RECESSIVE MUTANTS AT UNLINKED LOCI WHICH COMPLEMENT IN DIPLOIDS BUT NOT IN HETEROKARYONS OF *ASPERGILLUS NIDULANS*

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THE fungus *Aspergillus nidulans*, like some other fungi, offers the opportunity of comparing the interaction between genes when they are in the same nucleus (diploid condition) or in different nuclei sharing the same cytoplasm (heterokaryotic condition—in a heterokaryon each cell contains many nuclei from two different origins). Differences between the phenotypes produced by an identical genotype in these different cellular organizations were expected on general grounds (PONTECORVO 1950), and soon after it was possible to obtain heterozygous diploids of *Aspergillus* (ROPER 1952) the first example of such a difference was found (PONTECORVO 1952, pp. 228–229).

Thus far, differences between heterozygous diploids and heterokaryons which have the same genetical constitution have been reported in only eight cases. (For reviews, see PONTECORVO 1963; and ROBERTS 1964.) The usual test is a comparison of complementation between two recessive mutants when they are in the heterozygous diploid and in the corresponding heterokaryon. In most instances, only one or a few mutants were tested. In the case of three suppressor loci for a methionine requirement in *Coprinus lagopus* (LEWIS 1961 and unpublished results), some but not all combinations of mutants in different suppressor loci showed discrepancies when their phenotypes were compared in the dikaryon and the corresponding diploid. (In a dikaryon each cell contains only two nuclei, each from a different origin.) This paper deals with intergenic complementation among recessive mutants which complement in the heterozygous diploid but not in the corresponding heterokaryon. The mutants involved are the *fac* (fluoroacetate) mutants (APIRION 1962, 1965).

MATERIALS AND METHODS

General techniques used with *Aspergillus nidulans* are described by PONTECORVO, ROPER, HEMMONS, McDONALD and BUFTON (1953). Special techniques concerning *fac* mutants are described by APIRION (1965); (*fac* mutants were designated *f* in former publications [APIRION 1962; PONTECORVO 1963].) *fac* mutants are resistant to fluoroacetate when glucose is the carbon source and show only delayed “spidery” growth on acetate as the carbon source. The spidery growth is not due to leakiness, but to an alternative pathway for acetate utilization, unimpaired in *fac* mutants. *fac*⁺ strains are sensitive to fluoroacetate and grow well on acetate as the sole carbon source. *fac* mutants are indistinguishable from the wild type on glucose as the sole carbon

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source. They are located in three meiotically unlinked loci, *facA* in linkage group V and *facB* and *C* in linkage group VIII (APIRION 1965).

Synthesis of heterokaryons and diploids: For synthesis of heterokaryons, conidia of the two desired strains (each having a different conidial color marker and at least one growth factor requirement not present in the other) were mixed in tubes with 3 to 5 ml of liquid glucose minimal medium, and 2 to 3 drops of a complete liquid medium added (PONTECORVO *et al.* 1953). The tubes were incubated for 24 to 48 hours at 37°C until a layer of mycelium appeared on the surface. The mycelium was teased out onto minimal medium glucose agar, and on further incubation, a forced heterokaryon developed as a vigorous outgrowth due to establishment of a balanced heterokaryon.

To obtain diploids, conidia from a balanced heterokaryon were embedded in minimal-medium glucose agar at a density of 10^6 to 10^7 conidia per Petri dish (ROPER 1952). Diploid colonies which arose were isolated and purified by plating single conidia. The diploids could readily be identified since they were wild-type in conidial colour, prototrophic, and had a larger conidial diameter than haploid strains. They could also be haploidized (PONTECORVO, TARR-GLOOR and FORBES 1954; FORBES 1963) with recovery of the input markers.

Complementation tests: Several pieces (about 1 mm³) of vigorously growing hyphal tips of a desired heterokaryon were cut out of the minimal-medium glucose agar and transferred to slots in another minimal-medium glucose agar plate (control) and to plates containing acetate as the sole carbon source, with or without the growth requirements of both parental strains (test plates). Occasionally heterokaryotic hyphal tips were transferred also to liquid minimal or liquid acetate medium. The plates were incubated at 37°C for 2 to 7 days and were inspected for growth (Figure 1). Diploid strains were tested by inoculation of conidia instead of hyphal tips.

RESULTS

Diploids complement, heterokaryons do not: Thirty independently isolated *fac* mutants were employed in complementation tests, 15 previously located in the loci *facA*, *B* or *C*, and 15 which were not located. All the 15 located mutants and five of the unlocated mutants were tested individually against the wild-type alleles either in the heterokaryon or in the heterozygous diploid, and were recessive.

One mutant from each of the three loci was selected (*facA3*, *facB101* and *facC102*) and the three possible pairwise combinations were tested for complementation in heterokaryons and in the corresponding heterozygous diploids. All the heterokaryons failed to grow on acetate medium whereas the diploids grew (Table 1, Figure 1). In one case (*facA3/facB101*) the auxotrophic markers by which

TABLE 1

Test for complementation of unlinked fac mutants in heterokaryons and in heterozygous diploids

Combination*	Combination in respect of <i>fac</i> mutants	Heterokaryons		Heterozygous	Diploids	
		AM†	AM and requirements		AM	GM
<i>paba1</i> γ ; <i>facA3/w3</i> ; <i>pyro4</i> ; <i>facB101</i>	A3/B101	—	—	+	+	+
<i>ad23</i> ; <i>facA3/w3</i> ; <i>pyro4</i> ; <i>facC102</i>	A3/C102	—	—	+	+	+
γ ; <i>s1</i> ; <i>facB101/w3</i> ; <i>pyro4</i> ; <i>facC102</i>	B101/C102	—	—	+	+	+
<i>ad23</i> ; <i>facA3/\gamma</i> ; <i>s1</i> ; <i>facB101</i>	A3/B101	—	—	+	+	+

+ Indicates wild-type growth; — indicates mutant growth.

* *paba*, *pyro*, *ad*—requirements for para-aminobenzoic acid, pyridoxine, and adenine respectively; *s*—inability to utilize sulfate as the only source for sulphur; γ , *w*—conidial colour yellow and white respectively.

† AM—acetate medium; GM—glucose medium.

FIGURE 1.—Complementation in diploids and heterokaryons between two nonallelic *fac* mutants. The combination is between green strain *ad23; facA3* (designated *f3*) and white strain *w³; pyro4; facC102* (designated *f102*). Note spidery growth of the parental *fac* strains and their heterokaryon on acetate medium.

heterokaryons were forced were changed, but the pattern of complementation remained the same (Table 1).

Twenty-four other combinations of pairs of the 15 located *fac* mutants were tested, and again none of the heterokaryons grew on acetate medium whereas all the heterozygous diploids grew. The 15 unlocated *fac* mutants were also tested against the three *fac* mutants, *facA3*, *facB101* and *facC102*, and again none of the heterokaryons grew on acetate medium. None of the diploids or the heterokaryons between mutants in the same locus grew on acetate medium.

Since *fac* mutants grow slightly on acetate medium after 3 to 4 days of incubation (Figures 1, 2), it was possible to transfer hyphal tips from heterokaryons on acetate medium to minimal glucose and acetate medium. The transfers produced the same spidery growth on the acetate medium, but on the glucose medium vigorously growing heterokaryons were obtained after a day or two. These heterokaryons were comparable to those obtained when an established heterokaryon is

FIGURE 2.—A diploid sector growing out of a heterokaryon. The sector is indicated by an arrow. The heterokaryon is between the strains *paba1* γ ; *facA3* and *w3*; *pyro4*; *facB101* (*facA3* and *facB101* are unlinked). The plate contains acetate medium. Note the spidery growth of the heterokaryon.

transferred on glucose medium. Occasionally diploid sectors arose from the spidery growth of heterokaryons on acetate medium (Figure 2).

Diploids corresponding to the various tested heterokaryons were tested for resistance to fluoroacetate; as expected all combinations that grew on acetate medium were sensitive to fluoroacetate, and all that failed to grow on acetate medium were resistant. The heterokaryons were not tested for resistance to fluoroacetate, since the growth of *fac* mutants on a medium containing fluoroacetate is very limited, and further mutations to enhanced resistance appear frequently (APIRION 1965).

DISCUSSION

Are true heterokaryons being formed? If certain mutational defects blocked the process of heterokaryosis itself (at least under certain conditions) this would account for the apparent discrepancy between complementation in diploids and heterokaryons. In the case studied here, the fact that each *fac* mutation is recessive to its wild-type allele, when tested in the heterokaryon as well as in the diploid, shows that the phenomenon is not due to an effect of *fac* mutations on heterokaryosis under the conditions tested. Furthermore, as heterokaryons between *fac* mutants can be maintained on acetate medium (spidery growth) and even give rise to diploid sectors, the result cannot be explained as a failure to form proper heterokaryons.

Although heterokaryons are formed, maintenance and function of heterokaryons may lie within certain limits of nuclear ratios between the two components of the heterokaryon (PONTECORVO 1946; CLUTTERBUCK 1963). In the case studied here the possibility exists that the nuclear balance which is suitable for spidery growth of heterokaryons on acetate medium is unsuitable for wild-type growth of heterokaryons on the same medium. However, this seems unlikely since the pattern of complementation was not affected by varying the nuclear ratios of the components in the tested heterokaryons. This was attempted either by transferring hyphal tips which vary in respect to their size, age or in the amount of medium transferred.

Why does complementation fail in heterokaryons? PONTECORVO has suggested that the phenotypic difference between a heterokaryon and its corresponding diploid can be due to local concentrations of gene products which are too low to participate effectively in a complementation reaction in the heterokaryon. Such a phenotypic difference might be due to a nuclear limitation of gene products such as a nuclear protein or a repressor (PONTECORVO 1963). Alternatively, the gene products might be subunits of a protein which are formed in the cytoplasm; in the heterokaryon messengers coding for different subunits might lead to polypeptide chains too far from one another to permit efficient interaction (PONTECORVO 1963). Another possibility which might be expected to result in sharp differences of the kind reported here, is that the mutants involved are mutants in components of a heteromeric protein or a cellular organelle which is at least

partially assembled in the nucleus and then transferred to the cytoplasm. In the heterokaryon, there would then be two types of nuclei and thus two types of this organelle, both of them defective. Therefore, complementation would not occur. However, in the heterozygous diploid there would be only one type of nucleus and therefore one might expect to find four versions of the organelle: the two mutant types, a doubly defective type and a wild type. Therefore, complementation would occur (assuming a quarter of these organelles or less are enough to maintain growth).

One such organelle might be the ribosome which appears to be synthesized and assembled in the nucleus and transferred to the cytoplasm (GIRARD, LATHAM, PENMAN and DARNELL 1965; JOKLIK and BECKER 1965). Mutants in loci concerned with ribosomes might thus be expected to lead to results such as those reported here. Recently, certain suppressor mutations have been inferred to be ribosomal in their expression (DAVIES, GILBERT and GORINI 1964; APIRION 1966), and it may not be fortuitous that suppressor mutations are involved in most of the other cases of differences in intergenic complementation between heterokaryons and diploids. For example, in the case of suppression of the methionine requirement in the fungus *Coprinus* (LEWIS 1961), if some of the suppressor genes affect the ribosome, then a dikaryon homozygous for a methionineless mutation and heterozygous for two different suppressor genes (both of which suppress the particular methionine mutation involved), will produce two types of ribosomes. Both should permit formation of some of the enzyme that is lacking in the methionineless strain. Therefore, this dikaryon, as observed, will grow on a medium lacking methionine, i.e., show noncomplementation of the recessive suppressor mutations. On the other hand, in the corresponding diploid strain, four types of ribosomes might be found in each cell. At most only half of these should be capable of producing some active enzyme [negative complementation (FOLEY, GILES and ROBERTS 1965)]. Since suppressor strains frequently produce only a very low level of the effective enzyme in question (see, for example, YANOFSKY, HELINSKI and MALING 1961, Table 7; GAREN and SIDDIQI, 1962, Table 1), it is not surprising that when the amount of the active enzyme produced is further reduced at least by half, growth is abolished and there is no complementation.

In the case described here the products governed by the three *fac* genes are unknown; it is unlikely that they are ribosomal but they might be mitochondrial.

Further evaluation of the mechanism proposed here requires specific biochemical evidence about the mutations involved.

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SUMMARY

Mutants unable to utilize acetate and mapping at three distinct loci were tested in pairs for their ability to use acetate either in heterokaryons or in heterozygous diploids. While all diploids between mutants at different loci utilized acetate, the corresponding heterokaryons failed to do so.—It is proposed that the products governed by these loci are part of a particle that is assembled in the nucleus.

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