hydration, or metal shadowing were employed. Specimens often could be examined for 10 to 15 minutes before any detectable changes (drying out) appeared; even longer periods are possible with some other genera. Tissues near the bases of older leaves, exposed during dissection, were the first to show alterations and then these changes progressed toward the apex. Entire young leaves (Fig. 2) could be examined separately for a much longer time before alterations became apparent.

The SEM may prove to be extremely useful to plant morphogeneticists for examining the formative responses of meristematic tissues to microsurgery, to the application of growth-regulating substances, or to both. The responses of the plant tissue could be examined in considerable detail and with considerably less preparative effort than by more conventional methods.

RICHARD H. FALK
ERNEST M. GIFFORD, JR.
ELIZABETH G. CUTTER
Department of Botany,
University of California, Davis, 95616

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30 March 1970

Saccharomyces cerevisiae: A Diffusible Sex Factor

Abstract. *A hormone-like substance is secreted by α mating-type cells of heterothallic yeast strains. It induces in cells of the opposite mating type,* a, *a morphological change characteristic of the mating process. Secretion of this substance and mating ability have some common genetic determinants. In partially purified preparations, the substance has properties of an oligopeptide.*

The life cycle of heterothallic strains of the yeast *Saccharomyces cerevisiae* is characterized by a simple sexual mechanism which is under the genetic control of the mating-type locus (*1*). The two alternative allelic states of this locus are designated *a* and *α*. Haploid cells of opposite mating types conjugate to form a diploid zygote which, by mitotic division, will give rise to a clone of stable diploid cells that are heterozygous for mating type (*a*/*α*) (*2*). Under appropriate conditions such diploids undergo meiosis forming four haploid spores, two of each mating type.

The mating reaction does not require initial cell contact as the cells of mating-type *a* develop "copulatory processes" (*3*). Levi showed that this morphological response is caused by a diffusible substance when he placed cells of mating-type *a* on agar from which sexually reacting *a* and *α* cells had been removed.

We now confirm Levi's report. In addition we have partially purified a sex factor from filtrates of liquid cultures of cells of mating-type *α* and determined some of its physical, chemical, and additional biological properties. Production of this factor is controlled by the mating-type locus.

When haploid cells (*4*) of mating-type *a* are spread on a minimum agar medium (0.67 percent Difco yeast nitrogen base without amino acids, 2 percent glucose, and 2 percent agar) near a heavy streak of *α* cells, the division of *a* cells is inhibited, and they elongate to resemble those which Levi termed as having copulatory processes. The inhibition and elongation are especially pronounced when small numbers of *a* cells are streaked close to an excess of *α* cells (Fig. 1A).

Under these conditions the elongation becomes apparent after 3 to 4 hours. After 24 hours the *a* cells have formed long bizarre shapes which can reach more than ten times the length of a normal haploid cell. If the *a* cells are in excess, no elongation or inhibition is observed (Fig. 1B). Likewise, no combination of a haploid of either mating type with an *a*/*α* diploid produces this response. The inhibition and elongation, therefore, seem to be associated with the *a* phenotype in response to a diffusible factor which is secreted by the *α* cells. Identical results were observed with different, nonisogenic strains. Diploid cells which are homozygous for mating type (either *a*/*a* or *α*/*α*) behave like the corresponding haploid cells, whereas triploids, either *a*/*a*/*α* or *a*/*α*/*α*, behave like *a*/*α* diploids.

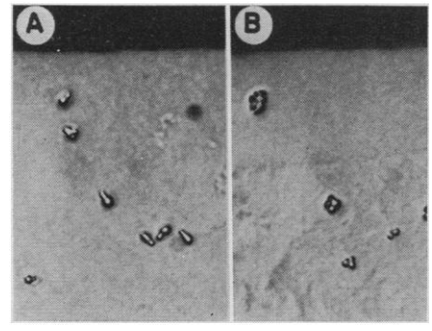

Fig. 1. (A) Cells of mating-type *a* spread near heavy streak (overnight growth) of mating-type *α* cells on minimum agar medium; (B) *α* cells near streak of *a* cells. Photographs taken after 7 hours of incubation at 30°C.

Further evidence for the correlation of the secretion of this sex factor with the *α* phenotype is provided by the properties of sterile mutants isolated from cultures of *α* cells. Haploid strains of mating-type *α*, carrying several nutritional genetic markers and the recessive allele for resistance to the arginine analog, canavanine, can^r (*5*), were exposed to ultraviolet light (374 erg/mm^2) from a germicidal lamp (General Electric). The irradiated cells (approximately 30 to 50 percent survivors) were mixed with a thousandfold excess of a mating-type *a* strain carrying complementing nutritional markers and the dominant CAN^s allele (sensitivity to canavanine). The mixture was incubated for 24 hours on YEPD agar medium (1 percent yeast extract, 2 percent Bacto-peptone, 2 percent glucose, and 2 percent agar) to allow mating. The cells were resuspended and plated on a synthetic medium that contained canavanine and the nutritional requirements of the *α* strain but lacking those of the *a* strain. Because of the dominance of the CAN^s allele, only unmated cells from the *α* strain formed colonies (*6*). Occasional diploids that had become homozygous for can^r were readily detected by their ability to grow on minimum medium. Isolates having the phenotype of the original *a* strain were tested for their ability to mate with the *a* strain by complementation.

Of 4652 clones tested, 93 were haploids that had lost the ability to mate at normal frequencies with cells of mating-type *a* and were still unable to mate with *α* cells. These sterile mutants were tested for their ability to stimulate elongation of *a* cells (Fig. 1). Sixty of them failed to effect any detectable response. The other 33 appeared to evoke varying amounts of elongation. These results strongly suggest that the ability to secrete the sex factor is under the genetic control of genes that also determine the ability of *α* cells to mate with *a* cells.

The *a*-cell response can be elicited by filtrates from liquid cultures of *α* cells. This observation has provided a method for assaying the activity, facilitating

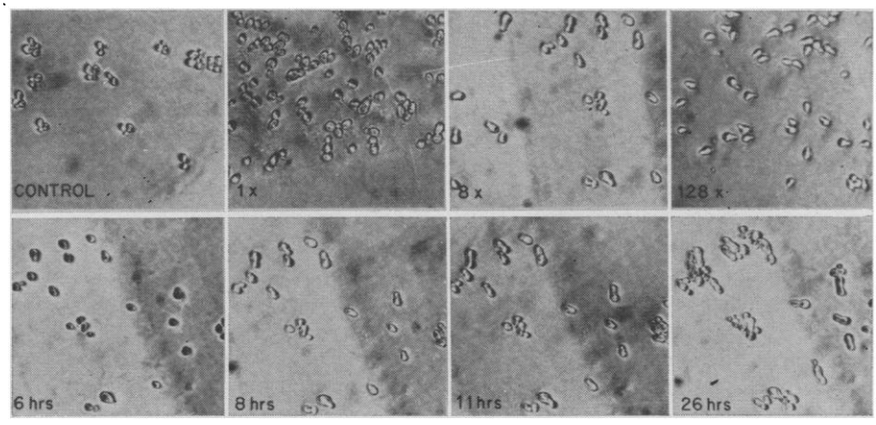

Fig. 2. (Top) Response of *a* cells to a dilution series of a concentrated and partially purified filtrate of α cells. The cells were streaked on minimum agar medium near wells containing the dilutions of the active fractions and incubated at 30°C. Photographs were taken after 8 hours. The control shows cells from the same plate streaked at some distance from the wells containing the active dilutions. (Bottom) Response of *a* cells to a single concentration of sex factor (8 units per milliliter). Photographs of the same group of cells were taken over a period of 24 hours.

isolation, and characterizing the factor. The activity is tested by exposing *a* cells on minimum agar medium to fractions placed in wells in the agar and observing the response directly under the microscope (Fig. 2). The photomicrographs were taken after the plate had been incubated for 8 hours at 30°C. The extreme elongation is especially impressive at higher concentrations, but even at a concentration of 1 unit per milliliter (defined as the lowest concentration achieved by twofold dilutions which will cause a detectable response) only a few buds are observed, whereas the control cells from the same plate have budded normally.

The sex factor can be concentrated from culture filtrates by adsorption to the weakly acidic cation exchanger, Amberlite CG-50, and elution with acid ethanol. After concentration of the eluate the active fraction is again adsorbed on a small column of the same resin. Impurities were removed by elution with 4.3*M* acetic acid. Elution with 8.6*M* acetic acid followed by concentration in a rotary evaporator yielded a concentrated solution of the factor. Further purification can be achieved by paper chromatography in a butanol, acetic acid, and water (4 : 1 : 5) system.

Physical and chemical properties of the factor have been inferred from studies of the influence of various conditions on its capacity to stimulate the elongation response. It has a molecular weight between 1000 and 2000, estimated by gel filtration on Sephadex G25. It resists boiling at slightly acid *p*H, but is unstable at alkaline *p*H even at room temperature. It is destroyed by strong acid hydrolysis or by proteolytic enzymes such as pronase or pepsin. It is not extractable by lipid solvents such as ether or chloroform and methanol, and it is strongly bound to cation-exchange resins. Preparations purified by paper chromatography give a weak or negative ninhydrin reaction but yield ninhydrin-positive material upon hydrolysis.

The properties described can be explained by the assumption that the factor contains several amino acids in peptide linkage that are necessary for its activity. They are not consistent with those of a steroid and, therefore, it is undoubtedly not the same as the steroids that cause expansion of cells (*7*).

The fact that this sex factor and the ability of α cells to mate can be affected by the same mutation implies that it plays an important role in the physiology of conjugation. The occurrence of sterile mutants that still produce the factor indicates that the ability of α cells to mate depends on additional gene products.

WOLFGANG DUNTZE*
VIVIAN MACKAY
THOMAS R. MANNEY
Department of Microbiology,
Case Western Reserve University,
Cleveland, Ohio 44106

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* Present address: Biochemisches Institut der Universität, 78 Freiburg, West Germany.

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Limb Movements in a Monotreme (Tachyglossus aculeatus): A Cineradiographic Analysis

Abstract. *In a walking echidna the principal movement of the humerus is long-axis rotation. The humerus remains approximately perpendicular to the sagittal plane, but the femur is directed anterolaterally at angles from 35° to 50°. In addition to long-axis rotation, the femur elevates and depresses in an arc which usually varies between 40° and 90°. The femoral angle, the femoral elevation and depression, and the plantar contact of the manus beneath the glenoid are features found also in generalized therians.*

Monotremes are survivors of a nontherian group of Mesozoic mammals (*1*) and retain many anatomical and physiological features indicative of a relatively primitive level of mammalian organization (*2*). Despite their numerous dietary and ecological specializations, monotremes are potentially useful as analogs in investigations of early mammalian evolution. Monotreme limb posture and movements are commonly cited as representing a primitive mammalian or even reptilian pattern (*3, 4*), although in fact no detailed functional analysis of monotreme limbs in vivo has been published. I have used cineradiography to analyze limb posture and movements in an Australian echidna (*Tachyglossus aculeatus*), which represents one of three extant monotreme genera.

The single adult echidna used in this