic acid is abundant in *Bacillus*, *S. faecium*, *M. lysodeikticus*, and *S. aureus*. Its removal is imperative, even in fractionated proteins (Table 2). The NH_2-terminal amino acid profiles that have been reported in *B. subtilis* without removal of esterified alanine (11), therefore, do not specifically represent the proteins. Because of the occasional interference in glutamic acid-aspartic acid determination, no NH_3 incubation would be preferable if teichoic acid is not appreciable, as for example in *C. kluyveri* and *Corynebacterium* (Table 1), whose levels of total and individual end groups are within usual ranges. Treatment of the gram-negative organisms with NH_3 was found to have no effect on their NH_2-terminal profiles.

End-group determinations were also made upon limited cell quantities of *Coxiella burnetti*, *Nostoc muscorum*, *Euglena gracilis*, and *Neisseria*

TABLE 1. *NH_2-terminal amino acids of cellular proteins in various microorganisms*

Organism	Relative NH_2-terminal level[a]					Total NH_2 termini as a percentage of all amino acid residues
	Glutamic and aspartic acids	Threonine	Serine	Alanine	Methionine	
PROCARYOTES						
Eubacteriales						
Herellea sp.	7	12	29	36	16	0.14
Corynebacterium sp.	4	11	23	52	10	0.13
Bacillus cereus	4	8	18	45	25	0.10
B. megatherium ATCC 13632	3	6	23	51	17	0.12
B. subtilis	3	4	24	56	13	0.10
Methanobacillus omelianksii	—[b]	7	15	30	38	
Clostridium kluyveri	6	5	22	23	44	0.12
Micrococcus lysodeikticus	7	12	19	54	8	0.10
Staphylococcus aureus	1	8	17	72	2	0.16
Streptococcus faecium ATCC 8043	2	12	22	41	22	0.16
Pseudomonadales						
Pseudomonas B-12	6	5	28	56	5	0.10
P. putida	13	5	29	48	5	0.12
Caulobacter crescentis CB 13	4	6	24	54	<0.3	0.14
Desulfovibrio vulgaris	3	9	29	55	4	
Rhodospirillum rubrum	10	10	28	52	<0.3	0.10
Hyphomicrobiales						
Rhodomicrobium vanniellii	5	11	23	56	5	0.12
Actinomycetales						
Nocardia corallina UC 2161	3	15	24	51	9	0.14
Mycoplasmatales						
Mycoplasma 880	10	4	30	56	<0.3	0.07
EUCARYOTES						
Saccharomyces carlsbergensis	20	6	18	50	<0.3	0.07
Penicillium sp. 2526	15	10	29	46	<0.3	
Chlorella vulgaris	21	3	10	66	<0.3	0.05
Tetrahymena pyriformis	30	6	22	32	<0.3	

[a] Leucine-isoleucine, valine, lysine, and glycine found in small amounts in individual species are not reported.

[b] The level of glutamic and aspartic acids was high and unverified by thin-layer chromatography. It was set arbitrarily at 10% for comparison of other amino acid levels.

sp. Alanine, serine, threonine, and generally glutamic and aspartic acids were clearly evident; methionine was either present in small amounts or absent.

Eucaryotes. The end groups of the eucaryotic cell proteins (Table 1) differ consistently from those of the procaryotes in the absence of methionine and in the heightened levels of acidic amino acids (or amides). It is noteworthy, however, that the remaining amino acids, alanine, serine, and threonine, continue essentially at unaltered relative levels. In unreported experiments, the level of total end groups in the eucaryotes *Crithidia fasciculata* and the L-1210 mouse leukemia amounted to 0.06 and 0.04% of the total amino acid residues and were within the range of the other two eucaryote values in Table 1. Thus, the eucaryote cell proteins are further distinguished in having less than half the procaryote level of free amino terminal end groups.

Enterobacteriaceae. End-group profiles of representatives of all current genera of the *Enterobacteriaceae*, as listed in Table 3, show greatest variation in methionine, which varies significantly between related species but retains a limited conformity at a tribal level. Methionine is lowest in the *Proteae* (9% of total end groups) and is most consistently high in the *Escherichiae* (25%). End-group methionine may also fall appreciably during cellular aging for 2 days in stagnant culture, as shown for *E. coli* (Table 3); in contrast, it does not decrease significantly in *S. faecium* (*unpublished data*). Independent effects of cellular aging would be anticipated from previous findings that some modification of the initiating terminus of the cell protein of *E. coli* may be delayed for a cell generation or more

after synthesis (16). Little of the differences in profile for the remaining species, however, can be attributed to aging, since duplicate end-group determinations agreed well in spite of limited differences in growth rate at the time of mid-growth harvest. The efficiency of removal of the initiating end group thus appears so varied phylogenetically as to be almost completely randomized within the *Enterobacteriaceae*. However, the amino acids exposed by its removal show extremely uniform profiles (Table 4). Divergences in the level of individual amino acids are minor,

TABLE 2. *Effect of 0.2 N ammonia treatment on the NH_2-terminal amino acid profile of whole cells and nonparticulate proteins of Bacillus cereus*

Prepn[a]	NH_2 terminal levels[b]										Total NH_2 termini as a percentage of all amino acid residues
	Glutamic and aspartic acids		Threonine		Serine		Alanine		Methionine		
	Amt	Per cent	Amt	Per cent	Amt	Per cent	Amt	Per cent	Amt	Per cent	
	μmoles		*μmoles*		*μmoles*		*μmoles*		*μmoles*		
Nonparticulate protein											
Unincubated	50	1	50	1	202	4	4,500	78	310	6	0.51
Incubated at *p*H 7	48	2	72	3	145	6	1,880	78	264	11	0.24
Incubated at *p*H 10–11	39	4	68	7	175	18	445	46	243	25	0.10
Unfractionated cells											
Incubated at *p*H 7	30	1	58	2	118	4	2,520	86	208	7	0.29
Incubated at *p*H 10–11	36	4	64	7	146	16	410	45	254	28	0.10

[a] Proteins were precipitated with trichloroacetic acid and dinitrophenylated immediately (unincubated). Unbuffered nonparticulate proteins or trichloroacetic acid-washed whole cells neutralized with NaOH were incubated overnight at 25 C without further treatment (*p*H 7.0) or were treated with 0.2 N ammonia (*p*H 10–11) before dinitrophenylation.

[b] In micromoles per mole of total amino acid residues, or as percentage of total NH_2 termini.

TABLE 3. *NH_2-terminal amino acids of cellular proteins of the Enterobacteriaceae and the Erwinieae*

Organism	NH_2-terminal amino acids[a] (%)				
	Glutamic and aspartic acids	Threonine	Serine	Alanine	Methionine
Escherichiae					
Escherichia coli B (young)	4	8	20	30	38
E. coli B (old)	5	5	21	48	21
Shigella alkalescens	5	6	25	43	21
S. dysenteriae ATCC 13313	6	6	24	42	22
Salmonelleae					
Salmonella paratyphi B	6	6	24	35	29
Citrobacter freundii ATCC 8030	7	9	26	45	13
Arizona arizonae ATCC 13314	7	7	33	47	6
Klebsielleae					
Enterobacter aerogenes ATCC 13018	5	5	25	37	28
Serratia marcescens ATCC 13880	5	6	23	43	23
Klebsiella pneumoniae ATCC 9590	5	8	31	48	8
Proteae					
Proteus morganii	7	8	30	40	15
P. mirabilis	7	8	30	45	10
P. rettgeri	8	8	33	43	8
P. vulgaris	7	7	31	47	8
Providencia sp. ATCC 12013	8	7	30	50	5
Erwineae					
Erwinia carotovora	5	3	21	44	26
E. amylovora	8	4	18	43	27

[a] Lysine levels are omitted.

TABLE 4. *Relative levels of nonmethionine end groups in the Enterobacteriaceae, Erwineae, and Bacillaceae*

Organism	End groups				
	Methionine (percentage of all end groups)	Percentage of nonmethionine end groups			
		Glutamic and aspartic acids	Threonine	Serine	Alanine
Enterobacteriaceae[a]					
Escherichia coli B	38	6	13	32	48
Salmonella-paratyphi B	29	8	8	34	49
Enterobacter aerogenes ATCC 13018	28	7	7	35	51
Serratia marcescens ATCC 13880	23	6	7	31	56
Shigella alkalescens	21	6	8	32	54
S. dysenteriae ATCC 13313	21	8	8	29	51
Proteus morganii	15	8	9	35	47
Citrobacter freundii ATCC 8090	13	8	10	30	52
P. mirabilis	10	8	9	33	44
P. rettgeri	8	9	9	36	47
P. vulgaris	8	8	8	34	47
Klebsiella pneumoniae ATCC 9590	8	5	7	33	51
Arizona arizonae ATCC 13314	6	7	7	35	50
Providencia sp. ATCC 12013	5	8	7	32	53
Average		7	9	33	50
Erwineae[a]					
Erwinia carotovora ATCC 495	26	7	4	29	60
E. amylovora ATCC 15580	27	11	6	25	59
Bacillaceae[b]					
Clostridium kluyveri	44	11	9	39	41
Bacillus cereus	25	5	11	24	60
B. megaterium ATCC 13632	17	4	7	28	61
B. subtilis	13	3	5	28	64

[a] Values are calculated from Table 3.
[b] Values are calculated from Table 1.

occasional, and not consistent when profiles are ranked in order of decreasing methionine content, as in Table 4, or in tribal order. The *Erwiniae* have until recently been allied taxonomically with the *Enterobacteriaceae*, which they resemble biochemically (*Bergey's Manual of Determinative Bacteriology*) but not immunologically (8). Two representatives of *Erwinia* (Tables 3 and 4) are dissimilar pathogens (*Bergey's Manual*). They have nearly the same end-group profile, which is similar to the average enterobacterial profile but clearly distinguishable from it. The percentages of at least three nonmethionine end groups in these *Erwineae* differ by more than 1 part in 10 from the enterobacterial averages (Table 1). There is thus an increase in alanine that exceeds 5%, a decrease in serine exceeding 3%, and a decrease in threonine greatly exceeding 1%. In comparison, deviations in any of the individual enterobacterial values are never greater than 1 in 10 and involve only one end group.

Bacillaceae. In spite of the paucity of representatives examined (Table 1), cell protein end groups varied far more among the *Bacillaceae* which comprise a heterogeneous assortment of physiological and morphological groups (*Bergey's Manual*), than among the *Enterobacteriaceae*. Profiles of the nonmethionine end groups of the different species of *Bacillus* bear no resemblance to those of *C. kluyveri* and differ significantly from one another.

DISCUSSION

End-group homology of cell protein would appear to be a particularly practical and convenient molecular criterion of familial homogeneity among the procaryotes, and might be examined among the *Protista* in general. If the *Enterobacteriaceae*, excluding *Erwinia*, are considered an examplary model, close or even identical homology in nonmethionine end groups among cellular proteins should serve in distinguishing the bounds of other bacterial families. The assay is rapid and accurate, can be tallied in direct chemical terms, appears to be of low ambiguity, and is probably not unduly affected by any single protein component (15, 21). Com-

parisons would appear valid only under standardized conditions of cellular cultivation and harvesting, and when nonprotein sources of end groups (11) are omitted. Although nonparticulate protein fractions produce clearer chromatograms than do whole cells, specific end groups might be selected for (21, 22) or even introduced as artifacts by autolysis.

Taxonomic variability in protein end groups is no doubt related to that in total amino acid composition (20), but end-group variation appears greater and hence more discriminating. These differences would perhaps originate in turn from differences in deoxyribonucleic acid (DNA) composition. End-group homology may usefully complement DNA homology, since the former property becomes discriminating at familial extremes where resolution of the latter property is largely dissipated (12). The same complementarity might also be predicted for immunological homologies (14).

Qualitatively, and in a roughly quantitative way, the favored NH_2-terminal amino acids show marked evolutionary conservation in the six major end groups common to procaryotes and the five common to eucaryotes, no new favored end groups having been found.

Methionine does not appear to be commonly repeated in nascent *E. coli* polypeptides next to the initiating *N*-formylmethionine residue (3). Therefore, the methionine in mature protein N termini should be considered in general to originate from the initiator rather than an exposed adjacent residue. The initiating residue itself does not have to arise from *N*-formylmethionyl-transfer ribonucleic acid, for it has been found in *S. faecium* that the formylation step can be dispensed with in the absence of folic acid (17). However, the near ubiquity of methionine in procaryote protein end groups would suggest a common evolutionary origin of methionine-based protein initiation, retained with minor modification among the procaryote representatives. Thus, *E. coli* must begin protein biosynthesis with a derivative of methionine (7, 17) and then retain a significant fraction of it. Rare procaryotes and all eucaryotes remove or exclude methionine from their mature protein end groups, but elimination of any of the remaining five most favored NH_2 termini is never found (Table 1). There would, therefore, be little rationale for an independent reacquisition of NH_2-terminal methionine in mature protein for a noninitiating purpose. Because of the uniform absence of appreciable NH_2-terminal methionine in the cell protein of eucaryotes, the question of methionine involvement in protein initiation in those organisms is still moot.

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ADDENDUM IN PROOF

The recent demonstration of an *N*-formylseryl-transfer RNA (W. S. Kim, Science, **163**:947–949) may alter the inferences that have been made of methionine as a general initiator of protein biosynthesis. Although methionine would appear to be the major initiator in *E. coli* (16), the question of its prevalence could be raised in particular in the two procaryotes (Table 1), in which it is absent as a major N-terminus.

LITERATURE CITED

1. Adams, J. M., and M. R. Capecchi. 1966. N-formylmethionyl-sRNA as the initiator of protein synthesis. Proc. Natl. Acad. Sci. U.S. 55:147–155.
2. Atkin, L., W. L. Williams, A. S. Schultz, and C. N. Frey. 1944. Yeast microbiological methods for determination of vitamins. Ind. Eng. Chem. 16:67–71.
3. Capecchi, M. R. 1966. Initiation of *E. coli* proteins. Proc. Natl. Acad. Sci. U.S. 55:1517–1524.
4. Cleland, C. F., and W. R. Briggs. 1967. Growing responses of the long-day plant *Lemna gibba* 63. Plant Physiol. 42:1553–1561.
5. Cohen-Bazire, G., W. R. Sistrom, and R. Y. Stanier. 1957. Kinetic studies of pigment synthesis by non-sulfur purple bacteria. J. Cell. Comp. Physiol. 49:25–68.
6. Cowperthwaite, J., M. M. Weber, L. Packer, and S. H. Hutner. 1953. Nutrition of *Herpetomonas (Strigomonas) culicidarum.* Ann. N.Y. Acad. Sci. 56:972–981.
7. Eisenstadt, J. M., and P. Lengyel. 1966. Formylmethionyl-tRNA dependence of amino acid incorporation in extracts of trimethoprim-treated *Escherichia coli.* Science 154:524–527.
8. Ewing, W. H. 1963. An outline of nomenclature for the family *Enterobacteriaceae.* Intern. Bull. Bacteriol. Nomen. Taxon. 13:95–110.
9. Fraenkel-Conrat, H., J. I. Harris, and A. L. Levy. 1955. Recent developments in techniques for terminal and sequence studies in peptides and proteins. Methods Biochem. Anal. 2:359–383.
10. Hakala, M. T., J. F. Holland, and J. S. Horoszewicz. 1963. Change in pyrimidine deoxyribonucleoside metabolism in cell culture caused by mycoplasma (PPLO) contamination. Biochem. Biophys. Res. Commun. 11:466–471.
11. Horikoshi, K., and R. H. Doi. 1968. The NH_2-terminal residues of *Bacillus subtilis* proteins. J. Biol. Chem. 243: 2381–2384.

12. Johnson, J. L., and E. J. Ordal. 1968. Deoxyribonucleic acid homology in bacterial taxonomy: effect of incubation temperature on reaction specificity. J. Bacteriol. 95:893–900.
13. Marcker, K. 1963. The formation of N-formyl-methionyl-sRNA. J. Mol. Biol. 14:63–70.
14. Marmur, J., S. Falkow, and M. Mandel. 1963. New approaches to bacterial taxonomy. Ann. Rev. Microbiol. 17:329–364.
15. Pine, M. J. 1963. Alcohol-soluble protein of microorganisms. J. Bacteriol. 85:301–305.
16. Pine, M. J. 1968. Kinetics of maturation of the amino termini of the cell proteins of *Escherichia coli*. Biochim. Biophys. Acta 174:359–372.
17. Pine, M. J., B. Gordon, and S. S. Sarimo. 1969. Protein initiation without folate in *Streptococcus faecium*. Biochim. Biophys. Acta. 179:439–447.
18. Randerath, K. 1963. Thin layer chromatography, p. 93–110. Academic Press Inc., New York.
19. Salton, M. R. J. 1964. The bacterial cell wall, p. 156–168. Elsevier Publishing Co., Amsterdam.
20. Sueoka, N. 1961. Correlation between base composition of deoxyribonucleic acid and amino acid composition of protein. Proc. Natl. Acad. Sci. U.S. 47:1141–1149.
21. Waller, J.-P. 1963. The NH_2-terminal residues of the proteins from cell-free extracts of *E. coli*. J. Mol. Biol. 7:483–496.
22. Waller, J.-P., and J. I. Harris. 1961. Studies on the composition of the protein from *Escherichia coli* ribosomes. Proc. Natl. Acad. Sci. U.S. 47:18–23.