derived from clone 929 of strain L and had been cultured for many years in chemically defined culture medium, it seemed possible that this type of medium failed to support replication of the virus. However, as previously mentioned, other cell lines initiated and continuously maintained on serum-free medium showed evidence of virus. It seems more reasonable to assume: (i) some heterogeneity in the original cell populations with respect to presence or absence of virus; (ii) failure of some cells to support continued virus replication; or (iii) sporadic contamination of cell lines from an extraneous source of virus—although the last seems rather improbable. With one exception, all of the cell lines showing evidence of leukemia virus had also undergone "spontaneous" neoplastic transformation in vitro. From these results, mouse leukemia virus cannot be excluded as a possible cause or influence in the neoplastic conversion of these particular cell lines.

The remaining 13 cell pools gave rise to lines that showed no evidence of contamination with leukemia virus, although many of these had undergone spontaneous neoplastic transformation in vitro. Others, such as lines 5415, 5433, 5434, 5435, and 5449, underwent conversion after the tests for CF antigens and viral recovery. These results suggest that spontaneous neoplastic transformation can occur in the absence of leukemia virus that is detectable by the two testing methods that we used. Eight of these cell lines also gave negative tests for the presence of 10 other murine viruses.

Since the demonstration of complement-fixing antigen in tissue culture cells infected with murine leukemia viruses, coupled with electron microscope observations, provides a means of detecting avirulent or poorly leukemogenic virus, it is now possible to select and monitor "virus-free" cell lines that are used in studies of preneoplastic and postneoplastic changes.

WILLIAM T. HALL
WILLIAM F. ANDRESEN
KATHERINE K. SANFORD
VIRGINIA J. EVANS
Laboratory of Biology,
National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014
JANET W. HARTLEY
Laboratory of Infectious Diseases,
National Institute of Allergy and
Infectious Diseases,
National Institutes of Health

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Growth and Sporulation of a Pyrimidine Spore Color Mutant of Sordaria fimicola

Abstract. *A nonautonomous spore color mutant of* Sordaria fimicola *is a pyrimidine auxotroph that produces hyaline nonviable ascospores. Uracil, uridine, and cytidine are more effective growth factors than cytosine and thymine and, in high concentrations, render the mutant self-fertile by inducing the ascospores to resume development and maturation. Crosses with the unlinked arginine nonautonomus spore color mutant* st-59 *yielded the double mutant* st-59 pyr *that requires both arginine and a pyrimidine for growth, which indicates a lack of suppression of the pyrimidine requirement by the arginine locus.*

Ascospore color mutants of the homothallic ascomycete *Sordaria fimicola* (*1*) are divided into two groups—the autonomous mutants that maintain their charcteristic spore color in the hybrid ascus and the nonautonomous whose ascospores in the hybrid ascus are of wild phenotype. In the second group the ascospores can resume their development and maturation as a result of gene complementation in the hybrid ascus. One of the nonautonomous mutants, *st-59* (*2*), is an arginine-deficient auxotroph; its nutritional requirement and spore color are the function of a single locus. However, neither supplementation with arginine at various concentrations nor complementation in a heterokaryotic mycelium could induce the ascospores to mature in selfed asci of the mutant. It is not yet clear whether the amount of arginine that reaches the ascus in either case is a limiting factor or whether arginine is merely a growth factor that plays no direct role in the development of ascospores. In the latter case, it would be assumed that pleiotropic effects of the locus are the result of a block in biosynthesis of a precursor required for two divergent pathways, one for growth that yields arginine and the other for development of spores.

Four other auxotrophs (*3*) have been found to be partially or completely blocked in self-fertility as a result of the same gene that governs nutritional requirements. Two of them, *a-3* and *st-412*, are partially arginine-deficient and are closely linked to *st-59*. Thus, nutritional requirements of such auxotrophic mutants, along with their centromere-locus intervals, not only provide the best criteria for selecting self-sterile mutants at complex loci but also indicate an approach to the study of the role of amino acids, pyrimidines, and purines in self-fertility of homothallic

Table 1. Effect of pyrimidines on growth of *pyr* mutant of *Sordaria fimicola*. Values are averages of triplicate assays representing dry weights of mycelium (in milligrams) grown in 50 ml of minimal medium for 6 days at 26° ± 1°C.*

Conc. of supplement (μg/ml)	Dry weight of mycelium (in mg) after addition of			
	Uracil	Uridine	Cytidine	Cytosine
100	212.3	158.0	189.0	14.1
50	196.6	98.8	114.8	9.4
20	110.6	60.8	62.9	11.6
10	77.2	38.3	44.3	9.3

* The mutant failed to grow on minimal medium; on medium supplemented with uracil (100 μg/ml) growth of both wild type and mutant was about the same.

fungi. This investigation is concerned with the effect of pyrimidines on growth and ascosporogenesis of a new self-sterile auxotroph. It is an x-ray-induced, pyrimidine-deficient, and nonautonomous spore color mutant that produces hyaline and nonviable ascospores (Fig. 1a) resembling those of the arginine mutant *st-59* (Fig. 1c). As in the wild-type strain, the ascospores are forcibly ejected, which indicates that the mechanism of spore dispersal is still operating in the mature ascus irrespective of the failure of spores to mature.

From crosses between the *pyr* mutant and the wild type, 38 asci were analyzed, and the progeny from each ascus gave rise to four mutant and four wild-type cultures. Thus, the mutant differs from the wild type by a single gene. In 25 out of 38 asci, segregation of the *pyr* locus occurred in the second meiotic division, and the centromere-to-locus interval is therefore 33 or more crossover units. Crosses with all available mutants have failed to indicate the exact location of the *pyr* locus in the linkage map.

The minimal medium and culturing procedures have been described (*3*). Of the four pyrimidine supplements tested, uracil yielded the best results in the experiments on growth, and the amount of growth was enhanced with increased concentration of each supplement (Table 1). The poor growth reported here with cytosine is in full accord with results obtained by Loring and Pierce (*4*) in *Neurospora* and by Fries (*5*) in *Ophiostoma multiannulatum*. Thymine was completely inactive during the 6-day period of growth, but when flask cultures were removed from the shaker and left stationary in the laboratory, growth commenced 1 to 4 weeks later, which indicates a phenomenon of adaptation similar to that reported by Bonner *et al.* (*6*). Growth response to uracil seems at variance with the conclusion, reached by Loring and Pierce (*4*) and by Fries (*5*), that the nucleosides uridine and cytidine are more efficient as growth factors for pyrimidine mutants than uracil. However, their conclusion was based largely on the observation of a lag phase with uracil followed by a progressively increasing rate of growth that eventually would be the same as the rate with uridine or cytidine. Furthermore, they did not use the dry weights of mycelium as a criterion for growth measurement. In my investigation the daily growth rate was not determined, and a lag phase with uracil was not noticeable.

The most interesting aspect of the *pyr* mutant is that when it is grown on media supplemented with certain pyrimidines at high concentrations, its ascospores resume development and maturation. For instance, on minimal medium supplemented with 1 mg of uracil, uridine, or cytidine per milliliter the ascospores that develop are phenotypically indistinguishable from those of the wild type (Fig. 1b) and approach the latter in viability (Table 2). Between this concentration and that in the cornmeal-dextrose agar supplemented with 1 g of yeast extract per liter there exists a gradation along which the ascospores that are formed are of various colors and have different degrees of viability. Since spore color is not a reliable criterion for

Table 2. Effect of pyrimidines on spore maturation in the *pyr* mutant of *Sordaria fimicola*. Figures represent percentages of germinated ascospores in samples of 200 to 300 taken from 2-week-old cultures grown on supplemented minimal medium (minimal medium contained 0.4 percent instead of 2.0 percent dextrose for better sporulation). Ascospores were allowed to germinate on cornmeal-dextrose agar medium containing 0.7 percent sodium acetate for 10 hours at 27°C. Ascospores of wild type grown on minimal medium showed 92.3 percent germination. Ascospores of *pyr* mutant grown on Difco cornmeal-dextrose agar plus yeast extract showed no germination.

Concentration of supplement (mg/ml)	Germinated ascospores (%); medium supplemented with		
	Uracil	Uridine	Cytidine
1	89.1	88.7	82.3
0.5	81.7	89.2	82.2
.25	85.1	9.0	27.0
.1	9.2	1.4	1.3

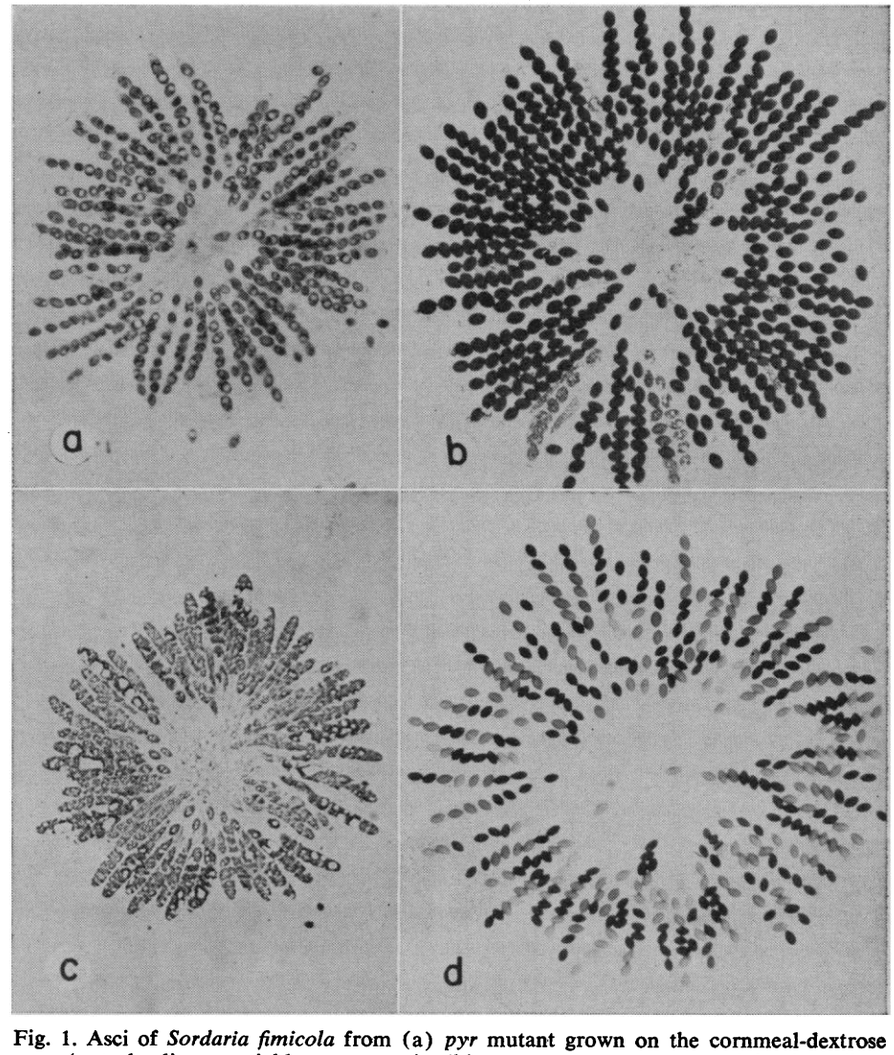

Fig. 1. Asci of *Sordaria fimicola* from (a) *pyr* mutant grown on the cornmeal-dextrose agar (note hyaline nonviable ascospores); (b) *pyr* mutant grown on minimal medium supplemented with 1 mg of uracil per milliliter and showing ascospores that are mature and viable; (c) *st-59* with hyaline nonviable ascospores; and (d) cross *st-59* × *pyr* with viable ascospores (*gray* is an independent autonomous color marker). (× 100)

determining spore viability, the ability of spores to germinate was adopted instead. As shown in Table 2, uracil at a concentration of 0.25 mg/ml is far most efficient in inducing spore maturation than either uridine or cytidine, and the mutant, therefore, might more properly be designated a uracil auxotroph. Ascospores that were tested had already been discharged and had accumulated on the inner side of the petri dish lids in cultures that were 2 weeks old. Maturation of spores can also be induced by adding the supplement in solution to cultures (6 days old) that have already produced perithecia, containing asci and immature ascospores, on the usual cornmeal-dextrose agar medium. On cytosine-supplemented medium only protoperithecia were formed at the highest concentration. The highest concentration of pyrimidines used in the experiment on growth was the same as the lowest concentration used in the experiment on ascospore germination.

Since the *pyr-3a* mutants in *Neurospora* can be suppressed either by certain arginine mutations or by an unlinked suppressor (*7*) that affects ornithine transcarbamylase and hence reduces the concentration of arginine in the mycelium (*8*), it seemed appropriate to search for such an interaction between *st-59* and *pyr*. Analysis of 40 asci from the cross *st-59* × *pyr* (Fig. 1d) yielded 6 parental ditype, 25 tetratype, and 9 nonparental ditype asci, which indicates that the two loci are not linked. The double mutants *st-59 pyr* recovered from this cross were deficient for both arginine and a pyrimidine and grew only in the presence of the two supplements. Lack of interaction between the two mutant loci is not surprising, since the *pyr-3a* mutants in *Neurospora* represent a minority among the *pyr-3* group (*9*). Furthermore, the *pyr-3a* mutants are blocked at an early step in the synthesis of pyrimidines prior to the appearance of ureidosuccinic acid (*8*), whereas the *pyr* mutant in *Sordaria fimicola*, unresponsive to orotic acid, is blocked at a later step. Regardless of whether interaction between arginine and pyrimidine mutants can be detected on the genetic level, arginine and pyrimidines are synthesized from a common precursor, carbamyl phosphate, along two divergent pathways, one leading to arginine by way of ornithine transcarbamylase and the other to pyrimidines by way of aspartate transcarbamylase. The role of amino acids and pyrimidines in ascosporogenesis and self-fertility of homothallic ascomycetes should be investigated further.

ARIF S. EL-ANI

Division of Laboratories and Research, New York State Department of Health, Albany

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Enzymatic Solubilization of Insoluble Proteins at Neutral pH

Abstract. *Insoluble epidermal proteins (possibly keratin), previously considered inert to enzyme action, were solubilized by either trypsin or chymotrypsin. Cleavage of the disulfide bonds prior to enzymatic action is not necessary. In addition, the enzymatic action on intact epidermis is not influenced by the presence or absence of endogenous lipids, soluble proteins, peptides, or amino acids. Solubilization of epidermal protein by chymotrypsin is inhibited by the supernatant solution of the homogenized epidermis.*

In normal epidermal keratinization, the epidermal proteins are considered to aggregate, forming tonofilaments, tonofibrils, and finally mature keratin. The study of epidermal proteins (*1*) and of the pathway by which these proteins become aggregate protein structures has been difficult because epidermal proteins (keratin?) are not easily solubilized, and when they are solubilized they are difficult to relate to the end product of keratinization (*2*). Soluble proteins have been obtained from insoluble epidermal proteins with urea (*3*), dilute alkali (*4*), LiBr (*5*), or citric acid (*6*).

Trypsin has been used to separate skin into its component parts, epidermis and dermis (*7*). The enzymatic action on the intracellular structures of the epidermis is thought to be minimum as the epidermis, after trypsin treatment, retains its initial ability to grow and keratinize in culture (*8*). Further evidence that viability of the epidermis is unaffected by enzyme action is found in experiments of Weiss and James (*9*). They treated chick embryo epidermis with trypsin and found that the dispersed cells, when incubated on dermis in culture, were able to reassemble into a normal growing and keratinizing epidermis. Many investigators routinely disperse epidermal cells by treatment with trypsin in order to obtain cells for culture studies.

In our work both trypsin and chymotrypsin separately have been used to solubilize the insoluble epidermal proteins of the epidermis. The enzymatic attack brings into solution more than 65 to 75 percent of the original insoluble epidermal protein. About 15 to 25 percent of the original epidermis remains insoluble after final enzyme action.

Whole normal human epidermis was pooled from abdominal skin of autopsy specimens 12 hours or less after death. The epidermis was separated from the corium by the stretch method (*10*).

Several different substrate preparations were tested for each of the enzyme reactions. These included homogenized residue, R_{II}; epidermis before and after defatting, E_N and E_D, respectively; and whole skin.

In some experiments the pooled epidermis was lyophilized, extracted with ether (*11*), and then homogenized in 0.1*M* phosphate buffer, *p*H 7.2 (*12*), with a Teflon Potter-Elvehjem tissue grinder. The epidermis, after three homogenizations at 0°C, was separated from the residue by centrifugation at 3000*g*. The final homogenate supernatant contained negligible amounts of protein, as measured by absorption at 280 mμ. The residue, R_{II}, was washed twice with water, dialyzed (two ½-hour periods), and then used as a substrate for enzymatic action.

In other experiments the lyophilized epidermis was used as a substrate before and after extraction with ether. When the undefatted epidermis was the substrate, it was reacted with chymotrypsin until no additional protein was solubilized, and then it was extracted with ether. Several additional enzyme incubations were then made until no further protein, as measured by absorb-