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Disruption of the *Dictyostelium* Myosin Heavy Chain Gene by Homologous Recombination

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The phenomenon of homologous recombination, which allows specific gene conversion and gene insertion, can be a powerful system for the study of eukaryotic cell biology. Data are presented demonstrating that integration of a transfected plasmid by homologous recombination occurs in the motile eukaryotic cell *Dictyostelium discoideum*. A plasmid carrying a G418 resistance gene and the amino terminal half of the myosin heavy chain gene was used to transfect *Dictyostelium*. A large fraction of the resultant G418-resistant cells had the plasmid integrated into the single genomic copy of the heavy chain gene. These cells, which fail to express the native myosin but express the myosin fragment, are defective in cytokinesis and become large and multinucleate. In spite of the absence of native myosin, these cells, termed *hmm* cells, exhibit many forms of cell movement, including membrane ruffling, phagocytosis, and chemotaxis. The *hmm* cells can aggregate but are blocked at a later stage in the *Dictyostelium* developmental cycle. The *hmm* cells revert to the wild-type phenotype. Reversion of the *hmm* phenotype is due to excision and loss of the transforming plasmid. The revertant cells express native myosin, are G418 sensitive, and have a normal developmental cycle. These results constitute genetic proof that the intact myosin molecule is required for cytokinesis and not for karyokinesis.

Dictyostelium discoideum IS A EUKARYOTE THAT SERVES AS A simple model system for the study of many cellular processes (*1*). It displays many forms of cell motility found in higher eukaryotic cells. For example, it extends filopodia and pseudopodia in its direction of travel like fibroblasts or macrophages and undergoes chemotactic movements like leukocytes. During mitosis, microtubules form a typical eukaryotic spindle (*2*), and the contractile ring associated with cytokinesis has been shown to contain actin and myosin filaments (*3*). *Dictyostelium* cells exhibit extensive endocytosis and exocytosis (*4*).

Dictyostelium has a well-characterized developmental cycle (*4, 5*). When nutrients are available, either in the form of bacteria or an axenic medium, the cells grow vegetatively as amebae. In this form they can be grown in large quantities for biochemical manipulations. When *Dictyostelium* cells starve, they enter a developmental program that involves the aggregation of many cells by chemotaxis toward cyclic adenosine monophosphate (AMP) signals. The culmination of the developmental program is the formation of a fruiting body that contains two cell types, the stalk cells and the spores. The spores can then germinate to complete the cycle.

Dictyostelium grows and accomplishes its developmental program as a haploid cell, but it can be induced to form diploid cells. This allows the use of classical genetics to map genes into the seven linkage groups of the *Dictyostelium* genome (*6*). Molecular biological approaches have been extensively applied to this organism, including DNA-mediated transformation, which has been accomplished by Firtel and his co-workers (*7*). This has opened the possibility of utilizing genetic manipulation of *Dictyostelium* for many cell biological studies.

In yeast, specific gene conversions and integration of known genetic elements into specific genes by homologous recombination have been successfully used (*8*). This approach is referred to as gene targeting (*9*). Gene disruption has been used in yeast for the analysis

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of cytoskeletal genes and their products (*10, 11*). However, many properties characteristic of animal cells are not exhibited by yeast. Gene targeting in mammalian cells, which are diploid, has recently been demonstrated, but it occurs at extremely low frequency (*9*). The development of a system for gene targeting in the haploid cell *Dictyostelium* would offer an ideal model for the analysis of the molecular basis of many cellular processes.

We now report the homologous integration of a plasmid carrying a portion of the myosin heavy chain gene (*mhc*A gene) (*12*) into the single genomic copy of that gene in *Dictyostelium*. This integration event eliminates the expression of the native myosin heavy chain gene and results in the expression of the myosin fragment coded for by the plasmid. The cells survive and undergo many forms of movement, but are defective in cytokinesis and development.

Structure of the plasmid used for transformation. The *Dictyostelium mhc*A gene has been cloned (*13, 14*) and sequenced (*15*). This 6.3-kb gene exists as a single copy and has no introns. The restriction map of an 8-kb genomic fragment containing the *mhc*A gene is shown in Fig. 1.

In order to explore the use of *Dictyostelium* as a eukaryotic expression system for myosin and its subfragments, we constructed a clone encoding a *Dictyostelium* myosin fragment equivalent to muscle heavy meromyosin (HMM). Muscle HMM is a proteolytic fragment of muscle myosin in which the heavy chain has been cleaved to about 130 to 150 kD. It consists of two globular heads and 400 to 600 Å of α-helical coiled-coil rod (*16*). HMM cannot assemble into thick filaments. The *Dictyostelium* myosin fragment expected to be expressed from the clone should give rise to an HMM-like molecule of 140 kD with a tail of about 500 Å [the length of the native *Dictyostelium* myosin tail is 1800 Å (*17, 18*)]. We refer to this expressed fragment as HMM-140.

The clone was constructed by deletion of 2.7 kb from the 3′ end of the coding portion of the *mhc*A gene. The deleted fragment codes for the 892 carboxyl-terminal amino acids of the myosin tail, and we term this fragment LMM (with analogy to light meromyosin of muscle myosin). The deletion was created with the use of a single-stranded copy of the 8-kb genomic fragment containing the *mhc*A gene in the vector pTZ-18 (Fig. 1).

A transformation plasmid carrying the HMM-140 coding region was constructed with the *Dictyostelium* transformation vector pA15TX (*19*). This plasmid is a pBR322 derivative that contains the neomycin phosphotransferase gene driven by the *Dictyostelium* actin-15 promoter and terminated by the *Dictyostelium* actin-15 terminator. This plasmid was chosen because it can replicate autonomously in *Escherichia coli*, but not in *Dictyostelium*. Therefore, any stable transformants would have to be the result of an integration event.

The HMM-140 construct was cloned into the transformation vector (Fig. 2). The new plasmid, pNEO-HMM140, contains 1.5 kb of 5′ flanking sequence, 3.6 kb of coding sequence (HMM), and 0.3 kb of 3′ flanking sequence of the *mhc*A gene.

Transformation of *Dictyostelium*. The transformation of *Dictyostelium* (*20*) was carried out with AX4 cells with modifications of the protocol described by Knecht *et al.* (*21*). AX4 cells were attached to plastic petri dishes in bis-tris-HL5 (*p*H 7.1), allowed to be in contact with the calcium phosphate precipitate of the transforming plasmid, and then treated with glycerol, as described (*21*). Penicillin at 100 U/ml and streptomycin at 100 μg/ml were present in all media, unless otherwise indicated. The medium was removed carefully by aspiration to avoid loosening the attached cells and replaced with bis-tris-HL5 (*p*H 7.1). The cells were allowed to recover overnight, and then the media was replaced with HL5 (*p*H 6.5) containing G418 at 10 μg/ml. After 3 days the media was replaced with fresh HL5 with G418. At the sixth day, colonies of transformants were visible under the microscope. These cells were harvested from the plate by pipetting and were washed in HL5 medium without antibiotics. Different amounts of transformants were plated onto SM plates (*22*) together with a lawn of live *Klebsiella aerogenes*. Plaques started to appear on the bacterial lawns after 2 to 3 days. Cells were harvested at that time from a plate containing about 500 plaques and then were washed free of remaining bacteria by several cycles of low-speed centrifugation and resuspension. During this nonselective growth period, the transformants that did not incorporate the plasmid into their genome lost the plasmid and became G418 sensitive. To select the cells that had stably incorporated the plasmid, 1×10^7 cells were plated again on plastic petri dishes and grown axenically under G418 selection for 6 to 8 days.

The cells in the population of transformants were cloned by dilution into microtiter wells. They were allowed to grow axenically under G418 selection until they formed colonies. Two distinct phenotypes of G418-resistant cells were apparent. A majority of the clones (70 percent) grew normally, and these cells had the normal

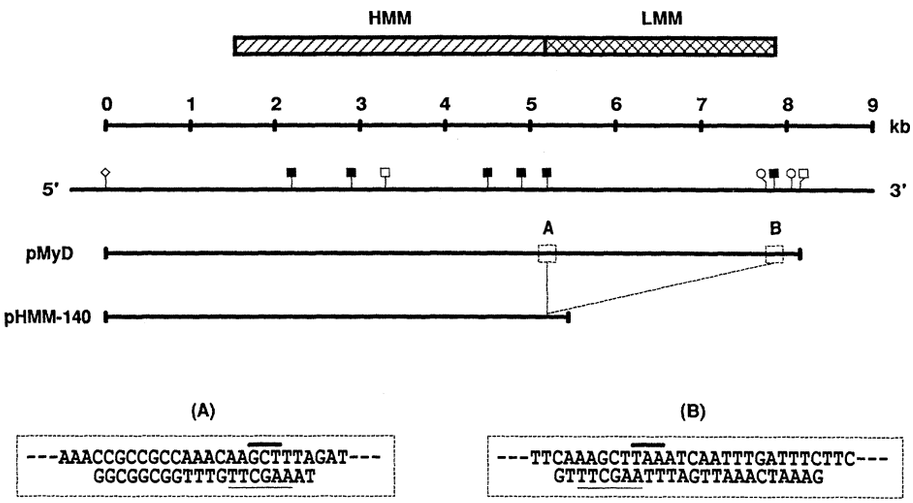

Fig. 1. Construction of the *Dictyostelium* HMM-140 clone. The bar at the top of the diagram represents the coding region of the *mhc*A gene divided into the HMM-140 portion (hatched bar) and the deleted portion (LMM) from the tail (crosshatched bar). The 8-kb Xba I–Bgl II fragment that covers the entire *mhc*A coding region and 5′ and 3′ flanking sequences was cloned into the vector pTZ-18 to give rise to the clone pMyD. A single-stranded copy of this plasmid was obtained as described (*28*). Two oligonucleotides were synthesized complementary to the sequences around the two Hind III sites indicated by the squares labeled A and B. The sequence of each oligonucleotide is shown at the bottom of the diagram, beneath the complementary sequence in the *mhc*A gene. The Hind III site is indicated by the line under both oligonucleotides. The oligonucleotides were annealed to the single-stranded pMyD clone and digested with Hind III. Digestion of this mixture can occur only at the Hind III sites in the regions of double-stranded DNA and not in the single-stranded regions. The digestion products were then ligated and introduced into *Escherichia coli*. Clones were screened for the deletion of the 2.7-kb Hind III fragment. This deletion causes the placement of the normal stop codon (indicated by a thick line in box B) and 3′ flanking sequence immediately after codon 1224 of the *mhc*A sequence (indicated by a thick line in box A) (*15*). The new clone, pHMM-140, is indicated below pMyD. Symbols: ◇, Xba; ■, Hind III; ○, Bcl I; and □, Bgl II.

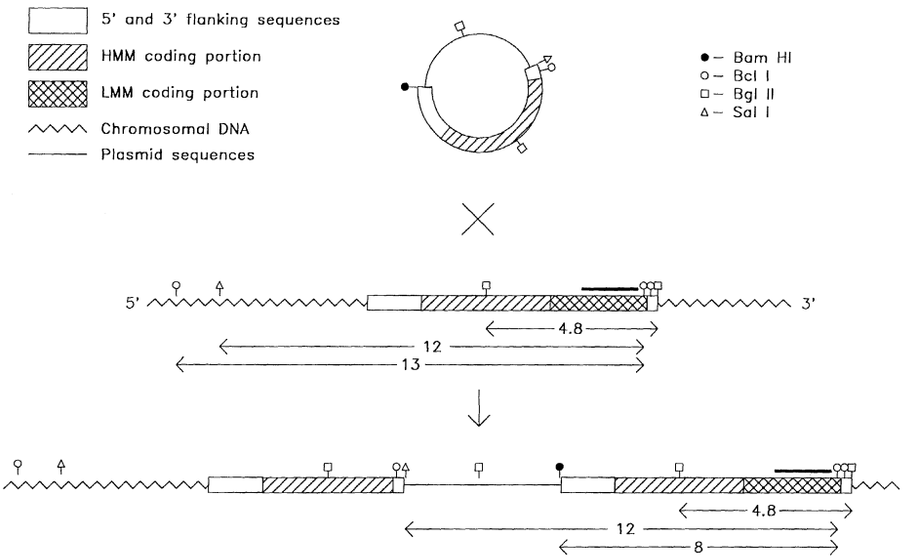

Fig. 2. Integration of pNEO-HMM140 into the *mhc*A gene. The circle represents the transformation plasmid pNEO-HMM140 that contains the HMM-140 coding region (hatched portion), the 5′ and 3′ flanking sequences (open portions), and the plasmid sequences of the vector pA15TX (continuous line). In the center of the diagram is the chromosomal region of the *mhc*A gene. The crosshatched portion represents the LMM coding region. Integration of one copy of pNEO-HMM140 into the *mhc*A locus would result in the map indicated below. The horizontal arrows indicate the size in kilobases of the restriction fragments that can be detected with a 1.5-kb probe (thick line) directed to the LMM coding portion. The restriction pattern of the *mhc*A locus with insertion of more than one plasmid can be easily predicted from this figure.

size and shape characteristics of the wild-type AX4 cells. We refer to these cells as transformants with a normal phenotype. A minority of the clones (30 percent) grew abnormally and were phenotypically aberrant in that the cells were large and multinucleated (Fig. 3). We refer to these transformants as *Dictyostelium hmm* cells.

Homologous recombination into the *mhc*A gene. Restriction maps of the *mhc*A gene from *Dictyostelium* wild-type clones and from several independent *Dictyostelium hmm* clones were generated using two different probes. The first probe hybridized with the LMM portion of the *mhc*A gene (Fig. 2). This portion is not present in the transformation plasmid, and thus it served as a specific probe for the *mhc*A locus. The second probe (the Xba I–Bcl I fragment in Fig. 1) hybridized with the entire gene.

The map obtained by hybridizing the LMM probe to the restriction digests of wild-type *Dictyostelium* DNA revealed a 4.8-kb Bgl II fragment, a 12-kb Bcl I–Sal I fragment, and a 13-kb Bcl I–Bam HI fragment (Fig. 4A, lanes a to c), as predicted in Fig. 2. This restriction pattern was altered in *Dictyostelium hmm* cells in that the Bcl I–Bam HI fragment shifted to 8 kb (Fig. 4A, lane f). This altered pattern is precisely that predicted if at least one copy of the plasmid pNEO-HMM140 integrated by homologous recombination into the *mhc*A gene (see Fig. 2).

With the Xba I–Bcl I probe, the pattern obtained in *Dictyostelium hmm* cells was again that predicted from a homologous recombination event (Fig. 4B). It is apparent from the intensity of the bands in the lanes from the transformed cells that they contain multiple copies of the plasmid pNEO-HMM140. The bands that derived from the plasmid (4.2- and 5.5-kb Bgl II, 9.8-kb Bcl I–Sal I, and 5.4-kb Bcl I–Bam HI) were darker than the bands that derived from the single copy of the disrupted *mhc*A gene (4.8-kb Bgl II, 12-kb Bcl I–Sal I and 10-kb and 7.7-kb Bcl I–Bam HI).

It has been shown (*21, 23*) that *Dictyostelium* transformation vectors integrate into the genome in tandem arrays ranging from a few up to several hundred. Our results are consistent with this observation. Comparison of the intensity of the multicopy bands with the single-copy bands for the three different restriction digests indicated that there are between five and ten copies of pNEO-HMM140 in the transformed cells.

The *Dictyostelium hmm* cells transcribe the integrated gene fragment. Analysis of RNA from wild-type *Dictyostelium* AX4 cells with the Xba I–Bcl I probe (Fig. 1) revealed a single messenger RNA (mRNA) species of about 7 kb, corresponding to the native *mhc*A mRNA (Fig. 5, lane a) (*13*). This 7-kb mRNA was absent from the *Dictyostelium hmm* cells where a new mRNA species of about 4.3 kb, corresponding to the HMM-140 mRNA, was present (Fig. 5, lane b). The HMM-140 mRNA comigrated with the 26*S* ribosomal RNA (4.1 kb) and thus, because of overloading, was not resolved as sharply as was the *mhc*A mRNA. The bands above the *mhc*A mRNA and the HMM-140 mRNA (Fig. 5) are due to contamination of the total RNA preparations with genomic DNA.

Integration of the plasmid into the *mhc*A locus results in the rearrangement of coding and 5′ flanking sequences (Fig. 2). The HMM-140 coding sequence is now downstream of the chromosomal 5′ flanking sequences and the *mhc*A coding region is downstream of the cloned 1.5 kb of 5′ flanking sequence. If the 1.5-kb fragment were active as a promoter, then the *Dictyostelium hmm* cells should contain mRNA's for both the *mhc*A and HMM-140 genes. The lack of expression of full-length *mhc*A mRNA in the *Dictyostelium hmm* cells indicates that the cloned 1.5 kb of 5′ sequence included in the transformation plasmid is not transcriptionally active. A possible explanation for this result is that sequences upstream of the 1.5-kb fragment are required for promoter function in a way similar to the enhancer elements described in other systems (*24*). Alternatively, it could be that the promoter for the HMM has suffered a mutation during clone manipulation that has rendered it inactive.

***Dictyostelium hmm* cells express the myosin fragment carried by the plasmid.** A polyclonal antibody specific for *Dictyostelium* myosin was used for protein analysis by Western blots of the various *Dictyostelium* strains (Fig. 6). Wild-type *Dictyostelium* expressed a single protein species of 243 kD, corresponding to the native myosin heavy chain (*13*) (Fig. 6, lane a). In contrast, the *Dictyostelium hmm* cells lacked the myosin heavy chain and instead expressed a new protein species of 140 kD, corresponding to the expected size of the HMM-140 encoded protein (Fig. 6, lanes b and c). Twenty clones of the *Dictyostelium hmm* phenotype were analyzed; all expressed HMM-140 and lacked the native myosin heavy chain. In contrast, 32 clones of transformants with the normal phenotype expressed only native myosin heavy chain and no HMM-140 (Fig. 6, lane d). This latter result is consistent with the lack of integration of the plasmid in the *mhc*A locus, and with the lack of a functional promoter in front of the HMM-140 gene in the plasmid. The apparent inability of the 1.5 kb of 5′ flanking sequence to direct expression of the HMM-140 construct is surprising and merits further consideration.

Recombination frequency of the transforming plasmid. The

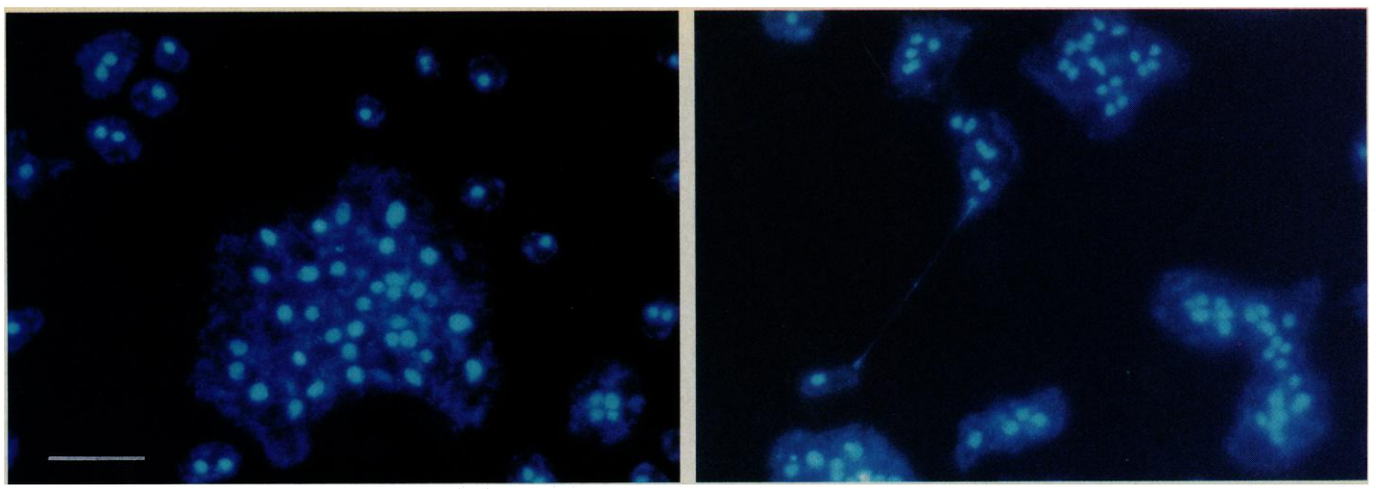

Fig. 3. Morphology of the *Dictyostelium hmm* cells. *Dictyostelium hmm* cells were allowed to attach to tissue culture chamber slides (Miles Scientific), fixed for 5 minutes in 1 percent formaldehyde in methanol at −15°C, rinsed for 5 minutes three times in phosphate-buffered saline (PBS) [50 m*M* sodium phosphate (*p*H 7.2), 150 m*M* NaCl], stained for 10 minutes in a fresh solution of DAPI at 0.1 μg/ml, and rinsed in PBS as before. The bar represents 25 μm.

fraction of cells (30 percent) that displayed the *hmm* phenotype in the experiment described above may not represent the true fraction of cells that underwent homologous recombination after transformation. It is possible, for example, that the *Dictyostelium hmm* cells, which have a growth disadvantage over the transformants with normal phenotype, initially constituted more than 30 percent. It is also possible that the *hmm* cells were enriched during some step of the transformation protocol. Given that there is a direct correlation between the homologous insertion of the plasmid and the expression of HMM-140 instead of native myosin heavy chain, we used the Western analysis as an assay to estimate the total fraction of homologous recombinants after transformation.

Dictyostelium AX4 cells were transformed as before, except that after the glycerol shock and recovery period the cells were diluted and plated into microtiter wells. In this way we scored the total number of transformants per transformation plate. Transformation efficiency ranged from 3×10^{-6} to 1×10^{-5}. Twenty six independent clones were analyzed by Western blot analysis and four showed the *hmm* phenotype, indicating a frequency of homologous recombination of 15 percent.

Given that these clones have not gone through the nonselective growth period, it is likely that some fraction of the G418-resistant clones that do not express HMM have not integrated the plasmid into their genome. Thus the frequency of homologous insertion among all the clones with integrated plasmids might be even higher.

Phenotype of the *Dictyostelium hmm* cells. The morphology of the *Dictyostelium hmm* cells is strikingly different from that of wild-type cells. The size of the *hmm* cells varies from one to ten times the diameter of wild-type cells. Staining of the *hmm* cells with DAPI showed that about half of the cells contain more than two nuclei, suggesting that they are defective in cytokinesis (Fig. 3). Time-lapse video microscopy was used to observe a population of *hmm* cells growing on a petri dish over a 48-hour period. Over this period, no

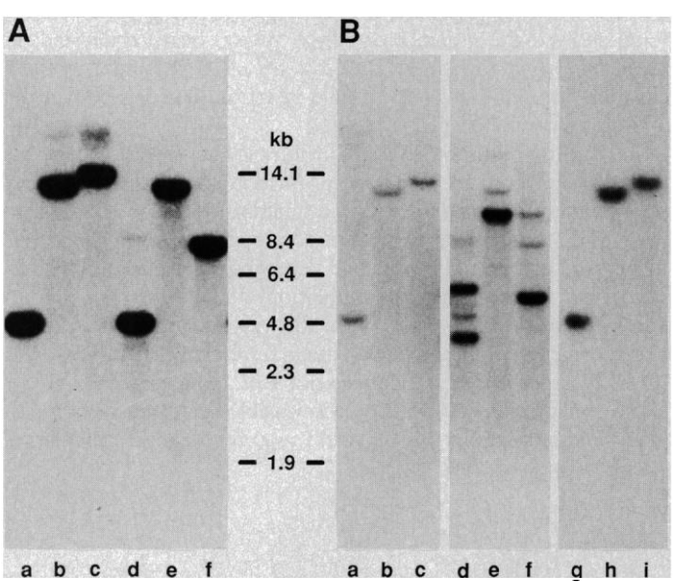

Fig. 4. Southern analysis of the *mhc*A gene in wild-type, *hmm*, and revertant cells. Total DNA from the indicated cells was digested with the indicated enzymes, separated on a 0.6 percent agarose gel, and transferred to nitrocellulose. The filter was hybridized to ^{32}P-labeled probes, washed, and exposed to x-ray film. (**A**) Hybridization with a 1.5-kb internal fragment of the LMM region indicated in Fig. 2. (**B**) Hybridization with the Xba I–Bcl I fragment indicated in Fig. 1. (lanes a to c) Wild-type cell DNA; (lanes d to f) *hmm* cell DNA; (lanes g to i) revertant cell DNA; (lanes a, d, and g) Bgl II digest; (lanes b, e, and h) Bcl I–Sal I digest; (lanes c, f, and i) Bcl I–Bam HI digest. A lighter exposure of the filter in (B) was scanned to determine the relative intensity of the single and multiple copy fragments.

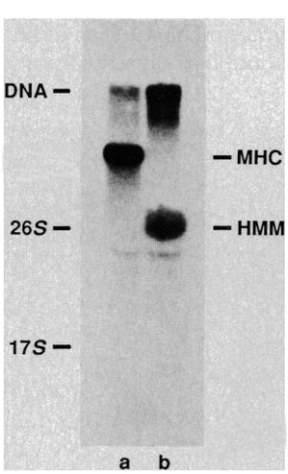

Fig. 5. Northern analysis of the myosin mRNA in wild-type and *hmm* cells. Total RNA was isolated from *Dictyostelium* wild-type and *hmm* cells as described (*20*). The RNA was separated on a 1 percent formaldehyde-agarose gel and transferred to nitrocellulose. The filter was hybridized with the same probe as in Fig. 4B. (Lane a) Wild-type cell RNA; (lane b) *hmm* cell RNA.

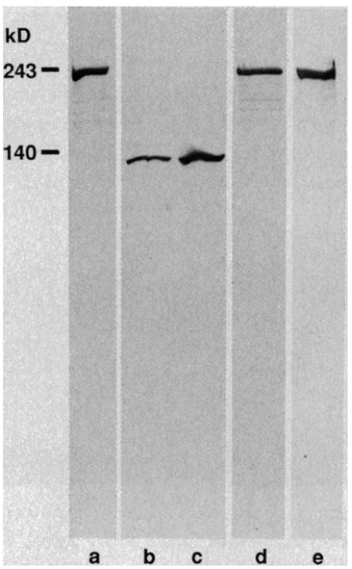

Fig. 6. Western analysis of wild-type, *hmm* and revertant cells. The indicated cells were grown axenically, washed, and lysed at a density of 10^7 cells per milliliter in a solution composed of 50 m*M* tris-Cl (*p*H 7.5), 20 m*M* sodium pyrophosphate, 5 m*M* EGTA, 5 m*M* EDTA, 1 m*M* phenylmethylsulfonyl fluoride, leupeptin at 1 μg/ml, pepstatin at 1μg/ml, 10 m*M* *p*-toluenesulfonyl-L-arginine methyl ester, 20 m*M* $NaHSO_3$, and 0.5 percent Triton X-100. The lysate was diluted 1:1 with SDS sample buffer and 20 μl of each sample was subjected to electrophoresis on a (7.5 percent running and 4 percent stacking) SDS-polyacrylamide gel. The proteins in the gel were transferred to nitrocellulose paper and incubated with a 1:1000 dilution of a polyclonal antiserum to *Dictyostelium* myosin (*29*). The filter was incubated with horseradish peroxidase–conjugated goat antiserum to rabbit antibody and then developed with 4-chloro-l-naphthol and hydrogen peroxide. (Lane a) Wild-type cells; (lane b) vegetative *hmm* cells; (lane c) developed *hmm* cells at the stage shown in Fig. 7; (lane d) transformed cells with normal phenotype; (lane e) revertant cells. The sensitivity of the polyclonal antibody was assessed by processing different amounts of wild-type cells on an identical gel. Myosin from as little as 100 cells could be detected in this experiment. This result indicates that there is less than 0.1 percent of contaminating myosin heavy chain in the *hmm* cells.

cytokinesis was observed in the *hmm* cells, whereas control wild-type cells underwent division several times over a similar period of time. Surprisingly, the *hmm* cells displayed a wide variety of cell movements, including dynamic membrane ruffling with extensions and retractions of filopodia and lamellopodia. With a periodicity that may correlate with nuclear division, the *hmm* cells were seen to round up and almost detach from the substratum and then flatten again to their maximum diameter. Sometimes a cell was seen "pinching off" a piece of cytoplasm that presumably was able to grow as an independent cell if a nucleus was included (Fig. 3, right panel). This phenomenon was only observed for cells that were flat and firmly attached to the substratum. It is possible that the population of *hmm* cells grows on plates by this type of illegitimate cell division. The observation that *hmm* cells, unlike wild-type cells, can only grow when attached to plates and not in a shaking culture is consistent with this hypothesis.

The response of the *hmm* cells to starvation conditions was studied by a modification of the streaming assay (*25*). *Dictyostelium hmm* or wild-type cells were grown axenically on plastic petri dishes until they reached confluency. The media and unattached cells were then removed carefully by aspiration and replaced with starvation buffer. The cells were then observed by time-lapse video microscopy. Wild-type cells start emitting cyclic AMP pulses around 6 to 8 hours of development and become elongated and polarized (*5*). Waves of cell shape changes, correlated with the cyclic AMP pulses, were seen in the time-lapse video. By 16 hours of development they formed large streams of cells (Fig. 7). However, the *hmm* cells exhibited delayed development. Around 16 to 24 hours, they displayed the waves of cell shape changes that presumably correlate with the cyclic AMP pulses. At 30 hours broken streams of cells were apparent (Fig. 7). Protein analysis by Western blot of the *hmm* cells at this stage showed that they contained HMM-140 and not myosin heavy chain (Fig. 6, lane c), indicating that the chemotactic aggregation of the *hmm* cells does not require an intact myosin molecule.

When *Dictyostelium hmm* cells were placed on the edge of a bacterial lawn on an agar plate, they advanced across the plate as they fed (Fig. 8). The rate at which the *hmm* cells advanced on the plate was considerably slower than that of wild-type cells. In the area behind the advancing edge, the cells starved and initiated their developmental program. They aggregated into multicellular mounds and these aggregates failed to differentiate further (Fig. 8).

Reversion of the *hmm* phenotype. An important observation was the appearance in the bacterial-lawn cultures of sectors that grew much faster than the rest of the leading edge. These areas gave rise to normal fruiting bodies (Fig. 8). When the spores from these fruiting bodies were germinated, they gave rise to cells with normal appearance and size. Four out of five of these apparent phenotypic revertants, each one arising from different *hmm* cell clones, proved to be G418 sensitive. One interpretation consistent with these results is that these revertants represent excision of the transforming plasmid from the genome and restoration of endogenous myosin heavy chain gene function. Restriction maps of the *mhc*A gene from three such revertants showed that the bands corresponding to the transforming plasmid are absent and that the wild-type pattern has been restored (Fig. 4B, lanes g to i). Western blot analysis of these clones showed the presence of native myosin heavy chain and no HMM-140 (Fig. 6, lane e). The loss of G418 resistance in these revertants implies that only a single insertion had occurred in the initial transforming event.

Concluding remarks. Should the homologous recombination we describe here prove to be a general phenomenon in *Dictyostelium*, the assessment of gene structure and function would be accessible in a system that undergoes a complex developmental pathway.

The potential that the observed reversion of the recombination process has for the genetic manipulation of *Dictyostelium* is significant. It may soon be possible to develop a system in *Dictyostelium* for specific gene deletion and replacement similar to that in yeast (*8*). Although other investigators have not seen insertional recombination events in *Dictyostelium* (*20*), it is possible that frequent homologous recombination in this organism requires a considerably larger

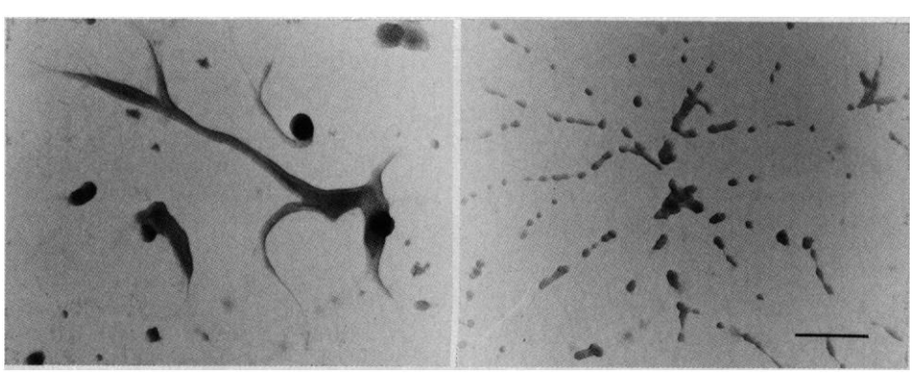

Fig. 7. Aggregation of wild-type and *hmm* cells. Wild-type (left) and *hmm* (right) cells were grown axenically on a plastic petri dish until they reached saturation. The medium and unattached cells were removed by aspiration and replaced with starvation buffer [20 m*M* 2(*N*-morpholino)ethanesulfonic acid (*p*H 6.8), 0.2 m*M* $CaCl_2$, 2 m*M* $MgSO_4$] (*29*). The bar represents 1 mm.

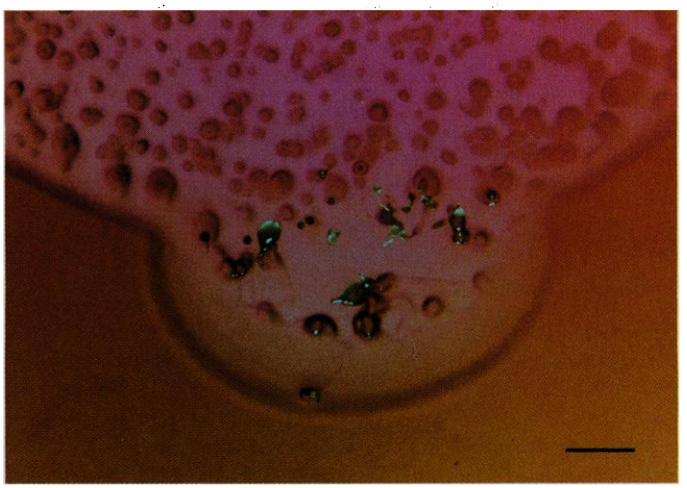

Fig. 8. Reversion of the *hmm* phenotype. *Dictyostelium hmm* cells were grown on an agar plate with a lawn of *Klebsiella aerogenes*. In this photograph the inoculum of *hmm* cells was at the top. As the cells fed on the bacteria, they advanced downward. The upper portion of the photograph shows the region where the *hmm* cells have depleted the bacteria and formed aggregates that did not develop further. A reversion event, recorded in the center of the photograph, is visible as an outgrowth from the leading edge. The reversion gave rise to cells that grew faster than the *hmm* cells and formed normal fruiting bodies. The bar represents 1 mm.

region of homology than that needed in yeast (*26*). The pNEO-HMM140 plasmid contains a large fragment (5.4 kb) of DNA that is homologous to the *mhc*A gene. It is also possible that within the 5.4-kb fragment there are sequences that induce a higher frequency of homologous recombination.

For those interested in the role of myosin in cell motility and development, this genetic approach has many advantages. We have demonstrated that the carboxyl-terminal 1300 Å of the 1800 Å myosin tail are not required for several forms of cell movement. Although the *hmm* cells are capable of chemotaxis and phagocytosis, their ability to carry out these functions is significantly impaired. It is clear that proper cytokinesis and normal development require the intact myosin molecule, but karyokinesis does not.

Although we have not established whether the HMM-140 being produced by the transformed *Dictyostelium* strains is functional, it is possible that some of the types of movements we observe in the *Dictyostelium hmm* cells require HMM-140. Knecht and Loomis (*27*), in experiments that preceded those we reported above, used a different approach to produce a *Dictyostelium* strain that lacked myosin. They eliminated the expression of myosin heavy chain by generating antisense RNA against the *Dictyostelium mhc*A mRNA. The resulting cells survive and become large and multinucleated due to a block in cytokinesis. Detailed comparisons of the behavior of these two different cell types, one that is essentially devoid of myosin heavy chain and one that contains the myosin heavy chain fragment HMM, are needed. Furthermore, using homologous recombination, one can now integrate other forms of myosin, and myosin or HMM modified in specific ways by site-directed mutagenesis, into the *Dictyostelium* genome and examine the resulting phenotypes of the cells.

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