abstracts by J. H. Healy and R. M. Hamilton; A. Ryall, G. Boucher, W. V. Savage, and A. E. Jones; F. A. McKeown, D. D. Dickey, and G. E. Brethauer; S. W. Smith; J. Evernden; and E. R. Engdahl, W. V. Mickey, S. R. Brockman, and K. W. King.
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32. Publication of this article is authorized by the director of the U.S. Geological Survey. We thank the Chevron Oil Company for its cooperation in carrying out the investigation at the Rangely Oil Field, in Colorado, and for permission to publish the pressure contours of Fig. 10; the Division of Nuclear Reactor Development and Technology of the U.S. Atomic Energy Commission for partial support of the investigations of microearthquakes in California; the Earthquake Mechanism Laboratory of the Environmental Science Services Administration for some of the data used to locate the epicenters shown in Fig. 2; and the Nevada Operations Office of the U.S. Atomic Energy Commission for its cooperation in carrying out the investigation of the aftershocks of the Benham underground nuclear explosion illustrated in Fig. 11.

Control of Specific Gene Expression in Higher Organisms

Expression of mammalian genes may be controlled by repressors acting on the translation of messenger RNA.

Gordon M. Tomkins, Thomas D. Gelehrter, Daryl Granner
David Martin, Jr., Herbert H. Samuels, E. Brad Thompson

It is generally acknowledged that the genetic information in most complete cells of a complex metazoan organism is identical with that of every other cell. Within a given organism the tremendous diversity of cell phenotypes must therefore derive from the fact that each cell expresses only a limited amount of its full genetic potential and that different cell types express different portions of their genome. A complete theory of metazoan cell biology must account not only for this differentiation of cell function, but also for the development of an adult organism from a single cell, a process which requires an orderly progression (and repression) of gene activities until the highly structured end state is reached.

Faced with complexity on this scale, biologists have turned to simpler nonnuclear systems—bacteria and their viruses—in which the control of individual genetic elements can be understood more easily. With these organisms, it has been established that DNA is the primary genetic material and that genetic information is expressed through an intermediate, messenger RNA, which acts as the direct template for protein synthesis.

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A bacterial gene is "active" only when its corresponding messenger is produced. Therefore, regulation of gene function depends on controlling the synthesis of specific messenger RNA's. In bacteriophage λ and the group of genes controlling lactose metabolism in *Escherichia coli*, the formation of the messenger is inhibited by the attachment of specific protein repressors to specific regulatory sites on the chromosome. The genes controlling lactose metabolism are activated by a specific "inducer" that combines with the repressor, causing the latter to detach from the DNA and permitting the messenger RNA to be synthesized (*1*, *2*).

The elegance of these ideas and the clarity with which they have subsequently been verified in microorganisms have led to their widespread acceptance as an explanation for gene regulation of higher organisms as well. This acceptance has been bolstered by the demonstration that the fundamental mechanisms of information flow in higher organisms are virtually identical with those in bacteria. Thus, in both cases, DNA is the primary genetic material; in both cases genetic information is expressed by transcription into RNA; and in both cases the codes assigning specific RNA triplets to specific amino acids are essentially identical (*3*).

However, certain features of the structure and function of the genetic apparatus of eukaryotic cells are very different from their bacterial counterparts; these differences raise the possi-

bility that the mechanisms which regulate gene expression in the two cases may also be significantly different. For example, the chromosomes of cells with a nucleus contain not only DNA but also numerous varieties of protein molecules, and perhaps RNA as well. Bacterial chromosomes, however, consist of naked, double-stranded DNA molecules. Despite the fact, significant in itself, that individual cells of a complex, differentiated organism synthesize only a limited number of types of protein molecules compared with their genetic potential, they display a striking lack of economy with respect to RNA synthesis. Thus, numerous studies (*4*) have suggested that large portions of the genome of animal cells are continuously transcribed into RNA in the nucleus, but only a small fraction of these ever reaches the cytoplasm. Therefore, a great variety of RNA molecules are synthesized and destroyed, never functioning as messenger RNA's even though they might have the potential to do so. Other differences in genetic organization between prokaryotes and eukaryotes have also been discussed (*5*).

The cells of higher organisms also appear, at times, to use more complex mechanisms for processing nascent polypeptides than bacteria do. For example, the initial product of translation of the poliovirus RNA seems to be a single, long polypeptide chain that is cleaved into the smaller viral components (*6, 7*). Furthermore, degradation or stabilization of active protein molecules can play a role in determining their intracellular concentration in animal cells [see (*8*) and below].

The reactions by which genetic information is converted into biologically active polypeptides are shown in Fig. 1, together with those processes, unique to eukaryotic cells, that might have a quantitative influence on the concentration of the products of specific genes. To investigate the mechanisms of gene regulation in mammalian cells we have been studying the hormonal induction of a specific protein.

Enzyme Induction in Mammalian Cells in Continuous Culture

When adrenal steroids are administered to intact animals, the liver enzyme tyrosine aminotransferase (E.C. 2.6.1.5), which catalyzes the rate-limiting reaction in tyrosine degradation, is induced (*9*). To investigate this hormonal regulation of gene activity in more detail, we have derived a line of rat hepatoma cells, HTC cells, that grow in continuous culture and in which the synthesis of the aminotransferase is stimulated by adrenal steroids (*10–12*). The similarity of the induction phenomena in vivo and in culture suggests that the process in HTC cells is a valid model for the normal biological control mechanism.

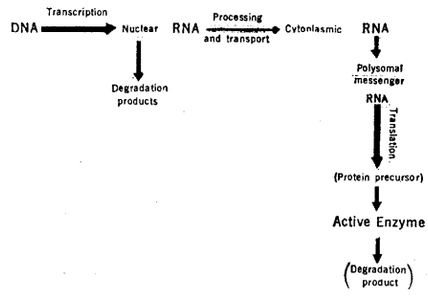

Fig. 1. Processing of genetic information in eukaryotic cells. The thick arrows marked "transcription" and "translation" refer to information flow from DNA to RNA, and from RNA to protein, respectively. "Transcription" is defined as the synthesis of RNA, the base sequence of which is specified by the DNA template. "Translation" refers to the initiation, elongation, and termination steps in protein synthesis. The thinner arrows refer to complex chemical conversions the mechanisms of which are largely unknown. Thus, nuclear messenger RNA might either be degraded or else transported to the cytoplasm where it directs protein synthesis. The hypothetical intermediate marked "protein precursor" refers to the possibility that certain biologically active proteins may be synthesized from larger inactive precursors by selective proteolysis.

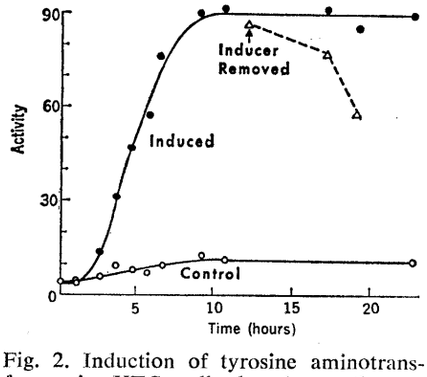

Fig. 2. Induction of tyrosine aminotransferase in HTC cells by dexamethasone phosphate. A culture of HTC cells, grown to a density of about 8×10^5 cells/ml, was resuspended in fresh medium and divided into two portions. To the first portion, dexamethasone phosphate, a synthetic adrenal steroid, was added to a final concentration of $5 \times 10^{-5}M$. The other portion was used as a control. Enzyme activity was assayed as described in (*22*) and is expressed as milliunits of enzyme per milligram of cell protein.

The population-doubling time of HTC cells, in suspension culture or attached to glass or plastic surfaces, is about 24 hours, and the cloning efficiency is close to 100 percent (*10*). In common with most other permanent lines of cells in culture, the karyotype is abnormal, with a modal chromosome number of about 63 (*10*).

For our purposes, the outstanding characteristic of HTC cells is their response to the adrenal steroids. For example, when $10^{-5}M$ dexamethasone phosphate is added either to a growing or stationary culture (Fig. 2), the activity of tyrosine aminotransferase rises rapidly, and after 6 to 12 hours the new steady-state is attained which is 5 to 15 times higher than the basal activity.

To analyze the mechanism of the induction, we first ascertained that the hormone-dependent rise in enzyme activity results from an increase in the rate of specific enzyme synthesis. To do this, tyrosine aminotransferase was purified from rat liver (*12–14*) and an antibody was prepared against it (*12*). We found that the aminotransferase of rat liver and that of HTC cells are immunologically identical (*12*), and that the steroid-induced increase in the activity of the enzyme in HTC cells is accompanied by a corresponding increase in the concentration of the enzyme, as determined by immunological precipitation (*12*). We next studied the rate of incorporation of radioactive amino acids into general cell proteins and into tyrosine aminotransferase, specifically precipitated immunologically. The steroids stimulate the rate of amino acid incorporation into the enzyme about 15-fold without noticeably affecting incorporation into general cell protein (*11, 12*).

Role of Enzyme Turnover

We have also investigated the extent to which the steroid-mediated induction of the aminotransferase might result from changes in the rate of degradation of the enzyme. Since the enzyme turns over rapidly, both in intact animals (*15*) and in HTC cells (*10, 11, 16*), an alteration in its rate of degradation could easily influence the intracellular concentration. Studies in intact rats had suggested that the inactivation of the enzyme might be especially com-

plicated because inhibitors of protein synthesis somehow inhibit enzyme degradation (*17, 18*). In addition, Reel and Kenney (*19*) have reported that actinomycin D can inhibit degradation of the enzyme in HTC and Reuber hepatoma cells in culture.

However, as a result of studies in random (*20*) and synchronized (*21*) populations of HTC cells, we find that, under normal growth conditions, the aminotransferase in HTC cells turns over with a half-time of from 3 to 7 hours and that its degradation is not affected when protein or RNA synthesis is inhibited. However, under "step-down" cultural conditions (where the nutritional value of the medium is suddenly decreased), the rate of tyrosine aminotransferase degradation is stimulated, and this "enhanced" rate is slowed toward the normal rate of degradation by inhibition of RNA or protein synthesis (*20*). We do not yet understand the mechanism of "enhanced" degradation of the enzyme, but since step-down conditions are not used in our usual experiments, neither RNA nor protein synthesis is required for "normal" enzyme degradation. Thus, the kinetics of the induction (Fig. 2) can be fully explained by an increase in the rate of enzyme sythesis. The constant induced activity achieved 6 to 12 hours after addition of steroid to HTC cells is a steady state in which the rate of enzyme synthesis equals its rate of degradation.

Experiments with Synchronized HTC Cells

The availability of bacterial mutants that differ from the wild type only with respect to specific regulatory functions has greatly facilitated the study of control mechanisms in these organisms. However, regulatory mutants have not yet been found in mammalian cells. Therefore, to study the effects of alterations in genetic activity on enzyme induction, we have examined the properties of the HTC cell generation cycle and the synthesis and inducibility of tyrosine aminotransferase in its different phases (*22*) (Fig. 3). Although the enzyme can be synthesized in all phases of the cycle, it can only be induced by the steroids during the periods indicated in Fig. 3—that is, in the latter two-thirds of the interval between mitosis and the onset of DNA synthesis and during DNA synthesis. During the period between DNA synthesis and mitosis, mitosis, and the first several hours of the interval between mitosis and DNA synthesis, synthesis of tyrosine aminotransferase is not influenced by the presence of the hormones. Additional experiments with synchronized cultures (*21*) have shown that early in the interval between mitosis and DNA synthesis, when the enzyme becomes inducible, a repressor of enzyme synthesis, which acts after transcription, is formed. The relation of this repressor to the mechanism of enzyme induction is discussed below.

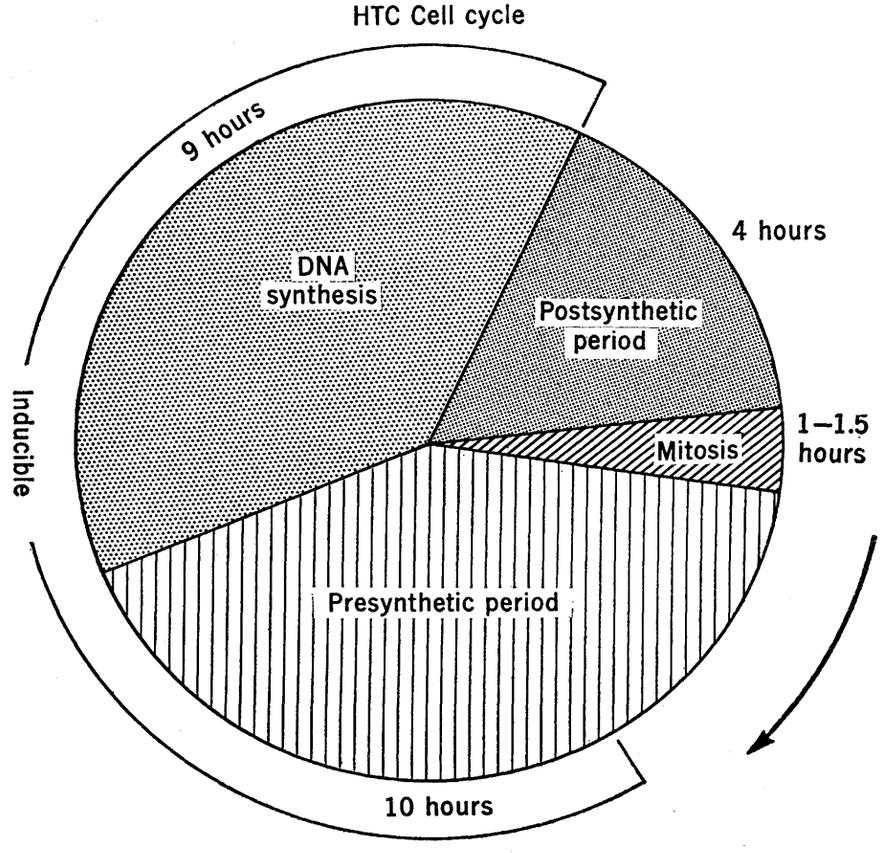

Fig. 3. The HTC cell cycle. The durations of these periods in the figure were determined as described in (*22*) as was the period during which tyrosine aminotransferase can be induced by adrenal steroids.

Theory of Enzyme Induction

Although the generally accepted explanation for hormonal enzyme induction is based directly on the Jacob-Monod model (*1*), various experiments in intact animals (*23*) and in HTC cells (*11*) had suggested that enzyme synthesis in mammalian cells cannot be controlled exclusively at gene transcription. To account for these results, we have proposed a model in which both transcriptional processes and processes after transcription might be regulated by the steroid inducer (*24*). In the light of our more recent findings, using populations of both random and synchronized cells, we present a more specific version of this model in which the steroids have only a single action, that is, to antagonize a posttranscriptional repressor which both inhibits messenger translation and promotes messenger degradation. In addition, we propose that during the noninducible phases of the cell-generation cycle, transcription of the tyrosine aminotransferase gene is repressed by a process insensitive to the steroid, and that this repression is lifted during the inducible periods of the cycle.

The model, presented in Fig. 4, entails two genes: the structural gene (G^S) for the induced enzyme and a regulatory gene (G^R). During the inducible phases of the cell cycle, the structural gene is transcribed continuously into messenger RNA, which, in turn, is translated into the final gene product, the enzyme.

During the inducible periods, the regulatory gene is also assumed to produce its product, the repressor, at a constant rate. We assume that the re-

pressor is a protein, and that both the repressor and its messenger RNA (not shown) are very labile relative to the other molecules depicted. The repressor is assumed to reversibly inhibit the translation of the messenger into the aminotransferase. Although this is symbolized in Fig. 4 as the combination of the messenger with repressor, we do not necessarily wish to imply this particular type of interaction, but merely the reversible effect of the repressor on messenger function.

As shown in Fig. 4, the messenger can only be degraded when its translation is inhibited by the repressor. Thus, the repressor both inhibits messenger translation and promotes its degradation. Finally, we postulate that the action of the repressor is directly or indirectly antagonized by the steroid inducers. Therefore, in the presence of an inducer, the repressor is inactivated, messenger translation occurs, and, at the same time, degradation of the messenger is prevented. Thus, the concentration of the messenger increases, because the transcription of the messenger gene is continuous.

During the noninducible phases of the cell cycle (the postsynthetic period, mitosis, and early in the presynthetic period), the transcription of both the structural and regulatory genes is assumed to be repressed by a process insensitive to the inducer (Fig. 4). Under these conditions, translation of the preexisting messenger continues, and because of the absence of the repressor, the messenger is more stable than during the inducible phases of the cycle. Obviously, in this state the steroids would have no effect on the system.

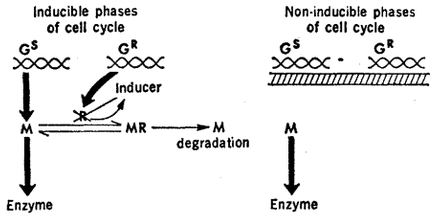

Fig. 4. Theory of enzyme induction in mammalian cells. The configuration shown on the left is assumed to exist during the inducible phases of the cell cycle (Fig. 3), while that on the right, during the noninducible phases (Fig. 3). The G^S refers to the structural gene for the inducible enzyme, while G^R refers to the regulatory gene. During the inducible periods, G^S is transcribed and the resulting messenger, M, can be translated to form the enzyme. The G^R is likewise transcribed and its messenger translated to produce the protein R. The R combines reversibly with M to produce the inactive complex MR which leads to M degradation. The R itself is labile, as shown by the thin arrow leading away from R. The inducer is indicated to inactivate R by an unknown mechanism. During the noninducible phases of the cycle, neither G^S nor G^R is transcribed, but M can be translated. Although for the case of tyrosine aminotransferase the degradation of the enzyme might also be depicted, we have not done so because its concentration is not regulated by changing the rate of its inactivation under constant cultural conditions (*20, 21*).

Tests of the Hypothesis with Unsynchronized Cell Populations

If the configuration of the genes is that which occurs during the inducible phases of the cell cycle, the model in Fig. 4 satisfactorily explains all the following observations made with random populations of HTC cells.

1) The steroid inducers stimulate the rate of enzyme synthesis (*11, 12*). The inducer somehow inactivates the repressor, preventing the conversion of the messenger to the repressed form. Thus, the inducer inhibits both the inactivation of the messenger and its degradation, allowing the concentration of the messenger to increase, thereby augmenting the rate of enzyme synthesis.

2) Enzyme-specific messenger RNA accumulates in the presence of the inducer, even when protein synthesis is inhibited (*11, 16, 25*). As shown in Fig. 4, the antagonism of the repressor by the inducer, be it direct or indirect, does not require protein synthesis. Once the repressor is neutralized, even though the messenger is not translated, the messenger accumulates because it is continually transcribed.

3) The constant presence of an inducing steroid is required to maintain the induced rate of enzyme synthesis (*10, 11, 20*). When the inducer is removed from the medium after induction or when a steroid that inhibits induction is added (*26*), the synthesis of the aminotransferase slows to the uninduced rate. According to Fig. 4, when the inducer is removed, the repressor rapidly becomes free to inhibit the translation of the messenger. Thus, the rate of enzyme synthesis is slowed to the basal value, and the repressed messenger is degraded.

4) Synthesis of RNA is required for enzyme induction (*16, 27*). Since the rate of enzyme synthesis is increased on induction because of the increase in messenger RNA concentration (caused by inhibition of its degradation), inhibition of the continuous transcription of the messenger RNA of tyrosine aminotransferase prevents the induction.

5) Continued RNA synthesis is not required to maintain enzyme synthesis at the basal or induced rates (*10, 11, 16, 20*). When the transcription of both the structural and repressor genes is inhibited, the repressor is inactivated and the messenger is stabilized at whatever concentration it had attained before RNA synthesis was interrupted. Therefore, enzyme synthesis continues at a fixed rate determined by the concentration of the stabilized template, the messenger.

6) If RNA synthesis is blocked after full induction, enzyme formation becomes constitutive—that is, the inducer may be removed but enzyme synthesis continues at the induced rate (*10, 11, 20*). When gene transcription is blocked, the repressor and its labile messenger are inactivated, freeing the aminotransferase messenger from rapid inactivation (and from subsequent degradation); this allows the translation of the messenger to continue in the absence of the inducer.

7) Actinomycin D "superinduces" the synthesis of the aminotransferase and increases its intracellular concentration (*10, 11, 16, 20*). Some time ago it was observed that rather high doses of actinomycin D apparently stimulated the synthesis of steroid-induced tryptophan oxygenase in the livers of intact rats (*23*). At that time, we proposed that this "paradoxical" effect of actinomycin D was due to a labile repressor of messenger RNA translation which was degraded in the presence of the inhibitor. More recently, we have observed a similar "superinduction" of tyrosine aminotransferase activity, when 1 to 5 μg of actinomycin D per milliliter are given to HTC cells either in the basal (*16*) or induced states (*11*).

Although it has been argued that superinduction could result from an inhibition of the aminotransferase degradation by actinomycin (*19*), we find (see above) that, under the usual conditions of our experiments, turnover of the enzyme in HTC cells is not inhibited by actinomycin D (*16, 20, 21*). Superinduction must therefore result from an increase in the rate of enzyme synthesis promoted by inhibiting RNA synthesis.

In addition to the two examples reported, many other cases of stimulation of either enzyme activities or synthesis by actinomycin D have subsequently been reported; this suggests that super-

induction is a fairly general phenomenon, at least in higher organisms (*28*). It occurs, according to our model, because, when gene transcription is inhibited, the repressor is inactivated and the repressed messenger can dissociate to liberate free, active messenger.

8) Induced enzyme synthesis, slowed by removing the inducer, may be reactivated by blocking RNA synthesis (*29*). This is illustrated in Fig. 5, which shows an experiment in which a culture of HTC cells had been exposed to $10^{-7}M$ cortisol for 17 hours. At time zero, the cell suspension was diluted tenfold into a warmed medium free of inducer; at intervals thereafter, portions of the culture were removed for assay of tyrosine aminotransferase activity. As early as 15 minutes after dilution of the inducer, the enzyme activity had fallen below the control value, and samples taken later show that the decline in activity continued with a half-time of about 5 hours. This immediate, rapid decline of enzyme activity indicates that, when the inducer is removed, the rate of enzyme synthesis abruptly decreases. When actinomycin D was added to the medium at the same time as the dilution into medium free of inducer, instead of falling, the enzyme activity was "superinduced," that is, rose above the control activity (Fig. 5). When the antibiotic was added 15 minutes after the inducer was removed, the activity again rose above the control value. Actinomycin D, given at 45 minutes, also caused an increase in enzyme activity (Fig. 5), although the maximum activity attained in this case was not as high as that produced when the inhibitor was given simultaneously with removal of the inducer. Whenever actinomycin D was given there was a reactivation of enzyme synthesis, indicated by the rise in activity. However, the longer the interval between administration of actinomycin D and removal of the inducer, the lower was the final maximum activity. Thus, when the inducer is removed, translation of the messenger is interrupted. However, when RNA synthesis is inhibited, translation can begin again, even in the absence of the steroid.

These findings are also explained by the model in Fig. 4, according to which removal of the inducer allows the rapid conversion of active messenger to inactive repressed messenger, preventing enzyme synthesis. When actinomycin D is given, repressor gene transcription is prevented and synthesis of the repressor stops. The concentration of the repressor then falls since it is rapidly degraded. The repressed messenger can then be converted to active messenger, and formation of the aminotransferase begins again. The fact that less enzyme-forming capacity can be "rescued" when actinomycin is given several hours after, rather than immediately after, removal of the inducer gives an indication of the rate at which the repressed messenger is degraded (roughly with a half-life of 3 hours). Thus, the rate of inactivation of the messenger, by combination with the repressor, occurs much more rapidly than the actual rate of messenger degradation. Therefore, a sizable pool of inactive, but "easily rescued," repressed messenger exists for some time after removal of the inducer. Since the repressed messenger seems to disappear more rapidly than the free messenger, we infer that the repressor somehow promotes the inactivation of the messenger, as suggested by the model.

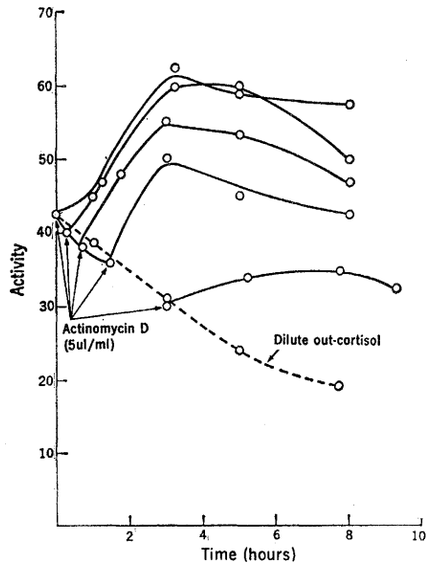

Fig. 5. "Messenger rescue" experiment. The HTC cell suspensions (800,000 cell/ml) were incubated in induction medium with $1 \times 10^{-7}M$ cortisol for 17 hours. At that time an 8-ml sample of cell suspension was added to fresh warmed induction medium free of steroid (37°C) and to warmed induction medium containing cortisol ($1 \times 10^{-7}M$). Both suspensions were further incubated at 37°C, and samples were removed from the culture free of steroid at the beginning of the incubation and at 15, 45, 90, and 180 minutes. Each sample was further incubated in the presence of actinomycin D (5 μg/ml), and, at the times indicated, samples were removed for enzyme assay. Portions were also removed for assay of tyrosine aminotransferase from the cell suspension containing no added steroid and from that containing cortisol. The activity is expressed as milliunits of enzyme per milligram of cell protein.

Whereas findings 1 through 4 can be explained by both the classical Jacob-Monod mechanism (*1*) and the posttranscriptional model proposed in Fig. 4, results 5 through 8 cannot be interpreted as an antagonism between the inducer and a stable repressor of gene transcription. However, the results of these experiments (and similar results in other systems) can be easily rationalized if we assume, as in Fig. 4, the existence of a labile repressor of messenger function operating at a site beyond that of gene transcription.

Test of the Hypothesis in Synchronized Cell Populations

In addition, the model in Fig. 4 accounts for the following results obtained with population of synchronized cells.

1) Tyrosine aminotransferase can be synthesized, but is not inducible, during the interval between DNA synthesis and mitosis, mitosis, and early in the interval between mitosis and DNA synthesis (*22*). In these noninducible phases of the cycle, the configuration of the genes is assumed to be that shown in the second part of Fig. 4, where both the structural and regulatory genes are repressed. The preexisting enzyme messenger continues to be translated and is stabilized since the repressor is not present. The steroids are inactive during these periods because of the absence of the repressor, and because the repression of transcription of the structural gene itself cannot be overcome by the inducer.

2) Preinduced cells, collected in mitosis, continue to synthesize the enzyme at the fully induced rate early in the interval between mitosis and DNA synthesis even in the absence of the inducer (*21*).

According to our model, the mRNA of the aminotransferase, accumulated on induction in random cells, is both stable and derepressed early in the presynthetic period because, during that period, the repressor gene is inactive so that formation of a new repressor is inhibited; and the preexisting repressor is degraded because of its lability (Fig. 4).

3) As preinduced synchronized cells enter hour 3 of the period between

mitosis and DNA synthesis, synthesis of tyrosine aminotransferase becomes repressed unless the inducer is added (*21*). According to the model, at hour 3 of the presynthetic period both the structural and repressor genes become activated, the repressor is synthesized, and, unless antagonized by the inducer, the translation of the enzyme messenger RNA is inhibited.

4) Inhibition of RNA synthesis before hour 3 of the presynthetic period preserves the "constitutive" state of tyrosine aminotransferase synthesis (*21*). This occurs because the expression of the repressor (and the structural) gene, which normally begins at this time, requires RNA synthesis. Thus, the repressor cannot be formed in the presence of actinomycin D, and whatever messenger RNA is present before hour 3 of the presynthetic period can be translated constitutively in the absence of the inducer.

Mechanism of Posttranscriptional Control

The posttranscriptional repressor could act at any site after the transcription of the messenger RNA.

Many chemical and physical reactions are required to transform the information encoded in nascent nuclear messenger RNA into the final polypeptide gene product, and there are, therefore, in principle as many potential loci of posttranscriptional regulation. In general, among the processes which might be regulated are: (i) specific protection or degradation of nascent nuclear messengers; (ii) transport of messengers from nucleus to cytoplasm; (iii) association of functional messengers bound to polyribosomes with specific regulatory proteins; (iv) activation or inactivation of ribosomes; (v) alterations in the folding of nascent polypeptide chains either by association with other proteins or with small molecules; and (vi) alterations in the function of specific factors involved in the initiation or termination of protein synthesis.

Certain types of posttranscriptional control, such as those operating in unfertilized eggs (*30*) or in mitotic cells (*31*) appear to be quite general because they inhibit virtually all cellular protein synthesis.

The proposed posttranscriptional regulator of tyrosine aminotransferase induction must be relatively specific because under normal conditions neither general protein synthesis nor growth of HTC cells is affected by the corticosteroids (*10, 11*). The rapidity with which induction can be terminated by removing the inducer suggests that the repressor may interfere in some way with polyribosome function. We obviously do not yet have enough information to propose with assurance any specific molecular model for repressor action. However, one reasonable possibility, among many others, might be that the repressor recognizes a specific base sequence at the 5′ end of the messenger, attaches to it, and, by preventing polyribosome formation, somehow allows more rapid messenger RNA degradation (*32*). Induction would result from detachment of the repressor from the messenger RNA, subsequent polysome formation, and stabilization of the messenger leading to its accumulation.

An apparently different, and less specific type of posttranscriptional regulation has also been recently discovered in HTC cells; a macromolecular fraction in serum stimulates the translation of the messenger RNA of the aminotransferase and, to a lesser extent, of other cellular messengers (*33*). The relationship of this type of regulation to the "posttranscriptional repressor" mechanism of Fig. 4 is not clear at present.

Mechanisms of Transcription Control

In principle, the posttranscriptional mechanism we propose could govern the expression of all the genes of differentiated cells, eliminating the necessity for any regulation over gene transcription.

However, considerable evidence has been presented for specific control of gene transcription in metazoans, particularly during development. For example, in insects only certain chromosomal loci synthesize RNA at a given time during development (*34*); and in amphibian embryos the synthesis of ribosomal RNA does not occur until a certain developmental stage has been reached, even though other classes of RNA are formed continuously (*35*).

Our results suggest that during certain phases of the HTC cell cycle both the structural and regulatory genes themselves become repressed and derepressed. This cyclic inhibition of gene transcription does not appear to be under control of the inducer, because the presence of the steroids during noninducible phases of the cell cycle does not stimulate synthesis of the aminotransferase.

The molecular basis of transcriptional regulation in eukaryotes is not well understood at present. Biochemical studies have shown that histones, the basic proteins associated with the DNA of eukaryotic chromosomes, inhibit the enzyme-catalyzed transcription in vitro of DNA and that chromatin is a much less effective template for RNA synthesis than purified DNA is (*36*). It has been suggested (*37*) that acidic chromosomal proteins, alone or together with the histones, may be responsible for the organ-specific restriction of DNA transcription in mammalian cells. Recent work has also shown that the specificity of RNA polymerase itself may be modified by the attachment of specific subunits (*38*). Finally, a theory of transcription control based on the redundant sequences of DNA in eukaryotic cells has been presented (*39*). In any event, it seems most likely that control of gene transcription is based on the specific interaction of macromolecules directly with the DNA.

Our prejudice at the present time is that transcriptional control is most important in the sequential gene activation which occurs during development, and in maintaining the differentiated state of metazoan cells. Posttranscriptional regulation and messenger stabilization might therefore be involved in finer regulation such as enzyme induction. One might well question the generality of the posttranscriptional mechanism proposed in Fig. 4. Unfortunately, relatively few other cases have been analyzed in sufficient detail to provide evidence for or against it. However, the frequency with which actinomycin D appears to stimulate the formation of specific proteins or makes their synthesis constitutive (*28*) suggests that specific labile repressors of messenger expression such as "the posttranscriptional repressor" may play a general role in biological control.

Site of Action of Steroid Hormones

In the model in Fig. 4, although the inducers are assumed to act by neutralizing the repressor, the precise mechanism is left open. Studies relating steroid structure with inducer action have led us to believe that the steroid receptors which mediate induction are

allosteric molecules, the conformation of which is regulated by the hormones (*26*). Direct binding studies (*40*) suggest that some of these receptors are located in the nucleus. Since the repressor appears to function in the cytoplasm to inhibit synthesis of the aminotransferase, it seems unlikely at first glance that the nuclear receptors themselves are the labile repressor.

Therefore, the mechanism by which the steroid-receptor complex influences repressor activity might well be indirect, for example either inhibiting repressor synthesis or transport, or else promoting its degradation.

Alternatively, the inducers might interact directly with the cytoplasmic repressor causing its translocation to the nucleus, where it could no longer inhibit aminotransferase synthesis. If this were true, the nuclear receptors would represent only an inactive form of the repressor. This possibility might be consistent with the presence, in the uterus, of cytoplasmic receptors which migrate to the nucleus after complexing with estradiol (*41*).

Relatively few results are available at this time which actually exclude conceivable mechanisms of hormone action. One set of such experiments (*26, 40*) shows that steroid metabolism is not required for binding to the specific corticosteroid receptors in HTC cells; and another study (*42*) has shown that neither cyclic adenosine monophosphate nor adenyl cyclase are present in these cells.

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Human Biological Adaptability

The ecological approach in physical anthropology.

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The theme of the U.S. effort in the International Biological Program is listed as "Man's survival in a changing world," and the whole of the International Biological Program has been described as focusing on ecology, especially human ecology.

The Human Adaptability Project is one of the principal aspects of the International Biological Program. Internationally about 50 nations are participating in the Human Adaptability Project studies of adaptation of many different peoples to a wide variety of environments. Of the integrated research programs constituting the U.S. contribution to the International Biological Program, five studies form the Human Adaptability group while eight constitute the environmental management group (*1*). The former consist of (i) the "International Study of Circumpolar Peoples Including Eskimos" involving adaptations to cold; (ii) the "Population Genetics of the American Indian" emphasizing adaptations to life under primitive conditions on the tributaries of the Amazon and Orinoco rivers; (iii) the "Biology of Human Populations at High Altitudes" in the Andes of Peru and in the Rockies but coordinated with studies in the Ethiopian highlands, and in the Himalayan and Tien Shan mountains; (iv) "Nutritional Adaptation to the Environment"; and (v) the "Ecology of Migrant Peoples." A sixth program in chronobiology is being prepared.