GENETIC STUDIES OF A DOME MUTANT AND ITS SUPPRESSOR GENE IN *SCHIZOPHYLLUM COMMUNE*

By S. T. Chang*

[Manuscript received 7 February 1972]

Abstract

A new morphological mutant of *S. commune*, *dm-10*, was induced by treatment with ultraviolet light. It was shown to be on a linkage group where neither *A* nor *B* incompatibility factor is located. A wild-type sector grown out from the dome mutant colony proved to be genetically controlled by a suppressor gene, *dm-10-s*, which can also affect another non-allelic dome mutant, *dm-9*, but not *dm-2*, *dm-5*, and *dm-7* dome-shaped mutants of the series. The nature and significance of *dm-10-s* and a possible mechanism for its operation are discussed.

I. Introduction

Different kinds of morphological mutants have been found in isolates of *Schizophyllum commune*, either treated or non-treated with mutagenic agents (Raper and Miles 1958; Raper, San Antonio, and Miles 1958; Miles 1970). A dome morphological mutant, showing a restricted and hemispherical colony, was first reported by Raper, Baxter, and Ellingboe (1960) in this fungus. During the past 3 years nine dome-like morphological mutants were induced by treatment with ultraviolet light and isolated independently. Among them, *dm-9* and *dm-2* were found to be linked to the *A* and *B* incompatibility factors respectively (Tang and Chang 1971; Chang and Wai 1971).

In *Neurospora crassa*, the band size of a clock mutant could be changed by a modifier gene, termed *mad* (Durkee, Sussman, and Lowry 1966). Sectors showing different morphology in the cultured colony are generally considered as signs of spontaneous mutations affecting the colony morphology. For example, the crinkled colonies of *Aspergillus nidulans* were due to the duplication of a chromosome. It showed vegetative instability by producing sectors which, in varying degrees, were caused by the loss of a variable amount of the chromosomal segments (Nga and Roper 1968). In *S. commune* a modifier gene, named *p-mod-1*, was identified by Miles (1970). It almost completely suppressed the morphology of the *puff* mutant.

The present study concerns the genetic analysis of a new dome mutant, *dm-10*, and a sector isolated from the *dm-10* colony, resembling wild type in growth rate and morphology. Genetic analysis showed that the sector was caused by a suppressor gene, *dm-10-s*.

* Biology Department of Chung Chi College, Chinese University of Hong Kong, Shatin, New Territories, Hong Kong.

II. Materials and Methods

(a) *Strains and Media*

The culture and the tester strains of *S. commune* were obtained from the collection of Professor J. R. Raper of Harvard University; the biochemical mutant, *ad-4*, was supplied by Dr. Y. Parag of the Hebrew University of Jerusalem; adenineless pink (*ad-p*), argininess ten (*arg-10*), and feather mutant (*fea*) were derived from strains in our laboratory.

The monosporous isolations were grown and mated on the complete medium. The biochemical mutants were tested on the minimal medium as well as the minimal medium supplemented with special biochemical nutrients. These media were prepared according to the formula described previously by Raper and Miles (1958).

(b) *Induction of* dm-10 *Mutation and Recovery of* dm-10-s *Gene*

The wild strain subjected to the mutagenic treatment carried incompatibility factors *A42, B42*. It was grown in liquid culture at 25°C for 4–6 days and then macerated and treated with ultraviolet light following the methods described by Chang and Wai (1971). The surviving colonies showing dome-shaped morphology were subjected to further tests.

Wild-type sectors were occasionally observed from the plate culture of dome-like colonies. These sectors were isolated and subjected to a series of tests in order to find out the cause of restoration of the wild-type morphology.

(c) *Testing Procedures*

Tests for mating types of homokaryons and the components of dikaryons were performed as suggested by Papazian (1950). The tests for assortment and recombination between mutants were based on the procedure described by Raper and Miles (1958). Details of these tests will be described with the respective experiments for which they are required. The *A* and *B* factors are compatible in all the tests; for the sake of convenience and simplicity, they will be omitted except where they play a role in the screening process.

III. Results

(a) *Segregation of* dm-10 *Mutant*

The 1 : 1 segregation of wild type and *dm-10* mutant (211 : 259 respectively in a total of four crosses, $\chi^2_{1:1} = 1{\cdot}59$, $P = 0{\cdot}23$) shows that the mutant character of the *dm-10* strain is inherited as a single gene. The single-spore progeny were grown for 5 days at room temperature after which their morphology, biochemical markers, and mating types were ascertained.

Table 1

Linkage study of *dm*-10 mutant

Mating	Total sample	No. of recombinations	Percentage recombination	Mating	Total sample	No. of recombinations	Percentage recombination
dm-10 × *A* factor	98	47	48·0	*dm-10* × *ad-4*	295	40	13·5
dm-10 × *B* factor	98	45	46·0	*dm-10* × *ad-p*	236	84	35·6
dm-10 × *arg-1*	76	41	53·8	*dm-10* × *arg-10*	117	51	50·0
dm-10 × *fea*	64	32	50·0				

(b) *Linkage Study of* dm-10 *Mutant*

Data of segregation and assortment between the *dm-10* mutant and the *A* and *B* incompatibility factors and other markers are given in Table 1. It is evident that

the *dm-10* mutant is linked to *ad-4*, 13·5 centimorgans (CM) apart, on a linkage group where neither *A* nor *B* factor is located.

(*c*) *Segregation of the Suppressor Gene*, dm-10-s

The wild-type sector (Fig. 1) from a *dm-10* mutant colony was isolated and mated with the compatible wild strain. The 3 : 1 segregation of wild type and dome-like mutant (797 : 277 respectively in a total of six crosses, $\chi^2_{3:1} = 0{\cdot}4$, $P = 0{\cdot}53$) indicates that the wild-type sector was caused by a suppressor gene, *dm-10-s*, and was not due to reverse mutation. If the sector were due to reverse mutation, the progeny of the mating should all be wild type. The mating of the sector with a *dm-10* tester strain showed 1 : 1 (wild : mutant) segregation as expected (117 : 105 respectively in a total of two crosses, $\chi^2_{1:1} = 0{\cdot}65$, $P = 0{\cdot}43$).

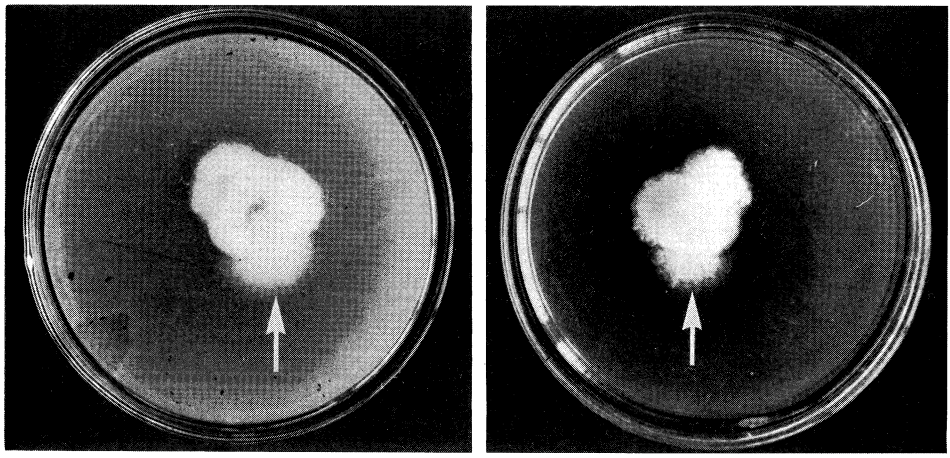


Fig. 1.—Wild-type sector of *dm-10* mutant colony. Left: reverse view of colony; right: surface view. Arrows indicate wild-type sector formation.

(*d*) *Isolation of* dm-10-s *Strain*

Three-quarters of the above 797 wild types segregating which were phenotypic in appearance should consist of three different kinds of genotypes: ++, *dm-10 dm-10-s*, and + *dm-10-s*. Thus one-third of these showing wild morphology should carry the *dm-10-s* gene. If this is so then this gene could be distinguished and isolated by the following test scheme:

Phenotype	Genotype	Testers	
		++	*dm-10* +
Wild	++	All wild	1 : 1*
Wild	*dm-10 dm-10-s*	3 : 1*	1 : 1*
Wild	+ *dm-10-s*	All wild	3 : 1*

* Wild : mutant.

The results of such an analysis (Table 2) show that there are only two classes (1 : 1 and 3 : 1) of segregation of wild type and mutant from the mating of wild-type

progeny with the *dm-10* tester. This was expected. The isolates MM5A-63, MM5A-69, MM5A-6, etc. in Table 2 whose progeny segregated in the ratio 3 : 1 (wild: mutant) are the strains which carry the *dm-10-s* gene.

TABLE 2

CLASSES OF SEGREGATION OBTAINED AS A RESULT OF MATING VARIOUS WILD-TYPE PROGENY WITH A MUTANT TESTER STRAIN

Various MM5A wild-type progeny [see Section III(*c*)] were each mated with *dm-10* tester strain M14

MM5A Progeny No.	Total sample	Wild type	Mutant	Expected ratio	χ^2	P
63	133	96	37	3 : 1	0·56	0·47
69	111	86	25	3 : 1	0·36	0·6
65	160	78	82	1 : 1	0·1	0·75
21	136	65	71	1 : 1	0·27	0·62
6	182	133	49	3 : 1	0·36	0·55
8	170	91	79	1 : 1	0·85	0·35
88	194	147	47	3 : 1	0·062	0·85
14	181	139	42	3 : 1	0·31	0·6
81	256	193	63	3 : 1	0·021	0·98
30	247	128	119	1 : 1	0·33	0·6
78	191	103	88	1 : 1	1·2	0·28
43	125	60	65	1 : 1	0·2	0·65
66	239	116	123	1 : 1	0·2	0·65
176	151	74	77	1 : 1	0·06	0·87
152	290	151	139	1 : 1	0·5	0·48
115	252	198	54	3 : 1	1·72	0·19
98	135	75	60	1 : 1	1·67	0·21
173	300	223	77	3 : 1	0·07	0·83
149	203	102	101	1 : 1	0·005	1
29	171	93	78	1 : 1	1·32	0·25
93	142	105	37	3 : 1	0·084	0·8
178	123	90	33	3 : 1	0·22	0·63
52	154	115	39	3 : 1	0·001	1
118	208	155	53	3 : 1	0·026	0·94
12	146	81	65	3 : 1	1·75	0·18
187	172	130	42	1 : 1	0·031	0·9
125	223	123	100	3 : 1	2·37	0·15
34	286	151	135	1 : 1	0·9	0·31
15	179	134	45	1 : 1	0·002	1
183	163	118	45	3 : 1	0·59	0·44
9	248	199	49	3 : 1	3·6	0·06

(*e*) *Can* dm-10-s *Suppress Other Dome-shaped Mutants?*

Seven *dm-10-s* strains (MM5A-6, MM5A-9, MM5A-14, MM5A-63, MM5A-69, MM5A-81, and MM5A-88—see Table 2) were mated at random with K29 *dm-2*, K13 *dm-5*, M11 *dm-7*, and M13 *dm-9* respectively. The results of these matings

(Table 3) demonstrate that the *dm-10-s* gene suppresses *dm-9*, but not any of the other three dome-shaped mutants.

TABLE 3

SEGREGATION IN THE MATING OF THE WILD-TYPE PROGENY BEARING THE *dm-10-s* GENE WITH FOUR OTHER DOME-SHAPED MUTANTS

Dome-shaped mutants	Progeny carrying *dm-10-s* gene						
	MM5A-6	MM5A-9	MM5A-14	MM5A-63	MM5A-69	MM5A-81	MM5A-88
K29 *dm-2*	1 : 1	1 : 1	1 : 1	—	1 : 1	1 : 1	1 : 1
n	152	151	166		132	377	104
χ^2	1·7	0·007	0·1		0·28	0·03	0·98
P	0·22	1	0·75		0·6	1	0·75
K13 *dm-5*	1 : 1	1 : 1	1 : 1	—	1 : 1	1 : 1	1 : 1
n	178	149	186		145	170	148
χ^2	0·2	0·54	0·01		0·56	0·09	0·68
P	0·65	0·48	1		0·45	0·8	0·4
M11 *dm-7*	1 : 1	—	1 : 1	1 : 1	1 : 1	1 : 1	1 : 1
n	154		199	161	126	111	211
χ^2	0·65		1·1	0·76	0·29	0·009	0·38
P	0·4		0·32	0·7	0·6	1	0·55
M13 *dm-9*	3 : 1	—	3 : 1	3 : 1	3 : 1	3 : 1	3 : 1
n	280		285	173	245	158	136
χ^2	3·05		3·53	0·1	2·75	0·01	0·04
P	0·08		0·06	0·75	0·1	1	1

IV. DISCUSSION

Dome-shaped mutants in *S. commune* give small, compact, and restricted colonies which makes them suitable for various genetical studies. Their use is potentially capable of increasing the efficiency of selection techniques. It has been shown above that these colonies are controlled by a single gene; hence they could provide suitable experimental material for studies on the effect of a single gene upon hyphal development.

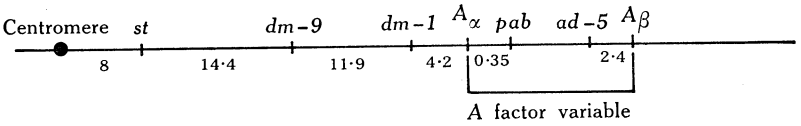
Morphological mutants in fungi have received considerable attention recently (Fuller and Tatum 1956; Brody and Tatum 1966, 1967*a*, 1967*b*; Bazinet, Fuscaldo, and Lechner 1967; Brody 1970; Miles 1970; Fuscaldo, Lechner, and Bazinet 1971; Lechner, Fuscaldo, and Bazinet 1971). Tatum and his associates and Bazinet and his co-workers respectively have identified the primary biochemical effect of certain single genes on morphological mutations in *N. crassa*. In *S. commune*, Miles and his associates have demonstrated that some of the morphological mutants differ from the wild type in cell wall composition (Wang, Schwalb, and Miles 1968), growth under microaerobic conditions (Schwalb and Miles 1967), and in response to an inhibitor of energy metabolism (Schwalb and Miles 1968). Chang, Srb, and Steward (1962) reported that the typical protein pattern of wild strains differs from that of the mutant strain in *N. crassa*.

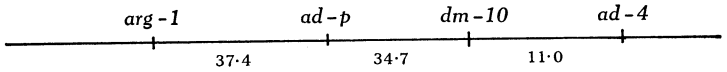
In crosses designed to yield data for mapping the *dm-10* gene, a wild-type sector from the compact and restricted dome colony (Fig. 1) was occasionally formed.

The macroscopical and microscopical features of the wild type and dome-mutant culture are distinguishable. The occurrence of the wild-type sector may be due to either contamination, reverse mutation of *dm-10* mutant, or presence of a suppressor gene which suppresses the colony size of the mutant. Since the original *dm-10* culture carried incompatibility factors *A42* and *B42*, a contamination of the wild-type culture with incompatibility factors different from *A42* and *B42* should be easily detected (e.g. by presence of clamp connections). But the contaminated wild-type culture carrying the same *A42* and *B42* factors is indistinguishable from the reverse mutation. Both of them produce all wild-type progeny when they are mated with a compatible wild-type tester, e.g. *A42 B42*+ × *A41 B41*+. However, when the wild-type sector isolated from the *A42 B42 dm-10* colony was mated with a wild tester strain, *A41 B41*, the 3 : 1 (wild : mutant) segregation was obtained [see Section III(*c*)]. This indicates that the sector was caused by a gene which could suppress the expression of *dm-10* gene function, thereby restoring the morphology of the wild-type colony.

The 3 : 1 segregation shown in Section III(*c*) indicates that *dm-10-s* is not linked to the *dm-10* mutant gene, because they segregate and assort independently. If *dm-10* and *dm-10-s* were linked, the segregation ratio of wild and mutant progeny should be much greater than 3 : 1. The locus of *dm-10-s* is, however, unknown as yet.

Further study of this suppressor gene has included attempts to determine whether the *dm-10-s* gene affects other dome-shaped mutants which are located on different chromosomes. The results reported in Table 3 indicate that *dm-10-s* is specific to *dm-10* and *dm-9* and that in a sense it is insensitive to *dm-2*, *dm-5*, and *dm-7*. *dm-10* and *dm-9* are not allelic, a fact which has been repeatedly confirmed. Partial maps of linkage groups compiled by Raper (1966), Tang and Chang (1971), and the present author would indicate that the relative loci of the *dm-9* and *dm-10* mutants are as follows:





Numerous examples were known where the effects of mutations were reversed by a second genetic change. One of such reversed changes is caused by a "suppressor gene" which effects suppression of mutation in other genes. In the present case, *dm-10-s* is a kind of intergenic suppressor which may be located at any point in the genome. It is suggested that the *dm-10* and *dm-9* mutant genes produce some repressor substances which could restrict the activities of the enzymes required for the development of wild-type morphology. Gene *dm-10-s* may produce an inducer substance which, in turn, deforms the repressors produced by *dm-10* and *dm-9* genes,

thereby restoring the dome-shaped colony to wild-type morphology. Each inducer substance produced by a suppressor gene must recognize and be recognized by a specific receptive site, which may represent a part of the sensitive gene (*dm-10* or *dm-9*) or its product (repressor substance). In *N. crassa*, two colonial mutants have been shown to possess defective enzymes, glucose-6-phosphate dehydrogenase (G6PD) in *col-2* and 6-phosphogluconic acid dehydrogenase (6PGD) in *col-3* (Brody and Tatum 1966; Fuscaldo, Lechner, and Bazinet 1971; Lechner, Fuscaldo, and Bazinet 1971). Both these enzymes catalyse steps in the hexose monophosphate (HMP) shunt. The restricted growth of these two colonial mutants (*col-2* and *col-3*) in *N. crassa* can be restored when grown on low glutamate and acetate, respectively (Brody 1970; Fuscaldo, Lechner, and Bazinet 1971; and Lechner, Fuscaldo, and Bazinet 1971). Glutamate and acetate are metabolized by alternate routes which generate NADPH, thereby alleviating the need for high G6PD and 6PGD activities. On account of the interest generated by the present paper, *dm-10* and *dm-10-s* are currently the subject of an extensive biochemical study.

V. Acknowledgments

I am grateful for financial support (Grant No. 2503–09) to the Institute of Science and Technology, Chinese University of Hong Kong, and for technical assistance of Mr. C. C. Wai.

VI. References

Bazinet, G., Fuscaldo, K. E., and Lechner, J. F. (1967).—Analysis of the glucose-6-phosphate dehydrogenase isozymes in *Neurospora crassa*. *Bact. Proc.* for 1967. p. 55.

Brody, S. (1970).—Correlation between reduced nicotinamide adenine dinucleotide phosphate levels and morphological changes in *Neurospora crassa*. *J. Bact.* **101**, 802–7.

Brody, S., and Tatum, E. L. (1966).—The primary biochemical effects of a morphological mutation in *Neurospora crassa*. *Proc. natn. Acad. Sci. U.S.A.* **56**, 1290–7.

Brody, S., and Tatum, E. L. (1967*a*).—Phosphoglucomutase mutants and morphological changes in *N. crassa*. *Proc natn. Acad. Sci. U.S.A.* **57**, 923–30.

Brody, S., and Tatum, E. L. (1967*b*).—On the role of glucose-6-phosphate in the morphology of *Neurospora*. In "Organizational Biosynthesis". (Eds. H. J. Vogel, J. O. Lampen, and V. Bryson.) pp. 285–310. (Academic Press: London.)

Chang, L. O., Srb, A. M., and Steward, F. C. (1962).—Electrophoretic separations of the soluble proteins of *Neurospora*. *Nature, Lond.* **193**, 756–9.

Chang, S. T., and Wai, C. C. (1971).—A dome morphological mutant linked to the *B* incompatibility factor of *Schizophyllum commune*. *Genetics, Princeton* **68**, 13–19.

Durkee, T. L., Sussman, A. S., and Lowry, R. J. (1966).—Genetic localization of the clock mutant and a gene modifying its band-size in *Neurospora*. *Genetics, Princeton* **53**, 1167–75.

Fuller, R. C., and Tatum, E. L. (1956).—Inositol-phospholipids in *Neurospora* and its relationship to morphology. *Am. J. Bot.* **43**, 361–5.

Fuscaldo, K. E., Lechner, J. F., and Bazinet, G. (1971).—Genetic and biochemical studies of the hexose monophosphate shunt in *Neurospora crassa*. I. The influence of genetic defects in the pathway on colonial morphology. *Can. J. Microbiol.* **17**, 783–8.

Lechner, J. F., Fuscaldo, K. E., and Bazinet, G. (1971).—Genetic and biochemical studies of the hexose monophosphate shunt in *Neuropsora crassa*. II. Characterization of biochemical defects of the morphological mutants *colonial 2* and *colonial 3*. *Can. J. Microbiol.* **17**, 789–94.

Miles, G. P. (1970).—The action of a modifier gene on the *puff* morphological mutant of *Schizophyllum commune*. *Can. J. Genet. Cytol.* **12**, 70–9.

Nga, B. H., and Roper, J. A. (1968).—Quantitative intra-chromosomal changes arising at mitosis in *Aspergilus nidulans*. *Genetics, Princeton* **58**, 193–209.

PAPAZIAN, H. P. (1950).—Physiology of the incompatibility factors in *Schizophyllum commune*. *Hull Bot. Gaz.* **112**, 143–63.

RAPER, J. R. (1966).—"Genetics of Sexuality in Higher Fungi." (The Ronald Press Co.: New York.)

RAPER, J. R., BAXTER, M. G., and ELLINGBOE, A. H. (1960).—The genetic structure of the incompatibility factors of *Schizophyllum commune*: the *A* factor. *Proc. natn. Acad. Sci. U.S.A.* **46**, 833–42.

RAPER, J. R., and MILES, P. G. (1958).—The genetics of *Schizophyllum commune*. *Genetics, Princeton* **43**, 530–46.

RAPER, J. R., SAN ANTONIO, J. P., and MILES, P. G. (1958).—The expression of mutations in common-*A* heterokaryons of *Schizophyllum commune*. *Z. VererbLehre.* **89**, 540–58.

SCHWALB, M. N., and MILES, P. G. (1967).—Morphogenesis in *Schizophyllum commune*. II. Effect of (1) Microaerobic growth. *Mycologia* **59**, 610–22.

SCHWALB, M. N., and MILES, P. G. (1968).—Morphogenesis in *Schizophyllum commune*. III. Activity of inhibitors of energy metabolism. *Pl. Cell Physiol.* **9**, 661–9.

TANG, C. Y., and CHANG, S. T. (1971).—Genetic study of *dm-9* mutant linked to the *A* incompatibility factor of *Schizophyllum commune*. (Abstr.) In "Incompatibility Items". (Ed. P. G. Miles.) p. 20. (State University of New York at Buffalo.)

WANG, C. S., SCHWALB, M. N., and MILES, P. G. (1968).—A relationship between cell wall composition and mutant morphology in the basidiomycete *Schizophyllum commune*. *Can. J. Microbiol.* **14**, 809–11.