Belcour, L. Loss of a cytoplasmic determinant through formation of protoplasts in Podospora.

In Podospora anserina, protoplasts of an average diameter of 5 µm can be obtained, as described for Neurospora (Bochmonn and Bonner 1959 J. Bacteriol. 78: 550) by treating young mycelia with snail juice. If plated on Petri dishes, a variable number (see Table I) can regenerate normal mycelia. The genetic analysis of very small quantities of cytoplasm mode possible by protoplast formation can reveal cytoplasmic heterogeneity which would not be easy to observe by other methods. Isolation from protoplasts of cytoplasmic (most probably mitochondrial) mutations displaying neither dominance nor suppressivity has already been reported (Belcour 1975 Genet. Res. Camb. 25: 155). We report here on the segregation of the two mutually exclusive cytoplosmic states (s) and (s^S), obtained by the use of protoplasts.

Table I. Isolation of (s^S) strains by regeneration of protoplosts from (s) strains.

experiment n°	strain genotype	protoplasts* regenerating (%)	no. of regenerated mycelia studied	no. of (s^S) mycelia	% of (s^S) mycelia
1	s mt+	10	194	12	6
2	s mt+	2	408	65	16
3	s mt+	7	173	27	16
4	s mt+	6	154	15	10
5	s mt-	15	609	41	7
6	s mt+	5	233	10	4
7	s mt+	3	198	2	1
8	s mt+	7	103	IO	10
9	s mt-	9	197	28	14

*Protoplasts were mode by a method derived from that of Bochmonn and Bonner (1959 J. Bacteriol. 78: 550) and described by Belcour (1975 Genet. Res. Comb. 25: 155).

The properties of the (s)/(s^S) system con be summarized as follows: S and s ore two alleles at one of the 9 well-known loci for protoplasmic incompatibility (Bernet 1965 Ann. Sci. Not. Bot. 6: 661). (s) and (s^S) represent the two possible alternative cytoplasmic states of a strain containing the allele s. When s strains ore cytoplasmically (s^S) th eys bw protoplasmic compatibility with strains of S nuclear genotype, while under the (s) cytoplosmic state they are incompatible with S strains. In (s) x (s^S) sexual crosses the (s^S) and (s) properties follow a strict cytoplasmic (maternal) inheritance. Finally, the (s) state is highly infectious with respect to the (s^S) state following anastomoses: the (s) → (s^S) conversion never occurs spontaneously during vegetative growth, but hos been obrewed after regeneration of conidiophores isolated by micromanipulation. Briefly the (s) state depends upon the presence of the s gene plus that of a cytoplosmic factor, assumed to be necessary for maintaining the activity of this gene. (Rizet 1952 Rev. Cytol. Biol. Veg. 13: 51; Beisson-Schecroun 1962 Ann. Gen. 4: 1).

A significant proportion of the protoplasts obtained from an (s) strain yield (s^S) mycelia after regeneration, as shown in Table I. The (s^S) mycelia thus obtained display all the properties of the (s^S) strain previously investigated, in particular the ability to transform to the (s) state following cytoplosmic contact. The percentage of (s^S) protoplosts varies from one experiment to another (1 % to 16%) and does not seem to be correlated with the rate of protoplast regeneration.

The simplest interpretation of these results is that a passive and random distribution of cytoplasm occurs during protoplast formation. Those protoplosts receiving the s cytoplasmic factor would yield (s) mycelia. Those not receiving it would yield (s^S) mycelia. A direct effect of the enzymatic treatment used for protoplast formation on the loss of the s factor may be excluded: experiments 3 and 4 have been carried out on two aliquots of the same culture, one treated with 5% enzyme (expt. n°3), the other with 20% enzyme (expt. n°4) bath far 4 hours. No significant difference in the ratio of (s^S) mycelia was noted.

The hypothesis of a random distribution of the cytoplasm in protoplasts, and hence of the s cytoplosmic factor, allows a rough estimotion of the concentration of s factors in the cytoplasm. Assuming that (s^S) mycelia are those that received no s factor, the Poisson law allows the estimation of the mean-number of s factors per protoplost. The numbers thus obtained vary from 1.8 to 4.6 s units per protoplast, depending on the experiment. The size of young protoplasts varies from 3 to 10µm in diameter. Assuming a diameter of

8 µm for those that regenerate (presumably the biggest ones) one reacher the following estimate: the cytoplasm contains 7 to 17 s "nits per 1000 μm^3. This low number of "nits is approximately equivalent to the number of nuclei in the some volume. This suggests that the factor s may be concerned with the regulation of the expression of genes for protoplasmic incompatibility.

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