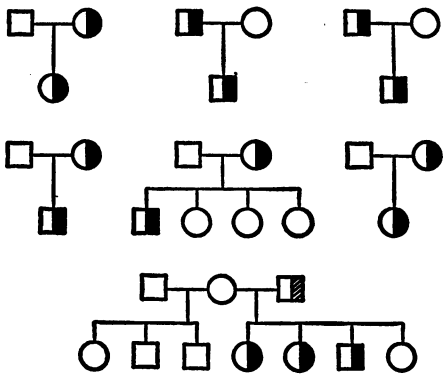

Fig. 2. A diagram of representative pedigrees. Individuals with variant mitochondrial GOT are indicated by half blacked-in symbols. The cross-hatched symbol represents a father who was not examined but who was presumed to be affected.

cludes the possibility that the observed patterns might have been due to oxidation of sulfhydryl groups, incomplete solubilization, or proteolysis of the enzymes. Mixture of the variants obtained by disrupting portions of different placentae with high-frequency sound showed, as expected, a superimposition of the individual variant patterns. Preparation of the samples and electrophoresis in the presence of L-aspartate, α-ketoglutarate, or pyridoxal 5-phosphate did not significantly alter the electrophoretic patterns, which suggests that the bands do not arise from stepwise loss of coenzyme nor from an equilibrium of the two postulated forms of mitochondrial GOT (the pyridoxal and the pyridoxamine forms) (*8*).

Family studies were performed with white blood cell preparations that were disrupted with high-frequency sound (Fig. 2). All three variants segregate in a simple Mendelian fashion, and the individuals with the variant are heterozygous for an autosomal mutant allele. Sex-linkage is ruled out by transmission from father to son in several families.

Of the 860 placental preparations examined, variant I occurred with a frequency of 1.7 percent among Caucasians (12/705) and 0.67 percent among Negroes (1/148). Variant II was detected almost exclusively among Negroes—of 11 individuals with the variant, 8 were Negroes; 1 was Puerto Rican; 1 was the product of a Puerto Rican father and a Negro mother; and 1 was white. This gives an incidence of 6.1 percent for the Negro sample, including the mixed mating (9/148) and 0.14 percent for the Caucasian sample (1/705). Variant III was found in three Negroes which is a frequency of 2.0 percent (3/148).

Thus, although variants of soluble GOT are extremely rare, there are at least three variants of mitochondrial GOT, all of which are common enough to be considered polymorphisms. The consistent triplet patterns support the hypothesis that both soluble and mitochondrial GOT exist as dimers. The differing patterns of variant I in the two buffer systems and the failure to differentiate between variants I and III in the phosphate-citrate buffer system emphasize the importance of altering electrophoretic conditions in the study of isoenzymes. Segregation of the variants according to simple Mendelian inheritance rather than maternal inheritance, as was the case with a variant of human mitochondrial malate dehydrogenase (*9*), provides a second example of a human mitochondrial enzyme that is under the control of nuclear DNA.

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Visualization of Bacterial Genes in Action

Abstract. *The morphology of active structural and putative ribosomal RNA genes was observed by electron microscopy after lysis of fragile* Escherichia coli *cells. Conclusions drawn are: most of the chromosome is not genetically active at any one instant; translation is completely coupled with transcription; the 16S and 23S ribosomal RNA cistrons occur in tandem, in regions which are widely spaced on the chromosome.*

Techniques developed for the visualization of the structure of active genes in a eukaryotic cell (*1*) were used to observe chromosomes of *Escherichia coli*. A mutant strain was utilized (*2*) which, under certain growth conditions, develops fragile cell walls. Fragile cells in the log phase of growth were osmotically burst by rapid dilution into water, then prepared for electron microscopy (Fig. 1).

At low magnification, the extruded contents of shocked cells appear as masses of thin fibers with attached strings of granules (200 to 250 Å in diameter); these granules are approximately the size of *E. coli* ribosomes (*3*). After osmotic rupture, treatment with deoxyribonuclease destroys the fibers, whereas ribonuclease removes the granular strings from the fibers. From these results, we conclude that the fibers are bacterial chromosomes and the granules are ribosomes which were translating messenger RNA (mRNA) molecules at the time of isolation. The contents are not completely removed from the cells by osmotic shock, although considerable amounts are extruded and spread to varying degrees around the cells. Consequently, we have been able to observe only portions of chromosomes rather than complete genomes.

The diameter of double-helix DNA is ~20 Å (*4*). In our preparations, the *E. coli* chromosome is ~40 Å in diameter. This suggests either that the DNA is combined with some nonhistone proteins in vivo (*5*) or that proteins at-

tach rather uniformly to the DNA during or after isolation.

The ribosomes are attached to the *E. coli* genome on mRNA molecules as monomers and in polyribosomes (Figs. 2 and 3). The longest polyribosome observed to date contained 40 ribosomes. In biochemical studies of the fragile strain, Mangiarotti and Schlessinger (*6*) demonstrated that all or very nearly all mRNA molecules are in polyribosomes within the cell. Our micrographs demonstrate that free polyribosomes are rare in our preparations, and these could possibly arise from shearing during cell rupture. Since our technique would allow the detection of significant numbers of naturally occurring free polyribosomes, we conclude that essentially all mRNA molecules are connected to the *E. coli* genome in vivo. This observation provides visual confirmation of the early predictions by Stent and others (*7*) that transcription and translation are intimately coordinated in prokaryotic cells.

Each polyribosome is attached to the chromosome at the site of an irregularly shaped granule ~75 Å in diameter. Although this is somewhat smaller than the most recently reported diameter for isolated RNA polymerase molecules (*8*), we suggest that these are polymerase molecules which were actively transcribing genes at the time of cell rupture. Ribosomes are closely spaced in polyribosomes, and the ribosome at the newly synthesized end of a messenger is almost always immediately adjacent to the putative RNA polymerase molecule on the chromosome.

The spacing of polyribosomes along the chromosome is irregular, suggesting that initiation of transcription does not usually exhibit regular periodicity. Messenger RNA chain initiation at regular intervals has been reported for the tryptophan operon in *E. coli* (*9*). In some instances, polyribosome lengths increase in a regular fashion along a portion of the chromosome. From this gradient of polyribosome length, one may estimate the location of the site of initiation of mRNA synthesis. In this position, a granule is often present which may correspond to an RNA polymerase at the promoter site (*10*) (Figs. 2 and 3).

The termination points for DNA segments being transcribed as units are difficult to estimate because of relatively wide variability in spacing of polyribosome attachment sites. The longest active segment of chromosome so far observed is about 3 μm and is shown in Fig. 2. A comparison with the estimated lengths of various bacterial operons suggests that such a segment probably is a polycistronic operon (*11*).

It is of interest to note that most regions of the extruded chromosomes do not have attached polyribosomes. This is consistent with the RNA-DNA hybridization experiments by Kennel (*12*) which indicate that a high percentage of the *E. coli* chromosome

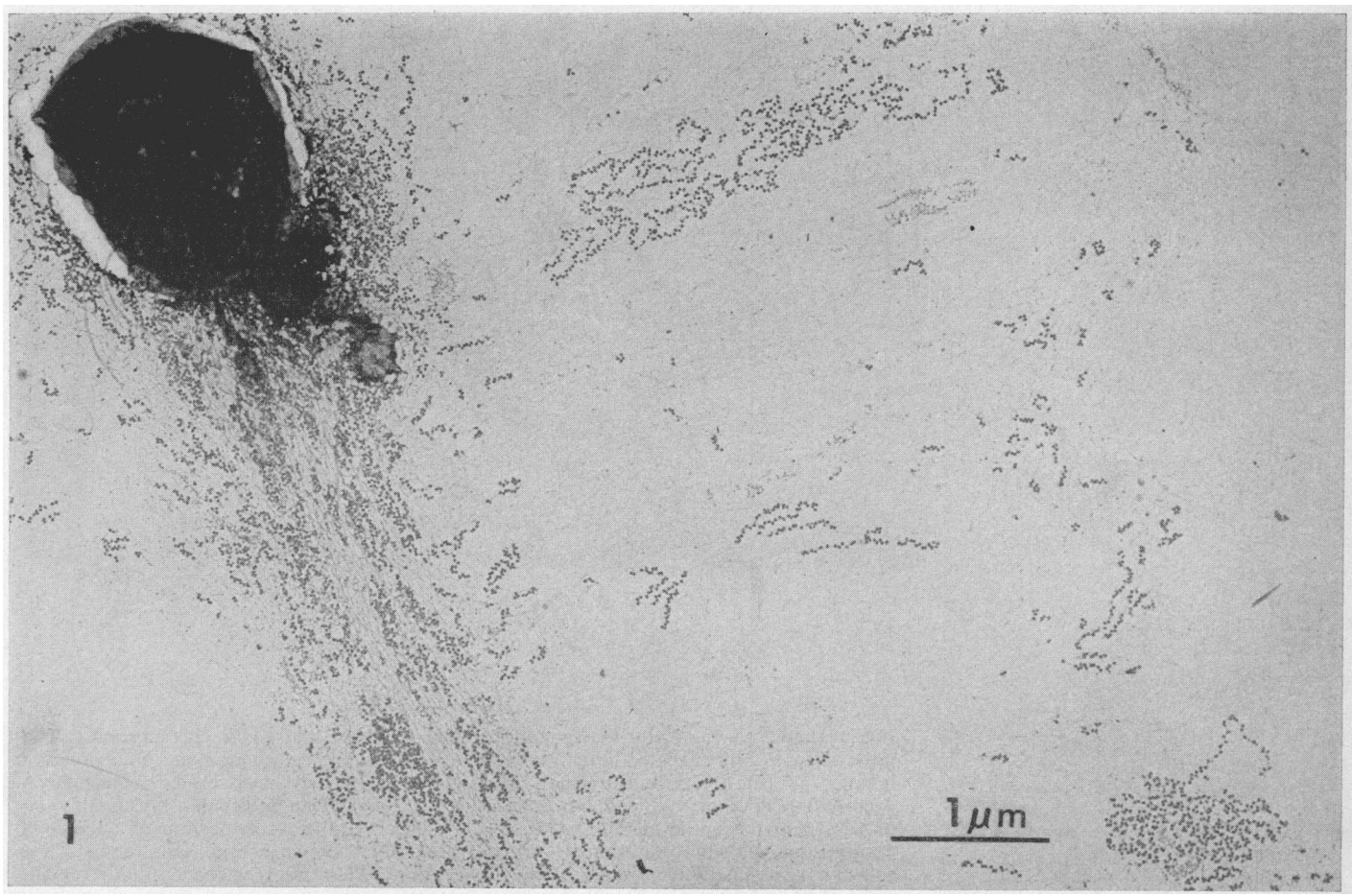
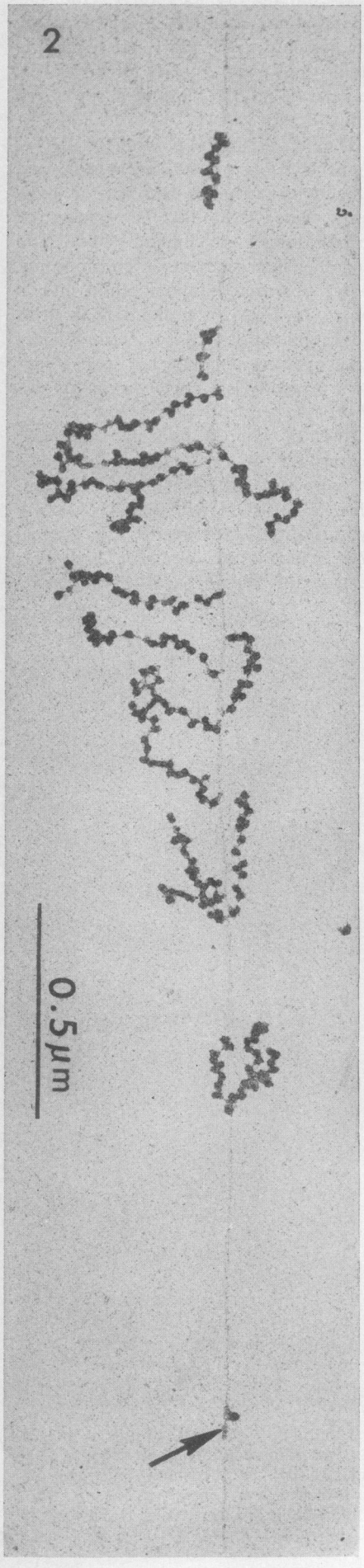
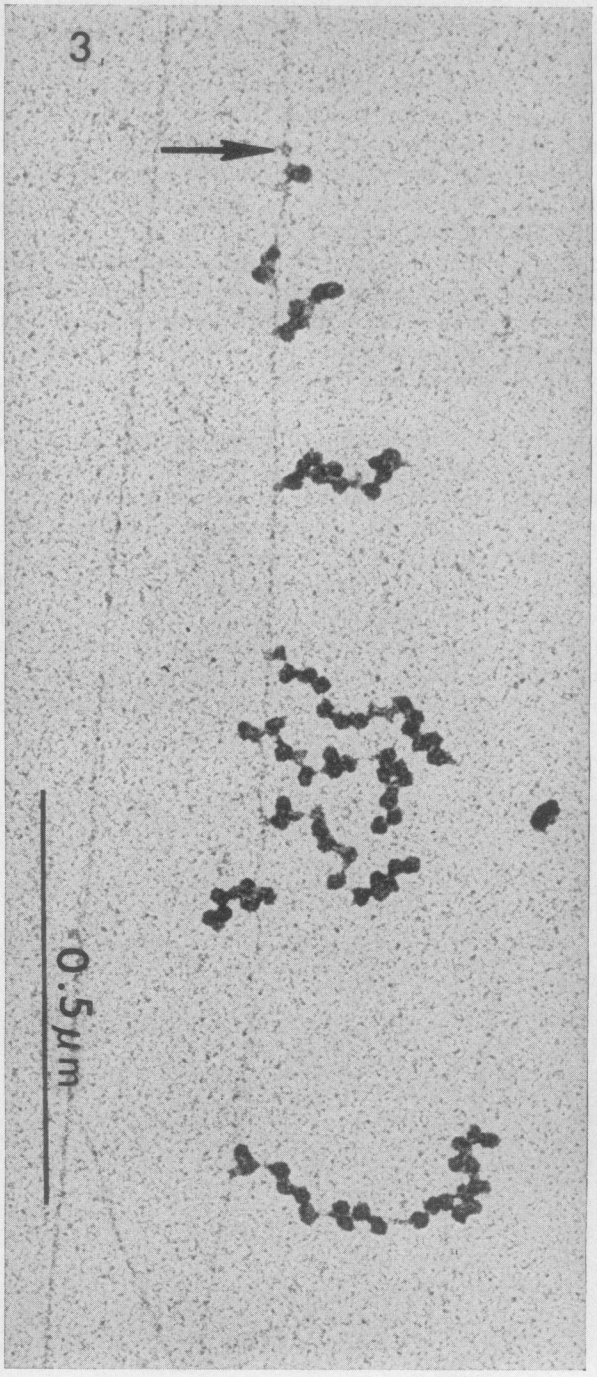
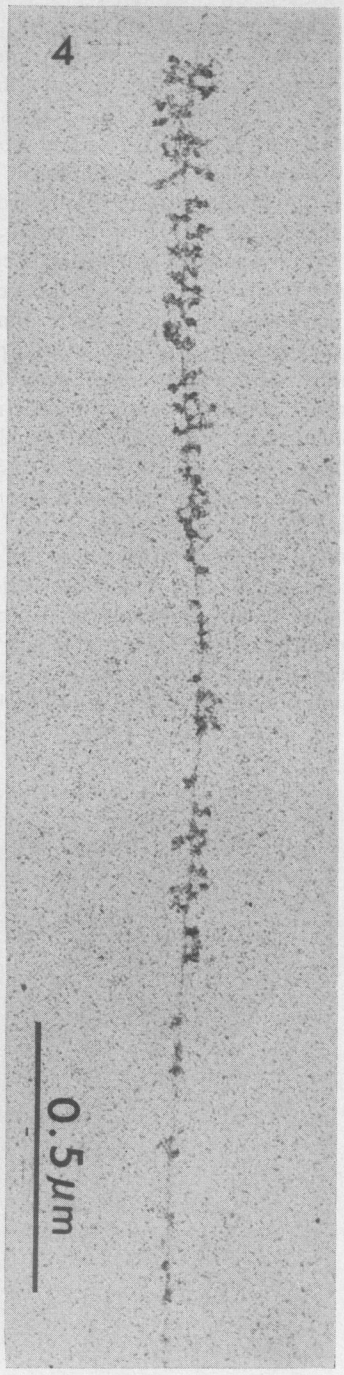


Fig. 1. Electron micrograph showing a portion of the extruded contents of an osmotically ruptured *E. coli* cell. Fragile cells (*2*) were burst by rapid dilution (1 : 50) into distilled water adjusted to *p*H 9. The burst cells were immediately centrifuged (3200 rev/min at 19.3 cm radius for 3 to 5 minutes) through a 0.1*M* sucrose plus 10 percent formalin (*p*H 8.5) cushion onto carbon-coated grids. The grids were rinsed in 0.4 percent Kodak Photo-flo, dried, and stained for 1 minute each in 1 percent phosphotungstic acid and 1 percent uranyl acetate, with both stains dissolved in 70 percent ethanol. The grids were then rinsed briefly in 95 percent ethanol, 100 percent ethanol, and isopentane and air-dried.







Figs. 2 and 3. Genetically active and inactive portions of *E. coli* chromosomes. The polyribosomes attached to the active segments exhibit imperfect gradients of increasing lengths. The shorter, most distal polyribosomes in Fig. 2 may have resuted from mRNA degradation. The arrows indicate putative RNA polymerase molecules presumably on or very near the initiation sites of these active loci. Fig. 4. A portion of an *E. coli* chromosome showing presumptive 16*S* and 23*S* rRNA loci in action. This segment was somewhat stretched during the isolation procedures. When not stretched, similar regions are ~ 1.5 μm long.

either is responsible for very rare species of RNA or is never transcribed. In regions where no polyribosomes are attached, numerous small granules the size of the putative RNA polymerase molecules are associated with the chromosome.

The kinetics of ribosomal RNA (rRNA) synthesis in *E. coli* indicate that the 16*S* and 23*S* cistrons are transcribed simultaneously by a relatively large number of RNA polymerase molecules as compared to the transcription of structural genes (*13*). In addition, ribosomal proteins, rather than ribosomes, become associated with the rRNA's as they are synthesized (*14*). Chromosomal segments with 60 to 70 attached fibrils have been observed (Fig. 4). Since the length of such segments is close to the length of DNA necessary to code for one 16*S* and one 23*S* rRNA (*15*), we suggest that these segments are rRNA genes. Studies with *Bacillus subtilis* (*16*) and *Proteus mirabilis* (*17*) have shown that the 16*S* and 23*S* cistrons are contiguous in those species.

Experiments on RNA-DNA hybridization (*18*) have shown that approximately 0.4 percent of the *E. coli* chromosome contains cistrons coding for rRNA. Taking into consideration the length of the chromosome and the amount of DNA necessary to code for the 16*S* and 23*S* rRNA molecules, this value indicates that the *E. coli* chromosome contains no more than six segments with tandem 16*S* and 23*S* cistrons (*19*). Our observations suggest that these sites are quite widely spaced on the chromosome. This conclusion is supported by biochemical data in another study using *E. coli* (*20*).

We believe that refinement of the techniques used in this study should provide a powerful tool for direct observation of specific active genetic loci in microbial systems, and that the procedures will prove generally useful for cytogenetic analysis at the molecular level.

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L-Dihydroxyphenylalanine: Effect on S-Adenosylmethionine in Brain

Abstract. *Forty-five minutes after intraperitoneal injection of a single dose (100 milligrams per kilogram) of* L-*dihydroxyphenylalanine, the concentration of* S-*adenosylmethionine in rat brain was lowered by 76 percent. As little as 10 milligrams of* L-*dihydroxyphenylalanine per kilogram decreased content of* S-*adenosylmethionine in the adrenal medulla by 51 percent, whereas 100 milligrams per kilogram did not significantly depress concentration of* S-*adenosylmethionine in the liver in this time interval. Concentration of* S-*adenosylmethionine in the brain varied diurnally;* L-*dihydroxyphenylalanine lowered this concentration whether administered at the daily peak or at the nadir.*

The efficacy of L-dihydoxyphenylalanine (L-dopa) in the treatment of Parkinson's disease has been correlated with the fact that this catechol amino acid is the physiological precursor for brain dopamine. Its administration to experimental animals causes an increase in content of dopamine in the brain (*1*). Moreover, brains of parkinsonian patients often contain subnormal amounts of dopamine (*2*), and the concentration of its chief metabolite, homovanillic acid (HVA), in their cerebrospinal fluid is depressed (*3*). Finally, the administration of L-dopa to human subjects elevates the HVA content of the urine and cerebrospinal fluid (*3, 4*), which indicates that significant quantities of the exogenous amino acid are converted to dopamine, as occurs with endogenous dopa.

After intraperitoneal administration, L-dopa is largely methylated to 3-*O*-methyl-dopa, which is then decarboxylated and converted to HVA. A surprisingly high percentage (more than half) of a dose of L-dopa is *O*-methylated within the first 20 minutes after administration (*5*). Conversion to central catecholamines is actually a very minor metabolic route of exogenous L-dopa. Since *S*-adenosylmethionine (SAMe) is the methyl donor in the *O*-methylation of L-dopa and dopamine (*6*), it seemed likely that large amounts of SAMe must be utilized in