incubated in the RNA preparation designated for its group. In addition, one group of mice received normal C3H spleen cells that had not been incubated with RNA, and one group received no spleen cells at all. Mice in all groups received a subcutaneous inoculum of 10^4 BP-4 cells in the flank, simultaneously with the first injection of spleen cells. All animals were observed for tumor development.

Recipients of intraperitoneal injections of spleen cells incubated with RNA from guinea pigs immunized with BP-4 evidenced a statistically significant inhibition of tumor development when compared with untreated mice or with mice receiving spleen cells that had not been incubated with RNA (Table 1). When RNA from pigs immunized with BP-4 was treated with ribonuclease, no inhibition of tumor development resulted. Groups of mice receiving spleen cells incubated with RNA from pigs immunized with Freund's adjuvant alone also exhibited no inhibition of tumor growth.

This inhibition of tumor development may represent the transfer of immunity to transplantation antigens with RNA extracted from lymphoid organs of heterologous, immunized animals. Mouse tumor cells, when injected into a guinea pig, constitute a xenograft. We had, therefore, not expected to note any tumor specificity in these responses, since, in the guinea pig, the contributions of tumor-specific transplantation antigens was expected to be inconsequential in the presence of strong, mouse transplantation antigens. However, RNA extracted from pigs immunized with tumor was significantly more effective in mediating responses than was RNA from pigs immunized with normal mouse tissues. This suggests that some degree of tumor specificity is present.

Alexander *et al.* have reported the regression of rat sarcomas after the direct injection of nucleic acids from lymphocytes of sheep immunized with the sarcoma (*9*). However, we believe our results represent the first reported instance of the transfer of immunity with spleen cells incubated with heterologous, rather than homologous, "immune" RNA. Cohen has suggested that genetic differences between lymphocytes and the RNA in which they are incubated might diminish or abrogate these types of responses (*6*). However, on theoretical grounds, the remarkable structural uniformity of nucleic acids from bacteria to man should not preclude the possibility of immune responses mediated by heterologous RNA. Molecular structures of nucleic acids are relatively constant throughout phylogeny, differences existing primarily in the number and sequence of their bases. Animals within the same order show the highest degrees of correspondence in the base compositions of their nucleic acids (*10*). Thus, it seems logical to assume that, within the rodent system herein described, immunity can be transferred with heterologous RNA.

It is possible that incorporation of informational "immune" RNA by the spleen cells during incubation is responsible for the conversion of these normal lymphoid cells to immunoreactive cells. However, Gottlieb and others have submitted convincing evidence that there is persistence of antigen (probably processed antigen) bound to RNA extracted from immune lymphoid cells (*11*). An RNA-antigen complex might sensitize lymphoid cells in a more efficient fashion than antigen not bound to RNA. This indeed was demonstrated with the antigen hemocyanin (*12*). Such RNA-antigen complexes of markedly increased immunogenicity have been termed "super antigens" by Friedman, and by Fishman and Adler (*13*). If immunologic specificity resides within this processed antigen, it would seem that, as long as the RNA does not alter antigenic specificity when binding to antigen, the species of origin of the RNA is not of prime importance. The role of RNA in such RNA-antigen complexes may be analogous to that of the protein in a hapten-protein conjugate, where the nature of the protein carrier in no way alters the antigenic specificity of the haptenic moiety. Whatever the mechanism of action of "immune" RNA, the fact that treatment of our RNA extracts with ribonuclease inactivated the preparations indicates that RNA is essential in this system of transferring immunity.

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DNA Synthesis during Yeast Sporulation: Genetic Control of an Early Developmental Event

Abstract. *In yeast the mating type alleles* a *and* α *also control sporulation; heterozygous strains* (a/α) *sporulate while homozygous strains* (α/α *or* a/a) *do not. Net DNA synthesis, an early event preceding sporulation, occurred normally in the heterozygous strains but did not occur in the homozygous strains. Therefore, the* α/a *alleles also influence early development well before spore formation begins.*

Sporulation in *Saccharomyces cerevisiae* is governed by the mating type alleles *a* and α located on chromosome III (*1*). This was demonstrated by the observation that diploid strains heterozygous for these alleles (that is, α/a) could sporulate, while strains homozygous for either one of these alleles (that is, α/α or a/a) failed to sporulate (*1, 2*). However, it is not known when during sporulation these alleles first begin to affect development. Since net DNA synthesis is one of the earliest events of the sporulation cycle (*3*), we examined the effects of the mating type alleles on this process. Our results show that the α/a alleles exert a profound influence early in development, well before the terminal steps of spore formation begin.

The strains of *S. cerevisiae* used and

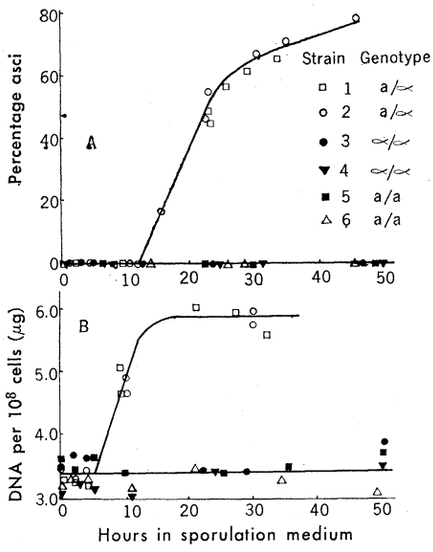

Fig. 1. Sporulation and DNA synthesis in yeast. Vegetable cells were harvested during early logarithmic growth, washed, and resuspended at zero hours in sporulation medium. At intervals samples were withdrawn for the determination of the percentage of asci (A) by phase contrast microscopy, and total DNA (B) with Burton's modification of the diphenylamine reaction.

their genotypes with respect to the mating type alleles are given in Fig. 1; each strain grew with a doubling time of about 3 hours in acetate presporulation medium (*4*). Strains 1 and 2 are heterozygous for the mating type alleles; strains 3 through 6 are homozygous for either the α or a allele. The ability of each of these strains to sporulate was examined by growth of the cells in presporulation medium followed by transfer of the cells to a potassium acetate sporulation medium free of nitrogen (*5*). All media were supplemented with 50 mg of adenine per liter. Strains 1 and 2 sporulated abundantly, while strains 3, 4, 5, and 6 failed to form asci (Fig. 1A). The cells of both the homozygous and the heterozygous strains increased about equally in mass during incubation in sporulation media, an indication that their ability to utilize acetate under these conditions was apparently normal (*6*).

Samples of cells from each of the above strains were harvested throughout the period of incubation in sporulation media, frozen, and subsequently analyzed for total DNA (*7*) (Fig. 1B). The total number of cells, including buds, was determined with a hemacytometer after the clumps were broken up by sonication. Cell numbers did not change appreciably (± 10 percent during the course of the experiment.

Upon inoculation into sporulation media (zero hours), each of the strains had approximately the DNA content expected of a growing diploid (*8*). Both heterozygous strains underwent net DNA synthesis which began 4 to 6 hours after the cells were placed in the sporulation media (*3*). In contrast, four homozygous diploids exhibited little if any net DNA synthesis during incubation in sporulation media (up to at least 50 hours).

Croes (*7*) first showed that DNA synthesis was an early event during sporulation, beginning about 8 hours before the first appearance of mature spores. When the net synthesis of DNA in the heterozygous strains is compared with the absence of synthesis in the homozygous strains, it is clear that the α/a alleles have already exerted a profound effect on development early in the sporulation cycle. This conclusion is further supported by our results which show that commitment to recombination, which also begins early in the development of heterozygous strains, is blocked in the homozygous strains (*9*, *10*).

Spore formation in yeast follows meiosis and recombination (*7*, *10*) and results in the segregation of the haploid nuclei to the individual ascospores. Synthesis of DNA is usually regarded as a prerequisite step in meiosis, which increases the diploid DNA content of the cell to the $4n$ level necessary for the formation of four haploid nuclei (*8*). Synthesis of DNA has also been implicated in aspects of the recombination process in yeast (*11*). Although DNA synthesis may be necessary for sporulation and recombination, the net synthesis of DNA, in itself, may not be sufficient to insure the completion of these processes. In our experiments the homozygous cells were harvested during logarithmic growth and were, therefore, heterogenous with respect to vegetative DNA replication; a small portion of them have surely undergone the DNA replication required for the ensuing division and, consequently, should contain enough DNA for meiosis. Nevertheless, the homozygous cells exhibited no sporulation whatever.

The simultaneous absence of DNA synthesis, recombination, and sporulation in the homozygous strains probably results from the blockade of some step, or steps, early in development which is controlled by the mating type alleles; this step need not be DNA synthesis itself.

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Starch Accumulation Associated with Growth Reduction at Low Temperatures in a Tropical Plant

Abstract. *Growth of* Digitaria decumbens *is severely reduced by night temperatures of 10°C or below. Ultrastructure of leaves and chemical analyses show a high starch content in chloroplasts of plants illuminated and kept at a temperature of 30°C. This starch disappears after a period in the dark at 30°C, but it remains if the temperature during the dark period is 10°C. The inhibition or slowing of starch translocation out of chloroplasts appears to account for reduced photosynthesis and growth at low night temperatures.*

Digitaria decumbens (Pangolagrass), a grass grown in tropical areas throughout the world, exhibits marked reductions in growth when subjected to low night temperatures (*1*). Since photo-