PHYLOGENIC DISTANCE BETWEEN PROKARYOTES AND EUKARYOTES AS EVALUATED BY RIBOSOMAL PROTEINS

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Between prokaryotes and eukaryotes, there is a gap involving many structural features. A gap does exist in the structure of prokaryote and eukaryote ribosomes. In prokaryotes, there are 55 different ribosomal proteins [1] while there are about 70 in eukaryotes [2–4]. Furthermore, the molecular weights of ribosomal proteins are lower in prokaryotes than in eukaryotes [5]. Owing to the numerous interactions of the proteins with each other (and with ribosomal RNAs), one would expect that molecular evolution of ribosomal components has been slow. This point has indeed been demonstrated in prokaryotes [6–9] and in eukaryotes [10, 11].

In eukaryotes, using two-dimensional (2-D) polyacrylamide gel electrophoresis [12], we have evaluated the phylogenic distance between various species. The evaluation was made in terms of ribosomal proteins, by means of a degree of proximity 'p' based on the number of co-migrating ribosomal proteins in various pairs of species [11]. When mammals were used as the reference source, p was found to be equal to 1 for mammals, birds and reptiles. This means that ribosomal proteins from mammals, birds and reptiles display similar 2-D finger-prints. For more distant species, the p value gradually decreased. Nevertheless, its lowest value was found for the pair mammal–plant, which was the most widely differing pair examined; p was equal to 0.40, which means that mammals and plants have a set of about 25 ribosomal proteins which co-migrate.

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It seemed pertinent to evaluate in the same way the degree of proximity between prokaryotes and eukaryotes, particularly in view of the gap which separates these organisms. So far, direct comparative studies of eukaryotic and prokaryotic ribosomal proteins have only been carried out one-dimensionally [5]. In the present study, we have found that only 5 proteins out of the total ribosomal proteins from *E. coli* are overlapped by guinea-pig ribosomal proteins. The overlapped proteins are L_{11}, L_{13}, L_{16}, L_{20} and S11 using the nomenclature of Kaltschmidt and Wittmann [1].

2. Materials and methods

Total ribosomal proteins from *E. coli* and guinea-pig were prepared as reported elsewhere [1, 10]. Separation of the subunits was not necessary as will be discussed below. Two-dimensional electrophoresis was carried out as previously described [10]. Proteins were separated according to their electrical charge in the first dimension and in the second dimension according to their molecular weight. For the co-electrophoresis of ribosomal proteins from *E. coli* and guinea-pig, we applied 1 mg protein from each source.

In order to evaluate the phylogenic distance between *E. coli* and guinea-pig in terms of ribosomal proteins, we could not apply the degree of proximity formula which we had previously worked out:

$$p = \frac{120 - n}{60} \qquad (1)$$

in which n is the total number of spots after co-electrophoresis or ribosomal proteins from two-species. It was not possible because the number of spots produced by *E. coli* ribosomal proteins is somewhat different from those produced by guinea-pig. Therefore, 60 cannot be taken as an average number. We calculated, however, an approximation p' of p. Assuming that the total number of spots for *E. coli* and guinea-pig is a plus b, respectively:

$$p' = \frac{(a+b) - n}{\frac{1}{2}(a+b)} \qquad (2)$$

$a = 51$ (spots corresponding to proteins S_1, S_7 and S_{12} were not visible, although they should have been within the limits of the gels; L20 and S11 overlap) and $b = 60$.

3. Results and discussion

Standard conditions for analysis of eukaryotic ribosomal proteins resulted in an important alteration of the finger-prints of *E. coli* ribosomal proteins. Along the vertical dimension, 11 proteins left the gel (proteins S20, S21, L26, L27, L28, L29, L30, L31, L32, L33 and L34). The other proteins remained within the gel but moved much further down than did the bulk of guinea-pig ribosomal proteins. These findings are in excellent agreement with the fact that ribosomal proteins have lower molecular weights in prokaryotes than in eukaryotes [5].

As for the electrical charge of ribosomal proteins, striking differences are exhibited by the so-called acidic proteins which move anodically in the first (horizontal) dimension. In *E. coli*, one protein (S1) out of the 11 acidic proteins was not visible although it should have been within the limits of the gels. In guinea-pig, we observed, as previously reported, only three weakly stained acidic spots: their ribosomal origin is not certain [11]. For the sake of homogeneity, we did not take them into account since we had neglected them in our previous phylogenic studies on the basis of arguments presented in these studies [11].

After co-electrophoresis of ribosomal proteins from *E. coli* and guinea-pig, the total number of spots was found to be $n = 96$ spots, thus taking no account of the three acidic spots from guinea-pig, which, parenthetically, do not overlap with *E. coli* acidic ribosomal proteins. To those 96 spots, we must add, however, the 11 proteins from *E. coli* which migrated outside the gel: those proteins could obviously not co-migrate with ribosomal proteins from guinea-pig which remain within the gel. Finally, n should be taken as equal to 107, so that:

$$p' = \frac{111 - 107}{\frac{111}{2}} = 0.07 .$$

Only 5 proteins of *E. coli* and guinea-pig co-migrate: proteins L_{11}, L_{13}, L_{16} from *E. coli* are overlapped proteins 21, 35 and 43 from guinea-pig respectively, according to a provisional nomenclature. Overlapping proteins L20 and S11 from *E. coli* are overlapped by protein 53 from guinea-pig (fig. 1). The fact that ribosomal proteins from *E. coli* and guinea-pig are so clearly resolved made it unnecessary that the ribosomal subunits be separated.

Although p' was not calculated exactly as had been p, it is much lower than the lowest value of p reported in eukaryotes. Thus, the proximity between *E. coli* and mammals in terms of ribosomal proteins can be considered unambiguously as much smaller ($p' = 0.07$) than that between plants and mammals ($p = 0.40$). Furthermore, it is not impossible that the 5 overlaps between *E. coli* and guinea-pig are random, while it is more difficult to make this point between plants and mammals where there are about 25 overlaps.

Between *Bacillus stearothermophilus* and guinea-pig ribosomal proteins, we have also found a very small number of overlaps which was of the order of magnitude of 5–6, as well as between *B. stearothermophilus* and *E. coli* (unpublished results). This confirms that the phylogenic distance between *E. coli* and *B. stearothermophilus* is important; as evaluated by our procedure, it is as important as that between *E. coli* and mammals. *E. coli* spots which overlap with spots from guinea-pig are different from those overlapping with spots from *B. stearothermophilus.*

Our results fits with the evolutionary tree: animals and plants have diverged 1.2×10^9 years ago [13] while prokaryotes and eukaryotes have done so 1.8×10^9 years ago [14].

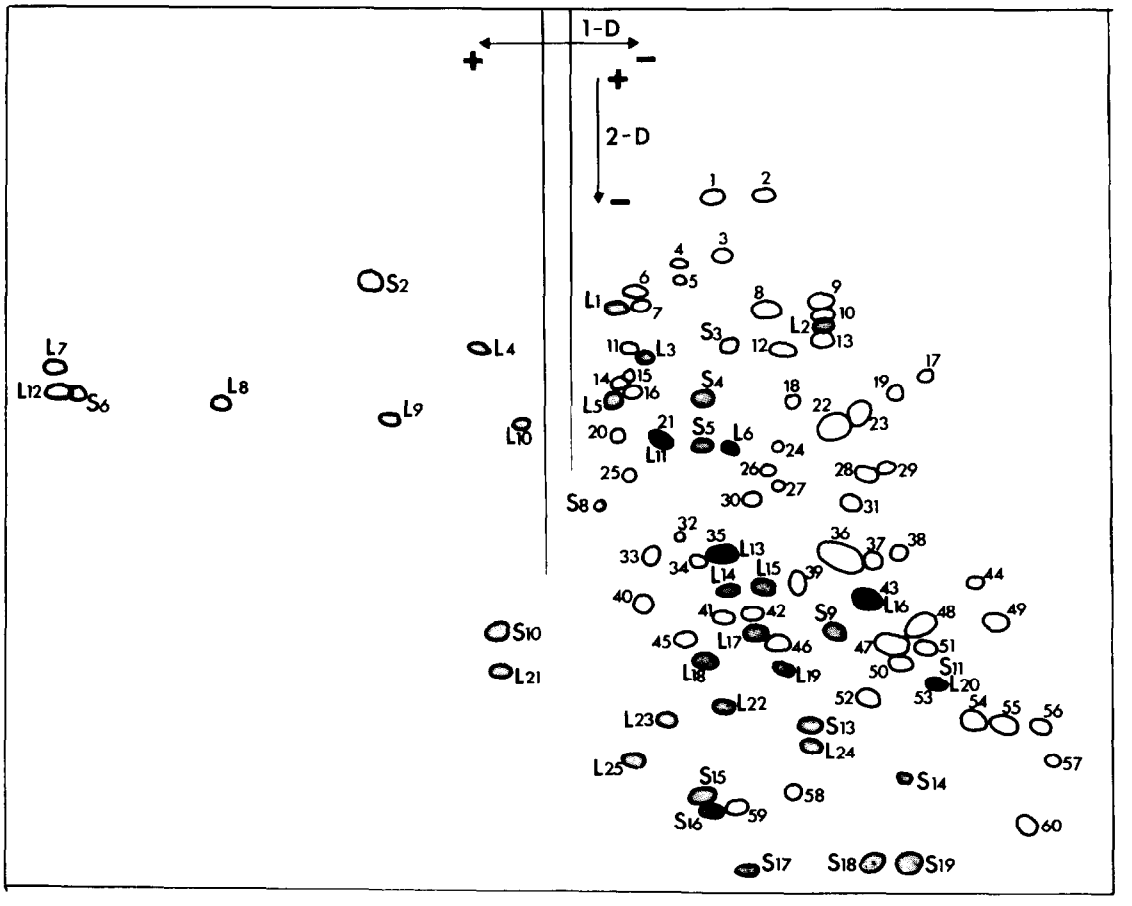

Fig. 1. Co-electrophoresis of total ribosomal proteins from *E. coli* (grey spots) and guinea-pig (clear spots). *E. coli* ribosomal proteins are numbered according to Kaltschmidt and Wittmann [1]. Proteins S2, S7 and S12 from *E. coli* are not visible. Ribosomal proteins from guinea-pig are numbered from 1 to 60. The dark spots represent overlapping proteins.

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