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Selection for *Escherichia coli* Mutants with Proteins Missing from the Ribosome

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Antibiotic-independent revertants of an erythromycin-dependent strain of *Escherichia coli* were isolated by spontaneous selection. Their ribosomal proteins were analyzed by two-dimensional polyacrylamide gel electrophoresis. In contrast to most ribosomally targeted selections, the specific absence of a certain protein from the ribosome, rather than alterations in ribosomal proteins, was observed. Mutants were found with protein S20, L11, L15, L28, L29, or L30 missing.

A large number of mutants of *Escherichia coli* have been isolated with alternatives in the genes coding for ribosomal proteins. Nearly all of the 53 protein genes have been mutationally altered (3, 7). However, instances in which a protein is missing from the ribosome are much rarer. Such mutants would be very useful in answering questions about the role of individual proteins in assembly, function, and regulation of activity. A selection for such mutants has now been devised based on an erythromycin-dependent strain.

A mutant with small ribosomal subunit protein S20 lacking from the ribosome (15) was the first reported case. Two different selective systems, which yield mutants affected in the entire array of ribosomal protein genes, also produce strains which lack protein S20 (3, 7). Mutants with small ribosomal subunit protein S9 missing from the ribosome (4) and those with large subunit protein L1 missing (2; E. Dabbs, unpublished data) have been found.

The study of these mutants is continuing. They were first detected by using two-dimensional polyacrylamide gels of ribosomal protein (8). This technique gives a very reproducible pattern of protein spots, in terms of both geometry and strength of staining of spots. The use of immunological techniques in the case of the mutants which lack ribosomal protein L1 confirmed that in these mutants the protein was missing from the isolated ribosome and that there was not any detectible cross-reacting material in the supernatant (G. Stöffler, unpublished data). In procaryotes other than *E. coli*, two instances of a missing ribosomal protein have been well documented. Mutants resistant to the antibiotic thiostrepton, of both *Bacillus subtilis* (B. Wienen, R. Ehrlich, M. Geisser, G. Stöffler, J. Smith, D. Wein, R. Vince, and S. Pestka, J. Biol. Chem., in press) and *Bacillus megaterium* (E. Cundliffe, P. Dixon, M. Stark, G. Stöffler, R. Ehrlich, and M. Geisser, J. Mol. Biol., in press), in some cases had a protein missing that was the homologue of *E. coli* protein L11.

However, for *E. coli*, the norm for antibiotic-resistant mutants is to have alterations in their ribosomal proteins rather than a protein missing (e.g., streptomycin [11], spectinomycin [1], and erythromycin [14]). The same is true for revertants from dependence on ribosomally targeted antibiotics (e.g., streptomycin [5, 6], spectinomycin [2]).

An erythromycin-dependent mutant has been isolated and described (10), but revertants from this or other such mutants have not been studied. Unlike aminoglycoside antibiotics, erythromycin acts on the 50S subunit (12). As a potential tool for obtaining new ribosomal mutants altered in the 50S subunit, erythromycin-dependent mutants were isolated from *E. coli* strain A19.

An antibiotic underlay technique was used to introduce cells mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine to the antibiotic (4). A concentration of 200 μg of erythromycin per ml was used in the selection. Erythromycin-dependent mutants constituted about 2% of the colonies which grew up when strain A19 (which is erythromycin sensitive) was used.

One dependent strain, termed AM, was selected for further study. Antibiotic-independent revertants were obtained from strain AM at a frequency of 3×10^{-5}. Of fifteen revertants analyzed on two-dimensional polyacrylamide gels (8), three had alterations in their ribosomal pattern. In mutant AM3, large subunit protein L28 was missing; in mutant AM10, protein L30 was missing; and in mutant AM13, protein S20 was

missing (Fig. 1). Further antibiotic-independent revertants were isolated from AM in several more experiments. A total of 120 revertants were analyzed, and the results are shown in Table 1. Examples of each type of mutant are shown in Fig. 1.

This selection produced only mutants which lacked a protein. In contrast to nearly all other ribosomally targeted selections, there were no detectable instances of altered proteins on the ribosome. Ribosomal subunits were also analyzed, in case a protein had moved so that its position on the gel was masked by a protein of the other subunit. No such instances were observed. Subunit analysis was necessary for the L11 mutants (Fig. 1h), since, in the gel system used, proteins S5 and L11 are coincidental.

Since erythromycin has as its target the 50S subunit, it is not surprising that most of the changes detected were in 50S proteins. S20 is no exception since it is present also on the 50S subunit (usually about 20% of the total) when ribosomes are dissociated, and so also has the designation L26 (13). S20-lacking mutants have been isolated in a number of other selections (3, 7, 15); the occurrence of L11-lacking mutants in this selection supports the findings from work with thiostrepton mutants of *Bacillus* (Cundliffe

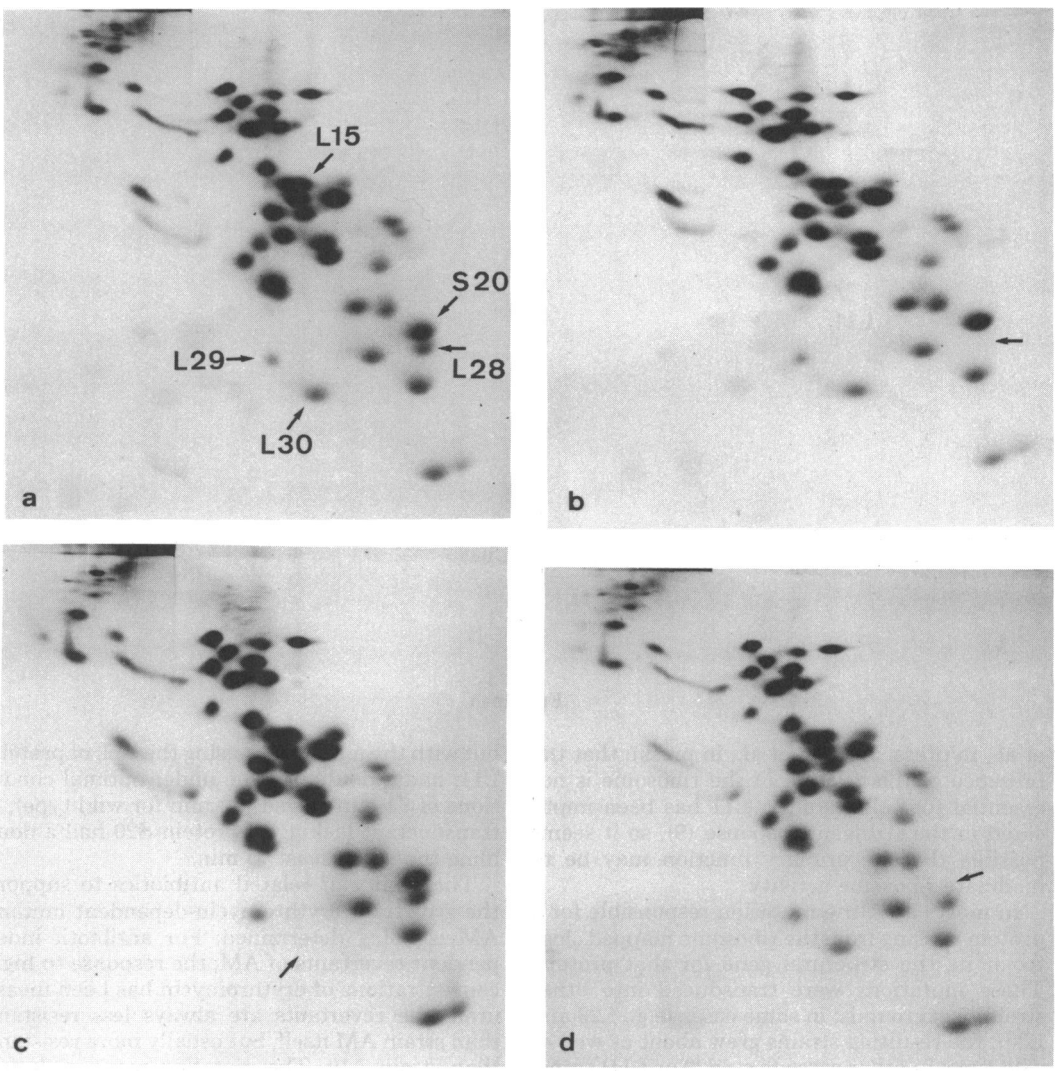

FIG. 1. *Two-dimensional gel electrophoresis of ribosomal proteins of the 70S ribosome (a through f) and of the 50S subunit (g through h). Proteins missing are indicated by arrows. a, Erythromycin-dependent strain AM (same as wild type); b, AM3; c, AM10; d, AM13; e, AM16; f, AM111; g, AM; h, AM76.*

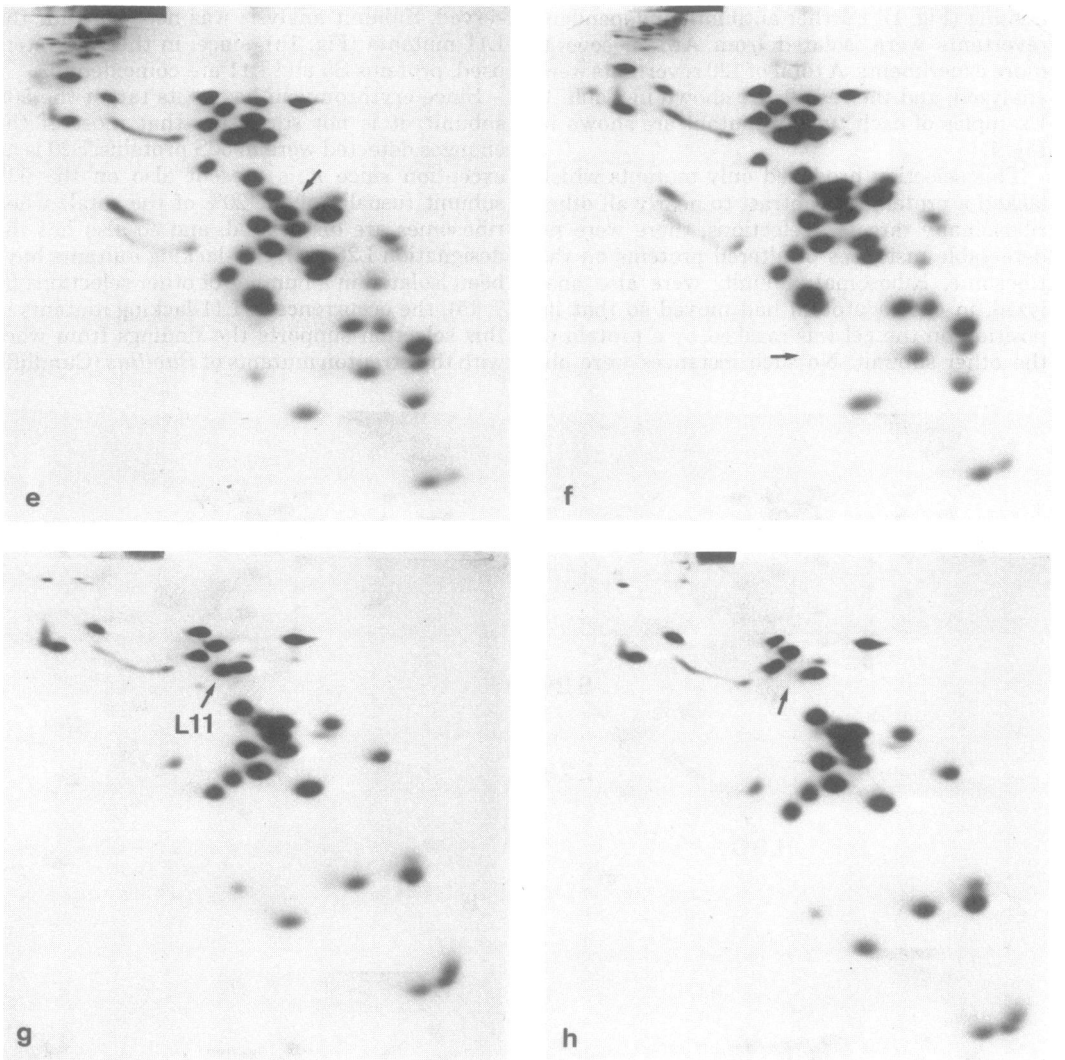

FIG. 1e–h.

et al., in press; Wienen et al., in press): that the presence of this protein in the ribosome is not essential for cell viability. L11 has been implicated in the stringent response (9), so it seems possible that its primary function may be to modulate ribosome activity.

In most cases, the mutation responsible for a protein missing from the ribosome mapped close to, or in, the structural gene for that protein. These mutations were transduced into other strain backgrounds; in some cases (e.g., L29 and L30), the resulting strains grew about as well as wild type; in other cases (e.g., S20 or L11) transductants possessing the mutation were much impaired in growth. A strain otherwise wild type, but with the mutation causing the lack of protein L11, had a doubling time under optimal conditions of 64 min (versus 22 min for wild type); a transductant lacking of protein S20 had a doubling time of at least 75 min.

The ability of related antibiotics to support the growth of erythromycin-dependent mutant AM is being determined. For antibiotic-independent revertants of AM, the response to high concentrations of erythromycin has been measured; the revertants are always less resistant than strain AM itself, but usually more resistant than strain A19. The mutation responsible for suppressing dependence in the revertants also lowers resistance.

TABLE 1. *Revertants of erythromycin-dependent mutant AM which have a protein missing from the ribosome*

Protein missing	No. of mutants	Mutants
S20 (L26)[a]	5	AM13, AM30, AM101, AM106, AM107
L11	3	AM68, AM76, AM77
L15	1	AM16
L28	4	AM3, AM21, AM81, AM108
L29	1	AM111
L30	3	AM10, AM46, AM98

[a] S20 is a protein occurring on both small and large ribosomal subunits. On the large subunit, it is designated L26. Conditions for mutant growth, isolation of ribosomes, preparation of ribosomal protein, and electrophoresis were as described in reference 6.

These mutants are being investigated further. They will prove a useful tool in investigating the role of these proteins in the ribosome with respect to various ribosomal properties, such as binding of amino-acylated tRNA and factors, assembly, and the interaction of the large and small subunits.

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