Appearance of Wild Type from Mixed Culture of Two Aggregateless Mutants in the Cellular Slime Mold

*Dictyostelium discoidium*

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The cellular slime mold *Dictyostelium discoideum* is an excellent organism for studying the mechanisms of cell differentiation and development. However, their sexual process is still obscure (Wilson 1952, 1953, Wilson and Ross 1957, Sussman 1961b, Olive 1963, Loomis and Ashworth 1968, Sinha and Ashworth 1969).

Since the cellular slime mold has a unique life cycle, a few general points regarding it should be mentioned. *D. discoideum* is an unicellular organism which has amoeboid shape. The cells grow by feeding on bacteria and reproduce themselves by mitotic division. On starvation, many cells come together to aggregate, and form a slug-like shaped mass, called a pseudoplasmodium. The slug migrates a while, and forms a fruiting body which consists of spores and stalk cells. In general, it has been believed that they were normally haplonts through whole their life cycle.

The writer isolated many stable mutants with developmental deficiencies, such as aggregateless, slugless or fruitless. Two kinds of aggregateless mutants were grown together in liquid nutrient medium with bacteria (Sussman 1961a) and left at 22°C for more than 30 days. It was found that all the aggregateless turned into wild type cells which aggregated and formed fruiting bodies.

**Material and methods**

Mutants employed in this experiment were aggregateless KA10, Ka20, Ka21 and Ka22, and slugless Kp8 and Kp12 but mainly KA10 and Ka20 were used. KA10 did not aggregate at all and the surface of their plaque was flat and smooth. Ka20 was an incomplete aggregateless and their plaque surface was rather rough.

Both mutants were isolated from a haploid wild type strain NC-4 by treating it with N-methyl-N′-nitro-N-nitrosoguanidine (Yanagisawa, Loomis and Sussman 1967). They were quite stable and back mutation from the aggregateless to the wild type was never observed. KA10 and Ka20 cells were mixed together in 1:1 ratio and cultured by shaking in liquid nutrient

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1 Kindly supplied by Dr. M. Sussman, Dept. of Biology, Brandeis Univ.
medium (10 ml medium in a 100 ml Erlenmeyer flask) was A. aerogenes at 22°C. Under these conditions, the cells grew and duplicated every 3.0-3.5 hours. When the cell density reached 2-3 × 10^6 cells/ml (still in an exponential growth phase), the culture was incubated statically at 22°C. Aliquots were taken from the culture at different times during the incubation and plated on nutrient agar with bacteria after proper dilution (20-30 cells per plate). The plates were incubated at 22°C until plaques appeared on the surface of the bacterial lawn. Each time, 500-600 plaques were examined.

Results

During the static incubation, the number of living cells in the culture decreased gradually with time. For example, after 30-40 days of incubation, the number of living cells decreased to about 1 × 10^4 cells/ml. All plaques formed on the plates were aggregateless, but after 30-40 days of incubation, 1-2 plaques which aggregated to form normal fruiting bodies appeared among 500-600 aggregateless mutants. Once the wild type appeared, the proportion of the wild type increased rapidly, and then keep certain proportions in the cell population (Figs. 1 and 2). Sometimes all cells become wild type (Figs. 3). This change took place usually in 2-3 weeks, and the total number of living cells was still decreasing gradually in this period. After this change, usually no aggregateless appeared again and all cells left in the culture were of the wild type. Clonal isolation and sub-culture of the cells from the wild type
botained from the mixed culture of the two kinds of aggregateless showed that the wild type was quite stable.

The experiments were repeated twenty times with KA10 and Ka20. Fourteen out of 20 of these experiments showed the appearance of the wild type after a certain period of static incubation. This period varied from Figs. 2, 3, and 4.

During static incubation of mixed culture of two different kinds of aggregateless mutant, the number of living cells decrease gradually with time, and aggregateless cell change to wild type ones after 30-40 days of the incubation. Open circles show total number of survivals. Solid circles indicate number of wild type appeared.
about a week to several months, but in most cases, it was 30-40 days. In several cases, the wild type appeared once and then disappeared after continuous incubation for several days (Fig. 4).

Incubation of either KA10 or Ka20 alone under the same conditions did not cause the appearance of the wild type.

Certain other mutant strains, such as Kp8 and Kp12, were also cultured together. In most cases the same result, appearance of the wild type, was obtained. However, in the incubation of the other strains, Ka21 and Ka22, the wild type had not appeared.

Discussion

There are several possible explanations for this directional change in combinations of strains which have developmental deficiencies. One possibility is the contamination of wild type cells in the culture, which eventually could cause overgrowth of the wild type through selection. Back mutants in the aggregateless population could result in the same situation. These two possibilities, however, can be easily dismissed by finding that no wild type cells appeared when each aggregateless mutant was cultured separately. Next possibility is intercellular complementation between mutants which compensate for their deficiencies. For example, substance A is missing in KA10 and substance B is missing in Ka20, thus neither strains can complete its development. But if the two mutants are mixed, they may complement each other and produce cells which are able to form fruiting bodies. In this case, however, the cells which form the fruiting bodies should be wild type in phenotype only. In other words, the spores of the sporocarps thus produced should give rise to the mutants rather than the wild type progeny. But our experiments show this is not the case.

Another possibility is fusion of two deficient mutant cells. This might result change of cell type from aggregateless to normal wild type.

Further experimental results are necessary to draw any final conclusion, and now the same experiment is being repeated by using mutant strains which have more than two genetic markers to confirm this result.

References


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