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Differential Action and Differential Expression of DNA Polymerase I during *Escherichia coli* Colony Development

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A mini-Tn*10* insertion in the *polA* cistron (*polA2099*) was isolated in a search for mutations that affect patterned Mud*lac* replication in colonies. The *polA2099* mutation had a dramatic effect on cell morphogenesis during the first few hours of microcolony development. Abnormal microcolonies containing filamentous cells were produced as a result of SOS induction. Despite gross abnormalities in early microcolonies, mature *polA2099* colonies after 2 to 4 days were morphologically indistinguishable from Pol$^+$ colonies, and 44-h *polA2099* colonies displayed a cell size distribution very similar to that of Pol$^+$ colonies. These results suggested the involvement of a protective factor produced during colony growth that compensated for the *polA* deficiency. The action of a diffusible substance that stimulates growth of *polA2099* microcolonies was shown by spotting dilute *polA2099* cultures next to established colonies. Differential transcription of *polA* during colony development was visualized by growing colonies containing *polA-lacZ* fusions on β-galactosidase indicator agar. When *polA-lacZ* colonies were inoculated next to established colonies, a diffusible factor was seen to inhibit *polA* transcription during the earliest stages of colony development. These results show that a basic housekeeping function, DNA polymerase I, is subject to multicellular control by the changing conditions which the bacteria create as they proliferate on agar.

There has been a growing awareness that in nature most bacteria exist in multicellular populations. In soil, for example, bacteria proliferate to form adherent biofilms and microcolonies (26). Under laboratory conditions, bacteria growing on agar plates form highly organized, differentiated colonies (17). The intricate structures of these colonies result from interactions between the component bacterial cells. These multicellular interactions are not limited to a few exotic species with specialized social behavior but in fact are exhibited by the classical objects of bacteriological research, such as *Escherichia coli* K-12 (20). Thus, if we are to understand prokaryotic biology in a comprehensive way, we need to investigate the populational aspects of control systems. The phenomena that we find to operate in colonies reveal some of the regulatory systems available to bacteria at different stages in their life histories.

E. coli colonies display both clonal (sectorial) and nonclonal (concentric) patterns of cellular differentiation and multicellular aggregation (17). Clonal and nonclonal patterns of differential gene expression can be visualized on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) indicator agar by utilizing *lacZ* fusion technology (15). The significance of these patterns is that spatially and temporally coordinated cellular differentiation occurs during colony development in response to changing conditions created by the bacteria themselves. The nonclonal patterns reflect the operation of control systems that coordinate the activities of cell groups which are not connected by common descent.

One interesting question concerns the behavior of basic housekeeping functions during colony development. Are they expressed constitutively, or are they also subject to multicellular control? Serendipitously, research in this laboratory provided an answer to this question for *E. coli* DNA polymerase I (PolI). During a study of periodic Mud*lac* replication-transposition (19), a *polA*::mini-Tn*10* mutation was isolated. Examination of the phenotypes related to this mutation extended previous observations on *polA* regulation (23) and revealed the control of PolI by one or more diffusible substances. In addition, the phenotypic effects of the *polA* lesion on cell division changed dramatically during growth on agar: filaments were produced in the first few hours, but filamentation was suppressed after 1 day. Thus, abnormal microcolonies produced normal colonies, indicating that there is no simple, fixed relationship between cell morphogenesis and colony morphogenesis in *E. coli* development.

MATERIALS AND METHODS

Bacterial strains. The standard *E. coli* K-12 strain used in this laboratory for studies of colony morphogenesis, M7124, is an F$^-$ *thi* Δ(*argF-lac*)*U169* descendant of Hfr3000 (1). MS1534 is an M7124 derivative that carries the MudII1681 replication-competent *lacZ* fusion element (3) near the *thyA* locus. At this position, the MudII1681 *lac* sequences are not expressed but β-galactosidase expression occurs during MS1534 colony development because cells are formed periodically in which the Mu*cts62* repressor is inactivated so that the MudII1681 element replicates and creates active *lacZ* fusions (19). Mini-Tn*10* insertions that affect β-galactosidase expression because of MudII1681 replication were obtained by infecting MS1534 with the λ1098 vector (24), plating on glucose–minimal salts–X-Gal indicator agar containing 20 μg of tetracycline per ml, and screening for colonies with altered staining patterns. MS2099 is one such mutant that harbors the *polA2099*::mini-Tn*10* allele.

λ*imm-21* bacteriophages carrying *polA-lacZ* translational fusions 14 and 24 were obtained from N. Murray (23). The fusions joined *polA* and hybrid *trpA-lacZ* open reading frames at distinct positions, but they gave rise to indistin-

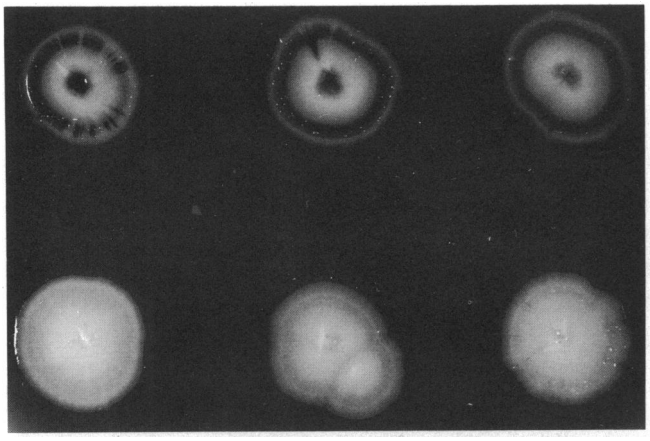


FIG. 1. Phenotype of Pol$^+$ and *polA2099*::mini-Tn*10* MudII1681 strains on X-Gal indicator agar. These colonies developed from toothpick inoculations and were incubated at 32°C and then at room temperature for 15 days prephotography.

guishable β-galactosidase colony staining phenotypes. To ensure that *lacZ* expression patterns in lysogens were not influenced by prophage induction and N-dependent transcription from the phage p_L promoter, λ*imm cII2003Nam213* recombinants of the fusion phages were isolated and lysogenized into M7124 with a λ *b2 red* helper phage to produce strains MS3051 and MS3052. Strain G1044, carrying the Δ*crp*::*CM* substitution that inactivates the cyclic AMP (cAMP) receptor protein and a Δ*cya* deletion that inactivates adenylate cyclase linked to an *ilv*::Tn*10* insertion, was obtained from S. Adhya of the National Cancer Institute, and strain GC3401, carrying the *sfiA*::Mud*Ap lac* insertion (8), was obtained from R. D'Ari of the Institut Jacques Monod, Paris, France. These mutations and the *polA2099*::mini-Tn*10* allele were each transduced into various strains with phage P1 and selection for (respectively) resistance to chloramphenicol (10 μg/ml), carbenicillin (25 μg/ml), and tetracycline (20 μg/ml). The phenotypes of the transductants were always verified by testing for microcolony phenotype and methyl methanesulfonate-UV sensitivity (*polA2099*::mini-Tn*10*), carbon source utilization (Δ*crp*::*CM* and Δ*cya*), and mitomycin C induction of β-galactosidase (*sfiA*::Mud*Ap lac*).

Kohara phage mapping of mini-Tn*10* inserts. The inserts in MS2099 and some other mini-Tn*10* mutants were mapped by Kohara phage clone transduction with testing for homologous recombination into the fragments cloned in one phage of the Kohara set (11). The entire set of overlapping phage clones was tested on six replica plates. Lawns of M7124 were infected with small spots of lysates of each of the Kohara phage clone set on tryptone (1%)-yeast extract (0.5%)-NaCl (0.5%) (TYE agar). After overnight growth, these plates were copied by replica plating onto fresh M7124 lawns, incubated, and then replica plated to TYE-tetracycline (20 μg/ml) agar spread with a lawn of a mini-Tn*10* strain. These plates were incubated to produce lawns containing spot lysates of each Kohara phage grown on the mini-Tn*10* strain and then replica plated to TYE-streptomycin (250 μg/ml) agar spread with a lawn of an *rpsL* λ lysogenic derivative of M7124. The streptomycin plate was then replica plated to tetracycline-streptomycin agar to see whether any of the spots were capable of transducing the mini-Tn*10* Tc marker. Positive results were confirmed by spotting the corresponding Kohara phage and controls carrying other fragments on a soft-agar overlay of the mini-Tn*10* strain, incubating them, eluting phage from the lysis spots, and testing for Tc transduction by liquid infection of the lysogen.

DNA analysis. A 16-kb *Bam*HI fragment carrying the *polA2099*::mini-Tn*10* allele was cloned from MS2099 DNA into spectinomycin resistance pSC101-based vector pGB2 (4) in both orientations by using selection for Tc^r. We were able to use *Bam*HI for the cloning because the mini-Tn*10* (Tc) element in λ1098 has no sites for this enzyme, in contrast to later constructions of the same transposon which do have *Bam*HI sites just inside the terminal repeats. Sequencing of plasmid DNA was carried out with the U.S. Biochemicals Sequenase kit by using primers complementary to unique sequences just inside the inverted repeats at each end of mini-Tn*10*: MINITCLEFT = CAGTGATCCAT TGCTGTTGAC for reading in the direction of *tetR* transcription, and MINITCRIGHT = GGCACCTTTGGTCACC AACGC for reading in the direction of *tetA* transcription (see Fig. 2).

Microbiological methods, microscopy, and photography. In general, microbiological methods, microscopy, and photography were performed as described previously (15, 16, 20). β-Galactosidase indicator agar contained the chromogenic substrate X-Gal at 30 μg/ml, except for the plates illustrated in Fig. 10, where 60 μg/ml was used to facilitate visualization of *polA-lacZ* expression. Several of the figures illustrating colony surface structure were photographed by using ceiling fluorescent light fixtures for illumination. This technique can be used to record the structure and development of living colonies at many different magnifications without special equipment. A 35-mm camera fitted with a 100-mm macro

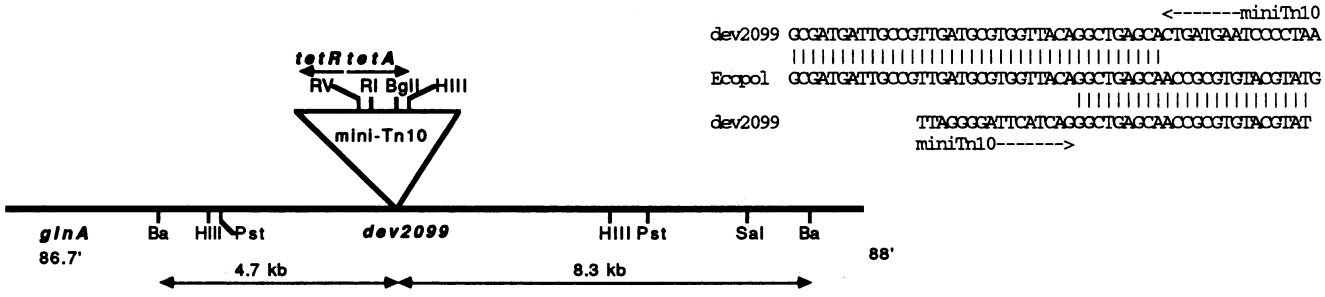


FIG. 2. Restriction map and sequence of the *polA2099*::mini-Tn*10* insertion. The map was derived from digests of plasmids pXJS3031 and pXJS3054 containing inserts of the 16-kb Tc^r *Bam*HI fragment cloned in each orientation from MS2099 DNA. The sequence CTGATGA ATCCCCTAA marks the left extremity of mini-Tn*10*, and TTAGGGGATTCATCAG marks the right extremity. Ecopol, *E. coli polA*.

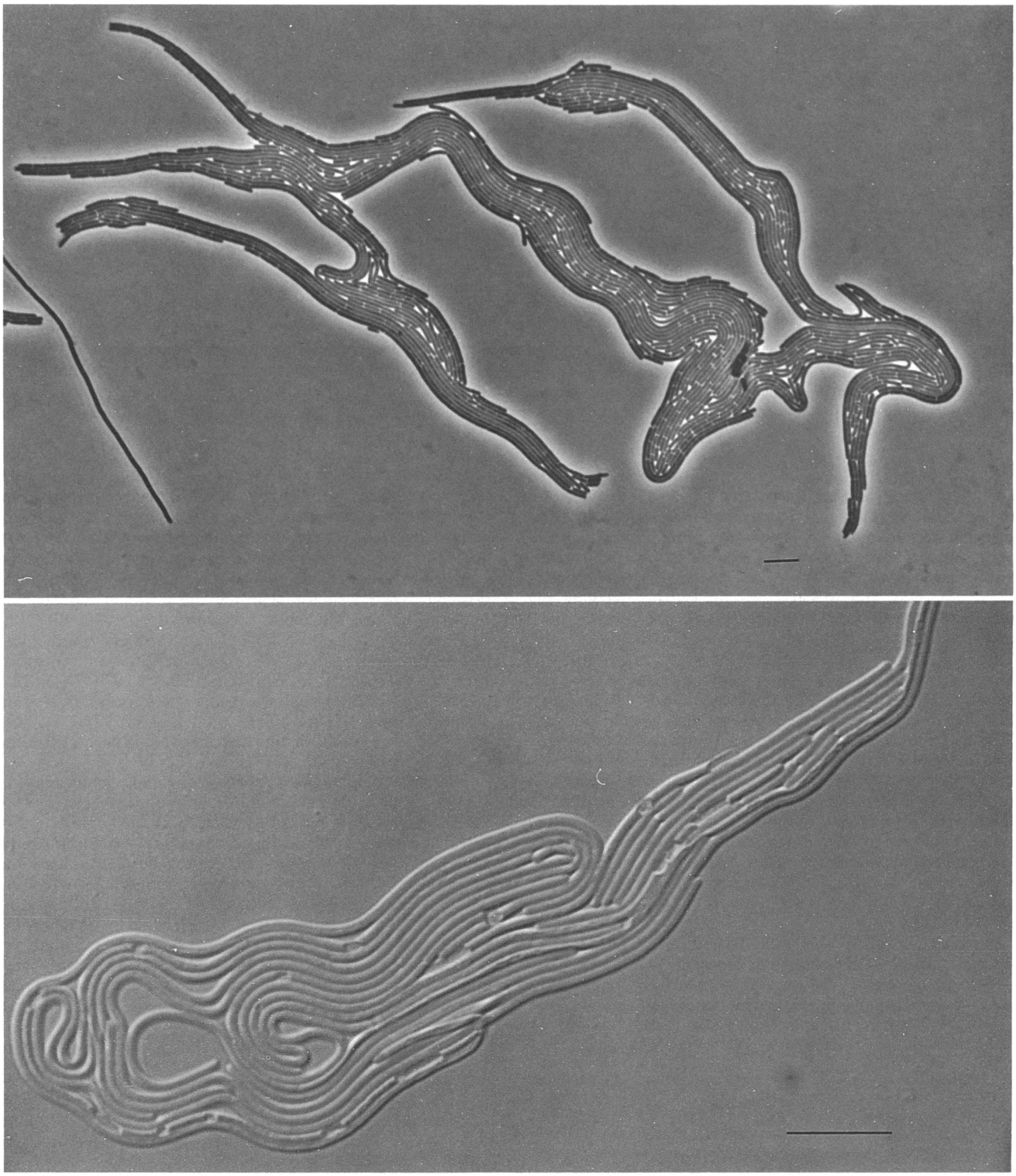


FIG. 3. Photographs of 6.5-h microcolonies of *polA2099*::mini-Tn*10* transductants of strain M7124. These microcolonies were grown on minimal-salts–glucose (0.4%) agar on a microscope slide under a coverslip at 37°C. Bars, 10 μm.

lens was mounted on a tripod so as to point down towards the petri dish at a sharp angle (10 to 20°C off the vertical). The position of the plate was adjusted so that the lid served as a mirror to reflect the image of the fluorescent ceiling fixture into the camera. The lid was then removed, and the camera was focused on the surface of the agar before the picture was taken. Since the surfaces of the agar and the colonies served as reflectors to illuminate the image, small

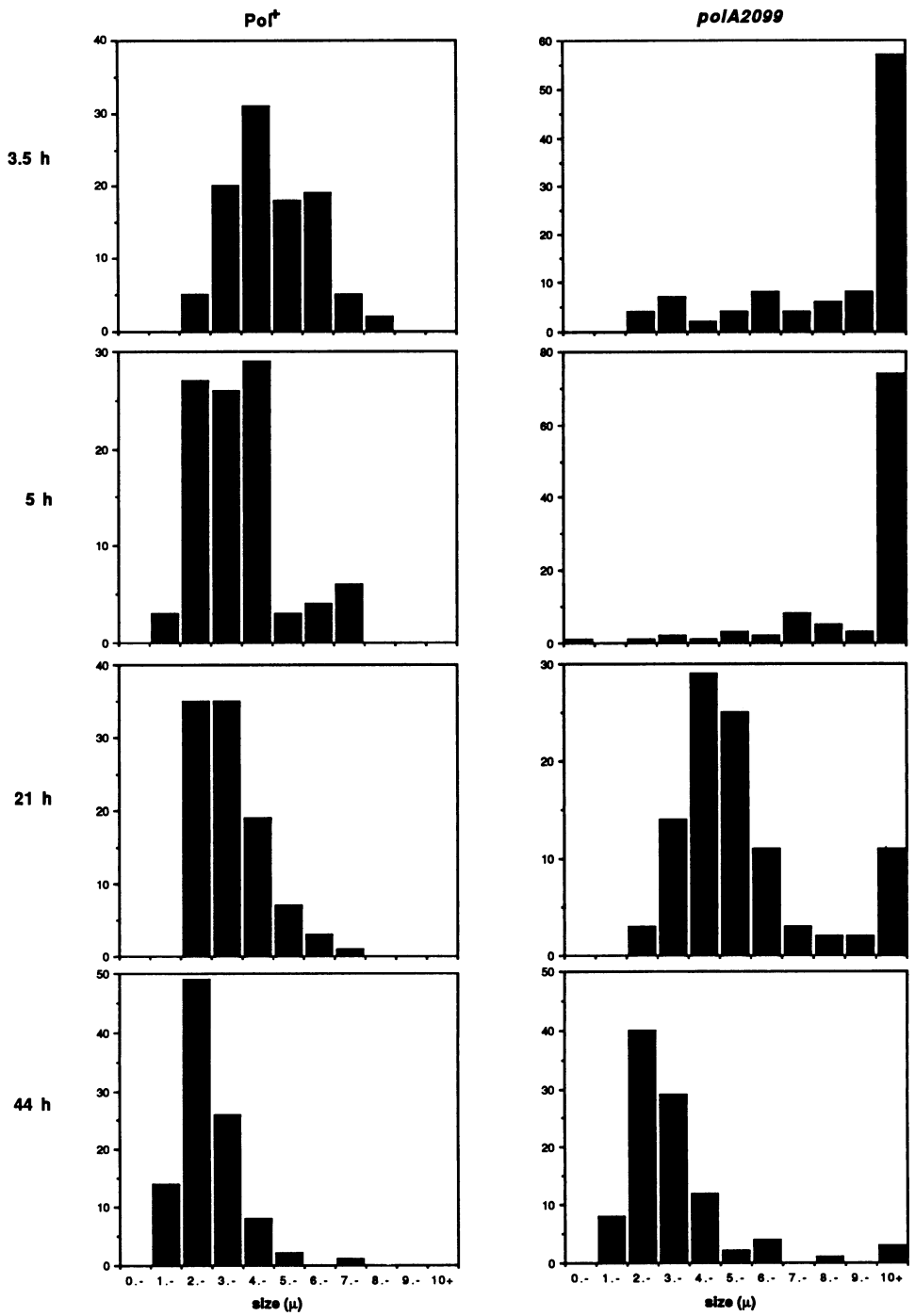


FIG. 4. Cell size distributions of Pol$^+$ and *polA2099* cells at different times during colony development. Cultures of M7124 and its *polA2099*::mini-Tn*10* transductant were inoculated on tryptone (1%)-yeast extract (0.5%)-NaCl (0.5%) agar and incubated for various times at 37°C before cells were suspended in sterile saline for microscopy. The 3.5- and 5-h samples were incubated as a lawn (i.e., as many close microcolonies which merge together, as described in reference 20) before resuspension. The 21- and 44-h samples were each taken from a single colony. The abscissa indicates cell sizes in 1-μm intervals, and the ordinate indicates numbers of cells measured in each cell length interval in a sample of 100 cells.

changes in surface angle and texture produced significant differences in contrast on the film. Cell length determinations were made by digitizing video images of phase-contrast micrographs (40× objective) with a QuickCapture frame grabber card (Data Translation, Inc., Marlboro, Mass.) attached to a Mac IIci computer and using the measurement tool of the *Image* program, version 1.22 (public domain software available from National Technical Information Service or via anonymous file transfer protocol from alw.nih.gov [128.231.128.251] in the directory/pub/image).

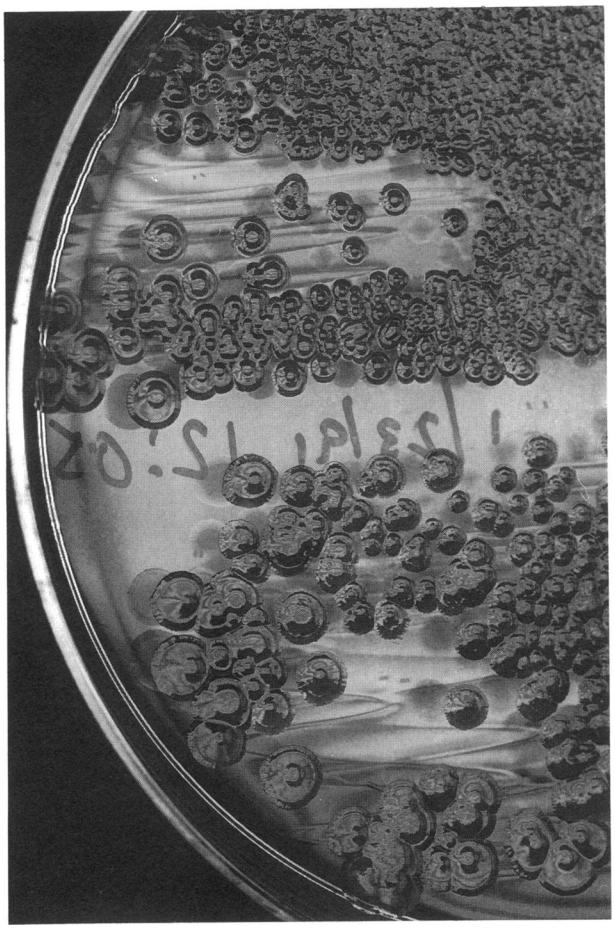


FIG. 5. Mature *polA2099* colonies are indistinguishable from Pol$^+$ colonies. The top streak is a Pol$^+$ strain, and the bottom streak is its *polA2099* transductant on minimal-salts–glucose–Casamino Acids agar after 4 days at 37°C.

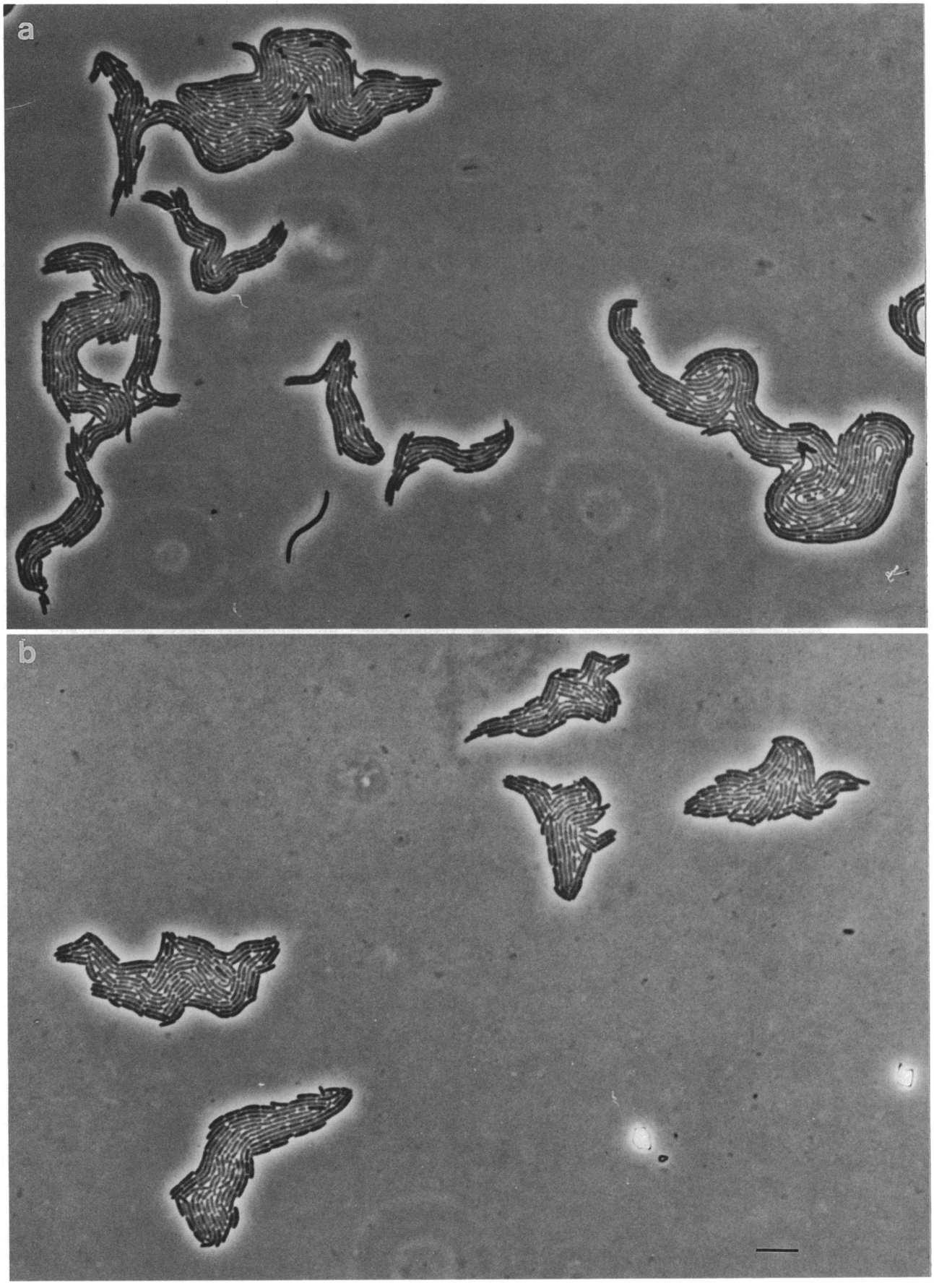
RESULTS

Isolation of the *polA2099*::mini-Tn*10* insertion. Colonies of *E. coli* strains harboring the transposition-competent MudII1681 *lacZ* fusion element display concentric rings of β-galactosidase expression on X-Gal indicator agar. These patterns are due to periodic formation of cell populations in which MudII1681 replication has been derepressed (19). To investigate the networks that regulate Mud*lac* activity during colony development, strain MS1534 was mutagenized by infection with λ1098, a mini-Tn*10* (Tc) vector (24). Among the mutants isolated was MS2099, which displayed severely inhibited MudII1681 replication (Fig. 1). The insert was mapped to the 87.2-min region of the *E. coli* chromosome by transduction with Kohara phage clone 547. Cloning of the MS2099 mini-Tn*10* insert, followed by restriction mapping and sequencing outwards through the mini-Tn*10* termini, showed that the transposon was inserted so that it duplicated *polA* base pairs 2604 to 2612 and created a TGA stop codon at bases 2 to 4 of the mini-Tn*10* terminus (Fig. 2; reference 10). The effect of this *polA2099*::mini-Tn*10* mutation was to remove the last 58 amino acids of the polymerase polypeptide. Strains carrying this insertion displayed increased UV and methyl methanesulfonate sensitivity, as expected for mutants defective in PolI activity. Even though the sequence indicates that the insertion should be capable of precise excision, strains carrying *polA2099*::mini-Tn*10* are remarkably stable when grown under a variety of conditions in the absence of tetracycline. Routine subculturing and purification of bacteria from colonies have not produced any Pol$^+$ Tc^s subclones.

The reason why *polA2099* has a strong negative influence on MudII1681 replication-dependent β-galactosidase synthesis in colonies is not known. Note that the MS2099 colonies in Fig. 1 display very faint LacZ expression at the appropriate position in the developing colonies, even after prolonged incubation. A delay in induced Mu*cts62* replication in a *polA* background has been reported (12), and a delay in the appearance of Mu*cts62*-dependent *araB-lacZ* fusion clones (21) has been observed in *polA2099* derivatives of the prefusion strain (unpublished data). But *polA* strains still produce 60 to 80% of normal Mu*cts62* yields after thermal induction (12), MS2099 is sensitive to thermal killing, and *polA2099* derivatives of the precursor strain for *araB-lacZ* fusions produce half as many fusion colonies as the standard precursor. Thus, Mu expression is not grossly dependent upon PolI activity, and it remains to be investigated whether the polymerase plays a regulatory role in MudII1681 derepression during colony development.

Colony development phenotypes of *polA*::mini-Tn*10* strains. Despite our ignorance about why the *polA2099*::mini-Tn*10* mutation inhibited MudII1681 derepression, it was possible to investigate the effects of this allele on colony morphogenesis, with interesting results. When the original MS2099 isolate or various transductant strains carrying *polA2099*::mini-Tn*10* were grown on agar medium, microcolonies characterized by filamentous cells were formed in the first few hours of growth. These microcolonies were generally very elongated and quite abnormal compared with microcolonies produced by Pol$^+$ strains (20). The abnormal cell length and microcolony phenotypes were expressed both on thin agar layers on microscope slides (Fig. 3) and on standard petri dish medium (Fig. 4). Despite the abnormal pattern of early microcolony development, mature *polA2099* colonies were structurally indistinguishable from PolA$^+$ colonies (Fig. 5), and the size distribution of cells taken from mature *polA2099* colonies was very similar to that of cells from PolA$^+$ colonies (Fig. 4). The filamentous growth in early stages of colony development appeared to be related to SOS induction, because introduction of an *sfiA*::Mud*Ap lac* insertion relieved abnormal cell and microcolony morphogenesis (Fig. 6 and 7). By comparing the cell size distributions of the young *polA2099* cultures in Fig. 4 and 7, it can be seen that there was a more extreme filamentation phenotype at 37°C than at 30°C, perhaps as a consequence of more rapid growth. Because of the stability of the *polA2099* mutation, we know that there was no selection for PolA$^+$ revertants during colony growth. Thus, the change in cell size was a

FIG. 6. Effect of the SOS cell division inhibitory function SfiA on *polA2099* microcolony development. The colonies shown were grown for 7.5 h at 30°C on tryptone (1%)-yeast extract (0.5%)-NaCl (0.5%) agar on a microscope slide under a coverslip. Panels: a, SfiA$^+$; b, *sfiA*::Mud*Ap lac*. Bar, 10 μm.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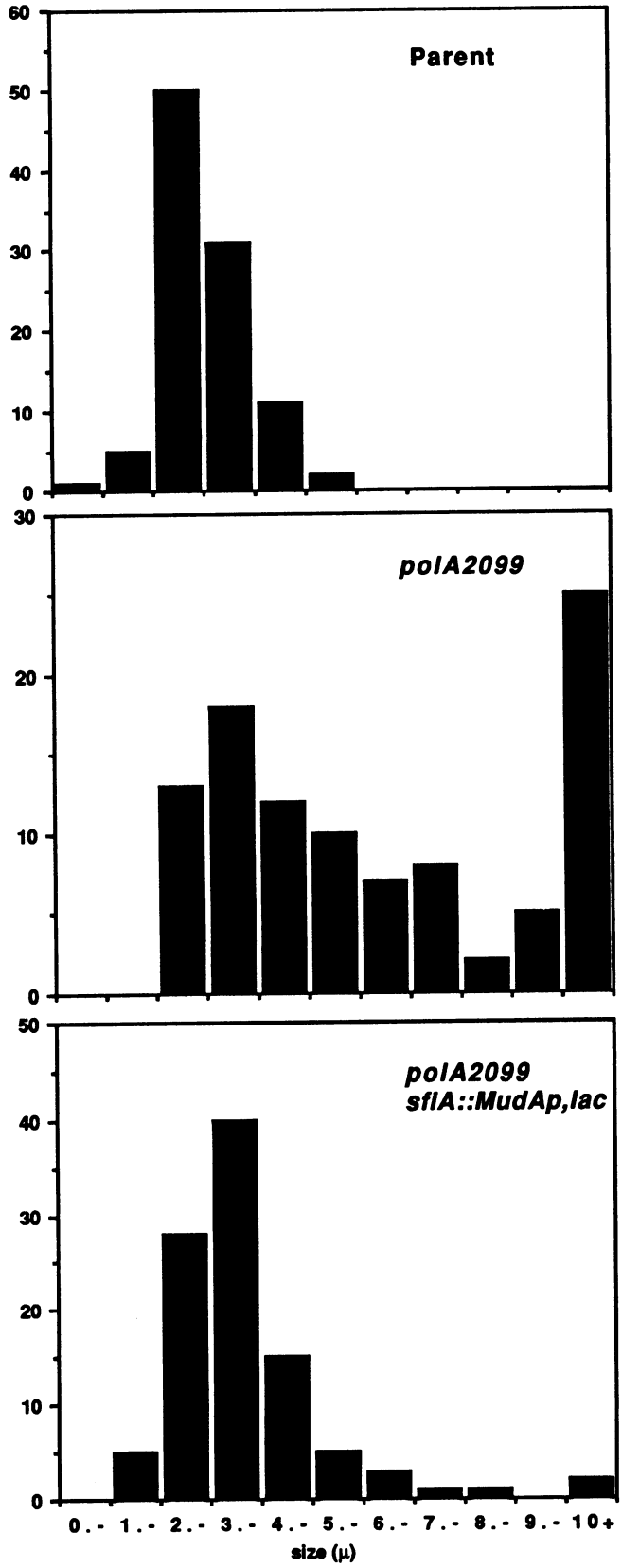

FIG. 7. Cell size distributions of Pol$^+$, *polA2099*, and *polA2099 sfiA*::Mud*Ap lac* cells in early microcolony development. Strains MS1534 (Pol$^+$), MS2099, and MS3103 (*sfiA*::Mud*Ap lac* transductant of MS2099) were incubated for 7.7 h at 30°C on tryptone (1%)-yeast extract (0.5%)-NaCl (0.5%) agar before measurement. For details see the legend to Fig. 4.

differential phenotype brought about by changing conditions within the developing colony.

A diffusible substance stimulates *polA2099* microcolony development. The finding that *polA2099* cells were indistinguishable from Pol$^+$ cells at later stages of colony development suggested that the colony might produce some substance which somehow compensated for the polymerase defect. This idea was investigated by inoculating 5-μl spots of *polA2099* cells at different distances from established colonies (generally about 2 days old) and then examining the spots periodically. In dozens of experiments, the result was always the same. The spots nearest the established colony displayed more extensive microcolony growth in the first 5 to 12 h of incubation, indicating that some substance diffusing from the established colony could facilitate *polA2099* proliferation (Fig. 8a). The microcolony morphologies and cell lengths were not readily distinguishable when stimulated regions near the established colony were compared with unstimulated regions far from the established colony, but a systematic study of the effect of the diffusible substance on cell length distributions was not performed. The results varied from experiment to experiment when the spots were examined after 1 or 2 days of incubation. In most cases, growth from the different spots was indistinguishable after 48 h, but on some plates the diffusible stimulatory effect could still be seen after longer incubation (Fig. 8b and c). No effect on Pol$^+$ microcolonies was discernible, indicating that a general stimulatory factor was not involved.

Since the test was begun to explain why cell morphogenesis changed within *polA* colonies as they got older, it was not surprising to find that either Pol$^+$ or *polA2099* colonies could exert the stimulatory effect (Fig. 8b and c). The presence of viable cells in the stimulatory colony was not necessary for the effect. If a block of sterile agar was excised from a petri dish next to an established colony, the block could also stimulate the growth of nearby *polA2099* spot inocula. The effect was not as strong as that seen with colonies, because the sterile agar was a weaker (and depleting) source of the stimulant, but spots next to the agar showed detectable growth while those farther away were almost invisible at low magnification in the first 12 h of incubation (Fig. 9). Thus, the diffusible substance was apparently stable enough to persist in the absence of a producer population.

Differential expression of *polA-lacZ* fusions. The differential requirement for PolI activity during colony development suggested the need to examine *polA* expression on agar. The original study of *polA-lacZ* fusions found no evidence for differential regulation in liquid culture (23). When noninducible N$^-$ derivatives of λ prophages carrying these same fusions were tested for expression during colony development on X-Gal agar, differential regulation was observed (Fig. 10). The colonies invariably displayed darker centers and lighter edges, and secondary rings of β-galactosidase activity were often seen. In addition, it was shown that *polA-lacZ* expression was inhibited by some diffusible signal emanating from an established colony (Fig. 11). The colony patterns of *polA-lacZ* fusion expression were the same in *polA2099* and Pol$^+$ backgrounds.

We and others have found that differential Mud*lac* repli-

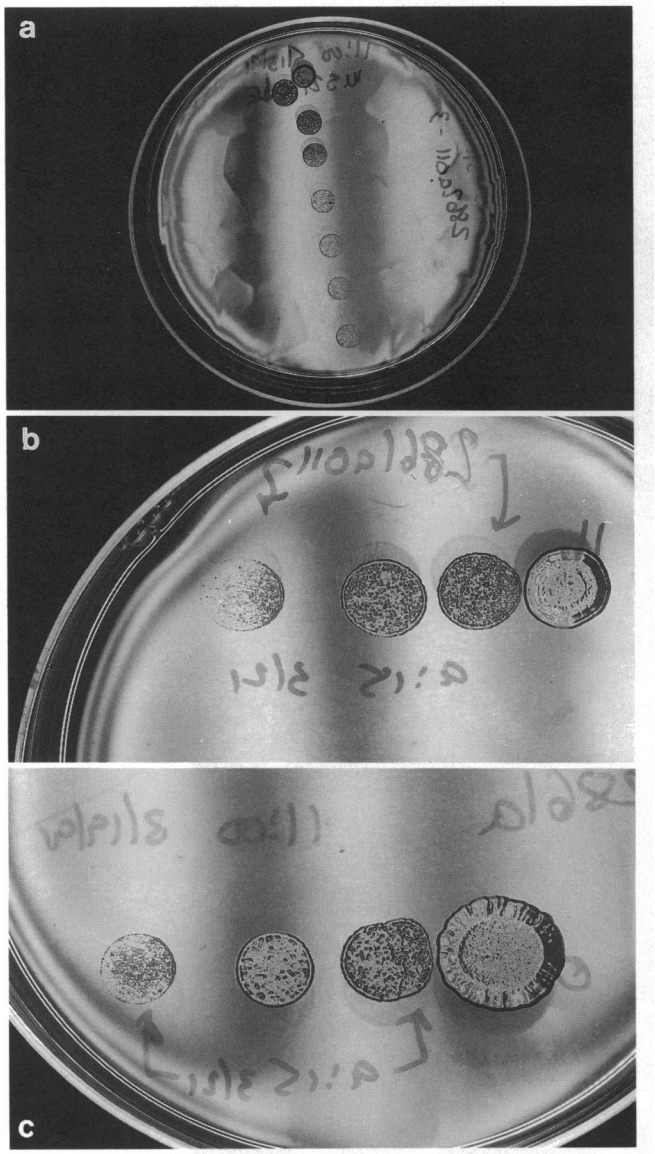

FIG. 8. Stimulation of *polA2099* microcolonies by a diffusible substance from an established colony on minimal-glucose (0.4%)–Casamino Acids (0.2%) agar. (a) The colony at the top of the plate was inoculated with a Δ*cya* strain 34.75 h prephotography. At 12.3 h prephotography, 5-μl aliquots of a 10^{-3} dilution of an overnight *polA2099*::mini-Tn*10* culture were spotted at different distances from the established Δ*cya* colony. The plate was incubated at 30°C. The Δ*cya* strain was tested because of the reported connection between the cAMP system and PolI activity (14) and to investigate the role of this global regulatory system on the production of diffusible signals that affect colony development. The adenylate cyclase defect did not affect excretion of the stimulatory factor. (b and c) The larger, dense colonies at the right were each inoculated 71.25 h prephotography, and spots of 10^{-3} dilutions of overnight *polA2099*::mini-Tn*10* cultures were inoculated at different distances from the established colonies 25.25 h prephotography. These plates were all incubated for 54 h at 37°C and then for 17.5 h at room temperature. The established colony was inoculated with the same *polA2099* culture as the dilution spots (b) or with a Pol$^+$ culture (c).

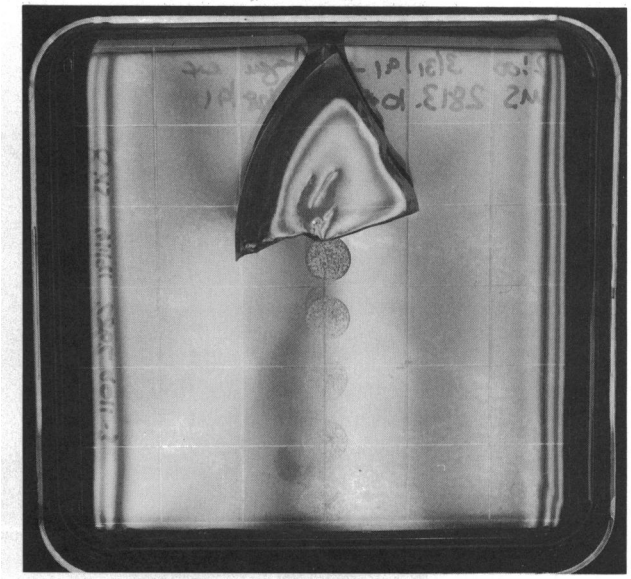

FIG. 9. Stimulation of *polA2099* microcolonies by a diffusible substance from sterile agar. The sector of agar at the top of the plate was excised from a plate inoculated 72 h previously with a Pol$^+$ culture. The sector was placed on this minimal-glucose–Casamino Acids agar plate 32.7 h prephotography. At 10.7 h prephotography, five spots of a 10^{-3} dilution of a *polA2099* culture were inoculated at different distances from the agar. This plate was incubated at 37°C. The last spot at the bottom of the plate is almost invisible.

cation-transposition is sensitive to diffusible signals and requires cAMP and the cAMP receptor protein (22a; unpublished data). There is also evidence that the cAMP-cAMP receptor protein complex negatively regulates DNA PolI activity indirectly: in the presence of cAMP, the cAMP receptor protein represses transcription of spot 42 RNA, and PolI activity is proportional to spot 42 accumulation (14). Thus, the effect of a Δ*crp*::*CM* mutation on *polA-lacZ* expression was tested. Although the Δ*crp*::*CM* lesion did alter the pattern of colony growth, it did not block differential *polA-lacZ* expression during development (Fig. 10c).

DISCUSSION

The results presented above indicate that full understanding of the expression and action of *E. coli* DNA PolI is possible only within the context of the changing conditions created by developing populations. Even a basic cellular housekeeping function such as PolI is subject to developmental and multicellular regulation.

It was something of a surprise to learn that DNA PolI acts differentially during colony development. Polymerase activity seems to be needed in early microcolony development to prevent SOS induction and promote normal cell morphogenesis (Fig. 6 and 7). One reasonable hypothesis is that repair is required to take care of oxidative damage to the genome (6, 9). Later in colony development, diffusible substances accumulate and eliminate the need for repair involving DNA PolI, perhaps by creating a reducing environment. This protective effect was seen in the stimulation of *polA2099* spot growth by substances diffusing from established colonies (Fig. 8). A similar multicellular protective effect against oxidative stress has been reported in the autoaggregation

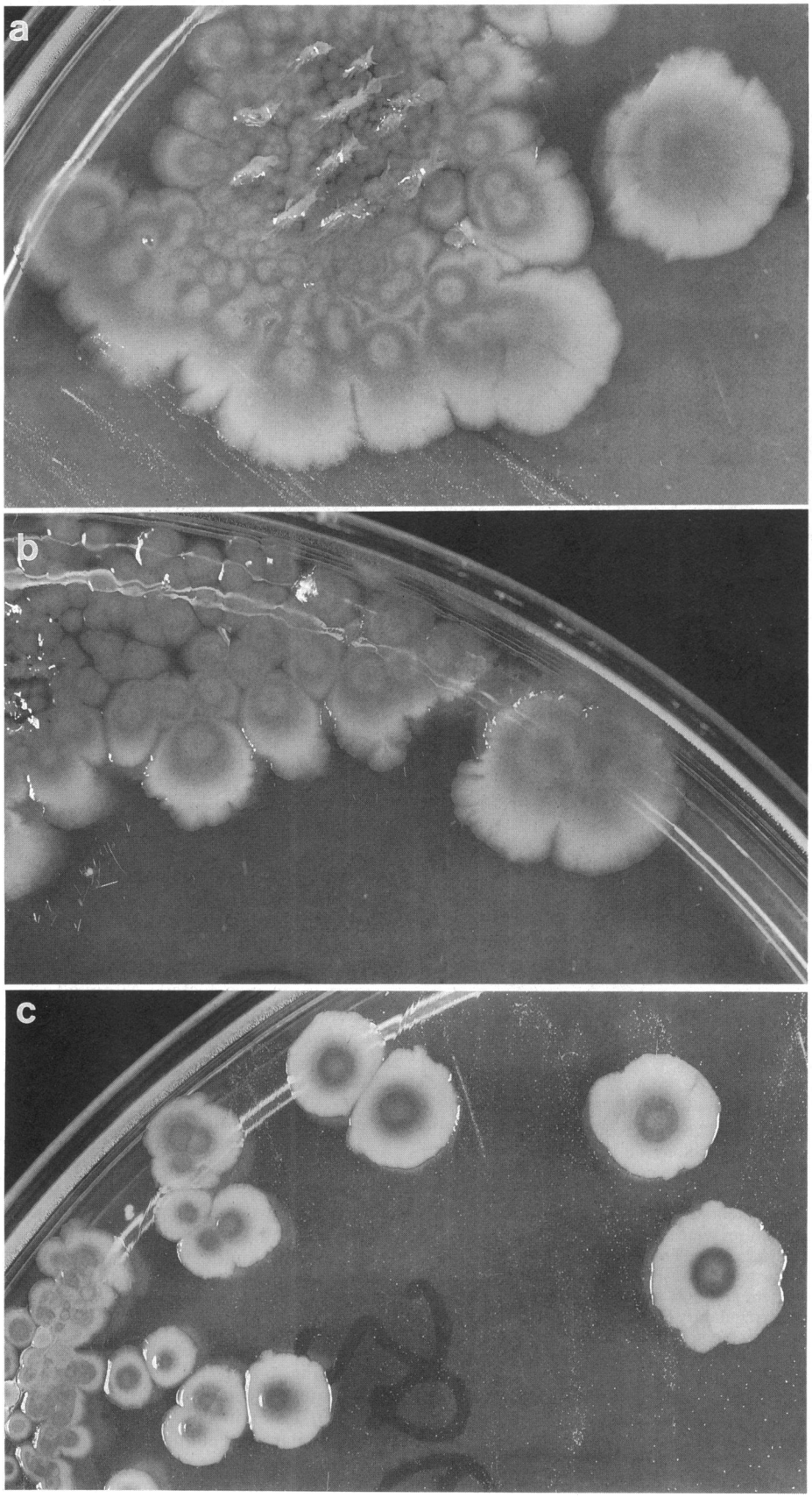

FIG. 10. Differential expression of *polA-lacZ* transcriptional fusions on X-Gal indicator agar. These colonies were grown for 5 days on minimal-salts–glucose–Casamino Acids agar containing 60 μg of X-Gal per ml. (a) Strain lysogenic for a phage carrying *polA-lacZ* fusion 14. (b) Strain lysogenic for a phage carrying *polA-lacZ* fusion 24. (c) Strain lysogenic for a phage carrying *polA-lacZ* fusion 14 with the Δ*crp*::*CM* mutation.

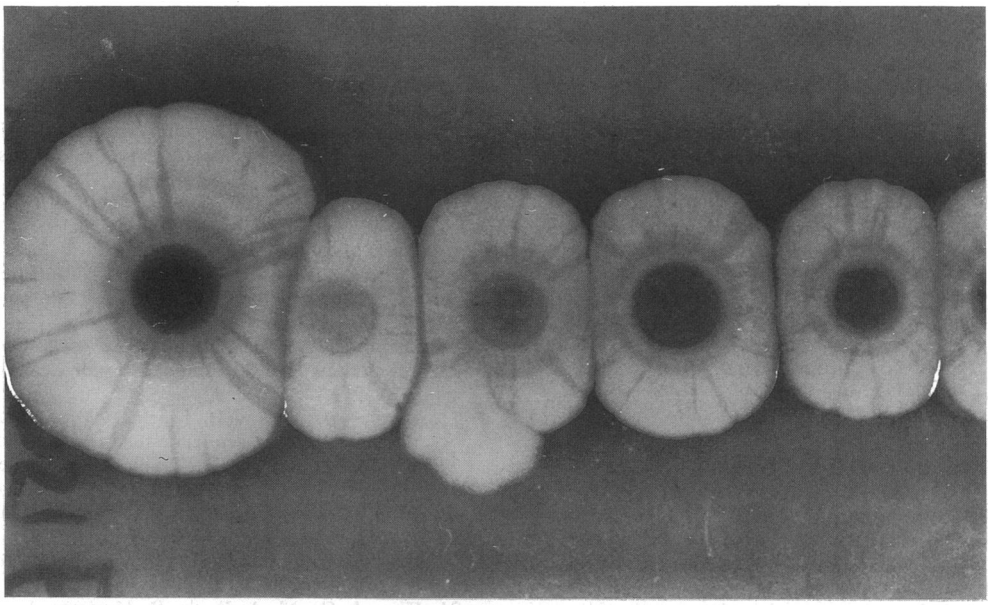

FIG. 11. Effect of a diffusible substance on *polA-lacZ* expression. Spots of a strain lysogenic for a phage carrying *polA-lacZ* fusion 24 on minimal-salts–glucose–Casamino Acids agar containing 30 μg of X-Gal per ml. The large colony on the left was inoculated 5 days prephotography, and the other colonies were inoculated 3 days prephotography.

behavior of motile *E. coli* in semisolid agar following H_2O_2 challenge (2).

Examination of X-Gal agar colonies harboring *polA-lacZ* fusion prophages showed that *polA* was differentially expressed during colony development and was under the regulation of one or more diffusible substances (Fig. 10 and 11). The simplest hypothesis is that there is oxidative stress induction of *polA* expression (for examples of other loci subject to oxidative stress induction, see reference 6) and that a diffusible reducing substance leads to both lower *polA* transcription and protection of *polA* cells. Ward and Murray reported that their *polA-lacZ* fusions were not subject to SOS regulation (23), but it is worth noting that the *sfiA*::Mud*Ap lac* fusion is also expressed much more strongly in colony centers than at the edges (unpublished data). In its response to the growth-related accumulation of an extracellular substance, *polA* expression would belong to a class of density-regulated systems, such as competence (reviewed in reference 5), bioluminescence (13), and sporulation (7, 25). In considering possible explanations for the role of PolI in MudII1681 derepression, it is worth noting that there does not seem to be an obvious correlation between the patterns of MudII1681 *lacZ* and *polA-lacZ* expression. Although PolI activity appears to be needed for full MudII1681 derepression, the zone of highest LacZ activity in the MS1534 colonies in Fig. 1 was near the edge, where *PolA* expression was generally found to be lowest (Fig. 10 and 11).

Perhaps the most interesting conclusion to come from the study of *polA2099* phenotypes is the finding that colony morphogenesis is not dependent on cell morphogenesis in a simple fashion. Although *polA2099* microcolonies are markedly different from Pol^+ microcolonies in both cellular size and overall shape (Fig. 3, 4, 6, and 7), the mature colony morphologies are indistinguishable (Fig. 5). These seemingly contradictory results fit with other data that indicate that bacterial colony development involves ongoing regulation, adjustment, and coordination to produce a regular structure, despite the inevitable vagaries of the growth process (18, 20, 22). Regularity is seen when the growth substrate is marred by creases (22), scratches (18), or small pieces of glass (unpublished data), and the present results show that regularity also obtains when early stages of morphogenesis are disrupted genetically. Thus, it is not unreasonable to consider colony development a regulative process in the sense that embryologists have used that term to describe compensation for perturbations of normal morphogenesis. If the comparison between embryonic development and colony development proves useful, then we can expect that our ability to manipulate *E. coli* cells and the *E. coli* genome will help make it possible to understand how morphogenetic regulation operates at the molecular, cellular, and multicellular levels.

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