determine the number of haploid cells in a clone, they derive conclusions from quantitative determinations of the amount of DNA per cell from only 50 individual cells, obtained from one NCD clone, and the same number of cells from three prototrophic clones, considered by them to be of CD type only by checking for prototrophy and not from their unstable phenotype. From our experience (10) these CD clones were certainly recombinants.

With respect to the criticisms raised by Hauser and Karamata, let us analyse a previous paper of ours (15) concerning the diploidy of the ‘recombinant progeny’ or the so-called ‘phenotypically recombinant progeny’ (15).

Their suggestion that Sanchez-Rivas and co-workers were dealing with syntrophic mixtures cannot be substantiated, since the primary and secondary recombinants described in that paper were all analysed after several purification protocols (re-isolation on minimal medium and sporulation) that are considered acceptable by Hauser and Karamata.

The phenotype of these recombinants cannot be explained by a simple transformation occurring in the plate. In fact such phenomena could not be understood unless both entire parental chromosomal were in the same cell, because of the intervals involved and the frequencies of recombination. Among the recombinants resulting from the same number of cross-overs, those involving the origin and terminus of replication were 100 times more frequent.

When these recombinants were analysed by transformation, diploidy was demonstrated for eight markers distributed around the chromosome. In addition, although the recombinants were phenotypically Tp+, the inactive wild-type Tp allele was also shown to be present.

Of the recombinants obtained from fusions in which only one parent was lysogenic for the 105 prophage, all (40/40) were lysogenic. The simple explanation of their occurrence by prophage induction is ruled out by the fact that induction of the prophage was not observed during the fusion procedure (14, 16).

Regarding the criticism of the poor burst-size reported with NCD clones containing the thermoinducible repressor mutation 1223 of prophage 105 (5), data from this (13) and other laboratories (J. Errington, 1982) (4), during his construction of 105 cloning vectors) indicate that this mutation is poorly inducible.

On the other hand, the above comments we feel justified in concluding that the experimental support of Hauser & Karamata (6) is insufficient to dismiss the polydiploidy of the fusion progeny.

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conclusions drawn from – in most instances – irreproducible experiments [see Discussion in (4)] will not advance the debate.

We note that Sanchez-Rivas and Levi-Meyrueis do not challenge our main new data, i.e. (i) the mixed nature of exfusant colonies, which exhibit the so-called diploid phenotype, (ii) the inadequacy of purification methods used in all previous experiments, and finally (iii) the validity of our reconstruction experiments with mixtures of whole cells. These experiments and their conclusion [i.e. that segregation of various parental and recombinant phenotypes from exfusants is due to the mixed nature of the original colonies and to transformation between various cells and possibly L-forms (P. M. Hauser, unpublished)] are not invalidated by any newer results. Therefore, we believe that our criticisms of the concepts of diploidy and chromosomal silencing remain valid.

We consider that the most plausible explanation proposed for the behaviour of the exfusants (4) is as follows. Multigenomic zygotes, obtained by protoplast fusion, allow recombination, as well as expression of all of the genomes. As long as such protoplasts or possible L-forms are able to grow in the absence of cell wall regeneration, complementation would be possible. However, cell wall regeneration seems to preclude the maintenance of more than one genome and thus of complementing diploids. The experiments of C. Karmazyn-Campelli and others (3), perfectly in line with this interpretation, were interpreted in favour of a correlation between resumption of peptidoglycan synthesis and chromosomal silencing.

The brief summary and assessment of previous results by Sanchez-Rivas and Levi-Meyrueis is revealing and hardly requires further comment. For instance, in a single paragraph of their letter, we read "Abundant experimental data support the diploidy of all fusion types", as well as "Every previous worker in this field has searched for the usual signs of diploidy without success". It is stated that even increased amounts of DNA could not be detected. Would this mean that silencing of a chromosome prevented its acid hydrolysis and detection of diphenylamine-staining material? It is further stated that diploidy was demonstrated by restoring the transforming activity of the silenced chromosome. The presence of identical values in two distinct papers [Table 3 in (1); Table VII in (2)] confirm that the evidence is based on results of a single experiment which we were never able to reproduce.

Inspection of the statements of Levi-Meyrueis and Sanchez-Rivas reveals that there does not seem to be much of a "controversy", as far as the data on exfusants is concerned; any controversy concerns only the belief as to their meaning. Our experiments (4) fully account for the "abundant experimental data which support the so-called diploidy of all fusion types" (see above), and have the advantage of not requiring an occult mechanism reducing the putative silent chromosome to an immaterial entity.

In conclusion, if a true diploid clone with a silenced chromosome could be obtained, it would provide an invaluable tool. However, until such a construct is available, experiments on B. subtilis protoplast fusion provide neither evidence for chromosomal silencing in prokaryotes, nor arguments for an evolutionary step in the passage from pro- to eukaryote. The latter conclusions will have to be abandoned, due to lack of unequivocal experimental support.

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