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NUTRIENT EFFECTS ON DNA AND CHROMATIN STRUCTURE

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CONTENTS

INTRODUCTION

Critical biological processes such as cellular differentiation, development, growth, and metabolic adaptation depend upon regulated gene expression, which in turn depends ultimately on components from the nutrient environment. The general sequential steps in gene expression are depicted in Figure 1. This review addresses the initial concepts of nutrient effects on the structure of DNA and chromatin. Nutritional effects on the regulation of gene expression is reviewed by Goodridge in this volume (34a). Specific nutrient involvement in transcription as well as in replication is reviewed by Wu & Wu, also in this volume (106). Nutritional effects on posttranscriptional and posttranslational modifications are reviewed by Rucker & Tinker (78).

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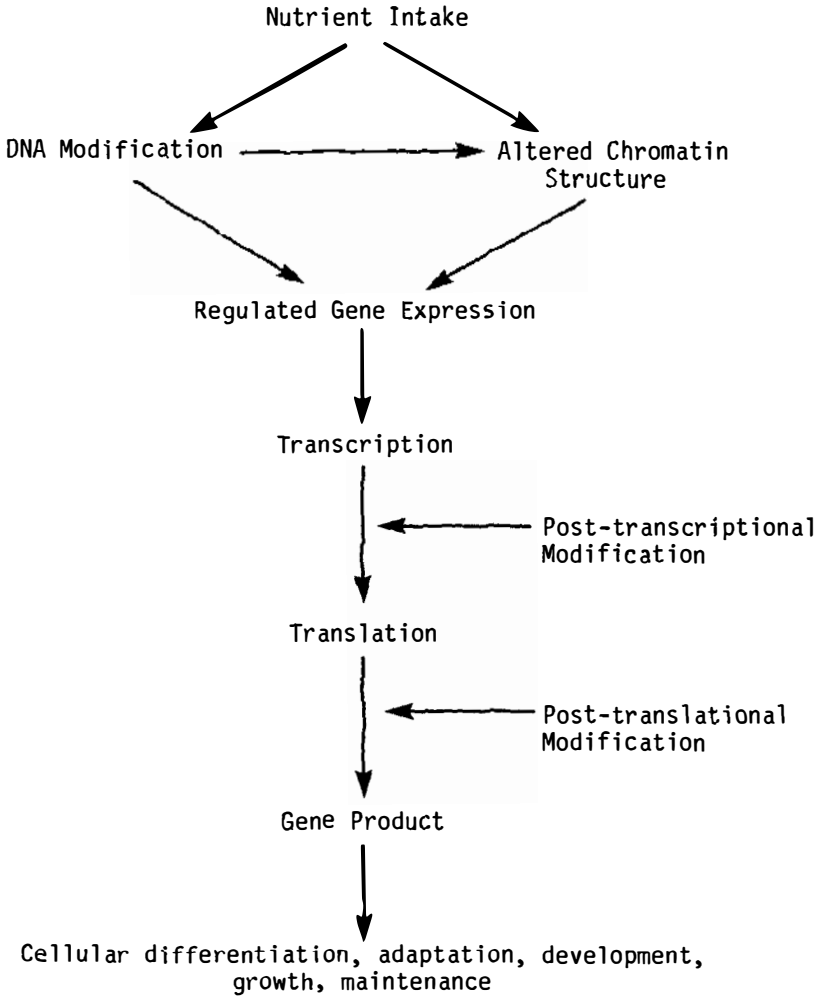

Figure 1 Steps in gene expression affected by nutrient intake.

ORGANIZATION OF DNA IN THE NUCLEUS

The eukaryotic nucleus is approximately 10 μm in diameter (7) and contains about 50 cm of DNA (65). The DNA must be condensed 50,000 times and yet still be spatially available for replication, transcription, recombination, and repair. The condensation of DNA is achieved by an elaborate hierarchy of organization (40, 54, 65).

In the first level of organization, 145 base pairs (bp) of the DNA helix are coiled around a protein core consisting of equimolar amounts of the histones H2A, H2B, H3, and H4 (40, 46, 54) to generate a disk-like structure called the nucleosomal core. An additional stretch of 20 bp of DNA yields a structure identified as a chromatosome (84). From one chromatosome to another are 20–60 bp of linker DNA to which a fifth histone, H1, is bound. The chromatosome plus linker DNA is called the nucleosome, the fundamental packaging unit that condenses linear DNA into a 10-nm fiber (69, 103). The 10-nm fiber is then organized into a 30-nm solenoidal fiber by a consecutive coiling of the fiber, with six nucleosomes per turn (32).

The higher-order arrangement of DNA in interphase chromatin and in metaphase chromosomes is incompletely understood and is a major issue in molecular biology. Generally, however, the 30-nm chromatin fiber appears to be organized into loops or domains that are defined units of DNA structure both in interphase nuclei (6, 64, 95) and in metaphase chromosomes (2). The DNA loop-domains are attached at nonrandom sites to the nuclear matrix, a protein network that functions in the organization of interphase chromatin (64, 95). The DNA loops are topologically constrained firmly to attachment sites on the matrix and function independently of one another. One such loop can condense DNA by 700-fold (65).

Roles in both replication and transcription are attributed to these loops. Pardoll et al (70) demonstrated that the nuclear matrix contains anchorage sites for the replication of DNA loops equivalent to the basic lengths of DNA synthesized as continuous units (39). In addition to playing a role in replication, the DNA loop-domains probably define transcribed sequences near regions that are anchored to the nuclear matrix (20, 23, 85).

Chromatin condensation is brought about by the addition of cations to the negatively charged chromatin fiber (51, 80). Thus, a major phenomenon in chromatin condensation is one of charge neutralization. Magnesium ions are required in the formation of a higher-order structure and condensation of chromatin (10, 55, 97).

Zinc ions also influence the structure of DNA and chromatin. Zinc complexes can bind directly to alternating purine-pyrimidine sequences to change DNA from a right-handed B-form helix to a left-handed Z-form in vitro (31). The zinc concentration alters not only the conformation of naked DNA but also the conformation of chromatin (59, 87, 90). Nutritional deprivation of Mg^{2+} or Zn^{2+} condenses chromatin and renders it more resistant to nuclease (15; C. E. Castro et al, unpublished results). This coincides with the state of overall transcriptional and translational quiescence characteristic of nutritional deficiencies of Mg^{2+} or Zn^{2+} and also with the inhibition of growth of mammary adenocarcinoma in rats (62, 63).

CHROMATIN STRUCTURE AND GENE EXPRESSION

The fine structure of chromatin within a gene and the higher orders of chromatin organization are both determinants of gene expression. The structure of transcribed chromatin is inherently distinct from nontranscribed chromatin. Nucleases or chemical probes often react selectively with transcriptionally active regions (9, 30, 57, 82). Weintraub & Groudine (100) were the first to demonstrate that the active globin genes in comparison with bulk DNA are preferentially digested by deoxyribonuclease I (DNAase I) in erythrocyte nuclei, but not in fibroblast or brain nuclei where the genes are not transcribed. Wood & Felsenfeld (102) extended these observations to show that the globin gene is 10- to 20-fold more sensitive to DNAase I, 6-fold more sensitive to DNAase II, and 3-fold more sensitive to micrococcal nuclease. The enhanced nuclease sensitivity of transcribed sequences indicates an alteration in gross chromatin conformation.

In comparison with these broad patterns of differential nuclease sensitivity, Wu et al (105) and others (24) demonstrated specific sites in chromatin that are even more sensitive to DNAase I. These regions are referred to as DNAase I–hypersensitive sites. DNAase I (or S1 nuclease)–hypersensitive sites are usually found in functionally important regions in chromatin such as at or near the 5' end of a gene (11, 104). Generally, these sites are necessary, but not sufficient, for transcription by RNA polymerase II in vivo (98).

NUTRIENT EFFECTS ON CHROMATIN STRUCTURE

Higher-Order Structure

METAPHASE CHROMOSOMES Early studies revealed that nutrient deficiencies were correlated with aberrations in the organization of metaphase chromosomes. The metaphase chromosomes of magnesium- and zinc-deficient pregnant rats exhibit aberrations such as terminal deletions, fragments, and gaps in maternal and fetal chromosomes (4). As has been pointed out, Mg^{2+} and Zn^{2+} are involved in chromatin condensation. It appears that nutritional deprivation of these ions in vivo may result in a general distortion of chromosome compaction.

Increasingly severe deficiencies of arginine and lysine in V79 Chinese hamster cells increase the rate of sister chromatin exchanges (SCE) (79). Certain of the histones are enriched in lysine and arginine residues. These data suggest that the arginine- and lysine-rich histones may be involved in maintaining the low frequency of SCE and that nutritional deprivation of the amino acids affects that role.

Ascorbic acid has been reported to affect chromosome integrity. Lo & Stich

(52) demonstrated a reduced mutagenic and chromosome-damaging effect of ascorbic acid when administered concurrently with nitroso compounds in cultured cells. In a human study, prophylactic administration of ascorbic acid for three months reduced the frequency of chromosomal aberrations (chromatid and chromosome breaks and chromatin and chromosome exchanges) in lymphocytes of individuals exposed to polyaromatic hydrocarbon and benzene (86). Contrasting results are reported in spermatocytes of mice exposed to influenza virus (71). The effect, if any, of ascorbic acid on chromosome integrity remains controversial.

Chromosomal damage resulting from folate or vitamin B_{12} deficiencies is well documented. Menzies et al (61) described aberrant chromosomal characteristics of patients with megaloblastic anemia such as chromatin breaks, reduced condensation, prominent centromeric constrictions, and prophase-arrested mitoses. Similar abnormal events occur in lymphocytes from folate-deficient patients, even after reversion of the deficiency (26, 47).

Folate-sensitive fragile sites on chromosomes have been identified and characterized by Sutherland (91, 92). These are morphological sites that show a nonstaining gap usually involving both chromatids, which are always at the same point in a particular chromosome and which are inherited in a Mendelian codominant segregation. Folic acid–sensitive fragile sites are induced in cultured cells by methotrexate, aminopterin (91), and 5-fluorodeoxyuridine (34). The addition of folate, thymidine, or bromodeoxyuridine to medium inhibits the expression of the folate-sensitive sites.

The expression of chromosomal aberrations and folate-sensitive fragile sites is partially ascribed to relative pool sizes of deoxyuridine triphosphate (dUTP) and thymidine triphosphate (dTTP). The enzyme thymidylate synthetase converts deoxyuridine monophosphate (dUMP) to thymidine monophosphate, which is readily phosphorylated to dTTP. The coenzyme for thymidine synthetase is 5,10-methylenetetrahydrofolate, which is lacking during folate or vitamin B_{12} deficiency and thereby results in decreased levels of dTTP (92). In addition, dUMP is readily phosphorylated to deoxyuridine triphosphate (dUTP), even though dUTPase normally regulates the accumulation of dUTP. If dUTP accumulates, it may be incorporated into DNA as easily as dTTP. One hypothesis proposes that folate or vitamin B_{12} deficiency results in a depletion of the dTTP pool and an accumulation of dUMP; this generates an overwhelming increase in the ratio of dUTP to dTTP such that uracil has a greater probability of incorporation into DNA (35). Further, this accelerated insertion of uracil instead of thymine into DNA may be the molecular event related to regions of excision and repair that could in turn generate chromosomal aberrations, expression of folate-sensitive fragile sites, and perhaps neoplastic transformation (35, 47).

INTERPHASE CHROMATIN The structure of interphase chromatin is affected by diverse nutritional conditions. Alteration in structure has been assessed primarily by two techniques, each having advantages as well as limitations. One technique involves isolation of nuclei, followed by incubation with one or several nucleases that cleave chromatin based on structural configuration rather than sequence composition. Another method for examining chromatin structure uses isolated nuclei or purified chromatin as the templates for measuring the incorporation of ^{3}H-UMP into RNA molecules.

Because of the higher-order structure as well as the subunit organization of chromatin, some regions of chromatin are more accessible than others to nucleases. Micrococcal nuclease (EC 3.1.31.1) catalyzes the cleavage of DNA and has been instrumental in the study of chromatin organization. In mammalian nuclei, approximately 50% of the DNA in chromatin can be digested by micrococcal nuclease into acid-soluble oligonucleotides (19). The enzyme initially cleaves internucleosomal "linker" DNA in transcriptionally active regions of chromatin.

We used these characteristics of micrococcal nuclease to determine whether the global configuration of nuclear chromatin changes with altered nutrient intake. The maximum amount of liver nuclear DNA that is digested by micrococcal nuclease varies as a function of short-term dietary condition (16). A stock diet of commercial chow renders 50% of the chromatin acid soluble. A fat-free diet with 60% glucose alters the structure of chromatin so that more than 70% of chromatin from rats fed this lipogenic diet is solubilized by micrococcal nuclease. This observation indicates that the chromatin is unusually disperse and suggests, indirectly, a general enhancement of transcriptional activity.

In contrast, nuclear chromatin from liver of rats fed a protein-free, low-carbohydrate diet assumes a relatively condensed configuration not conducive to high levels of transcription, and only 39% of total chromatin is solubilized by micrococcal nuclease (16). Likewise, nuclear chromatin from liver of zinc-deficient rats (15) and magnesium-deficient rats (C. E. Castro, et al, manuscript in preparation) assumes a relatively condensed configuration and is resistant to micrococcal nuclease. Stankiewicz et al (87) demonstrated that zinc deficiency in *Euglena gracilis* increases by 10- to 30-fold the resistance of chromatin to micrococcal nuclease. The heightened condensation of chromatin associated with zinc deficiency in rodents and in a lower eukaryote is probably biologically significant since it has been evolutionarily conserved.

Micrococcal nuclease has proved to be instrumental in dissecting both the higher-order and the fine structure of chromatin. It is, however, not without limitation. Although the enzyme predominantly recognizes tertiary structure, it also recognizes nucleotide composition and sequence, with some preference for A-T-rich regions (28, 38). A sequence-neutral enzymatic or chemical

probe for chromatin structure would be more ideal. Still the enzyme offers great utility in the study of the structure of bulk chromatin.

Another approach for studying diet-mediated alteration in chromatin structure is to measure the incorporation of ^{3}H-UMP into RNA. Differential rates of incorporation are then ascribed to an altered number of RNA chains being synthesized, an altered rate of chain elongation, or a combination of both. The level of ^{3}H-UMP incorporation into nascent RNA molecules is measured under conditions in which initiation or reinitiation by eukaryotic polymerases is inhibited. Using this procedure, Porter et al (72) quantitated the RNA chain number and elongation rate in nuclei isolated from testes of retinal-deficient or normal rats. In isolated testicular nuclei, RNA was synthesized in the presence of 5-adenosylhomocysteine and rifamycin to inhibit initiation by RNA polymerase. Within four hours after an oral dose of retinyl acetate to deficient animals, RNA synthesis directed by polymerase II significantly increased. Alkaline hydrolysis of the newly synthesized RNA indicated that the increased synthesis was due to a greater number of actively transcribing polymerase II molecules on the DNA template. To substantiate this observation, the chromatin templates from retinyl-acetate-treated and -deficient rats was transcribed by *E. coli* polymerase. The number of sites recognized by this nonhomologous polymerase was two-fold greater after retinyl acetate administration. It appears that retinyl-acetate-induced changes are due in some degree to altered chromatin structure.

Several investigators have examined the effects of protein restriction or controlled feeding schedules on RNA synthesis and chromatin structure. A protein-restricted (5.5% casein) diet fed to pregnant rats from conception up to the adult stage of their offspring was associated with an increased in vitro incorporation rate of ^{3}H-UMP into RNA molecules of glial nuclei. The increased incorporation resulted from an increased total number of RNA initiation sites present in the glial chromatin template (45). In contrast, protein restriction in young male rats is associated with an unchanged number of chains synthesized by RNA polymerase II in liver (3, 96). After the use of heparin, which stimulates transcription of blocked enzymes, an increased number of short chains were synthesized at a lower rate of incorporation (96). Discrepancies observed in relative numbers of RNA initiation sites associated with protein restriction may be due in part to the different cell populations examined and to the inherent difficulties of the techniques (see below).

Starvation of rats decreases the number of transcribing polymerase II molecules in liver (25). In this study, RNA synthesis appeared to be regulated at the level of RNA polymerase initiation by a mechanism not linked to protein synthesis. An alteration in template structure may be involved because starvation induces a greater degree of condensation of the chromatin, manifested in a decrease in binding sites for the polymerase.

The synthesis of RNA in isolated nuclei is an extremely complex reaction. The quantity of synthesized RNA may vary with the concentration of nuclei and the salt and metal ion concentrations. Consequently, the technique is limited to comparing the relative rates of RNA synthesis and elongation and the number of transcribing molecules. Absolute quantitation of nuclear events is questionable (93). A disadvantage in using isolated chromatin instead of intact nuclei as a substrate for determining template activity is the limited solubility of chromatin. Hence, the use of purified chromatin and exogenously added RNA polymerase is technically more difficult to interpret than the use of intact nuclei.

Chromatin Subunit Organization

Alterations in higher-order chromatin structure may likely depend on subtle changes occurring at the nucleosomal level, since even minute variations in repeat length would change the orientation of adjoining nucleosomes and, hence, the structure of the chromatin fiber. The nucleosome repeat length is the average combined lengths of the "linker" DNA (DNA joining adjacent nucleosomes) and the core DNA (146 base pairs of DNA wrapped around a protein core).

The nucleosome repeat length is 6–10% shorter in liver of rats fed a lipogenic diet than in liver of rats fed a basal or protein-free diet (13). In addition to the lipogenic state, diverse biological processes—such as the differentiation of cortical neurons (41), early stages of embryogenesis (18), increased progression to malignancy (50), and increasing age in human diploid fibroblast-like cells (27)—are associated with decreased nucleosomal repeat length. Subtle changes in nucleosomal spacing likely has important biological impact.

Raul & von der Decken (73) demonstrated a reduction in oligonucleosome size in intestinal epithelial chromatin during starvation and a particular enrichment of nucleosome particles (13S) in crypt cells. The enrichment of nucleosome particles occurred in the absence of exogenously added nuclease and was reversible by the refeeding of sucrose.

Nutrient Effects on Nuclear Proteins

The molecular basis for modified chromatin structure at the nucleosome or higher-order level is not completely understood. One view is that DNA-binding nuclear proteins primarily dictate the configuration of chromatin (81). Nutritional modulation of nuclear proteins occurs under many circumstances. Lysine deficiency (12) and a protein-free diet (17) increase the mass ratio of nonhistone protein to DNA in liver chromatin, while the ratio of histone to DNA remains unchanged. However, a 5% casein diet significantly decreases the mass ratio of nonhistones to DNA, compared with a 20% casein diet (74).

Resolution of such discrepancies must await experiments using more highly purified and characterized protein fractions.

Intake of food by newly hatched chicks causes an increase in the initiation of RNA synthesis in liver accompanied by an increased sensitivity of nuclei to micrococcal nuclease. Salt extraction and reconstitution experiments revealed that the high mobility group (HMG) of nonhistone proteins and 0.35-M NaCl extracts are responsible for the enhanced sensitivity to micrococcal nuclease. The nuclear HMG protein content in fed chicks was greater than that of fasted chicks (68).

Nutritional deprivation of Mg^{2+} or Zn^{2+} causes abrupt alterations in liver chromatin accompanied by changes in the relative proportions of histone H1 variants. Histone H1 variants have a potential significance in promoting variegated chromatin structures, which may be important in the regulation of gene expression and cell differentiation (49, 99). The three major subtypes of H1 in rodent liver are H1.1, H1.2, had H1° (77). The differences are due to changes in the amino acid sequences located in the C-terminal half of the molecule (21). Thus it is likely that each member of the H1 family is derived from a corresponding gene.

The relative abundance of the H1 subtypes often changes during a variety of cellular events. For example, one molecular subtype of H1, H1°, is decreased in adult tissue during regenerative growth (33). Zinc deficiency in rats results in a relative decrease in the amount of histone H1° (14). Magnesium deficiency also is associated with an alteration in the pattern of histone H1 in rat liver (C. E. Castro et al, manuscript in preparation).

NUTRIENT EFFECTS ON DNA METHYLATION

The most commonly modified base in vertebrate DNA is 5-methylcytosine (5-mC), although other minor bases such as N^6-methyladenine and N^4-methylcytosine have been identified (1). From 4 to 5% of all cytosine residues in mammalian DNA are methylated as 5-mC, with more than 90% of the total 5-mC in the dinucleotide CpG (29, 36), which is underrepresented in the eukaryotic genome (42). The degree of methylation is often detected by restriction endonucleases such as MspI, which cleaves C-C-G-G and C-^{m}C-G-G, and its isoschizomer, HpaII, which cleaves only C-C-G-G. The pattern of methylation is postulated to be transmitted to daughter cells by a "maintenance methylase" that locates the methylated site on the parental strand and methylates the newly synthesized daughter strand shortly after replication (43, 76).

There is a strong correlation between gene expression and hypomethylation in many in vivo models (75). Many genes, such as those coding for chicken ovalbumin (58), mouse metallothionein I gene (22), chicken vitellogenin

(60), prolactin (30), and phosphoenolpyruvate carboxykinase (5), are hypomethylated in the cells in which they are expressed but not in other cells.

Herbert (37) has suggested that deficiency of folate or vitamin B_{12} (or any other means of failing to methylate DNA) activates malignancy by hypomethylation of oncogenes, whereas methylating oncogenes inhibits malignancy by silencing them. The transfer and utilization of one-carbon moieties, which are mediated by lipotropes including folate and vitamin B_{12}, are certainly essential to the synthesis and methylation of DNA (47, 67). However, investigations into the role of lipotropes in transformation and malignancy reveal conflicting associations. Lipotrope deficiency alone induces hepatocellular carcinoma (66, 71a), yet in conjunction with many, but not all, carcinogens it enhances the tumorigenesis processes (67).

Lipotrope deficiency decreases the levels of 5-mC in rat liver. Wilson et al (101) fed a methyl-deficient diet to rats for 4, 8, or 22 weeks and then measured deoxynucleosides by high-performance liquid chromatography. After 8 and 22 weeks, the level of 5-mC in hepatic DNA is significantly decreased. In confirmation of these results, Newberne & Rogers (67) showed a decrease in 5-mC in rat liver after feeding a lipotrope-deficient diet for six months. A similar decrease is observed if ethionine, an antimetabolite of methionine, is fed to rats for 10 weeks (83).

Whether DNA methylation is a key element in gene regulation or whether it has a minor role is unclear. There is not a strict correlation between demethylation and gene expression. McKeon et al (56) demonstrated that the 5' end of the α 2(I) collagen gene is demethylated whereas the rest of the gene is methylated, whether or not the gene is expressed. Likewise, consistently unmethylated domains have been observed at the 5' end of the mouse dihydrofolate reductase gene, regardless of transcriptional activity (88).

Kunnath & Locker (48) showed that the albumin gene is actively transcribed in 18-day fetal liver when it is heavily methylated, as well as in adult liver when it is unmethylated. Although the albumin gene is completely methylated over its entire length in cells not producing albumin (kidney), extensive demethylation of C-C-G-G sequences over the entire transcription unit is observed during rat liver developmental stages in which there is no marked change in gene expression (94).

The degree of control that DNA methylation confers on gene expression is variable. Hypomethylation at some region of a gene may be a required but not sufficient event for transcription. Bird (8) suggests that expression of a gene begins while the gene is still in a methylated state. Activation leads to demethylation, which relaxes control of the gene. In this case, methylation is a means of continuing expression once activation has occurred. It still holds that DNA methylation may exert its effects, although variable, on gene

expression by modifying both specific and nonspecific interactions between DNA and nuclear proteins (44).

The ability of methyl-group deficiency to decrease the DNA methylating events may be highly significant. Methylation of the *mos* oncogene suppresses its transforming ability (53), and highly methylated Moloney leukemia virus is noninfectious in a DNA transfection assay (89).

CONCLUSIONS

Many of the intricacies of genome structure and function are not fully understood. Much less is known of the interaction of nutritional state on chromatin structure and gene expression. Early research on the effects of specific nutrients (e.g. zinc, magnesium, folate) on metaphase chromosomes revealed that deficiencies of these essential nutrients are highly correlated with chromosomal breaks, deletions, or fragile sites. More recent studies demonstrate alterations in the higher-order configuration of interphase chromatin caused by imbalances in the carbohydrate, fat, or protein components of the diet. Others have observed nutrient-mediated alterations in the fine structure of chromatin, such as altered length of DNA in nucleosomal spacing, or quantitative or qualitative changes in structural nuclear proteins.

In view of the dominant role that genome structure plays in gene expression, studies that lead to a clearer understanding of nutrient-related changes in chromatin structure should continue in concert with studies of gene expression. Structural changes in higher-order chromatin may be the coarse mechanism of gene expression that precedes the events of fine regulation such as binding of promoters. Finally, nutrition-mediated changes in structure may alter the accessibility of chromatin to a variety of effector molecules such as mutagens, xenobiotics, or toxigens and may help clarify the molecular basis of nutrient interaction in disease processes.

Literature Cited

1. Adams, R. L. P., Burdon, R. H. 1985. *Molecular Biology of DNA Methylation*, pp. 1–8. New York: Springer-Verlag. 247 pp.
2. Adolph, K. W., Cheng, S. M., Laemmli, U. K. 1977. Role of nonhistone proteins in metaphase chromosome structure. *Cell* 12:805–16
3. Åström, S., Arrhenius, E. K., von der Decken, A. 1981. Reduced transcription activity of rat liver chromatin after protein restriction and selective digestion of nuclei with micrococcus nuclease. *J. Nutr.* 111:1258–64
4. Bell, L. T., Branstrator, M., Roux, C., Hurley, L. S. 1975. Chromosomal abnormalities in maternal and fetal tissues of magnesium- or zinc-deficient rats. *Teratology* 12:221–26
5. Benvenisty, N., Mencher, D., Meyuhas, O., Razin, A., Reshef, L. 1985. Sequential changes in DNA methylation patterns of the rat phosphoenolpyruvate carboxykinase gene during development. *Proc. Natl. Acad. Sci. USA* 82:267–71
6. Benyajati, C., Worcel, A. 1976. Isolation, characterization, and structure of the folded interphase genome of *Drosophila melanogaster*. *Cell* 9:393–407

7. Berlin, J., Castro, C. E., Bailey, F., Sevall, J. S. 1982. Adaptation of rat parenchymal hepatocyte to nutritional variation: quantitation by stereology. *Nutr. Res.* 2:51–63
8. Bird, A. P. 1984. DNA methylation—how important in gene control? *Nature* 307:503–4
9. Bode, J., Pucher, H.-J., Maass, K. 1986. Chromatin structure and induction-dependent conformational changes of human interferon-β genes in a mouse host cell. *Eur. J. Biochem.* 158:393–401
10. Borochov, N., Ausio, J., Eisenberg, H. 1984. Interaction and conformational changes of chromatin with divalent ions. *Nucleic Acids Res.* 12:3089–96
11. Burch, J. B. E., Weintraub, H. 1983. Temporal order of chromatin structural changes associated with activation of the major chicken vitellogenin gene. *Cell* 33:65–76
12. Canfield, L. M., Chytil, F. 1978. Effect of low lysine diet on rat liver nuclear metabolism. *J. Nutr.* 108:1336–42
13. Castro, C. E. 1983. Nucleosomal repeat length in rat liver nuclei is decreased by a high carbohydrate, fat-free diet. *J. Nutr.* 113:557–65
14. Castro, C. E., Alvares, O. F., Sevall, J. S. 1986. Zinc deficiency decreases histone H1° in rat liver. *Nutr. Rep. Int.* 34:67–74
15. Castro, C. E., Armstrong-Major, J., Ramirez, M. E. 1986. Diet-mediated alteration of chromatin structure. *Fed. Proc.* 45:2394–98
16. Castro, C. E., Sevall, J. S. 1980. Alteration of higher order structure of rat liver chromatin by dietary composition. *J. Nutr.* 110:105–16
17. Castro, C. E., Sevall, J. S. 1982. Analysis of rat liver chromatin and nuclear proteins after nutritional variation. *J. Nutr.* 112:1203–11
18. Chambers, S. A. M., Vaughn, J. P., Shaw, B. R. 1983. Shortest nucleosomal repeat lengths during sea urchin development are found in two-cell embryos. *Biochemistry* 22:5626–31
19. Clark, R. J., Felsenfeld, G. 1974. Chemical probes of chromatin structure. *Biochemistry* 13:3622–28
20. Cockerill, P. N., Garrard, W. T. 1986. Chromosomal loop anchorage of the kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. *Cell* 44:273–82
21. Cole, R. D. 1984. A minireview of microheterogeneity in H1 histone and its possible significance. *Anal. Biochem.* 136:24–30
22. Compere, S. J., Palmiter, R. D. 1981. DNA methylation controls the inducibility of the mouse metallothionein-I gene in lymphoid cells. *Cell* 25:233–40
23. Cook, P. R., Brazell, I. A. 1980. Mapping sequences in loops of nuclear DNA by their progressive detachment from the nuclear cage. *Nucleic Acids Res.* 8:2895–2906
24. Costlow, N. A., Simon, J. A., Lis, J. T. 1985. A hypersensitive site in *hsp* 70 chromatin requires adjacent not internal DNA sequence. *Nature* 313:147–49
25. Coupar, B. E. H., Davies, J. A., Chesterton, C. J. 1978. Quantification of hepatic transcribing RNA polymerase molecules, polyribonucleotide elongation rates and messenger RNA complexity in fed and fasted rats. *Eur. J. Biochem.* 84:611–23
26. Das, K. C., Herbert, V. 1978. The lymphocyte as a marker of past nutritional status: Persistence of abnormal lymphocyte deoxyuridine (dU) suppression test and chromosomes in patients with past deficiency of folate and vitamin B_{12}. *Br. J. Haematol.* 38:219–33
27. Dell'Orco, R. T., Whittle, W. L., Macieira-Coelho, A. 1986. Changes in the higher-order organization of DNA during aging of human fibroblast-like cells. *Mech. Ageing Dev.* 35:199–208
28. Dingwall, C., Lomonossoff, G. P., Laskey, R. A. 1981. High sequence specificity of micrococcal nuclease. *Nucleic Acids Res.* 9:2659–73
29. Doskočil, J., Šorm, F. 1962. Distribution of 5-methylcytosine in pyrimidine sequences of deoxyribonucleic acids. *Biochim. Biophys. Acta* 55:953–59
30. Durrin, L. K., Weber, J. L., Gorski, J. 1984. Chromatin structure, transcription, and methylation of the prolactin gene domain in pituitary tumors of Fischer 344 rats. *J. Biol. Chem.* 259:7086–93
31. Fazakerley, G. V. 1984. Zinc Z-DNA. *Nucleic Acids Res.* 12:3643–48
32. Finch, J. T., Klug, A. 1976. Solenoidal model for super-structure in chromatin. *Proc. Natl. Acad. Sci. USA* 73:1897–1901
33. Gjerset, R., Gorka, C., Hasthorpe, S., Lawrence, J. J., Eisen, H. 1982. Developmental and hormonal regulation of protein H1° in rodents. *Proc. Natl. Acad. Sci. USA* 79:2333–37
34. Glover, T. W. 1981. FUdR induction of the x chromosome fragile site: Evidence for the mechanism of folic acid and thymidine inhibition. *Am. J. Hum. Genet.* 33:234–42
34a. Goodridge, A. G. 1987. Dietary regu-

lation of gene expression: enzymes involved in carbohydrate and lipid metabolism. *Ann. Rev. Nutr.* 7:157–85
35. Goulian, M., Bleile, B., Tseng, B. Y. 1980. Methotrexate-induced misincorporation of uracil into DNA. *Proc. Natl. Acad. Sci. USA* 77:1956–60
36. Grippo, P., Iaccarino, M., Parisi, E., Scarano, E. 1968. Methylation of DNA in developing sea urchin embryos. *J. Mol. Biol.* 36:195–208
37. Herbert, V. 1983. The inhibition of some cancers and the promotion of others by folic acid, vitamin B_{12} and their antagonists. In *Nutritional Factors in the Induction and Maintenance of Malignancy*, ed. C. E. Butterworth, Jr., M. L. Hutchinson, pp. 273–87. New York: Academic. 316 pp.
38. Hörz, W., Alterburger, W. 1981. Sequence specific cleavage of DNA by micrococcal nuclease. *Nucleic Acids Res.* 9:2643–58
39. Huberman, J. A., Riggs, A. D. 1968. On the mechanism of DNA replication in mammalian chromosomes. *J. Mol. Biol.* 32:327–41
40. Igo-Kemenes, T., Hörz, W., Zachau, H. G. 1982. Chromatin. *Ann. Rev. Biochem.* 51:89–121
41. Jaeger, A. W., Kuenzle, C. C. 1982. The chromatin repeat length of brain cortex and cerebellar neurones changes concomitant with terminal differentiation. *EMBO J.* 1:811–16
42. Josse, J., Kaiser, A. D., Kornberg, A. 1961. Enzymatic synthesis of deoxyribonucleic acid. VIII. Frequencies of nearest neighbor base sequences in deoxyribonucleic acid. *J. Biol. Chem.* 236:864–75
43. Kautiainen, T. L., Jones, P. A. 1985. DNA methylation in mammalian nuclei. *Biochemistry* 24:5575–81
44. Keshet, I., Lieman-Hurwitz, J., Cedar, H. 1986. DNA methylation affects the formation of active chromatin. *Cell* 44:535–43
45. Knoll-Kohler, E., Handke, A. 1980. Effect of life-long dietary protein restriction on RNA synthesis of fractionated rat brain nuclei. *Nutr. Rep. Int.* 21:147–55
46. Kornberg, R. D., Thomas, J. O. 1974. Chromatin structure: oligomers of the histones. *Science* 184:865–68
47. Krumdieck, C. 1983. Role of folate deficiency in carcinogenesis. See Ref. 37, pp. 225–45
48. Kunnath, L., Locker, J. 1983. Developmental changes in the methylation of the rat albumin and α-fetoprotein genes. *EMBO J.* 2:317–24
49. Lennox, R. W. 1984. Differences in evolutionary stability among mammalian H1 subtypes. Implications for the roles of H1 subtypes in chromatin. *J. Biol. Chem.* 259:669–72
50. Leonardson, K. E., Levy, S. B. 1980. Organizational changes in chromatin at different malignant stages of Friend erythroleukemia. *Nucleic Acids Res.* 8:5317–31
51. Lewis, C. D., Laemmli, U. K. 1982. Higher order metaphase chromosome structure: Evidence for metalloprotein interactions. *Cell* 29:171–81
52. Lo, L. W., Stich, H. F. 1978. The use of short-term tests to measure the preventive action of reducing agents on formation and activation of carcinogenic nitroso compounds. *Mutat. Res.* 57:57–67
53. McGeady, M. L., Jhappan, C., Ascione, R., Van de Woude, G. F. 1983. *In vitro* methylation of specific regions of the cloned Moloney sarcoma virus genome inhibits its transforming activity. *Mol. Cell. Biol.* 3:305–14
54. McGhee, J. D., Felsenfeld, G. 1980. Nucleosome structure. *Ann. Rev. Biochem.* 49:1115–56
55. McGhee, J. D., Rav, D. C., Charney, E., Felsenfeld, G. 1980. Orientation of the nucleosome within the higher order structure of chromatin. *Cell* 22:87–96
56. McKeon, C., Ohkubo, H., Pastan, I., de Crombrugghe, B. 1982. Unusual methylation pattern of the α2(I) collagen gene. *Cell* 29:203–10
57. McKeon, C., Pastan, I., de Crombrugghe, B. 1984. DNase I sensitivity of the α2(I) collagen gene: Correlation with its expression but not with its methylation pattern. *Nucleic Acids Res.* 12:3491–3502
58. Mandel, J. L., Chambon, P. 1979. DNA methylation: Organ specific variations in the methylation pattern within and around ovalbumin and other chicken genes. *Nucleic Acids Res.* 7:2081–2103
59. Mazus, B., Falchuk, K. H., Vallee, B. L. 1984. Histone formation, gene expression, and zinc deficiency in *Euglena gracilis*. *Biochemistry* 23:42–47
60. Meijlink, F. C. P. W., Philipsen, J. N. J., Gruber, M., Geert, A. B. 1983. Methylation of the chicken vitellogenin gene: Influence of estradiol administration. *Nucleic Acids Res.* 11:1361–73
61. Menzies, R. C., Crossen, P. E., Fitzgerald, P. H., Gunz, F. W. 1966. Cytogenetic and cytochemical studies on marrow cells in B_{12} and folate deficiency. *Blood* 28:581–94
62. Mills, B. J., Broghamer, W. L., Higgins, P. J., Lindeman, R. D. 1984. In-

hibition of tumor growth by magnesium depletion of rats. *J. Nutr.* 114:739–45
63. Mills, B. J., Broghamer, W. L., Higgins, P. J., Lindeman, R. D. 1984. Inhibition of tumor growth by zinc depletion of rats. *J. Nutr.* 114:746–52
64. Mirkovitch, J., Mirault, M. E., Laemmli, U. K. 1984. Organization of the higher-order chromatin loop: Specific DNA attachment sites on nuclear scaffold. *Cell* 39:223–32
65. Nelson, W. G., Pienta, K. J., Barrack, E. R., Coffey, D. S. 1986. The role of the nuclear matrix in the organization and function of DNA. *Ann. Rev. Biophys. Biophys. Chem.* 15:457–75
66. Newberne, P. M., de Camargo, J. L. V., Clark, A. J. 1982. Choline deficiency, partial hepatectomy and liver tumors in rats and mice. *Toxicol. Pathol.* 2:95–109
67. Newberne, P. M., Rogers, A. E. 1986. Labile methyl groups and the promotion of cancer. *Ann. Rev. Nutr.* 6:407–32
68. Oka, T., Han, J. P., Natori, Y., Hasegawa, N., Kanai, M., Watari, N. 1985. Enhancement of RNA synthesis in chick liver by food intake: Possible role of high mobility group nonhistone proteins. *J. Nutr.* 115:1504–14
69. Olins, A. L., Olins, D. E. 1973. Spheroid chromatin units (ν bodies). *J. Cell Biol.* 59(2):252a
70. Pardoll, D. M., Volgelstein, B., Coffey, D. S. 1980. A fixed site of DNA replication in eucaryotic cells. *Cell* 19:527–36
71. Pichan, K., Polasa, H. 1982. Lack of effect of vitamin C on the incidence of chromosome anomalies induced by influenza virus in germ cells of mice. *Hum. Genet.* 61:259–61
71a. Poirier, L. A., Wilson, M. J., Shivapurkar, N. 1986. Carcinogenesis and DNA hypomethylation in methyl-deficient animals. In *Biological Methylation and Drug Design*, ed. R. T. Borchardt, C. R. Creveling, P. M. Ueland, pp. 151–61. Clifton, NJ: Humana. 457 pp.
72. Porter, S. B., Ong, D. E., Chytil, F. 1986. Vitamin A status affects chromatin structure. *Int. J. Vitam. Nutr. Res.* 56:11–20
73. Raul, F., von der Decken, A. 1985. Changes in chromatin structure and transcription activity by starvation and dietary sucrose in mature and immature intestinal epithelial cells of the rat. *Cell. Mol. Biol.* 31:299–304
74. Ravi, R. J., Ganguli, N. C. 1980. Effect of dietary protein restriction on chromatin proteins of rat mammary gland. *J. Nutr.* 110:1144–51
75. Razin, A., Riggs, A. D. 1980. DNA methylation and gene function. *Science* 210:604–10
76. Riggs, A. D. 1975. X inactivation, differentiation, and DNA methylation. *Cytogenet. Cell Genet.* 14:9–25
77. Roche, J., Girardet, J.-L., Gorka, C., Lawrence, J. J. 1985. The involvement of histone H1° in chromatin structure. *Nucleic Acids Res.* 13:2843–53
78. Rucker, R., Tinker, D. 1986. The role of nutrition in gene expression: A fertile field for the application of molecular biology. *J. Nutr.* 116:177–89
79. Schempp, W., Krone, W. 1979. Deficiency of arginine and lysine causes increase in the frequency of sister chromatid exchanges. *Hum. Genet.* 51:315–18
80. Sen, D., Crothers, D. M. 1986. Condensation of chromatin: Role of multivalent cations. *Biochemistry* 25:1495–1503
81. Sevall, J. S. 1983. DNA-binding nonhistone proteins. In *Chromosomal Nonhistone Proteins*, ed. L. S. Hnilica, 1:25–46. Boca Raton: CRC Press
82. Shimada, T., Nienhuis, A. W. 1985. Only the promoter region of the constitutively expressed normal and amplified human dihydrofolate reductase gene is DNase I hypersensitive and undermethylated. *J. Biol. Chem.* 260: 2468–74
83. Shivapurkar, N., Wilson, M. J., Poirier, L. A. 1984. Hypomethylation of DNA in ethionine-fed rats. *Carcinogenesis* 5:989–92
84. Simpson, R. T. 1978. Structure of the chromatosome, a chromatin particle containing 160 base pairs of DNA and all the histones. *Biochemistry* 17:5524–31
85. Small, D., Nelkin, B., Vogelstein, B. 1985. The association of transcribed genes with the nuclear matrix of *Drosophila* cells during heat shock. *Nucleic Acids Res.* 13:2413–31
86. Šrám, R. J., Dobiáš, L., Pastorková, A., Rössner, P., Janča, L. 1983. Effect of ascorbic acid prophylaxis on the frequency of chromosome aberrations in the peripheral lymphocytes of coal tar workers. *Mutat. Res.* 120:181–86
87. Stankiewicz, A. J., Falchuk, K. H., Vallee, B. L. 1983. Composition and structure of zinc-deficient *Euglena gracilis* chromatin. *Biochemistry* 22:5150–56
88. Stein, R., Razin, A., Cedar, H. 1982. *In vitro* methylation of the hamster adenine phosphoribosyltransferase gene inhibits

its expression in mouse L cells. *Proc. Natl. Acad. Sci. USA* 79:3418–22
89. Stuhlmann, H., Jahner, D., Jaenisch, R. 1981. Infectivity and methylation of retroviral genomes is correlated with expression in the animal. *Cell* 26:221–32
90. Supakar, P. C., Kanungo, M. S. 1984. Conformational changes in the chromatin of the brain of developing rats and its modulation by zinc chloride. *Mol. Biol. Rep.* 9:253–57
91. Sutherland, G. R. 1979. Heritable fragile sites on human chromosomes I. Factors affecting expression in lymphocyte cultures. *Am. J. Hum. Genet.* 31:125–35
92. Sutherland, G. R. 1983. Fragile chromosomes. See Ref. 37, pp. 63–73
93. Tsai, M.-J., Tsai, S. Y., O'Malley, B. W. 1982. Expression of eucaryotic genes: Transcription and analysis. In *Laboratory Methods Manual for Hormone Action and Molecular Endocrinology*, ed. W. T. Schrader, B. W. O'Malley, pp. 13–1 to 13–31. Houston: Houston Biol. Assoc. 14–35 pp.
94. Vedel, M., Gomez-Garcia, M., Sala, M., Sala-Trepat, J. M. 1983. Changes in methylation pattern of albumin and α-fetoprotein genes in developing rat liver and neoplasia. *Nucleic Acids Res.* 11:4335–54
95. Volgelstein, B., Pardoll, D. M., Coffey, D. S. 1980. Supercoiled loops and eucaryotic DNA replication. *Cell* 22:79–85
96. von der Decken, A., Åström, S., Arrhenius, E. K. 1981. Increased transcription activity of rat liver chromatin after protein restriction and limited digestion of nuclei with micrococcal nuclease. *J. Nutr.* 111:1249–57
97. Watanabe, K., Iso, K. 1984. Magnesium binding and conformational change of DNA in chromatin. *Biochemistry* 23:1376–83
98. Weintraub, H. 1985. High-resolution mapping of S1– and DNase I–hypersensitive sites in chromatin. *Mol. Cell. Biol.* 5:1538–39
99. Weintraub, H., Flint, S. J., Leffak, I. M., Groudine, M., Grainger, R. M. 1978. The generation and propagation of variegated chromosome structures. *Cold Spring Harbor Symp. Quant. Biol.* 42: 401–7
100. Weintraub, H., Groudine, M. 1976. Chromosomal subunits in active genes have an altered conformation. Globin genes are digested by deoxyribonuclease I in red blood cell nuclei but not in fibroblast nuclei. *Science* 193:848–56
101. Wilson, M. J., Shivapurkar, N., Poirier, L. A. 1984. Hypomethylation of hepatic nuclear DNA in rats fed with a carcinogenic methyl-deficient diet. *Biochem. J.* 218:987–90
102. Wood, W. I., Felsenfeld, G. 1982. Chromatin structure of the chicken β-globin gene region. Sensitivity to DNase I, micrococcal nuclease, and DNase II. *J. Biol. Chem.* 257:7730–36
103. Woodcock, C. L. F. 1973. Ultrastructure of inactive chromatin. *J. Cell Biol.* 59(2):368a
104. Wu, C. 1980. The 5' ends of *Drosophila* heat shock genes in chromatin are hypersensitive to DNase I. *Nature* 286:854–60
105. Wu, C., Bingham, P. M., Livak, K. J., Holmgren, R., Elgin, S. C. R. 1979. The chromatin structure of specific genes: I. Evidence for higher order domains of defined DNA sequence. *Cell* 16:797–806
106. Wu, Y.-H., Wu, C.-W. 1987. Zinc in DNA replication and transcription. *Ann. Rev. Nutr.* 7:251-72