Dikaryosis: Genetic Determination in Schizophyllum

Abstract. *A dominant gene,* dik+, *has been identified as responsible for the establishment of the dikaryon in the basidiomycetous fungus* Schizophyllum commune. *Interaction between compatible homokaryons, both of which carry the recessive allele* dik, *leads to the formation of a diploid mycelium that closely resembles the homokaryon in morphology.*

In the life cycle of every sexually reproducing eukaryotic organism, there is an alternation of two nuclear phases: one contains a single set of genes, whereas the other contains two associated sets. In a few primitive plants and animals, the phase containing two sets of genes endures for only a single nuclear generation, but in a vast majority of all organisms this phase is protracted and encompasses the vegetative plant or somatic animal body. In any case, the phase with two genomes is a balanced, complementary system that permits an organization and function that transcends the summed capacities of its two associated genomes. In all groups of organisms save one, the two associated genomes are contained within a single nucleus, the diploid nucleus. The exceptional group, the higher fungi —the Euascomycetes and the Basidiomycetes—have, instead of diploid nuclei, pairs of haploid nuclei that divide synchronously and retain their structural integrity for many nuclear generations.

In the Basidiomycetes (*1*), this system of associated genomes in separately maintained nuclei, the dikaryon or dikaryophase, constitutes an independent vegetative phase prior to nuclear fusion and the immediate onset of meiosis during the reproductive cycle. The dikaryophase of these forms is the genetic and physiological equivalent of the diplophase of other organisms.

The perpetuation of this seemingly primitive type of nuclear association only in the fungi is intriguing in view of the prevalence of diploidy throughout the eukaryotes. Two opposing views have been considered: (i) the fungi have not "learned" to manage the intricacies of routine mitotic divisions of the diploid nucleus (*2*); (ii) the dikaryon is a more plastic type of balanced system than is the diploid, and its greater versatility in the biology of the higher fungi has assured its retention and stabilization in this group (*3*). The recent discovery of a dominant gene, *dik*+, that is required for stable and persistent dikaryosis gives strong support to the second of these ideas relating to the evolutionary significance of the dikaryon.

Reproduction in a typical higher Basidiomycete, for example *Schizophyllum commune,* is by haploid spores. Each spore gives rise to a haploid vegetative mycelium made up of uninucleate cells, devoid of clamp connections, and capable of indefinite vegetative propagation in isolation. Mating between two compatible haploid strains leads by means of a reciprocal exchange of nuclei to the establishment, in both mated mycelia, of the dikaryon, a highly specialized type of heterokaryon that eventually produces the fruiting body and completes the life cycle. Each cell of the dikaryon contains two nuclei, one of each parental type, and in growth the two associated nuclei divide synchronously. This process of conjugate division involves a lateral appendage, the clamp connection, that persists at each septum and serves as a highly reliable telltale of the dikaryophase.

In one exceptional mating between compatible strains, the product of the interaction had the morphology of the normal haploid rather than that of the dikaryotic mycelium. The mating, between strains carrying seven mutations, including nonallelic deficiencies for adenine and uracil, was repeated several times, and each time the result was the same. The product of this interaction was either a haploid mycelium or a close mimic thereof: there were no clamp connections, the septa being simple as in the haploid; the nuclear distribution in a sample of 380 cells was also typical of the haploid, 91 percent of the cells being uninucleate and only 4 percent binucleate. Certain characteristics, however, suggested from the first that this apparent haploid was not what it seemed.

1) Single-celled hyphal tips, each containing only a single nucleus, grew into prototrophic mycelia. Haploid strains could thus not have resulted from a simple sectoring of the original auxotrophic mates. Prototrophy might be explicable in haploid strains resulting from somatic recombination within a transient dikaryon, but somatic recombinants have never been found in dikaryons with such promptness and regularity or in such high frequency (*4*).

2) Each of these prototrophic mycelia was fully compatible with both parental strains, an all but impossible result for any haploid strain that could be somatically derived from the mating.

Both of these characteristics suggested that the mycelia are diploid. despite their essential haploid morphology. Diploidy has been known for a few years in this and related forms, but diploids had previously been recovered only in low frequency under intense selective pressure and never before between fully compatible strains (*5*). The further characterization of these anomalous mycelia provided supporting evidence of diploidy.

3) Fruiting occurred in several prototrophic mycelia derived from uninucleate, one-celled hyphal tips, and, in each case, there was segregation of the alternate alleles of all seven known marker loci of the two original mates.

4) The putative diploid mycelium mated universally with haploid strains to establish "haploid-diploid" dikaryons, within which, however, the "diploid" nucleus appeared to be unstable. Most of the fruiting bodies originating from such dikaryons contained only partial arrays of markers from the "diploid." Even so, the progenies of several fruiting bodies—each thought to originate from a single cell of the dikaryon (*1*)—contain all known markers of the three genomes involved. Surprisingly, the segregants from such crosses appeared to be truly haploid; that is, no evidence of aneuploidy has been seen. We suspect that instability of the "diploid" nucleus in association with a haploid nucleus may reduce the "haploid-diploid" dikaryon to a mosaic of two haploid-haploid dikaryons in the fruiting body prior to the production of spores.

The evidence for stable and obligatory diploidy was sufficient to serve as the basis of the following working hypothesis for the further exploration of the situation: Dikaryosis requires a dominant gene, *dik*+, in one or in both partners; in its absence, the dikaryon is unstable and its members rapidly fuse to establish stable diploid nuclei. To test this hypothesis, four specific predictions were tested with the following results.

1) The progeny of an outcross between either of the two strains that originally gave the anomalous interaction (*A* and *B*) and a common mate (*C*)

when tested with the other original strain should yield dikaryons and "diploids" in equal frequency. This expectation was realized: Segregants from the cross of original *A* and *C*, when mated with original *B*, produced 26 dikaryons and 24 "diploids" ($P = .8$ to .9). Segregants from the cross of original *B* with *C*, when mated with original *A*, produced 13 dikaryons and 20 "diploids" ($P = .2$ to .3).

2) All of the progeny of these two outcrosses should yield dikaryons when mated with a "wild-type" strain. This was confirmed in matings with a strain chosen at random from the stock collection.

3) All isolates of both outcrossed progenies should be assignable either as dominant, *dik*$^+$, or as recessive, *dik*, from their interactions with the alternate original strains. When mated among themselves, homozygous dominant and heterozygous pairings should yield dikaryons, whereas homozygous recessive pairings should yield diploids. No exceptions were found to these expectations.

4) Although the two strains in which the recessive allele *dik* was originally found carried various mutations, a series of outcrosses should transfer the recessive allele to strains that are otherwise normal, and in these this allele should elicit the same effects as in the original mutant strains. These expectations have been confirmed: in matings between compatible strains, diploids were readily established whenever each of the two opposed strains carried the recessive allele *dik*.

Whether the recessive alleles of the two strains that were crossed by chance in this instance had a common origin is uncertain; both strains were generated from x-rayed material in laboratory crosses for which no comprehensive pedigrees were maintained. One thing is apparent, however: the recessive allele is not common either in strains collected from nature or in our laboratory strains. In the tens of thousands of different matings that have been made in this laboratory during the past dozen or so years, only this single homozygous recessive pairing was recognized. The dominant allele is overwhelmingly predominant, and thereon depends a unique and most characteristic feature of the higher fungi, the dikaryon.

The *dik*$^+$ gene suppresses karyogamy within the vegetative mycelium to limit the diploid phase to a single nuclear generation in the specialized cells, the basidia, in which meiosis normally occurs. The genetic stabilization of the dikaryon assures for these fungi all of the benefits of the diploid phase while retaining for the dikaryotic components certain prerogatives of the haploid.

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Protein Synthesis: Differential Stimulation of Cell-Specific Proteins in Epithelial Cells of Chick Oviduct

Abstract. *Immunofluorescent and radioautographic studies demonstrate that ovalbumin and avidin are cell-specific proteins synthesized by different epithelial cells in the chick oviduct mucosa. The mechanism of the selective induction of ovalbumin synthesis by estrogen and of avidin synthesis by progesterone may be through stimulation of specific target cells by these hormones.*

The differentiation of specialized epithelial cells that occurs during normal sexual maturation of the hen oviduct mucosa can be reproduced by administration of exogenous hormone (*1*, *2*). Estrogens cause the single layer of primitive stem cells in the immature chick oviduct mucosa to differentiate into three morphologically distinct epithelial cells: (i) tubular gland cells, (ii) ciliated cells, and (iii) goblet cells which are interspersed with the ciliated cells in the surface layer of duct epithelium. Concomitant with the cytodifferentiation, estrogens induce the synthesis of ovalbumin and stimulate general protein synthesis (*3*). The initiation of ovalbumin synthesis coincides with the appearance of discrete secretory granules in the cytoplasm of the tubular gland cells (*2*).

In the chick treated with estrogen, progesterone induces synthesis of the egg-white protein avidin, although general protein synthesis remains unchanged (*4*). This induction by progesterone is accompanied by microscopic evidence of increased secretory activity by the goblet cells (*2*). These observations suggest that ovalbumin and avidin are secreted by different epithelial cells of the oviduct mucosa. We have localized ovalbumin and avidin to different epithelial cell types by means of immunofluorescent and radioautographic techniques.

Five-day-old female Rhode Island Red chicks were divided into two groups. One group received 5 mg of diethylstilbestrol (DES) in sesame oil subcutaneously for 17 days. The other group received 5 mg of DES for 12 days and then 5 mg of progesterone in sesame oil subcutaneously for 5 days. The chicks were killed at regular intervals. The magnum area of the oviduct was excised immediately. A portion was fixed in Zenker-formalin and embedded in paraffin for conventional histology. The remainder was rapidly frozen for special studies.

For immunofluorescent studies, rabbit antiserums to avidin and ovalbumin (*4*) were conjugated to fluorescein isothiocyanate (*5*) and absorbed twice with acetone powder of chicken liver. Frozen sections of oviduct were heat-fixed on glass slides and placed in petri dishes containing moistened filter paper. A dilution (1:2) of the fluorescein-conjugated antiserum in 0.01*M* phosphate-buffered saline was layered on the tissue for 20 minutes before removal by flooding with the same buffer.

Fluorescent staining from antiserum to ovalbumin was localized in the epithelial cells of tubular glands in the oviducts of chicks treated with DES (Fig. 1B). This was present both before and after administration of progesterone. In contrast, oviduct tissue from chicks treated with DES alone showed essentially no localization of antiserum to avidin. However, after treatment with progesterone, fluorescence from labeled antiserum to avidin was clearly localized