Nonhistone Chromosomal Proteins and Gene Regulation

Nonhistone chromosomal proteins may participate in the specific regulation of gene transcription in eukaryotes.

Gary S. Stein, Thomas C. Spelsberg, Lewis J. Kleinsmith

Of the many functions of the cell nucleus, one of the most important is the process of gene transcription whereby information encoded in the DNA genes is utilized in the synthesis of complementary molecules of RNA. It is through this synthesis of RNA that the genetic information stored in the nuclear DNA is ultimately transmitted to the protein synthesizing machinery in the cytoplasm, where it is used to code for the synthesis of specific proteins.

Specific regulation of the process of gene transcription is extremely important both during the development and normal functioning of cells of higher organisms. For example, differentiated cells that perform different functions utilize characteristic sets of genetic information, and so must use different regions of the genome for the synthesis of RNA. Since the DNA of each cell in a multicellular organism appears to be the same, this means that various cell types use the information contained in this DNA differently. Specific regulatory mechanisms must, therefore, be available for activating and inactivating particular regions of the genome for RNA synthesis, depending on the needs of the cell. The mechanism by which this selection and regulation of genetic potential is accomplished in higher organisms is still largely unknown, and presents one of the most challenging problems in modern biology.

Considerable progress has been made on this problem in recent years in microbial systems. In the best understood situation, namely the *Escherichia coli* *lac* operon, it has been shown that a specific repressor protein can be isolated which regulates DNA transcription by binding to a specific site in the bacterial DNA (*1*). The binding of this repressor to DNA can be regulated by small molecules (lactose and its analogs) which act as allosteric effectors, inducing changes in the conformation of the repressor protein which cause it to lose its affinity for DNA (*2*).

Because of the complexity of the organization of the genome in nucleated (eukaryotic) cells, however, analogous proteins and regulatory mechanisms have not been found. Unlike the bacterial chromosome, which is composed primarily of DNA, the eukaryotic chromosome is a complex structure called chromatin which, in addition to DNA, contains large amounts of histone and nonhistone proteins and small amounts of RNA. Histone polypeptides contain large amounts of the basic amino acids, arginine, lysine, and histidine, in contrast to the nonhistone proteins that are acidic in character. Very little is known about the specific functional roles played by these various chromatin components, but recent evidence suggests that the molecules responsible for specific gene regulation are to be found among the chromosomal proteins.

In 1943, Stedman and Stedman (*3*) deduced from the apparent amounts (*4*) of histones in actively growing and nongrowing tissues that these proteins function as biological repressors. However, the first definitive biochemical studies on the effects of histones on DNA function were performed in the early 1960's by Huang and Bonner (*5*) and Allfrey *et al.* (*6*). In these studies it was shown that histones inhibit the ability of DNA to serve as a template for RNA synthesis. These findings, which have been confirmed in a variety of systems, led to the speculation that histones serve as specific repressors of gene transcription in a fashion analogous to that of the bacterial repressors.

Unfortunately, the early excitement caused by these findings was soon tempered by increasing amounts of data that gave rise to serious doubts about the ability of histones to function as specific repressors. Crude preparations of histones could be fractionated into only five major classes, the proportions of which did not vary significantly in different tissues, different organisms, or under different physiological states (*7*, *8*). Indeed, there is a difference in only two amino acid residues in one histone that has been compared in pea seedlings and calf thymus (*9*). Thus, histones exhibit a uniformity and lack of specificity which makes it appear very unlikely that they are the molecules primarily responsible for the recognition of unique gene loci.

Attention has recently turned to the nonhistone chromosomal proteins as potential regulators of specific gene transcription. However, it is interesting to note that Stedman and Stedman (*10*) proposed that the nonhistone chromosomal proteins (chromosomin) were involved in the regulation of gene expression in 1943. According to these authors, the chromosomin represented the chemical basis of inheritance, and although we now realize that DNA, and not protein, constitutes the genetic material, they may have been correct in predicting that a physical association of nonhistone chromosomal proteins and nucleic acids is required for the synthesis of specific proteins. These early investigations also revealed that many of the nonhistone chromosomal proteins are enriched in aspartic acid and glutamic acid residues (*10*), and the term "acidic nuclear proteins" has frequently been used to describe them. Subsequent studies have shown that these proteins are made in the cytoplasm (*11*), and are more actively synthesized and turned over than histones (*12*, *13*).

The studies that focused attention on the possibility of nonhistone chromosomal proteins being regulators of gene expression in eukaryotic cells were

Dr. Stein is assistant professor in the Department of Biochemistry at the University of Florida, Gainesville 32601. Dr. Spelsberg is a consultant in the Department of Endocrine Research at the Mayo Clinic, Rochester, Minnesota 55901. Dr. Kleinsmith is associate professor in the Department of Zoology at the University of Michigan, Ann Arbor 48104.

those of Paul and Gilmour (*14*) who demonstrated that nonhistone chromosomal proteins are responsible for tissue-specific gene readout. Utilizing the techniques of chromatin reconstitution (as illustrated in Fig. 1) and DNA-RNA hybridization analysis of the transcription products, these workers established that nonhistone chromosomal proteins interact with DNA and modify transcription in a manner characteristic of the tissue of origin. In contrast to the histones, which are present in similar amounts in active and inactive tissues and chromatin (*7*), nonhistone chromosomal proteins are found in increased amounts in active tissues and chromatin (*7*). Also, nonhistone proteins are highly heterogeneous and possess tissue and species specificity (*15–17*); they can stimulate the synthesis of RNA in cell-free systems (*16, 18–24*); some of them bind specifically to DNA (*16, 23*); and the synthesis of particular classes of these proteins is associated with the induction of gene activity (*25*).

In this article we summarize some of the evidence for nonhistone chromosomal proteins being regulators of gene expression in eukaryotic cells. In particular, we discuss several systems that we have explored and in which differential regulation of gene transcription occurs. We also discuss some mechanisms by which the nonhistone proteins may interact with the genome and thereby initiate, modify, or augment the transcription of specific molecules of RNA.

Nonhistone Proteins and Control of Cell Proliferation

Two general types of model systems have been employed in attempts to study the regulation of gene expression during the cell cycle: (i) continuously dividing cells and (ii) resting cells that have been specifically induced to divide by the application of an appropriate stimulus. In both types of systems, a complex and interdependent series of biochemical changes precedes the actual processes of DNA synthesis and mitosis (*26*). Because the capacity of the genome for RNA synthesis fluctuates during the cell cycle, it has been suggested that prior to division there is a specific activation of the transcription of genes which code for the synthesis of macromolecules essential for DNA replication and mitosis. In its broader sense, this question of the mechanism by which defined regions of the genome are regulated during the cell cycle is relevant and potentially applicable to the general problem of the mechanism of gene regulation in eukaryotic cells (*26*).

The cell cycle is divided into four distinct phases: G1, or the prereplicative phase, is the period between the completion of mitosis and the onset of DNA synthesis; S is the period when DNA replication occurs; G2 is the period between completion of DNA synthesis and the onset of the fourth phase, mitosis.

If nonhistone chromosomal proteins are, in fact, responsible for the activation of those regions of the genome which contain the information required for a cell to replicate its DNA and divide mitotically, one would expect variations to occur in the rates of nonhistone chromosomal protein synthesis and turnover, as well as in the specific classes of nonhistone chromosomal proteins synthesized and associated with the DNA during defined periods of the cell cycle. In several models of stimulated DNA synthesis, such stage-specific differences in the nonhistone chromosomal proteins have been observed. In mouse salivary glands stimulated to proliferate by the synthetic catecholamine, isoproterenol (*27*), mouse kidneys stimulated to proliferate by folic acid (*28*), nondividing monolayers of cells stimulated to proliferate by a change of medium (*29, 30*), lymphocytes stimulated to proliferate by phytohemagglutinin (*31*), and in regenerating liver following partial hepatectomy, increased rates of labeled amino acid incorporation into nonhistone chromosomal proteins occurs early during the prereplicative phase of the cell cycle, with maximal rates of incorporation evident prior to the onset of DNA synthesis.

Because, in many of these studies, fluctuations in the size and specific activity of the acid-soluble amino acid precursor pool was taken into consideration, the increased prereplicative incorporation of amino acids into nonhistone chromosomal proteins reflects actual increased rates of synthesis. That specific classes of nonhistone chromosomal proteins are synthesized and associated with chromatin during restricted periods of the cell cycle in quiescent cells stimulated to proliferate is suggested by stage-specific differences in the polyacrylamide gel electrophoretic profiles of these proteins. Such varia-

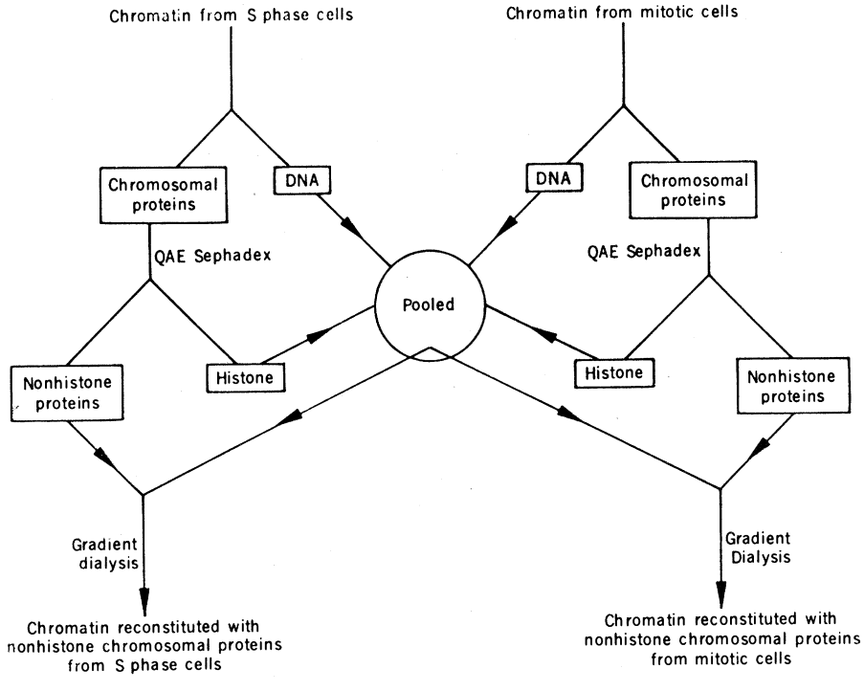

Fig. 1. Flow diagram for chromatin dissociation and reconstitution. Chromatin from S phase and mitotic HeLa S_3 cells was dissociated into DNA and chromosomal proteins with 3*M* NaCl, 5*M* urea, 0.01*M* tris(hydroxymethyl)aminomethane, *p*H 8.3. The chromosomal proteins were fractionated into histones and nonhistone chromosomal proteins by QAE Sephadex chromatography and the S phase and mitotic DNA and histones were pooled. Chromatin was then reconstituted by gradient dialysis, using the pooled DNA and histones from S phase and mitotic chromatin and either S phase or mitotic nonhistone chromosomal proteins (*21*).

tions occur early during G1 (*30, 32, 33*), as well as during S phase (*32*). In addition, cells exposed alternately to labeled and then nonlabeled amino acids (pulse-chase studies) in several models of stimulated DNA synthesis indicate that the nonhistone chromosomal proteins synthesized early during the prereplicative phase exhibit a faster rate of turnover than similar proteins in nonproliferating cells (*28, 29*).

Nonhistone chromosomal proteins may also play a significant role in modifying genome activity during the cell cycle in continuously dividing cells. Short-term and continuous exposure of cells to ^{3}H- and ^{14}C-labeled amino acids have demonstrated that nonhistone chromosomal proteins of specific classes are actively synthesized at different stages throughout the cell cycle (*34*). The highest rates of turnover of most molecular weight classes of nonhistone chromosomal protein occur during mitosis, and the lowest during S phase (*35*). Recent findings also indicate that the rates of phosphorylation of nonhistone chromosomal proteins fluctuate during the cell cycle, and that the different classes of these proteins are phosphorylated selectively during different stages of the cell cycle (*36*).

The synthesis of these nonhistone proteins may be regulated at least in part at the translational level. In quiescent cells stimulated to proliferate, treatment with actinomycin D, a potent inhibitor of DNA-dependent RNA synthesis, at a concentration which completely blocks the synthesis of messenger RNA (mRNA), is ineffective in reducing the increased rate of nonhistone chromosomal protein synthesis that occurs early during G1 (*27*). However, synthesis during later stages of the cell cycle is sensitive to the antimetabolite. The implication is that the initial synthesis of nonhistone chromosomal proteins which occurs following the stimulation of cellular proliferation takes place on preformed "stable templates." Consistent with these findings is the recent observation that treatment of synchronized continuously dividing HeLa S_3 cells with actinomycin D late in G2 is ineffective in suppressing the synthesis of several major classes of nonhistone chromosomal proteins in the subsequent G1 period (*37*). Inasmuch as there is a disaggregation of polyribosomes during mitosis as well as a cessation of mRNA synthesis, it is evident that those classes of nonhistone chromosomal proteins that are unaffected by the actinomycin D during G1 are synthesized on a stable species of mRNA transcribed sometime prior to mitosis and "reactivated" during G1.

In contrast to the situation found in bacterial or viral systems, chromosome replication in eukaryotic cells entails considerably more than synthesis of an identical copy of the DNA. The genome of eukaryotic cells contains the information for its own replication, and the expression of this information is apparently regulated in a manner similar to that in which other cellular differentiated functions are controlled. In continuously dividing cells, as well as in quiescent cells stimulated to proliferate, histone polypeptide synthesis is restricted to the S phase of the cell cycle and immediately ceases if DNA replication is inhibited (*38*); also, the mRNA for these basic chromosomal proteins is associated with polyribosomes and is translated concomitantly with DNA synthesis (*39*). In contrast, nonhistone chromosomal protein synthesis continues throughout the cell cycle in continuously dividing cells (*33–35, 37*), as well as in quiescent cells stimulated to proliferate (*27–32*), and does not appear to be dependent on DNA replication. This lack of coupling of nonhistone chromosomal protein synthesis and DNA replication is strongly suggested by the inability of cytosine arabinoside and hydroxyurea to reduce the incorporation of labeled amino acids into the nonhistone chromosomal proteins during S phase (*32, 34, 35*).

To investigate directly the influence of nonhistone chromosomal proteins on the transcriptional properties of the genome during the cell cycle, advantage was taken of two experimental situations where marked differences in gene expression are evident during the cell cycle. In one situation, continuously dividing HeLa S_3 cells, the isolated chromatin has a restricted capacity for DNA-dependent RNA synthesis during mitosis, compared to its capacity during S phase (*21*). In the other situation, in quiescent cells stimulated to proliferate, an increase in chromatin template activity occurs early during G1 (*20, 40*). The approach utilized in both situations was one of dissociating chromatin into its constituent DNA and protein components, fractionating the chromosomal proteins and subsequently reconstituting chromatin in a selective fashion. Support for the fidelity of chromatin reconstitution is provided by several lines of evidence (*14, 41*).

That nonhistone chromosomal proteins are responsible for variations in transcription during the cell cycle of continuously dividing cells is suggested by the reduced template activity of chromatin reconstituted with mitotic nonhistone chromosomal proteins, in comparison to chromatin reconstituted with S phase nonhistone chromosomal proteins (*21*) (Fig. 1). These findings are consistent with the observation that chromatin reconstituted in an analogous fashion with nonhistone chromosomal proteins from human diploid fibroblast cells of strain WI-38 1 hour after stimulation to proliferate has a higher template activity than chromatin reconstituted with nonhistone chromosomal proteins from nondividing WI-38 cells (*20*). In both systems, chromatin reconstituted with histones isolated from various stages of the cell cycle do not exhibit differences in template activity (*20, 21*).

Although it is apparent that nonhistone chromosomal proteins may play a key role in the control of gene expression during the cell cycle, little has been said concerning the specific manner in which this may be achieved. Recently it has been observed that, in addition to a decreased capacity of mitotic compared to S phase chromatin for DNA-dependent RNA synthesis (*21*), histones are more tenaciously bound to DNA during mitosis than during S phase (*42*). This conclusion is based on studies where the binding of histones to DNA was assayed by using sodium deoxycholate at concentrations which selectively dissociate histones from chromatin without removing DNA and nonhistone chromosomal proteins (*42, 43*). A given concentration of the ionic detergent dissociated a greater amount of histone from S phase than from mitotic chromatin (*42*). Taken together with the decreased template activity of chromatin reconstituted with mitotic rather than with S phase nonhistone proteins (*21*), one can speculate that nonhistone chromosomal proteins regulate transcription during the cell cycle by mediating the binding of histones to DNA. This is further suggested by (i) variations in the nonhistone chromosomal proteins synthesized and associated with chromatin during both stages of the cell cycle (*44*), (ii) differences in the phosphorylation of S phase and mitotic nonhistone chromosomal proteins (*36*), and (iii) the lack of any apparent variations in S phase and mitotic histones. To test this hypothesis, chromatin was reconstituted

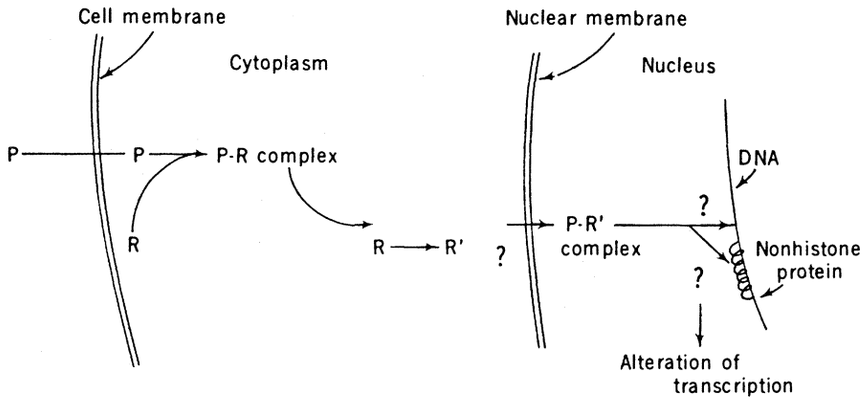

Fig. 2. Model of the intracellular fate of progesterone in the oviduct target cell. Progesterone (P) enters the target cell and binds receptor protein (R) that is located somewhere in the cytoplasm. The P-R complex is transported to the nucleus, during which time the receptor protein undergoes some modification (R'). The P-R' complex then binds to the chromatin, and as a result of this binding the transcriptional process becomes altered.

with either S phase or mitotic nonhistone chromosomal proteins see Fig. 1). It was found that, in agreement with the decreased ability of sodium deoxycholate to extract histones from DNA in native mitotic compared to native S phase chromatin, there was also a difference in histone binding between chromatin reconstituted with mitotic nonhistone chromosomal proteins and chromatin reconstituted with S phase nonhistone proteins (*42*). These results are indeed consistent with the proposed involvement of nonhistone chromosomal proteins in determining the specific manner in which histones are associated with DNA.

Nonhistone Proteins and Steroid Hormone Action

There is much evidence that the action of steroid hormones is mediated at least in part at the level of gene transcription. The picture that has emerged is that steroid hormones associate with specific cytoplasmic receptors which then transport the steroid to the nucleus; this leads ultimately to an alteration in DNA-dependent RNA synthesis (*45*) (see Fig. 2). This general model now seems to hold for a wide variety of steroid hormones including estrogen (*46*), progesterone (*47*), aldosterone (*48*), hydrocortisone (*49*), and androgens (*50*).

Attempts have been made recently to determine which of the components of chromatin participate in the binding of steroid hormones. Evidence that both histone (*49, 51*) and nonhistone chromosomal proteins (*48, 52–55*) represent the site of binding has been reported from studies in vivo. However, even the DNA has not been ruled out as the "acceptor site" that binds the hormone receptor translocated from the cytosol. A rewarding investigation by Tsai and Hnilica (*54*) confirmed the studies in vivo and in vitro showing that a small amount of the hormone cortisol is bound to the arginine-rich histones (one molecule of cortisol per 66,000 histone molecules). However, the cortisol was found to be associated with a trypsin-resistant fraction of this arginine-rich histone fraction. These workers suggest that since the arginine-rich histones contain significant amounts of nonhistone chromosomal proteins which are difficult to remove (*56*), and since the nonhistone chromosomal proteins are comparatively resistant to trypsin digestion, the cortisol is probably bound to a nonhistone protein associated with the arginine-rich histones. These results are supported by more direct evidence obtained by Barker (*57*) who found that labeled estradiol is associated in vivo in ovariectomized rat uteri with an acidic protein which contaminates the arginine-rich histone.

The acceptor role of nonhistone chromosomal proteins has been well studied in the binding of the steroid progesterone in the chick oviduct. The addition of labeled progesterone to oviduct cytosol (the supernatant fraction of oviduct homogenates centrifuged at 100,000*g*) results in the formation of a hormone-receptor complex (*47, 58, 59*). When this complex is incubated with isolated oviduct nuclei, a nuclear uptake of the labeled hormone is observed. The hormone, the cytosol receptor, and nuclei of oviduct are all required for this reaction to take place. Little uptake of the hormone occurs when the nuclei are incubated with hormone alone. Likewise, the substitution of either the cytosol or nuclei of other organs of the chick for those of the oviduct results in lower uptake of the progesterone-receptor complex (*59*).

Further studies of the nature of the specific sites in the nuclear binding were conducted in vitro with isolated progesterone-receptor (P-R) complex (the oviduct cytosol containing labeled progesterone) and with chromatin from oviduct nuclei. Several interesting results were obtained: (i) progesterone complexed to its cytosol receptor bound more extensively to chromatin than did the free hormone, which explains why progesterone in the presence of liver or spleen cytosols (containing no receptor) displays little binding; (ii) the bound progesterone could be reextracted from oviduct chromatin still as a complex with its receptor; (iii) the association of the P-R complex was sensitive to conditions of *p*H, ionic strength, and temperature; and (iv) the P-R complex displayed more extensive binding to oviduct chromatin than to the chromatins of other (nontarget) tissues (*60*). The fourth result does not necessarily imply a specificity of the oviduct chromatin to bind the P-R complex. It does, however, explain the specificity of hormone binding by oviduct nuclei (*59*) and gives evidence that the target tissue chromatin contains specific "acceptor sites" for the P-R complex.

To examine directly the role of chromosomal proteins in the binding of steroid hormones by target tissue chromatin, the dissociation and reconstitution of chromatin as described previously was utilized. When the histones together with one fraction of the nonhistone chromosomal proteins [fraction AP_1 (*61*)] were exchanged between the chromatins of different tissues by the method of Spelsberg *et al.* (*61*), there was no alteration in the extensive binding of the P-R complex by the chromatin containing the oviduct DNA and nonhistone chromosomal proteins (*60*). The binding to the reconstituted oviduct chromatin from which the nonhistone chromosomal proteins were removed was markedly reduced compared to the binding to reconstituted oviduct chromatin containing the nonhistone chromosomal proteins (*60*). Hence, the nonhistone chromosomal proteins but

not the histones appear to be involved in the specific binding of the P-R complex.

Three further pieces of evidence support this view. First, binding of P-R complex to oviduct dehistonized chromatin (containing most of the nonhistone chromosomal proteins) was much greater than binding to pure DNA or intact chromatin (*60*). Second, when the nonhistone chromosomal proteins were exchanged between chick oviduct and hen erythrocyte chromatin, the capacity for extensive binding of the P-R complex was also exchanged—that is, the reconstituted erythrocyte chromatin containing the nonhistone chromosomal proteins from the oviduct chromatin displayed the extensive binding formerly displayed by the oviduct chromatin and vice versa. The third line of evidence comes from experiments involving the dissociation of oviduct chromatin, the removal of certain subfractions of the nonhistone chromosomal proteins, followed by reconstitution (*62*). The binding of the P-R complex to these reconstituted chromatins deficient in one or more of the nonhistone chromosomal protein subfractions revealed that the "acceptor sites" for the hormone-receptor complex are primarily localized in fraction AP_3 (*62*). When subjected to polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS), this AP_3 fraction demonstrates a heterogeneous protein pattern (10 to 12 bands). Amino acid analysis indicates an acidic nature of this group of proteins. It is interesting that SDS-polyacrylamide gel electrophoresis (*63*) and the immunochemical technique of microcomplement fixation (*64*) have demonstrated a tissue specificity of the proteins of the AP_3 fraction. Further purification of the "acceptor molecules" in the AP_3 fraction requires alternative fractionation techniques. Unfortunately, attempts to activate chromatin and nuclear polymerases with this system have failed (*65*). Consequently, the "nativeness" of the interactions in vitro are subject to some skepticism. Other studies in which estradiol (*66*) as well as androgens are used (*67*) provide evidence that these hormones interact with nonhistone chromosomal proteins on their target cell chromatin.

At present, what is generally known about the fate of steroid hormones in target cells is depicted in Fig. 2 for progesterone in the chick oviduct. Progesterone enters the target cell and binds receptor protein. The intracellular localization of this receptor is unknown. The steroid hormone complex is transported into the nucleus by a temperature-sensitive process. There is evidence with estrogens that this process involves some modification of the receptor protein. The hormone-receptor complex then binds to the chromatin probably via a nonhistone protein, the "acceptor," but some binding function by the DNA cannot be ruled out. The intranuclear localization of this binding—that is, whether the complex binds to the proteins of eu- or heterochromatin or even to chromatin associated with the nuclear envelope, is not known. As a result of this binding, however, there is an alteration of the transcriptional process by an unknown mechanism. Whether the nonhistone protein "acceptors" are involved only in recognizing the cytoplasmic hormone receptor or are also involved in the changes of transcription remains to be determined.

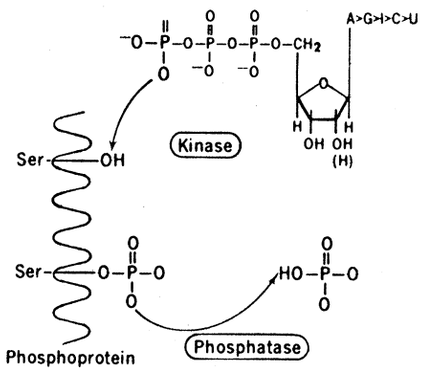

Fig. 3. Model summarizing the metabolic relationships of the nonhistone chromosomal phosphoproteins. Serine (and threonine) residues in the protein are phosphorylated in a kinase reaction utilizing the terminal phosphate groups of various nucleoside and deoxynucleoside triphosphates. In a separate enzymatic reaction, phosphate groups are cleaved from the protein and released in the form of inorganic phosphate. The relative activities of the phosphate donors (top right) are: adenosine triphosphate (*A*) > guanosine triphosphate (*G*) > inosine triphosphate (*I*) > cytidine triphosphate (*C*) > uridine triphosphate (*U*).

Phosphorylation of Nonhistone Proteins and Gene Expression

One striking property of the nonhistone chromosomal proteins which may help to elucidate the chemical nature of the regulation of gene action in higher organisms is the extensive phosphorylation that occurs in this protein fraction. The existence of phosphoprotein fractions which rapidly incorporate radioactive phosphate has been known in a variety of tissues for many years (*68*), and more recently phosphorylation and dephosphorylation of protein molecules have been shown to be important regulatory mechanisms for a variety of protein classes, including enzymes (*69*), membrane proteins (*70*), microtubular proteins (*71*), and ribosomal proteins (*72*). However, phosphorylated cellular proteins occur in highest concentrations in the cell nucleus, where both histone and nonhistone chromosomal proteins are phosphorylated (*73–75*). More than 90 percent of the nuclear protein-bound phosphorus is associated with the nonhistone chromosomal proteins, from which can be extracted a class of proteins which contain about 1 percent of phosphorus by weight. This phosphorus is present mainly as the phosphorylated amino acid phosphoserine, which is present in high enough quantities to account for 5 out of every 100 amino acid residues present (*73, 74*).

Metabolic studies on isolated nuclei have shown that nuclei are capable of rapidly phosphorylating nonhistone chromosomal proteins in a reaction that requires adenosine triphosphate as a source of energy, but occurs independently of protein synthesis (*73*). This latter observation demonstrates that phosphate groups are linked to preexisting proteins rather than being incorporated as part of protein synthesis. Once such phosphate groups have been put onto the nonhistone proteins they are not stable, but are instead subject to a rapid turnover. Current evidence suggests that separate enzymes are involved in phosphorylation and dephosphorylation of nonhistone chromosomal proteins, as is indicated in the model summarized in Fig. 3.

That phosphorylation of nonhistone chromosomal proteins is involved in gene activation is suggested by (i) the correlations between the phosphorylation of nonhistone chromosomal proteins and gene activity that have been observed in a number of different systems, including lymphocytes stimulated by phytohemagglutinin (*76*); (ii) changes in gene activity that are associated with maturation of avian red blood cells (*77*); (iii) the stimulation of gene activity that is induced in the prostate by testosterone (*78*); (iv) the activation of mammary glands by prolactin (*79*) and of ovaries by chorionic gonadotropin (*80*); (v) differences in gene activity in compact and diffuse chro-

matin (*81*); and (vi) the changes that occur during the cell cycle of synchronously dividing HeLa cells (*36*). In all of these situations, the activation of chromatin for RNA synthesis is associated with an increased rate of phosphorylation of nonhistone chromosomal proteins.

By means of acrylamide gel electrophoresis, it has been shown that the nonhistone chromosomal phosphoprotein fraction is highly heterogeneous, and although the overall patterns for different tissues share many features in common, each tissue has a unique, reproducible pattern which differs quantitatively and qualitatively from the others (*16, 17*). Labeling with ^{32}P has shown that most of the bands are phosphorylated proteins, and the phosphorylation patterns have been shown to be tissue specific. This observed heterogeneity and tissue specificity is in sharp contrast to the histones, of which there are a limited number of types, none of which vary substantially from tissue to tissue.

If the phosphorylation of nonhistone chromosomal proteins is directly involved in gene regulation, then one might expect to find an effect of these proteins on cell-free RNA synthesis. Several investigators have reported the stimulation of cell-free RNA synthesis by the addition of nonhistone chromosomal phosphoproteins (*16, 22, 74, 82, 83*). In one instance, the removal of some of the phosphate groups bound to nonhistone chromosomal proteins abolished this stimulatory effect on RNA synthesis, thus directly implicating the phosphorylation of nonhistone chromosomal proteins as playing a key role in modulating RNA synthesis (*22*). One of the most interesting aspects of the effects of nonhistone chromosomal phosphoproteins on cell-free RNA synthesis is the template specificity of the effect. If rat liver DNA and rat liver RNA polymerase are used to synthesize RNA, then the rate of the reaction is enhanced by the addition of rat nonhistone chromosomal phosphoproteins. Such a stimulation is not observed, however, if a bacterial DNA is employed as a template for RNA synthesis in the same system (*22*).

These results suggest the possibility of a specific recognition of DNA sequences by nonhistone chromosomal phosphoproteins. Indeed, it is well known that specific regulators of gene expression in bacteria, such as the *lac* operon repressor, can recognize and bind to specific base sequences in DNA (*2*), and it might be anticipated that specific gene regulators in eukaryotic cells exhibit a similar property. It has been shown both by DNA cellulose chromatography (*23, 84*) and sucrose gradient centrifugation (*16*) that a small fraction of the nonhistone chromosomal phosphoproteins will in fact bind to DNA. These phosphorylated proteins bind less efficiently to foreign DNA's than to host DNA, thereby demonstrating the specificity of the interaction. At physiological ionic strength, binding sites on the DNA are saturated at a value of approximately 1 microgram of phosphorylated protein per 100 μg of DNA. The DNA-binding proteins are still heterogeneous, as demonstrated by acrylamide gel electrophoresis, and are primarily in the molecular weight range of 30,000 to 70,000. Although there is no agreement on how much of the total nonhistone chromosomal phosphoprotein fraction is capable of such specific recognition of DNA sequences, the existence of such recognition is certainly consistent with the proposed role of these phosphorylated proteins in the specific regulation of gene activity.

When chromatin is reconstituted from its individual components, it is the source of the nonhistone chromosomal protein which determines the specific pattern of RNA synthesis (*13, 14, 18–21*). All the methods for preparing nonhistone chromosomal proteins for such experiments include some of the phosphorylated nonhistones in the final preparation. However, in such a mixture it is difficult to determine whether or not it is the phosphorylated components which are responsible for the specificity of reconstitution. In one instance, it has at least been shown that the addition to chromatin of a nonhistone chromosomal protein fraction rich in protein-bound phosphorus can confer specificity of gene readout (*24*). Thus, the data from such experiments are consistent with the proposal that phosphorylation of nonhistone chromosomal proteins is involved in specific gene control, although it is not yet possible to rule out alternative interpretations.

If the phosphorylation of nonhistone chromosomal proteins is involved in gene regulation, then how is the phosphorylation of these proteins in turn regulated? A large number of protein kinases that are associated with chromatin and that specifically phosphorylate different species of nonhistone chromosomal proteins have recently been discovered (*85, 86*). It is most interesting that these different protein kinase fractions exhibit varying responses to adenosine 3′,5′-monophosphate (cyclic AMP), some being inhibited while others are stimulated (*85*). Since cyclic AMP is known to act as a "second messenger" mediating the effects of many hormones and other agents on cell activity, the varying responses of these different kinases to various concentrations of cyclic AMP suggests at least one possible way in which the phosphorylation of nonhistone chromosomal proteins can be modulated in response to external stimuli and changing needs of the cell.

The complexity of the phosphorylated nonhistone chromosomal proteins, and of the enzymes involved in their phosphorylation and dephosphorylation, make it difficult to create a simple model of gene regulation based on these proteins. It was originally speculated that the negatively charged phosphate groups on the nonhistone chromosomal proteins might interact with the positively charged histones, thereby displacing the inhibitory histones from the DNA-histone complex and thus allowing the DNA to become active as a template for RNA synthesis (*73*). More recent studies that have shown that histones stimulate the phosphorylation of nonhistone chromosomal proteins have led to a refinement of this hypothesis (*87*).

According to this newer model, the increase in phosphorylation of nonhistone chromosomal proteins induced by the presence of histones would result in an increased negative charge on the nonhistone chromosomal proteins, and would thus serve to strengthen the ionic bonding between the nonhistone chromosomal phosphoproteins and the positively charged histones. Thus, when nonhistone chromosomal phosphoproteins and histones come together in vivo, one would expect this interaction to lead to phosphorylation of the nonhistone chromosomal proteins, resulting in a rapid increase in the strength of attraction between phosphoprotein and histone. Such an increased attraction might be sufficient to displace the histone from the DNA double helix, thereby allowing gene transcription to take place. Some evidence which supports this hypothesis can be found in the previously mentioned studies on the cell cycle, where it has been shown that S phase nonhistone chromosomal proteins, which have a higher rate of phosphorylation than the nonhistone pro-

teins from mitotic cells, cause histones to be more loosely bound to DNA than do mitotic nonhistone chromosomal proteins.

Although this model has certain attractive features, it also has a number of serious limitations. For example, it is difficult to understand how the recognition of specific genes occurs. Although we have already cited data which show that some of the phosphorylated nonhistone chromosomal proteins can bind to specific types of DNA sequences in purified systems, such binding has not yet been reported in systems employing DNA covered with histone. Another observation that is difficult to reconcile with this model is the previously mentioned stimulation of RNA synthesis by nonhistone chromosomal phosphoproteins in the absence of any histone at all. It is thus possible that some of these phosphorylated nonhistone chromosomal proteins interact with DNA or RNA polymerase directly. In terms of the latter possibility, the report that the sigma factors which regulate the specificity of bacterial RNA polymerases can also be phosphorylated (*88*) leads to the suggestion that some of the phosphorylated nonhistone chromosomal proteins may function in an analogous manner. Thus, in view of the great heterogeneity present among the phosphorylated nonhistone chromosomal proteins, it is most likely that no single model will explain all the experimental data in a satisfactory fashion, and that these phosphorylated proteins are in fact exerting their regulatory effects at a number of different levels and in a number of different ways.

Conclusions

Evidence from several model systems suggests that nonhistone chromosomal proteins may regulate gene expression in eukaryotic cells. The data indicate that the synthesis of new species of nonhistone chromosomal proteins as well as modifications of preexisting nonhistone chromosomal proteins are involved in the control of transcription. However, from the vast number of proteins included in this class, it is apparent that, in addition to regulating the transcription of defined genome loci, the nonhistone chromosomal proteins include enzymes that have a general function, proteins that are involved in determining the structure of chromatin, as well as proteins that serve as recognition sites for binding of regulatory macromolecules. The presence of a nucleoplasmic pool of nonhistone chromosomal proteins which may exchange with the chromatin has also been reported (*89*). While it is clear that the nonhistone chromosomal proteins play a key role in the regulation of gene expression, the exact manner in which they interact with the genome to initiate, modify, or augment the transcription of specific RNA molecules remains to be resolved.

References and Notes

1. J. R. Beckwith and D. Zipser, *The Lactose Operon* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1970); W. Gilbert and B. Müeller-Hill, *Proc. Natl. Acad. Sci. U.S.A.* **56**, 1891 (1966).
2. K. Adler, K. Beyreuther, E. Fanning, N. Geisler, B. Gronenborn, A. Klemm, B. Müeller-Hill, M. Pfahl, A. Schmitz, *Nature (Lond.)* **237**, 323 (1972).
3. E. Stedman and E. Stedman, *Nature (Lond.)* **152**, 556 (1943).
4. Although Stedman and Stedman (*3*) were correct in postulating a repressor function for the histones, their conclusions were based on the histone contents of active and inactive tissues which have subsequently been shown to be inaccurate.
5. R. C. Huang and J. Bonner, *Proc. Natl. Acad. Sci. U.S.A.* **48**, 1216 (1962).
6. V. G. Allfrey, V. C. Littau, A. E. Mirsky, *ibid.* **49**, 414 (1963).
7. C. W. Dingman and M. B. Sporn, *J. Biol. Chem.* **239**, 3483 (1964); J. H. Frenster, V. G. Allfrey, A. E. Mirsky, *Proc. Natl. Acad. Sci. U.S.A.* **50**, 1026 (1963); J. H. Frenster, *Nature (Lond.)* **206**, 680 (1965); V. C. Littau, V. G. Allfrey, J. H. Frenster, A. E. Mirsky, *Proc. Natl. Acad. Sci. U.S.A.* **52**, 93 (1964).
8. L. S. Hnilica and H. Busch, *J. Biol. Chem.* **238**, 918 (1963); L. Hnilica, E. W. Johns, J. A. V. Butler, *Biochem. J.* **82**, 123 (1962).
9. R. J. DeLange, D. M. Fambrough, E. L. Smith, J. Bonner, *J. Biol. Chem.* **244**, 5669 (1969).
10. E. Stedman and E. Stedman, *Nature (Lond.)* **152**, 267 (1943).
11. G. S. Stein and R. Baserga, *Biochem. Biophys. Res. Commun.* **44**, 218 (1971).
12. M. Daly, V. G. Allfrey, A. E. Mirsky, *J. Gen. Physiol.* **36**, 173 (1952); V. G. Allfrey, M. Daly, A. E. Mirsky, *ibid.* **38**, 415 (1955); P. Byvoet, *J. Mol. Biol.* **17**, 311 (1966); R. Hancock, *ibid.* **40**, 457 (1969).
13. V. Holoubek and T. Crocker, *Biochim. Biophys. Acta* **157**, 352 (1968).
14. J. Paul and R. S. Gilmour, *J. Mol. Biol.* **34**, 305 (1968); R. S. Gilmour and J. Paul, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **9**, 242 (1970).
15. A. J. MacGillivary, D. Carroll, J. Paul, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **13**, 204 (1971); J. Loeb and C. Creuzet, *Bull. Soc. Chim. Biol.* **52**, 1007 (1970); S. Elgin and J. Bonner, *Biochemistry* **9**, 4440 (1970); K. H. Richter and C. E. Sekeris, *Arch. Biochem. Biophys.* **148**, 44 (1972); J. A. Wilhelm, A. T. Ansevin, A. W. Johnson, L. S. Hnilica, *Biochim. Biophys. Acta* **272**, 220 (1972); T. Y. Wang, *Exp. Cell Res.* **69**, 217 (1971); K. R. Shelton and J. M. Neelin, *Biochemistry* **10**, 2342 (1971); B. Dastugue, L. Tichonicky, J. Penit-Soria, J. Kruh, *Bull. Soc. Chim. Biol.* **52**, 391 (1970).
16. C. S. Teng, C. T. Teng, V. G. Allfrey, *J. Biol. Chem.* **246**, 3597 (1971).
17. R. D. Platz, V. M. Kish, L. J. Kleinsmith, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **12**, 38 (1970); D. Rickwood, P. G. Riches, A. J. MacGillivary, *Biochim. Biophys. Acta* **299**, 162 (1973).
18. T. C. Spelsberg and L. Hnilica, *Biochem. J.* **120**, 435 (1970); T. Y. Wang, *Exp. Cell Res.* **53**, 288 (1968); M. Kamiyama and T. Y. Wang, *Biochim. Biophys. Acta* **228**, 563 (1971).
19. T. C. Spelsberg and L. S. Hnilica, *Biochim. Biophys. Acta* **228**, 550 (1971).
20. G. S. Stein, S. C. Chaudhuri, R. Baserga, *J. Biol. Chem.* **249**, 3918 (1972).
21. G. S. Stein and J. Farber, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2918 (1972).
22. M. Shea and L. J. Kleinsmith, *Biochem. Biophys. Res. Commun.* **50**, 473 (1973).
23. L. J. Kleinsmith, J. Heidema, A. Carrol, *Nature (Lond.)* **226**, 1025 (1970).
24. N. Kostraba and T. Y. Wang, *Biochim. Biophys. Acta* **262**, 169 (1972).
25. C. Teng and T. Hamilton, *Proc. Natl. Acad. Sci. U.S.A.* **63**, 465 (1969); K. Shelton and V. G. Allfrey, *Nature (Lond.)* **228**, 132 (1970); V. Enea and V. G. Allfrey, *Nat. New Biol.* **242**, 265 (1973).
26. G. S. Stein and R. Baserga, *Adv. Cancer Res.* **15**, 287 (1972); G. S. Stein, in *The Pathology of Transcription and Translation*, E. Farber, Ed. (Dekker, New York, 1972), p. 21; R. Baserga, *Cell Tissue Kinet.* **1**, 167 (1968).
27. G. S. Stein and R. Baserga, *J. Biol. Chem.* **245**, 6097 (1970).
28. G. S. Stein, in preparation.
29. G. Rovera and R. Baserga, *J. Cell. Physiol.* **77**, 201 (1971).
30. A. Tsuboi and R. Baserga, *ibid.* **80**, 107 (1972).
31. R. Levy, S. Levy, S. Rosenberg, R. Simpson, *Biochemistry* **12**, 224 (1973).
32. G. S. Stein and C. L. Thrall, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, in press.
33. R. Baserga and G. S. Stein, *Fed. Proc.* **30**, 1752 (1971).
34. G. S. Stein and R. Baserga, *Biochem. Biophys. Res. Commun.* **41**, 715 (19[illegible] G. S. Stein and T. W. Borun, *J. Cell Biol.* **52**, 292 (1972).
35. G. S. Stein, T. W. Borun, L. Pegoraro, *Fed. Proc.* **30**, 457 (abstr.) (19[illegible] T. W. Borun and G. S. Stein, *J. Cell Biol.* **52**, 308 (1972).
36. R. Platz, G. S. Stein, L. J. Kleinsmith, *Biochem. Biophys. Res. Commun.* **51**, 735 (1973).
37. G. S. Stein and D. E. Matthews, *Science* **181**, 71 (1973).
38. J. Spalding, K. Kajiwara, G. Mueler, *Proc. Natl. Acad. Sci. U.S.A.* **56**, 1535 (19[illegible] E. Robbins and T. W. Borun, *ibid.* **57**, 409 (1967).
39. T. W. Borun, M. D. Scharff, E. Robbins, *ibid.* **58**, 1977 (1967).
40. J. Farber, G. Rovera, R. Baserga, *Biochem. J.* **122**, 189 (19[illegible] A. Novi and R. Baserga, *J. Cell Biol.* **55**, 554 (1972); R. Hirschhorn, W. Troll, G. Brittinger, *Nature (Lond.)* **222**, 1247 (1969); M. Thaler and C. Villee, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 2055 (1967); S. Bannai and H. Terayama, *J. Biochem.* **66**, 289 (1969); J. Mayfield and J. Bonner, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 7 (197[illegible] K. Barker and J. Warren, *ibid.* **56**, 1298 (1966); C. Teng and T. Hamilton, *ibid.* **60**, 1410 (1968).
41. I. Bekhor, G. Kung, J. Bonner, *J. Mol. Biol.* **39**, 351 (1969); J. Paul and I. More, *Nat. New Biol.* **239**, 134 (1972).
42. G. S. Stein and G. Hunter, *Fed. Proc.*, in press.
43. J. Smart and J. Bonner, *J. Mol. Biol.* **58**, 651 (1971).
44. G. S. Stein, G. Hunter, L. Lavie, *Biochem. J.*, in press.
45. J. Gorski, D. Toft, G. Shyamala, D. Smith, A. Notidies, *Recent Prog. Horm. Res.* **24**, 45 (1968); J. D. Wilson and R. E. Gloyna, *ibid.* **26**, 309 (1970); B. W. O'Malley, W. L. McGuire, P. O. Kohler, S. G. Korenman, *ibid.* **25**, 105 (1969); T. H. Hamilton, *Science* **161**, 649 (1968).
46. E. V. Jensen and E. R. DeSombre, *Annu. Rev. Biochem.* **41**, 789 (1972).
47. B. W. O'Malley, M. R. Sherman, D. O. Toft, T. C. Spelsberg, W. T. Schrader, A. W. Steggles, *Adv. Biosci.* **7**, 213 (1971).
48. G. E. Swanek, L. L. H. Chu, I. S. Edelman, *J. Biol. Chem.* **245**, 5382 (1970).
49. J. D. Wilson and P. M. Loeb, in *Developmental and Metabolic Control Mechanisms and Neoplasia* (Williams & Wilkins, Baltimore, 1965), p. 375.
50. T. Liang and S. Liao, *Biochim. Biophys. Acta* **277**, 590 (1972); G. S. Harris, *Nat. New Biol.* **231**, 246 (1971).
51. M. Sluyser, *J. Mol. Biol.* **19**, 591 (1966).
52. D. D. Fanestil and I. S. Edelman, *Proc. Natl. Acad. Sci. U.S.A.* **56**, 872 (1966).
53. G. Shyamala and J. Gorski, *J. Biol. Chem.* **244**, 1097 (1969).
54. Y. H. Tsai and L. S. Hnilica, *Biochim. Biophys. Acta* **238**, 277 (1971).
55. M. Beato, D. Biesewig, W. Braendle, C. E. Sekeris, *ibid.* **192**, 494 (1969); R. Maurer and R. Chalkley, *J. Mol. Biol.* **27**, 431 (1967).
56. R. B. J. King, J. Gordon, A. W. Steggles, *Biochem. J.* **114**, 649 (1969); L. S. Hnilica

and L. G. Bess, *Anal. Biochem.* **12**, 421 (1965); R. H. Stellwagen and R. D. Cole, *J. Biol. Chem.* **243**, 4452 (1968).
57. K. L. Barker, *Biochemistry* **10**, 284 (1971).
58. M. R. Sherman, P. L. Corvol, B. W. O'Malley, *J. Biol. Chem.* **245**, 6085 (1970).
59. B. W. O'Malley, D. O. Toft, M. R. Sherman, *ibid.* **246**, 1117 (1971).
60. T. C. Spelsberg, A. W. Steggles, B. W. O'Malley, *ibid.*, p. 4188.
61. T. C. Spelsberg, L. S. Hnilica, A. T. Ansevin, *Biochim. Biophys. Acta* **228**, 550 (1971).
62. T. C. Spelsberg, A. W. Steggles, F. Chytil, B. W. O'Malley, *J. Biol. Chem.* **247**, 1368 (1972).
63. T. C. Spelsberg, W. M. Mitchell, F. Chytil, E. M. Wilson, B. W. O'Malley, *Biochim. Biophys. Acta*, in press.
64. F. Chytil and T. C. Spelsberg, *Nat. New Biol.* **233**, 215 (1971).
65. T. C. Spelsberg, unpublished data.
66. R. J. B. King, J. Gordon, A. W. Steggles, *Biochem. J.* **114**, 649 (1969); A. Alberga, N. Massol, J. P. Raynaud, E. E. Baulieu, *Biochemistry* **10**, 3825 (1971).
67. T. Liang and S. Liao, *Biochim. Biophys. Acta* **277**, 590 (1972).
68. J. N. Davidson, S. C. Frazer, W. C. Hutchinson, *Biochem. J.* **49**, 311 (1951); R. N. Johnson and S. Albert, *J. Biol. Chem.* **200**, 335 (1953); E. P. Kennedy and S. W. Smith, *ibid.* **207**, 153 (1954); J. F. Kuo, B. K. Krueger, J. R. Sanes, P. Greengard, *Biochim. Biophys. Acta* **212**, 79 (1970).
69. E. G. Krebs, R. J. DeLange, R. G. Kemp, W. D. Riley, *Pharmacol. Rev.* **18**, 163 (1966); D. L. Friedman and J. Larner, *Biochemistry* **2**, 669 (1963); T. C. Linn, F. H. Pettit, F. Hucho, L. J. Reed, *Proc. Natl. Acad. Sci. U.S.A.* **64**, 227 (1969).
70. R. N. Zahlten, A. A. Hochberg, F. W. Stratman, H. A. Lardy, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 800 (1972); D. Bownds, J. Dawes, J. Miller, M. Stahlman, *Nat. New Biol.* **237**, 125 (1972); R. O. Williams, *Biochem. Biophys. Res. Commun.* **47**, 671 (1972); J. M. Trifaro, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **23**, 237 (1972); T. P. Dousa, H. Sands, O. Hechter, *Endocrinology* **91**, 757 (1972); L. de Meis, *Biochemistry* **11**, 2460 (1972).
71. A. W. Murray and M. Froscio, *Biochem. Biophys. Res. Commun.* **44**, 1089 (1971); D. B. P. Goodman, H. Rasmussen, F. DiBella, C. E. Guthrow, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **67**, 652 (197→ B. A. Eipper, *ibid.* **69**, 2283 (1972).
72. D. Kabat, *Biochemistry* **9**, 4160 (1970); J. E. Loeb and C. Blat, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **10**, 105 (197→ D. Kabat, *Biochemistry* **10**, 197 (1971); C. Eil and I. G. Wool, *Biochem. Biophys. Res. Commun.* **43**, 1001 (1971).
73. L. J. Kleinsmith, V. G. Allfrey, A. E. Mirsky, *Proc. Natl. Acad. Sci. U.S.A.* **55**, 1182 (1966); L. J. Kleinsmith and V. G. Allfrey, *Biochim. Biophys. Acta* **175**, 123, 136 (1969).
74. T. A. Langan, in *Regulation of Nucleic Acid and Protein Biosynthesis*, V. V. Koningsberger and L. Bosch, Eds. (Elsevier, Amsterdam, 1967), p. 233.
75. M. G. Ord and L. A. Stocken, *Biochem. J.* **98**, 888 (1966); T. A. Langen, *Science* **162**, 579 (1968).
76. L. J. Kleinsmith, V. G. Allfrey, A. E. Mirsky, *Science* **154**, 780 (1966).
77. E. L. Gershey and L. J. Kleinsmith, *Biochim. Biophys. Acta* **194**, 519 (1969).
78. K. Ahmed and H. Ishida, *Mol. Pharmacol.* **7**, 323 (1971).
79. R. W. Turkington and M. Riddle, *J. Biol. Chem.* **244**, 6040 (1969).
80. R. A. Jungmann and J. S. Schweppe, *J. Biol. Chem.* **247**, 5535 (1972).
81. T. A. Langan, personal communication.
82. D. Rickwood, G. Threlfall, A. J. MacGillivary, J. Paul, *Biochem. J.* **129**, 50p (1972).
83. M. Kamiyama, B. Dastugue, J. Kruh, *Biochem. Biophys. Res. Commun.* **44**, 1345 (1971); M. Kamiyama, B. Dastugue, N. Defer, J. Kruh, *Biochim. Biophys. Acta* **277**, 576 (1972).
84. L. J. Kleinsmith and V. M. Kish, in *Methods Enzymol.*, in press; L. J. Kleinsmith, in preparation.
85. V. M. Kish and L. J. Kleinsmith, *J. Cell Biol.* **55**, 138a (1972).
86. M. Takeda, H. Yamamura, Y. Ohga, *Biochem. Biophys. Res. Commun.* **42**, 103 (1971); R. W. Ruddon and S. L. Anderson, *ibid.* **46**, 1499 (1972).
87. P. B. Kaplowitz, R. D. Platz, L. J. Kleinsmith, *Biochim. Biophys. Acta* **229**, 739 (1971).
88. O. J. Martelo, S. L. C. Woo, E. M. Reimann, E. W. Davie, *Biochemistry* **9**, 4807 (1970).
89. G. S. Stein and C. L. Thrall, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, in press.
90. This work was supported by research grants DRG-1138 from the Damon Runyon Memorial Fund for Cancer Research, GB-23921 and GB-38349 from the National Science Foundation, F73UF-6 from the American Cancer Society, CA-14920 from the National Institutes of Health, and a grant from the Mayo Foundation. T.C.S. is a fellow of the National Genetics Foundation. We thank Drs. Paul Byvoet, Rowland Davis, Carl Feldherr, Rusty Mans, John Paul, Owen Rennert, and Janet Swinehart for their helpful and stimulating suggestions.

Prevention of Food-Processing Wastes

Processes can be changed to reduce wastes, maintain product quality, and improve product yield.

Sam R. Hoover

Although technology for treating wastes resulting from food processing is available, and is moderately successful by today's standards, it does not meet national goals set forth in the Clean Water Restoration Act of 1972. This act, the culmination of the several clean water acts passed since 1962, provides that discharge of pollutants into navigable waters be eliminated by 1985. Therefore, in recent years a new look has been taken at food processing, with some notable successes and the promise of more. The question is, "Can we change existing processes so that less waste is produced, while maintaining or improving product quality?" The following is a discussion of major processing steps.

The author is retired from the U.S. Department of Agriculture, where he was active in food processing and treatment of dairy waste. His address is 2017 Hillyer Place, NW, Washington, D.C. 20009. This article is adapted from a paper presented at the AAAS-CONACYT meeting, Mexico City, 21 June 1973.

Peeling

The Western Regional Research Laboratory of the Agricultural Research Service, U.S. Department of Agriculture (USDA), decided to study first the peeling of white potatoes; more potatoes, by weight, are produced in this country than any other vegetable (*1*). There were many plants processing a million pounds a day (1 pound = 0.45 kilogram), with a 5-day biochemical oxygen demand (BOD_5), equivalent to that of a city of 300,000 people. About 75 percent of this BOD was directly associated with the peeling process.

Traditionally, potatoes were peeled by dipping them in a 16 to 20 percent lye solution at 95° to 120°C for 3 to 5 minutes, followed by a 2- to 5-minute holding period at the boiling point. They were then peeled in a rotating reel with high-pressure water jets.

After studying the variables involved in the process, the Western Laboratory developed a process which it put to use in a pilot plant. The new process consisted of a 1-minute dip in 12 percent lye, a 3- to 5-minute holding period, a 1-minute heating with infrared, and mechanical peeling with rotating rolls that have ½-inch rubber studs. It is called, not absolutely accurately, dry-caustic peeling. The peel is thrown off the rubber-tipped rolls and accumulates as a pumpable, 25 percent solid residue (Fig. 1). The peeled potatoes go through a finisher, which uses wire brushes with water spray to remove gelatinous (cooked) material from the