We have also isolated histones from rat thymus. It has been reported (*8*) that thymus and spleen histones differ from those of other rat tissues. The histone patterns of thymus from both 40-g and 250-g rats (Fig. 1*F*) appear very similar to those of the organs of newborn animals and are therefore different from those of brain, liver, and kidney of the adult animal. The histone pattern of rat thymus is also quite distinct from that of calf thymus.

Similarity in the histone composition of various tissues of the same species has been pointed out (*7, 9*). Our studies of histone patterns of brain and other organs, obtained by electrophoresis in polyacrylamide gels, are consistent with this conclusion. The complexity of the histone pattern appears to vary among species, from the relatively simple pattern found in the guinea pig and rabbit to the more complex patterns of rat and mouse. This observation may be of interest in view of the highly complex patterns reported for calf thymus histones (*8, 10*) and of the speculation that this complexity may be related to histone function (*11*). Our results with rat tissues suggest changes in histone composition with maturation; changes in which the histones of the thymus gland do not participate. Our findings impose considerable restrictions as to the function of histones in the regulation of information transfer from DNA. Whereas our findings, and those of other laboratories demonstrating similarity of histones in various tissues, do not exclude a direct role for these basic proteins in regulating DNA expression, they point to the need for additional mechanisms to provide the specificity required to control the transcription process. It would seem that such a control mechanism is not reflected in a differential rate of breakdown and resynthesis of individual histones in the adult animal.

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Transcription of a Repressed Gene: Evidence That It Requires DNA Replication

Abstract. *It is known that the synthesis of DNA is not essential for constitutive or induced enzyme synthesis. Our studies indicate that DNA synthesis is required for the basal level synthesis (that is, for synthesis in absence of inducer) of two inducible enzymes, a finding which supports an earlier speculation that a messenger-RNA transcription event may normally accompany DNA replication. Studies with cultures of* Escherichia coli *TAU-bar in which DNA replication is synchronized suggest that the β-galactosidase gene is transcribed at a particular time in the sequential replication of the bacterial chromosome.*

The induced synthesis of a number of inducible enzymes has been demonstrated in bacteria under conditions of thymine starvation (*1–3*). As initially reported by Cohen and Barner (*1*), the induction process itself can occur in the absence of thymine in a thymine-requiring bacterium. Thus, the synthesis of DNA is not essential to either the induction process or to induced enzyme synthesis. However, the *rate* of induced enzyme synthesis is dependent on the number of copies of the particular gene in the cell (*4*) and there is some evidence from synchronous growth studies that the enzyme synthesizing *capacity* doubles as the particular gene is duplicated (*5*). In the studies described here we examined specifically the synthesis of an inducible enzyme in the *repressed* state and the dependence of its synthesis on the simultaneous replication of DNA.

Escherichia coli strain TAU-bar (*6*) was cultured aerobically in a glucose-salts synthetic medium at *p*H 7.4 and 37°C with the required supplements (*7*). Changes in the medium were accomplished by the rapid filtration technique (*8*). Induced synthesis of β-galactosidase required the use of 0.5 percent lactose in place of 0.5 percent glucose as energy source in the growth medium. Bacteria were lysed for enzyme assay by treatment of a 5-ml suspension for 1 minute at 0°C with a Branson Sonifier adjusted for resonance at power setting No. 7. Control studies indicated better than 95 per-

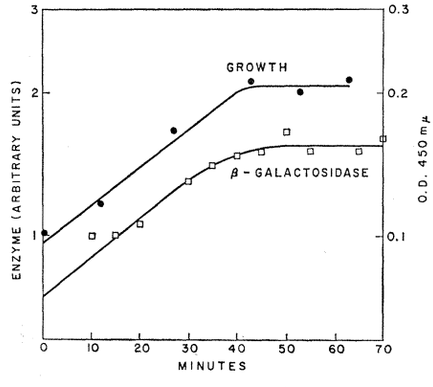

Fig. 1. Effect of thymine deprivation on induced enzyme synthesis and growth in *E. coli* TAU-bar. Culture grown in lactose medium. Thymine removed at 0 minutes. Growth followed by optical density (O.D.) readings (*15*). β-Galactosidase activity in samples from lysed culture determined by hydrolysis of ONPG (*16*) and optical density readings at 420 mμ (*17*).

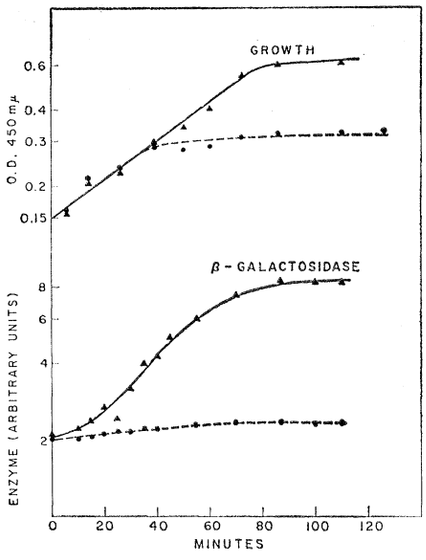

Fig. 2. Effect of thymine deprivation on repressed enzyme synthesis and growth. At 0 minutes an exponential culture in glucose medium was filtered and equal portions were resuspended in medium with thymine (solid line) and without thymine (dashed line). Assays as described for Fig. 1.

cent lysis by this procedure and no inactivation of either β-galactosidase or alkaline phosphatase.

In the presence of thymine in an exponentially growing culture the induced enzyme concentration increases in proportion to the amount of growth. Figure 1 shows that in the absence of thymine, both the cell mass and the concentration of β-galactosidase doubled in amount, in agreement with Nakada (*3*), even though the synthesis of DNA was inhibited (*1*). In striking contrast to the result for the induced enzyme, the result of a similar experiment in which the enzyme was repressed is shown in Fig. 2. The average amount of enzyme was of the order of 1 or 2 molecules per cell in this condition, a factor of about 1000 less than the concentration in the induced state (*9*). Nevertheless, in the presence of thymine this basal level of enzyme paralleled growth (see Fig. 2). When thymine was withheld enzyme synthesis did not follow growth and, in fact, showed less than a 10 percent increase as the optical density doubled. A similar result was obtained when basal level alkaline phosphatase (repressed by 0.05*M* inorganic phosphate) was examined (*10*). In the presence of thymine it followed growth but in the absence of thymine the concentration of alkaline phosphatase remained essentially constant as growth continued. The synthesis of both enzymes resumed when thymine was restored to the growth medium.

The small number of enzyme molecules present in the repressed state suggests that the messenger-RNA transcription event which specifies their synthesis may occur at a unique time in the cell cycle. Furthermore, since the single bacterial chromosome replicates sequentially with only one growing point (*11*), it would seem reasonable to suppose that this transcription event occurs at or near the point of replication. This idea has also been suggested from the evidence that a messenger-RNA synthesis event participates in the phenomenon of death due to absence of thymine (*7*). The inhibition of DNA replication (for example, by thymine deprivation) might then be expected to interfere with such a transcription event.

To test this hypothesis we examined the synthesis of β-galactosidase in a culture in which the DNA replication cycle was synchronized. In such a culture, we could determine whether the synthesis of β-galactosidase occurs as a particular region of the genome is replicated. The results of this experiment are shown in Fig. 3. All of the cells in the population were brought to the end of a cycle of chromosome synthesis by inhibiting the synthesis of protein (*8, 12, 13*). When the required amino acids were again added (culture *A*, Fig. 3) growth resumed with no lag, but both DNA synthesis and enzyme synthesis resumed gradually, such that balanced growth was not re-established until the cell mass had doubled. As previously noted for this condition, the resumption of the DNA replication cycle does not occur simultaneously in all cells; instead, a variable period of protein synthesis must be permitted to reinitiate the cycle in different cells (*12*). For culture *B* (Fig. 3) a doubling in cell mass was permitted in the absence of DNA synthesis to align the population with respect to initiation of the next round of synthesis. The amount of enzyme level did not change in the absence of thymine. It also did

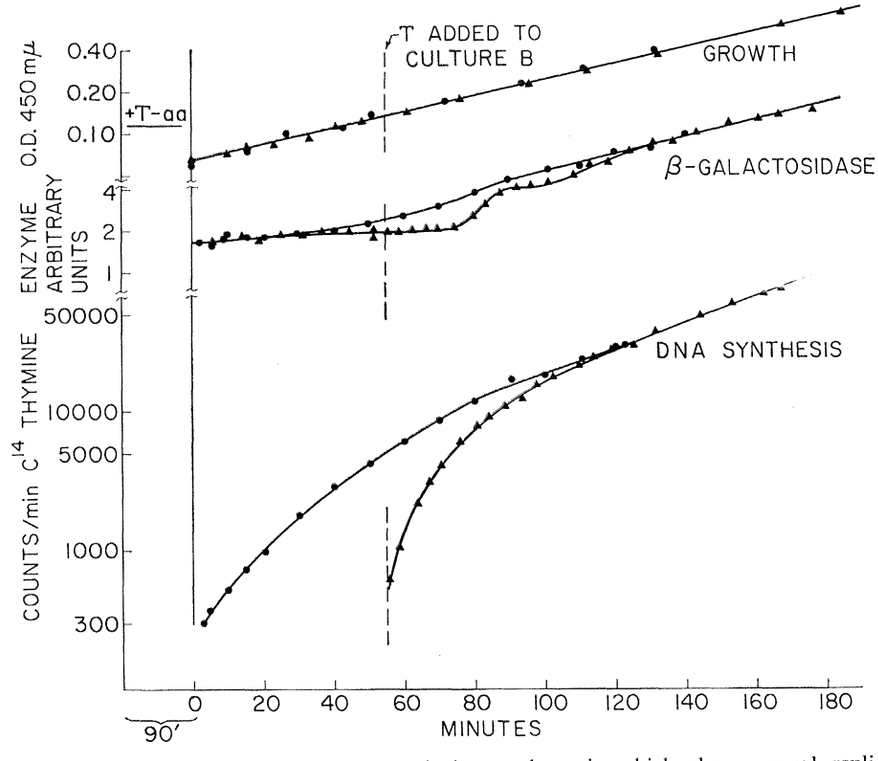

Fig. 3. Repressed β-galactosidase synthesis in a culture in which chromosomal replication is synchronized. An exponential culture in glucose medium was filtered and resuspended in medium lacking the amino acids (−*aa*) but containing thymine (+*T*). After 90 minutes the culture was again filtered and split into cultures *A* and *B*. Culture *A* (circles) was resuspended with all required supplements. Culture *B* (triangles) was subjected to a 55-minute period of growth with all supplements except thymine, and then thymine was added as indicated. Assays as described for Fig. 1. In addition, DNA synthesis was followed by incorporation of C^{14}-thymine into trichloroacetic acid insoluble aliquots. For culture *B*, if C^{14}-activity is plotted against optical density, a straight line is obtained, indicating balanced growth. A similar plot for culture *A* confirms the gradual resumption of DNA synthesis previously reported (*8*).

not change for about 20 minutes after the addition of more thymine but it then doubled in the next 15 minutes. This result was obtained in four separate experiments. It is consistent with the hypothesis that the transcription event for the synthesis of this enzyme in the repressed state occurs at a particular time in the replication of the chromosome.

Our model would suppose that the entire bacterial genome is transcribed at least once in the course of its replication, as supported by evidence of McCarthy and Bolton (*14*), and that this event accounts for basal levels of inducible enzymes.

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Mechanoreceptors in the Cuticle of the Honey Bee: Fine Structure and Stimulus Mechanism

Abstract. *The distal nerve process of hair plate sensilla and campaniform sensilla contains a special terminal structure in the form of a bundle of tubules herein designated the "tubular body." Physiological and morphological results indicate that compression at the site of this body probably acts as the stimulus at the cellular level. A ciliary structure separates an outer segment of the distal nerve process from the remaining distal fiber.*

Recent investigations show that the hair plates of insects function as position receptors in the joints and in this way can act as gravitation receptors (*1*, *2*). An electrophysiological and microscopic study of the single receptor elements brought results which related to the mechanism of stimulation in the nerve endings (*3*). In the study reported here the morphological basis of the primary receptor processes was investigated by electron microscopy. The objects studied were the cervical hair plates of the honey bee (*2*, *3*) and, for comparison, the campaniform sensilla of the head.

The sensilla of the hair plates and the campaniform sensilla each contain only one receptor cell of the bipolar type. The distal process of the cell enters through a canal in the cuticle and connects with the joint membrane in the hair plate sensillum, and with the cap membrane in the campaniform sensillum (Fig. 1). These cuticular structures probably consist of the rubber-like protein resilin (*4*), as indicated by their special staining properties, by their extraordinary deformability in the joint membrane, by their stress-birefringence, and by their digestability by pepsin. Both types of sensillum have a nearly bilaterally symmetrical form: the hairs in their resting position lean against an overhang of the cuticle at one side and can be freely pivoted only in the opposite direction; the campaniform sensilla have an elliptic form, when observed from above.

With the electron microscope (*5*) it is possible to distinguish in both types of sensilla an outer segment of the distal nerve process from the remaining distal fiber; the outer segment and the fiber are connected by a ciliary structure (Fig. 1). In each type of sensillum the outer segments are of roughly constant length (6 to 8.5 μ in the hair sensilla, 3.2 to 4.2 μ in the campaniform sensilla) independent of the thickness of the surrounding cuticle. Within the conical ending of the outer segment lies a prominent dense body (Figs. 2 and 3*A*) consisting of 50 to 100 tubules lying parallel to one

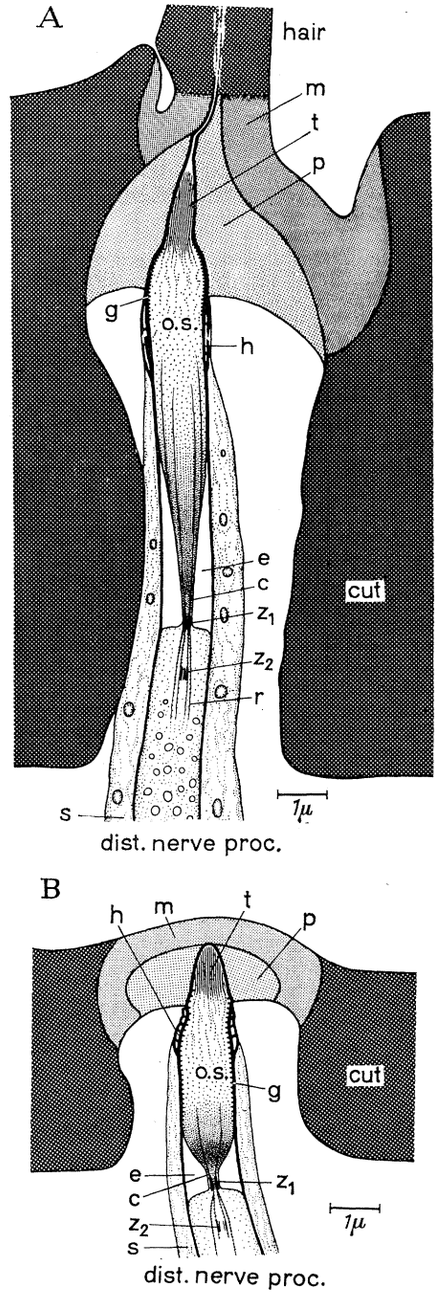

Fig. 1. Diagram of hair plate sensillum (*A*) and campaniform sensillum (*B*). Abbreviations: *c*, ciliary structure; *e*, extracellular space; *g*, granules; *h*, cuticular sheath; *m*, joint membrane (*A*), cap membrane (*B*); *o.s.*, outer segment; *p*, cap; *s*, Schwann cell; *r*, root fiber; *t*, tubular body; *cut*, cuticle; z_1, z_2, centrosome-like structures.