26. C. D. Winker, *Gulf Coast Section Soc. of Econ. Paleontol. Mineral. 5th Annual Research Conference* Austin, TX (1982), p. 109.
27. In depths shallower than about 50 m, we would expect to see more evidence of wave activity; in depths >100 m, the coarse bed would be even more remarkable.
28. We estimated the shear velocity (U_*) necessary to erode and transport a clast 20 by 100 cm in cross section (in the DMC section, Fig. 2) by calculating the force necessary to push this block across the bed; with a clast density of 2.0 g/cm^3 and a coefficient of static friction of 0.5, the necessary U_* is 35 to 100 cm/s, depending on how much the clast is projecting above the surface. The U_* necessary to initiate motion of typical clasts (5 to 20 cm diameter) in the basal sandstone, over a rough bed (1 cm characteristic roughness), is at least 15 cm/s, which can be used as a minimum requirement [method of P. L. Wiberg and J. D. Smith, *Water Resour. Res.* **23**, 1471 (1987)]; a shear velocity of about 25 cm/s and a wave period of 50 min are sufficient to suspend enough sand to form a 20-cm-thick sand layer with climbing ripples; sediment concentration profiles calculated after J. D. Smith [in *The Sea*, E. D. Goldberg, Ed. (Wiley, New York, 1977), vol. 6, pp. 539–577]; P. L. Wiberg and J. D. Smith, *Continental Shelf Res.* **2**, 147 (1983).
29. This size of sediment (about 0.06-mm diameter) goes directly into suspension when the critical shear stress is exceeded. Therefore, the wave ripples must be generated by deposition under an oscillatory velocity field with a subcritical shear stress (Table 1). For wave ripples of 15-cm wavelength (*22*) to form, the minimum wave height to produce the appropriate orbital diameter in 50-m water depth is 1 m for 10-s waves or 3 m for 8-s waves; in 100-m water depth 2 m for 12-s waves or 6 m for 10-s waves [technique of P. L. Wiberg and J. Bourgeois, *Soc. Econ. Paleontol. Mineral. Annual Midyear Mtg. Abstr.* **3**, 116 (1986); see also H. E. Clifton and J. R. Dingler, *Mar. Geol.* **60**, 165 (1984)]. Thus the occurrence of these ripples is indicative of waves with long period and low wave height.
30. By turbidity current, we mean a normal, discrete, sediment-laden current flowing along the bed, which would produce unidirectional current indicators. The turbulence generated by a tsunami of the scale postulated would suspend enough sediment to produce density gradients in the water column, which would cause sediment advection, for example, of sandy sediment from close to shore toward the site of deposition.
31. Large storm waves could generate shear stresses of the magnitude necessary to produce the bed only if the environment were shallow, about 10- to 20-m water depth; if this were the case, other evidence of storm waves would be present in the 12 m of bracketing mudstone (which would likely have several sandy intervals and would be coarser).
32. After initial turbulence, the sand (settling velocity of about 0.25 cm/s) would settle out of 100 m of water in about 12 hours; the silt and clay would take a few days to settle out, depending on the residual turbulence.
33. See, for example, R. A. Heath and M. M. Creswell, Eds., *Tsunami Research Symposium 1974* (Unesco Press, Paris, 1974); K. Iida and T. Iwasaki, Eds., *Tsunamis: Their Science and Engineering* (Reidel, Dordrecht, 1981); for a review of the scale of submarine slides, see D. G. Moore, in *Rockslides and Avalanches*, vol. 1, *Natural Phenomena*, B. Voight, Ed. (Elsevier, Amsterdam, 1978), pp. 563–604.
34. F. M. Bullard, *Volcanoes of the Earth* (University of Texas, Austin, 1984); T. Simkin and R. S. Fiske, Eds., *Krakatau, 1883—The Volcanic Eruption and Its Effects* (Smithsonian Institution, Washington, DC, 1983).
35. G. W. Moore and J. G. Moore, *Geol. Soc. Am. Abstr.* 17, 668 (1985).
36. The effects of tsunamis generated by impacts are reviewed by D. E. Gault and C. P. Sonett, *Geol. Soc. Am. Spec. Pap.* **190**, 69 (1982).
37. These calculations are meant to indicate only order-of-magnitude conditions; no theory is established for the behavior of large tsunamis, particularly in shelf depths.
38. But see Maurrasse (*10*) and Borella (*13*).
39. In the present Gulf of Mexico, typical fair-weather wave periods are 3 to 6 s; storms produce waves with periods of 8 to 10 s. Differing paleogeography, that is, greater fetch, could produce larger waves, as in the modern Pacific, which has the largest measured waves.
40. We thank W. Gose, G. Keller, and W. V. Sliter for sharing unpublished data; J. D. Smith for discussions about waves and sediment-transport calculations; B. Atwater for reviewing an early version of this manuscript; and A. D. Donovan and J. Reinhardt for sharing information about the Braggs, Alabama, locality. This research was supported in part by donors to the Petroleum Research Fund of the American Chemical Society (to J.B.) and NSF grant EAR-8411202 (to T.A.H. and E.G.K.).

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DNAs of the Two Mating-Type Alleles of *Neurospora crassa* Are Highly Dissimilar

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The mating-type alleles *A* and *a* of *Neurospora crassa* control mating in the sexual cycle and function in establishing heterokaryon incompatibility in the vegetative cycle. The *A* and *a* alleles were cloned, and they were shown to encode both the sexual functions and vegetative incompatibility. The mating-type clones contain nonhomologous DNA segments that are flanked by common DNA sequences. *Neurospora crassa* and all heterothallic and pseudohomothallic *Neurospora* species contain a single copy of one mating-type sequence or the other within each haploid genome. The six known self-fertile homothallic isolates contain an *A* homolog, but only one species also contains *a* homologous sequences. Homothallism in these species is not due to mating-type switching, as it is in *Saccharomyces cerevisiae*.

Neurospora crassa is a haploid heterothallic filamentous fungus; its two mating types are designated *A* and *a* (*1, 2*). Fusion of cells of opposite mating type initiates a series of developmental events leading to the formation of a fruiting body (perithecium) containing many asci, each with ascospores that bear the haploid products of meiosis. There is no visible difference between strains of the two mating types, and otherwise nearly isogenic strains will mate provided that they are of

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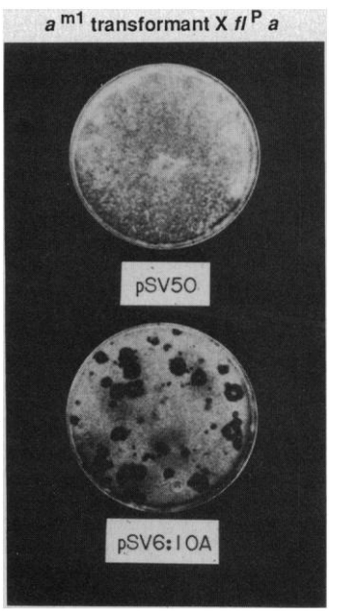

Fig. 1. Transformation of a^{m1}, *ad-3B*, *cyh-1* (FGSC 4564) (*8*) spheroplasts (*6*) by pSV50 and pSV6:10A. DNA (2.5 μg) of pSV50 (encoding an altered β-tubulin that confers resistance to benomyl) (*6, 9*) or PSV6:10A (encoding both altered β-tubulin and *A* mating-type function) were introduced by transformation. Transformation plates containing 0.5 μg/ml benomyl were incubated at 30°C for 4 days after plating. Whatman 1 filter paper was placed on the agar surface, and transformants were allowed to grow into the filters for 12 hours. The filters were then transferred to plates containing an *a* mating-type tester inoculated 4 days previously with the highly fertile nonconidiating strain fl^P *a* (FGSC 4347). The plates were incubated at 25°C for 4 days. Transformants carrying pSV6:10A express the *A* mating-type function and induce the formation of black perithecia in the fl^P *a* lawn. The location of the mating-type *A* function was determined by transformation of a^{m1} spheroplasts with restriction fragments of pSV6:10A. Cosmid pSV6:10A was digested with various restriction enzymes and the digests were used in conjunction with the benomyl resistance vector, pSV50, to cotransform the a^{m1} recipient. Previous studies demonstrated that approximately 80% of transformed *Neurospora* spheroplasts are cotransformed when two separate DNA fragments are used simultaneously; a selectable marker is necessary in only one of the fragments (*6*). Benomyl-resistant transformants that receive a functionally intact *A* gene (that is, one not disrupted by cleavage with a restriction enzyme) initiate perithecium development when crossed to *a* mating-type protoperithecia. Restriction fragments that induced perithecium development were subcloned into pGem vectors (Promega Biotec, Inc.).

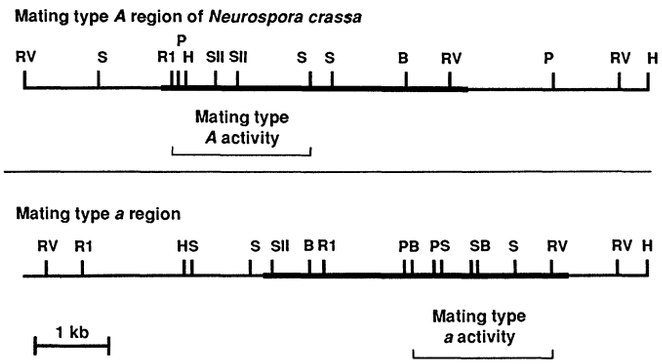

Fig. 2. Comparison of the restriction maps of the cloned *A* and *a* mating type–specific regions of *N. crassa*. All sites cut by the enzymes used are indicated. B, Bam HI; R1, Eco RI; RV, Eco RV; H, Hind III; P, Pst I; S, Sal I; and SII, Sst II. The *A* and *a* mating-type regions defined by nonhomology are represented by bold lines in the figure. Regions defined as having *A* or *a* mating-type activity are restriction fragments from the *A*-specific or *a*-specific regions that confer the capacity to induce perithecium formation when transformed into sterile mutants.

opposite mating type. In addition to their role in sexual reproduction, the mating-type alleles determine vegetative incompatibility; if cells of opposite mating type fuse on medium inappropriate for mating, the resulting heterokaryotic cells die (*3*). No mutations or recombinational events converting one mating type to the other or to a state of self-fertility have been observed (*4*). This contrasts with the situation in *Saccharomyces cerevisiae*, in which mating-type switching makes yeast operationally "self-fertile" or homothallic (*5*).

The genus *Neurospora* includes heterothallic species (mating-type alleles present in separate ascospores), pseudohomothallic species (both mating types present in a single ascospore, but in separate nuclei, thus giving the appearance of being self-fertile), and true homothallic species (each haploid nucleus generates a self-fertile organism) (*1*). We describe here the cloning and the physical characterization of the *A* and *a* mating loci of *N. crassa* and the allele distribution in heterothallic and homothallic *Neurospora* species.

The mating-type alleles of *N. crassa* provide no obvious basis for phenotypic selection in DNA-mediated transformation. Therefore, we cloned *A* DNA by exploiting its proximity to its closest selectable genetic marker, the temperature-sensitive mutation *un-3*. One cosmid from a clonal *A* mating-type library (*6*), pSV6:10A, allowed the *un-3* recipient to grow at the restrictive temperature. The chromosomal origin of the *N. crassa* DNA insert in pSV6:10A was located by restriction fragment length polymorphism (RFLP) mapping (*7*); the polymorphism segregated with mating type in all progeny. The presence of a functional *A* allele on pSV6:10A was demonstrated by its ability to confer *A* mating behavior by DNA-mediated transformation of a sterile recipient [Fungal Genetic Stock Center (FGSC) 4564] mutated at the mating-type locus (*8*) (Fig. 1). A 4.4-kbp fragment from pSV6:10A (Fig. 2) contains DNA unique to *A* cells and has all the information necessary to transform the a^{m1} sterile mutant to function as mating type *A*: namely, to form perithecia in crosses with *a* strains.

DNA encoding the wild-type *a* mating determinant was detected in a bacteriophage λJ1 library that contains randomly sheared fragments from an *a* strain (FGSC 3460). The cosmid pSV6:10A was used to probe for DNA sequences common to *a* and *A* that flank the DNA that is unique to *A*. Phage DNA that hybridized to pSV6:10A transformed an *A* recipient (FGSC 2338) to *a* mating-type behavior, that is, it acquired the ability to mate with an *A* partner. The *a* mating-type sequences responsible for perithecium induction reside on a 4.5-kbp fragment unique to *a* (Fig. 2). Insertion of the *A*-specific fragment or the *a*-specific fragment into appropriate *N. crassa* recipients by transformation confers the ability to mate with the specificity corresponding to the inserted DNA. Fragments containing the functional *A* and *a* mating-type regions are structurally distinct (Fig. 2). The *a*-specific and *A*-specific DNA segments do not hybridize to each other except under very low stringency (0.6*M* NaCl, 37°C). Not all of the *A*-specific or *a*-specific sequence is necessary for conferring the ability to form perithecia. Transformation by a 1.7-kbp Pst I–Sal I fragment from within the 4.4 kbp of *A*-specific DNA is sufficient to confer *A* mating-type function on a sterile recipient; a 2.0-kbp Eco RV–Bam HI fragment from within the *a*-specific mating-type region is sufficient to confer *a* mating-type function (Fig. 2). Transformation by other restriction fragments from the *A* and *a* mating-type regions into *N. crassa* sterile mutants does not induce any detectable mating reactions.

A central question about the mating system of *N. crassa* is whether a strain of a given mating type contains unexpressed genetic information of the opposite mating type. Mating behavior in *S. cerevisiae* is determined by the gene present at the expressed mating-type locus (either **a** or α). Switching of mating types occurs by a mechanism in which information from a "silent" copy of the opposite mating type is moved from elsewhere in the genome into the mating-type locus (*10*). In *N. crassa*, however, the mating type is stable because information for the opposite mating type is physically absent from a haploid genome (Fig. 3). In addition, the *A* or *a* mating-type sequences are present only as a single copy; probes made from the internal *A* and *a* mating-type regions hybridize only to bands that correspond to internal restriction fragments of the mating-type region. The region of nonhomology between *A* and *a* extends from a 1.0-kbp Eco RI–Sal I fragment to a 0.6-kbp Eco RV–Pst I fragment, a span of 4.4 kbp (Fig. 2). Although close sequence similarity of *A* and *a* genomes is reestablished on either side of the nonhybridizing *A* and *a* mating-type regions, restriction site differences do exist in the homologous regions flanking these sequences, even between isolates of the same mating type.

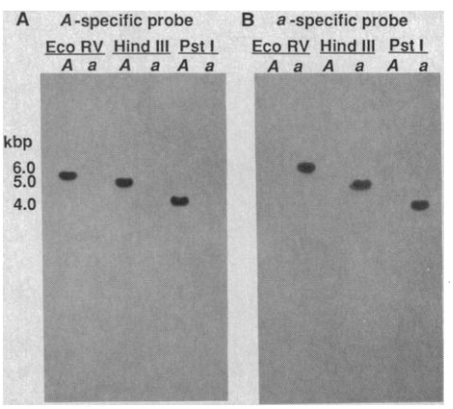

Fig. 3. (**A**) DNA blot of 74-OR23-IV*A* (FGSC 2489) and OR*S-a* (FGSC 2490) genomic DNA (*13*) cut with Eco RV, Hind III, and Pst I endonucleases, subjected to agarose gel electrophoresis, then blotted to Bio101 Genescreen, and probed with the 1.6-kbp Hind III–Sal I fragment from the *A*-specific mating-type region (Fig. 2). The 1.6-kbp Hind III–Sal I fragment had been isolated from an agarose gel and labeled with ^{32}P (*14*). The blots were washed in 0.1× saline sodium citrate (SSC) and 0.1% SDS at 60°C, and the autoradiogram was exposed at −70°C overnight with an intensifying screen. (**B**) DNA blot from (A) probed with a 2-kbp Eco RV–Bam HI fragment from the *a*-specific mating-type region (Fig. 2). The radioactive probe was stripped from the blot in (A) by washing at 42°C in 0.4*N* NaOH. The *a*-specific Eco RV–Bam HI fragment was isolated and labeled with ^{32}P by random priming (*15*). Blots were hybridized overnight in 5× SSC, 50% formamide, and 0.25% dry milk and then washed in 2× SSC and 0.1% SDS at 42°C. The autoradiogram was exposed as before at −70°C. Blots washed in 0.1×SSC and 0.1% SDS at 65°C yielded identical results.

The majority of perithecia that develop from crosses of either *A* or *a* mating-type transformants to protoperithecial lawns are devoid of asci and ascospores, that is, they are barren. Approximately 1 to 5% of the perithecia are fertile, and the ascospores they produce are viable. The integration pattern in the fertile progeny we have examined is explicable as a gene replacement of the a^{m1} allele with a segment from the transforming *A* cosmid. Although the efficiency of gene replacement is low, all of the information required for the completion of the sexual cycle apparently is contained in the *A* and *a* mating-type clones.

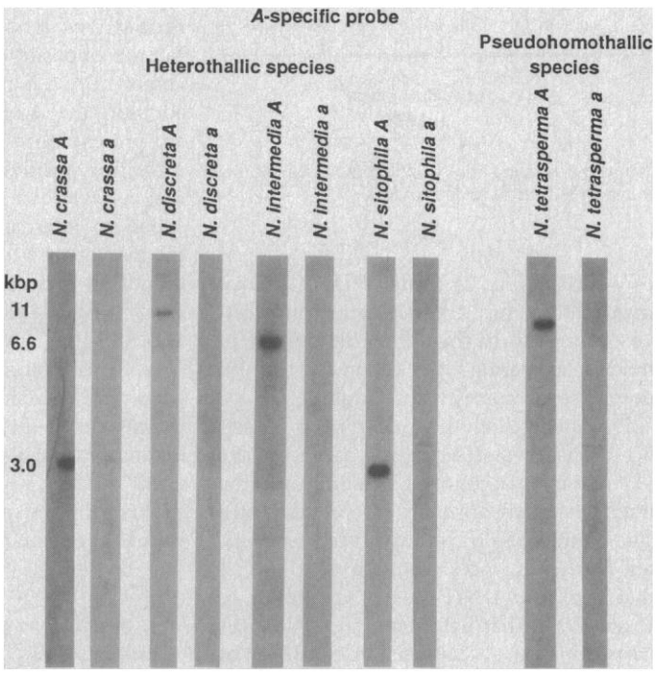

Fig. 4. Genomic DNA (*13*) from heterothallic and pseudohomothallic species of *Neurospora* was digested with Pst I–Bam HI endonucleases and subjected to agarose gel electrophoresis. DNA fragments were transferred to Bio101 Genescreen and probed with a nick-translated (*14*) Hind III–Sal I fragment from the *A*-specific mating-type region. The blot was hybridized, washed, and exposed as described in Fig. 3A. The hybridizing 3.0-kbp band is the Pst I–Bam HI fragment of the *A*-specific region (see Fig. 2). The blot was stripped and reprobed with a 2.0-kbp Eco RV–Bam HI fragment from the *N. crassa a*-specific region. Hybridization was detected only in the *a* genomes of the heterothallic and pseudohomothallic species (data no shown).

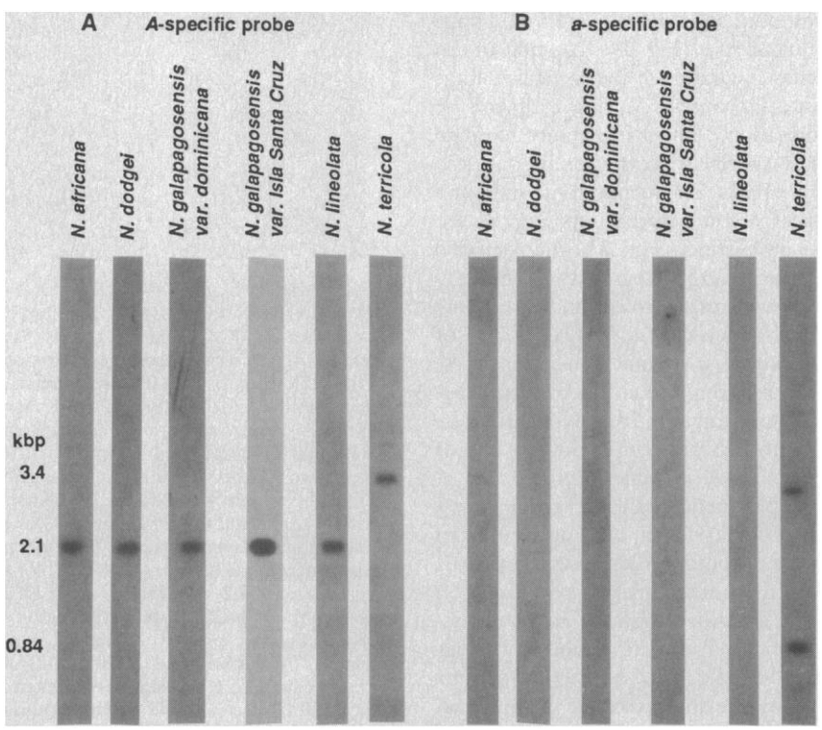

Fig. 5. (**A**) Genomic DNA (*13*) from homothallic species of *Neurospora* was digested with the restriction enzymes Pst I and Bam HI, and DNA blots were prepared and probed as in Fig. 3A. The probe was a nick-translated (*14*) 1.6-kbp Hind III–Sal I fragment from the *A*-specific mating-type region. (**B**) The radioactive probe was stripped from the blot in (A), and the membrane was rehybridized with a 2.0-kbp Eco RV–Bam HI fragment from the mating-type *a*-specific region as in Fig. 3B.

The heterokaryon-incompatibility function associated with the mating-type alleles is expressed in the vegetative stage; if cells of the opposite mating type fuse, the resulting heterokaryotic cells are inhibited in their growth. Attempts to resolve the heterokaryon-incompatibility function from the fertility function of the mating-type locus by recombination have failed (*4*), and mutants selected for heterokaryon compatibility are almost always sterile [a single exceptional mutant, a^{m33}, was isolated that is heterokaryon-compatible and fully fertile (*8*)]. Reversion of sterile mutants to fertility is accompanied by the restoration of incompatibility. Introduction of the 1.7-kbp *A* mating-type Pst I–Sal I fragment (Fig. 2) into spheroplasts from an *a* mating-type strain (*qa-2;aro-9a*) reduces transformation efficiencies 100-fold as compared to the results with an *A* mating-type recipient (*qa-2;aro-9A*). Similarly, transformation by the 2.0-kbp Eco RV–Bam HI *a* mating-type fragment reduces transformation frequency of the *A* mating-type recipient compared to the transformation frequency when the recipient is *a* mating type. An unlinked suppressor of mating-type vegetative incompatibility, *tol* (*11*), restores the high transformation frequency of *a* fragments in *A*. Therefore, the 1.7-kbp *A* and 2.0-kbp *a* mating-type fragments that induce perithecium formation also contain the heterokaryon-incompatibility function associated with the mating-type locus. Most of the transformants that grow when either the *A* or *a* mating-type sequences are used to transform spheroplasts of the opposite mating type contain disrupted copies of the ectopic mating-type sequences. However, a minority of transformants that contain intact ectopic mating-type sequences mate as both *A* and *a* and exhibit the growth-inhibited phenotype associated with mixed mating-type heterokaryons. When placed on mating media, these transformants produce perithecia, although ascospores are not formed.

The genus *Neurospora* includes species that have various mating strategies. Like *N. crassa*, three heterothallic species, *N. discreta*, *N. sitophila*, and *N. intermedia*, have two distinct mating types (*1*). A pseudohomothallic species, *N. tetrasperma*, usually harbors both mating types within a single ascospore, but in separate haploid nuclei. Such mixed mating-type heterokaryons are easily resolved into two self-sterile strains of opposite mating type. DNA from each mating type of *N. sitophila*, *N. intermedia*, *N. discreta*, and *N. tetrasperma* was probed at high stringency with cloned *A* and *a* DNAs of *N.*

crassa. A single DNA fragment in the *A* mating-type strains of *N. discreta, N. intermedia, N. sitophila*, and *N. tetrasperma* hybridized to the *A* probe from *N. crassa* (Fig. 4). Hybridization of the *N. crassa a*-specific probe to *A* and *a* genomic fragments could be detected only in the *a* genomes of the related heterothallic and pseudohomothallic species. Our data show that the two mating-type sequences are conserved among *Neurospora* heterothallic and pseudohomothallic species, although there is some variability of restriction sites within and around the mating type–specific regions. As in *N. crassa*, there is only a single copy of a mating-type sequence in any haploid, homokaryotic strain of the related heterothallic and pseudohomothallic species, and no unexpressed copy of the opposite mating type.

Six homothallic strains, denominated as five species, have been placed in the genus *Neurospora* (*1, 12*). Haploid ascospore isolates from any of these species are capable of completing the sexual cycle. In the homothallic yeasts, mating occurs by the rapid switching of the **a** or α cassette to the active locus by a transposition event (*10*). To determine whether such a switching mechanism could also be responsible for homothallism in the genus *Neurospora*, we isolated DNA from the six homothallic strains and probed restriction digests at high and low stringency with the cloned nonhomologous segments of the *A* and *a* mating-type regions of *N. crassa*. Five homothallic strains contained fragments that hybridize only with the *A*-specific *N. crassa* mating-type probe (Fig. 5A); no hybridization to *a*-specific sequences was detected (Fig. 5B). In all of these homothallic species the size of the fragment that hybridizes to the *A*-specific probe of *N. crassa* is identical. Thus, in five of the six homothallic strains all the events involved in sexual development can occur with the genetic information associated with only the *A* mating type of heterothallic species; the products of the *a* and *A* genes are not both required for sexual development. The genome of the sixth homothallic strain, *N. terricola*, contains a segment that hybridizes to the *A*-specific probe and a second segment that hybridizes to the *a*-specific probe (Fig. 5, A and B). However, unlike the situation in yeast, there is only one copy of each mating type–specific sequence. This suggests that homothallism may function by two different mechanisms in the genus *Neurospora*, neither of which involves mating-type interconversion. In addition, our findings suggest that expression of *A* may be sufficient to activate the genes or gene products necessary for perithecium formation and meiosis in at least some homothallic strains.

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HIV-1–Infected T Cells Show a Selective Signaling Defect After Perturbation of CD3/Antigen Receptor

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The binding of antigen or monoclonal antibody to the T cell receptor for antigen or the closely associated CD3 complex causes increases in the concentration of intracellular ionized calcium and subsequent cell proliferation. By measuring second messenger production in primary cultures of human immunodeficiency virus (HIV-1)–infected T cells stimulated with monoclonal antibodies specific for either CD3 or CD2, a specific impairment of membrane signaling was revealed. The HIV-1–infected T cells were unable to mobilize Ca^{2+} after stimulation with anti-CD3, whereas CD2-induced calcium mobilization remained intact. Furthermore, the HIV-1–infected cells proliferated poorly after CD3 stimulation, although the cells retained normal DNA synthesis in response to interleukin-2 stimulation. These results show that the signals initiated by CD2 and CD3 can be regulated independently within the same T cell; uncoupling of signal transduction after antigen-specific stimulation provides a biochemical mechanism to explain, in part, the profound immunodeficiency of patients with HIV-1 infection.

THE MOST CONSPICUOUS ABNORMALITY associated with HIV-1 infection is a quantitative deficiency of the CD4 helper/inducer T cell (*1*). The CD4 cell is a major source of lymphokines and is important for most immunologic functions, including interactions with nonlymphoid cells. Among the immunologic defects associated with HIV-1 infections are cutaneous anergy and decreased T cell proliferative responses after stimulation by soluble antigens such as tetanus toxoid (*1*). This T cell defect appears to be qualitative in that immunologic unresponsiveness in HIV-1–seropositive patients can occur despite normal numbers of circulating CD4 T lymphocytes (*2*). We have investigated early transmembrane signaling events in an effort to further understand the basis for the impaired response of HIV-1–infected T cells to antigen.

In T lymphocytes, the binding of monoclonal antibody (mAb) to the CD3 complex mimics activation via the antigen receptor, resulting in the production of inositol 1,4,5-trisphosphate (IP_3), increased intracellular ionized Ca^{2+} concentration ($[Ca^{2+}]_i$), and

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