Effects of cannabinoids on gene expression

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Et al.

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INTRODUCTION

In this article we will consider approaches that have been taken and can be taken to assess the influence of cannabinoids and other abused substances on the genome and on gene expression. This is a problem central to understanding drug-induced effects on a broad spectrum of biological processes since numerous modifications in cell structure and function, which have been reported to be associated with abused substances, either a) affect expression of genetic sequence or b) are a reflection of modifications in gene expression. Within this context we should emphasize that drug-induced perturbations in gene expression can result from alterations in the genome itself or from modifications in the transcription, processing, or translation of genetic information.

This article will be divided into three parts. First, by way of introduction, we will summarize the experimental basis for our current concepts of the eukaryotic genome and eukaryotic gene control. Second, we will review approaches that nave been taken to address the influence of cannabinoids on gene expression. We will then consider approaches, which can be taken and should be pursued, to further define in molecular terms cannabinoid-induced effects on the structure, organization, and regulation of specific genes.

It is our strong conviction that there are many long-standing and to date unresolved questions related to cannabinoid-induced effects on genes and gene control. Answers to these questions are essential to understand the influence of abused substances from the standpoints of immediate health hazards and, perhaps even more important, of hereditary effects. It is encouraging tnat during the past several years our understanding of genes and gene regulation in cells has evolved dramatically, largely through a number of highly innovative cellular and molecular approaches that have been taken to address the organization and regulation of eukaryotic genes. We are therefore now in a position, conceptually and technologically, to apply these approaches to assessing the effects of abused substances on the genome and on gene expression--particularly in human cells.

I. Genes and Gene Regulation

Several of the experimental observations which historically have
served as the basis for our current concept of gene expression are
summarized in table 1. While in general terms, these classical
observations have a direct bearing on the manner in which
eukaryotic genes are controlled, a number of subtle qualifications
based on recent results provide explanations for long-standing
inconsistencies in our understanding of eukaryotic gene regulation.

TABLE 1

Gene Expression in Eukaryotic Cells

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1. All diploid cells in an organism contain the same amount of
   DNA.

2. All diploid cells contain identical genetic information.

3. Limited expression of genes in all cells.

4. Differences and similarities in expression of specific genes
   in differentiated cells.

5. Ability to modify expression of specific genes.

The initial experimental Observations which led to models for
eukaryotic gene control were that all diploid cells of an organism
contain the same amount of DNA and that the DNA sequences present
in all diploid cells are identical. Equally important were the
observations that all cells express only a limited number of
genetic sequences and that those genes expressed reflect general
metabolic requirements shared by all living cells as well as
specialized requirements of differentiated cells. For example,
almost all cells express genes encoding enzymes involved in
intermediary metabolism while expression of globin genes is
restricted to erythropoietic cells. Superimposed upon this
preferential expression of specific genes, which permits cells to
execute their specialized biological/biochemical functions, is the
flexibility to permit variation in those genes expressed in
response to modifications of cellular activities or cellular
requirements.   It was these observations that led to experimental
pursuit of the mechanisms by which defined genetic sequences are
selectively expressed while others are held in a nontranscribed
structure, conformation, and transcriptional state.   What we must
now additionally take into consideration is that expression of
genes can be associated with modifications in the organization
and/or the representation of genetic sequences.

Our views of eukaryotic genes and eukaryotic gene regulation are constantly evolving. Structural and functional properties of genes are largely inseparable, as reflected by a functional relationship between the organization and expression of genetic sequences. The eukaryotic genome is a protein-DNA complex, both chromosomal proteins and DNA being essential for genome structure, and alterations in the interactions of chromosomal proteins with DNA in turn affect transcription or the transcriptional potential of specific genes. It is becoming increasingly apparent that the eukaryotic genome is not a static macromolecular complex, but rather is subject to modifications in organization, structure, and conformation which influence expression. There are different types of genes, those which encode proteins and those for which the products are ribosomal or transfer RNAs. Moreover, there are substantial differences in the organization of various genetic sequences, ranging in complexity from genes whose encoded proteins are represented by contiguous nucleotide sequences to genes from which the transcripts must undergo numerous splicing steps to generate functional messenger RNAs. It has been well documented that different genes are under different types of regulation. Likewise, there may be some differences in the structure and regulation of the same genes in different biological situations.

It therefore follows that to address regulation of eukaryotic genetic sequences it is necessary to consider control at several levels, which have been delineated in table 2. By definition gene expression encompasses an extensive range of cellular structures

TABLE 2

Regulation of Gene Expression

NUCLEUS	DNA	TRANSCRIPTION .Deletion-Addition .Rearrangement .Amplification .Methylation
	Nucleoplasm	TRANSCRIPT PROCESSING .Splicing .5' Capping .3" Polyadenylation .Methylation .RNA-Protein complexes
CYTOPLASM		TRANSPORT TO CYTOPLASM TRANSLATION POST-TRANSLATIONAL MODIFICATIONS

and biochemical processes, beginning in the nucleus at the DNA
double helix and terminating with a completely processed and
functional protein or RNA molecule. This presents a problem of an
extremely complex nature, and cannabinoid-induced lesions may
reside at any one or a combination of cellular levels.

Within the nucleus key steps in control of gene readout reside at
the level of the genome and in the nucleoplasm. Cannabinoids may
influence the structure and/or function of DNA nucleotide
sequences which constitute structural genes or their components,
in which case regions of the genome coding for defined proteins
would not be transcribed or the transcripts would not be appro-
priately processed and translated into functional proteins. In
addition, cannabinoid-induced alterations in genetic sequences
coding for the synthesis of ribosomal RNAs, tRNAs, or purported
"regulatory RNAs" must be considered. Cannabinoio-induced
alterations may also become apparent in the nucleotides contained
within regulatory sequences or within those sequences involved in
punctuating the genetic code. In an overall evaluation of tne
mechanisms by which cannabinoids may modify genes, one must bear
in mind that there are four general categories of changes in the
nucleotide bases which are prevalent--base substitutions,
modifications of preexisting bases, base additions, and base
deletions. Recent evidence for additions, deletions, and
amplification of nucleotide sequences, as well as rearrangements
of genetic sequences in conjunction with expression, necessitates
serious consideration of quantitative and qualitative modifica-
tions in DNA as potential regulatory events, and hence targets for
drug-induced perturbations in gene expression. Within this
context drug-mediated effects on DNA methylation, which has been
implicated in structural/transcriptional properties of genetic
sequences, should not be overlooked.

In evaluating the implications of cannabinoid-associated DNA
sequence modifications, one must critically determine the
influence of these drugs on the capability of the cell to repair
its DNA correctly. The repair process may itself introduce or
amplify errors.

Cannabinoid-induced modifications in gene expression may also
result from changes in macromolecules, principally chromosomal
proteins, which interact with DNA and are intimately involved with
the structural and transcriptional properties of the genome.
Variations of these proteins and their mode of association with
other genome components may be attributable to alterations in
amino acid sequences as well as to post-translational modifica-
tions such as acetylation, methylation, phosphorylation, and
ADP-ribosylation. It should be kept in mind that cannabinoid-
induced changes in the metabolism of acetate, methyl, phosphate,
and ADP-ribose groups may be caused by variations in genetically
coded enzymes which are responsible for the addition and removal
of these moieties from genome-associated proteins. In addition,
some of these post-translational modifications of chromosomal
proteins may occur, at least in part, by nonenzymatic mechanisms.

Another class of macromolecules which possess the ability to influence readout as as function of cannabinoid treatment are the RNA polymerases. Here, cannabinoid-induced changes may reside in any one or several of the polymerases, in any one or several of the subunits of the given polymerase, or in "factors" which influence the specificity or efficiency of the enzyme.

A complex system which contains numerous focal points for cannabinoid-induced lesions in the expression of genetic information is that which is utilized in the processing of RNA molecules. This is a multicomponent system consisting of: a) endo- and exonucleases which cleave and degrade ribonucleotide sequences during RNA precursor processing; b) enzymes modifying ribonucleotide bases; c) nucleotidyl exotransferases which utilize the 3' and 5' ends of RNA molecules as primers for addition of nontemplated ribonucleotides; and a) proteins which complex with RNAs or precursors thereof and are involved with enzymatic modifications of transcripts, export of transcripts from the nucleus to the cytoplasm, or assembly of functional translational complexes. Such processing occurs in the three principal classes of RNA molecules--ribosomal RNAs, messenger RNAs, and transfer RNAs. While these reactions generally occur in the nucleoplasm, they have also been reported to take place, to some extent, in the cytoplasm.

Cannabinoid-induced aberrations in gene expression may also result from perturbations in the equally complex cellular protein synthe-sizing and processing machinery which resides primarily in the cytoplasm. This may involve lesions in ribosomal and transfer RNAs, in ribosomal proteins, in the extensive range of "transla-tional factors," and in enzymes involved in the assembly and/or activation of proteins. Enzymes involved with protein turnover constitute targets often overlooked when considering potentially important sites for cannabinoid-induced lesions in gene expression.

From the preceding discussion it should be apparent that cannabinoid-induced modifications in gene expression may result from perturbations in a broad spectrum of macromolecular, biosynthetic processes in the nucleus as well as in the cytoplasm. Any step in the elaboration and processing of genetic information is a potential target for a drug-induced lesion. Do cannabinoids modify the structure or composition of the genome? Do cannabinoids modify which genes are transcribed and which remain silent? Do cannabinoids affect the efficiency or fidelity of transcription? Are RNA processing steps modified by cannabinoids? Do these drugs act at the translational level? The key to addressing these questions is availability of high resolution procedures for detecting cannabinoid-induced changes in gene expression at various levels, and equally important, for determining if drug-induced perturbations in gene expression are functional or nonfunctional.

II.   Effect of Cannaoinoids on the Genome

A.   Composition of the Genome

The eukaryotic genome exists in the form of a protein-DNA complex
(Stein et al. 1974, 1975); hence, an assessment of cannabinoid-
induced effects on the composition of the genome requires
evaluating the influence of cannabinoids on both DNA and
chromosomal proteins. It is also necessary to consider the
influence of cannabinoids on both chromatin and on chromosomes
since these represent interchangeable modes of genome packaging.

Several laboratories have investigated the effects of cannabinoids
on chromosome morphology and on the cellular representation of
specific chromosomes. Yet, to date this remains an area where
considerable controversy exists. The critical issues are whether
cannabinoids exhibit clastogenic activity, that is, induce
chromosome breaks, and/or whether cannabinoids act as mitotic
poisons. The latter effect would imply drug-induced action,
direct or indirect, on the mitotic apparatus or on the region of
the chromosome where attachment of spindle fibers occurs--
centromeric DNA or centromere-associated chromosomal proteins.
The mutagenic nature of cannabinoid-induced chromosomal lesions
also remains to be resolved. An indepth review of these
chromosome-related effects of cannabinoids is covered in the
chapter by Morishima in this volume.

An examination of the influence of cannabinoids on chromosomal
proteins indicates that the relative composition of both histones
and nonhistone chromosomal proteins is not significantly altered.
However, psychoactive and nonpsychoactive cannabinoids appear to
bring about a dose-dependent decrease in the synthesis of some
chromosomal polypeptides (Mon et al. 1981a,b). These results tend
to suggest that while cannabinoids do not affect the relative
cellular levels of specific histones, which are the molecules
primarily responsible for DNA packaging, these drugs may affect
the ability of cells to express genes which code for histone
proteins and/or affect histone protein turnover. Nonhistone
chromosomal proteins, which are involved in structural, enzymatic,
and regulatory action at the level of the genome, may be similarly
affected following cannabinoid treatment. Variations observed in
the extent to which chromosomal proteins are acetylated following
cannabinoid treatment can be related to changes in the nature of
chromosomal protein-DNA interaction, which may in turn reflect
drug-induced modification in chromatin structure and/or in
transcriptional properties of the genome. The studies carried out
to date do indeed suggest possible drug-induced changes in genome
composition, structure, and function but the data are of a
correlative nature. Although as discussed above, cannabinoid-
induced alterations in gene organization are not an unrealistic
expectation, experimental data to substantiate or eliminate such a
possibility are lacking.

B. Gene Expression

Two approaches have been undertaken in several laboratories,
including ours, to study cannabinoid-induced effects on gene
expression (Blevins and Regan 1976; Carchman et al. 1976a,b;
Desoize et al. 1979; End et al. 1977; Green et al. 1983; Nahas et
al. 1974a,b, 1977; Lemberger 1973; McClean and Zimmerman 1976; Mon
et al. 1978, 1981a,b; Nahas and Desoize 1974; Nahas and Paton
1979; White et al. 1976; Zimmerman and McClean 1973; Zimerman and
Zimmerman 1976; Zimmerman et al. 1979). Early in vivo studies
suggested that cannaoinoid treatment brings about dose-dependent
inhibition of ^{3}H-thymidine incorporation into DNA, ^{3}H-uridine
incorporation into RNA, and ^{3}H-leucine incorporation into
protein. However, these results, particularly the ^{3}H-uridine
and ^{3}H-leucine results, are complicated by the influence of
cannabinoids on the ribonucleotide and amino acid precursor pools--
perhaps in part a reflection of cannabinoid-induced effects on
cellular membranes. In vitro transcription stuaies carried out
using isolated nuclei, DNA, or chromatin suggest that such
preparations from untreated control and cannabinoid-treated cells
do not differ significantly with respect to their ability to
synthesize RNA. Interpretation of the latter studies is not
complicated by drug-related effects on precursor pools; however,
from these in vitro experiments it is possible to conclude only
that the overall transcriptional capacity of the genome is
refractory to cannabinoid treatment, and no indication of possible
cannabinoid-induced effects on the qualitative nature of gene
transcription can be gleaned. Furthermore, caution should be
exercised in interpreting results from in vitro studies because
the fidelity of the transcription process and the transcripts by
necessity must be carefully evaluated.

Recently, to assess more definitively the influence of
cannabinoids on gene expression, we examined the effect of
Δ**9**-THC on the representation of RNA transcripts from two defined
genetic sequences, histone genes and ribosomal genes, in several
human cell lines. Levels of cellular histone mRNAs and ribosomal
RNAs were assayed by hybridization with cloned genomic human
histone and ribosomal genes under conditions where quantitation
was not influenced by nucleotide precursor pools. Our results
suggest that Δ**9**-THC causes a dose-dependent reduction in the
cellular representation of histone mRNA sequences. This
drug-induced reduction is at least to some extent selective
because cellular levels of ribosomal RNAs are not affected. We
have also observed that the cannabinoid-induced effect on histone
gene expression is less pronounced in human cells with active
drug-metabolizing systems.

Human histone and ribosomal genes represent two distinct types of
genetic sequences which differ with respect to their organization,
regulation, and functions. Human nistone genes are a family of
moderately reiterated genetic sequences--approximately 40 copies
per haploid genome. Each histone mRNA is transcribed from a set
of contiguous nucleotide sequences (unspliced), and histone gene

expression is related to cell proliferation. The gene products,
the histone proteins, are required for packaging several yaras of
DNA into "nucleosomes" where they are contained in a nucleus only
several microns in diameter. These histone proteins are necessary
for genome replication (to package newly replicated DNA) and
additionally play a role in the control of gene expression. The
human ribosomal genes are represented as a reiterated set of
sequences and the final gene products are the major structural RNA
species associated with large and small ribosomal subunits. In
contrast to the histone genes, where the primary transcripts
undergo a minimal amount of processing, the 5.8S, 18S, and 28S
ribosomal RNAs are derived from a 45S precursor via a series of
post-transcriptional cleavages.

Initially, the steady state levels of histone mRNAs were
determined in exponentially growing human cervical carcinoma
cells, HeLa S3 cells, following treatment with increasing
concentrations of Δ^9-THC. Total cellular RNAs were fractionated
electrophoretically in 1.5% agarose gels (Rave et al. 1979),
transferred to nitrocellulose (Southern 1975) and hybridized with
^{32P}-labeled [nick-translated (Maniatis et al. 1975)] cloned
genomic human histone sequences (Sierra et al. 1982). The levels
of histone mRNAs were then assayed autoradiographically.

Isolation of total celluar RNA permits greater than 90% recovery,
circumventing loss of RNA through nuclease activity and physical
manipulations which generally occur during subcellular frac-
tionation. Because the hybridization probe is radiolabeled
in vitro rather than the cellular RNAs in vivo, quantitation of
RNAs is not complicated by the intracellular ribonucleotide
precursor pools. RNA samples are quantitated spectrophoto-
metrically prior to electrophoretic fractionation and the extent
of transfer to nitrocellulose is monitored by ethidium bromide
staining and/or ultraviolet shadowing prior to and following
diffusion transfer. The efficiency of transfer to nitrocellulose
by the procedure used in these experiments has been monitored by
transfer of ^{32P}-labeled DNA and shown to be greater than 95%.

The data in figures 1 and 2 clearly indicate that Δ^9-THC brings
about a dose-dependent decrease in the representation of mRNAs for
the four core histone proteins, H2A, H2B, H3, and H4. Shown in
figure 1A is a hybridization signal obtained when 50 µg of
nitrocellulose-immobilized, total cellular HeLa cell RNAs from
control, and Δ^9-THC treated, cells are hybridized with a cloned
human DNA sequence (pFF435) encoding H2A, H2B, and H3 histone
mRNAs. While the levels of H2A, H2B, an H3 histone mRNAs
isolated from cells treated with 10 µMΔ^9-THC are not below
those from nondrug-treated or vehicle-treated controls, a marked
inhibition (greater than 80%--see table 3) is observed in cells
treated with 30 µM and 40 µM drug concentrations. Verification
that equivalent amounts of all' RNA samples were fractionated can
be gleaned from figure 1B which shows similar levels of ethidium
bromide staining of all RNAs and from figure 1C which shows
similar levels of all RNAs by ultraviolet shadowing. It should be

TABLE 3

Effect of ^{9}-THC on Cellular Levels
of Human (HeLa) Histone mRNAs

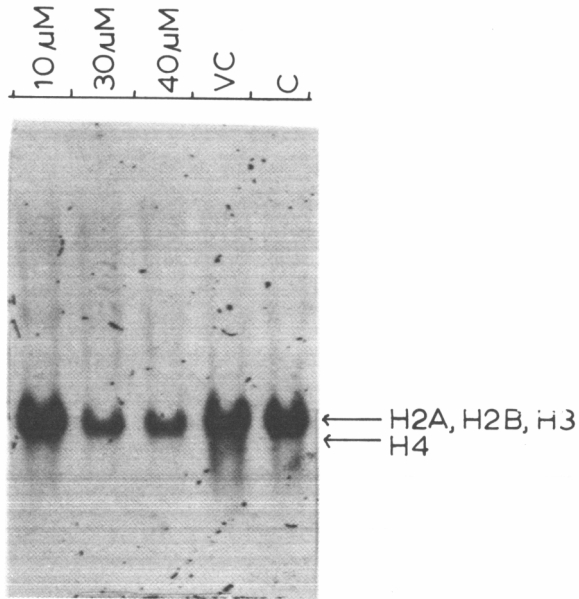| Treatment	Drug Conc.	% Inhibition
Δ^9-THC	10 µM	0.0
Δ^9-THC	30 µM	78.1
Δ^9-THC	40 µM	81.0
Vehicle Control	0	0.0
Control	0	0.0

FIGURE 1A

A) Effects of varying concentrations (10 µM, 30 µM, 40 µM, VC-
vehicle treated control and C-control) of Δ^9-THC on the representa-
tion of mRNAs for three of the four core histone proteins, H2A, H2B,
and H3. The signals shown were obtained when 50 µg of electro-
phoretically fractionated nitrocellulose-imobilized total cellular
HeLa cell RNAs were hybridized to a cloned human DNA sequence
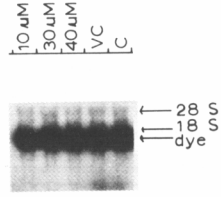(FF435) encoding H2A, H2B, and H3 histone mRNAs.

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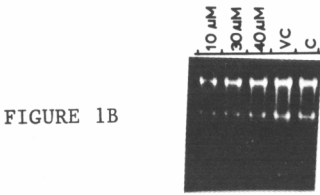
FIGURE 1B

FIGURE 1C

B.) Ethidium bromide stain of 1.5% (w/v) agarose gel with 6% (w/v) formaldehyde, containing 10 µg of each of the Δ^9-THC treated and control samples of total cellular RNAs from HeLa cells. The gel was stained for one hour in 0.1 M ammonium acetate containing 0.1 µg/ml ethidium bromide and destained overnight in water. The gel was placed on a shortwave ultraviolet transilluminator and photographed with Polaroid type 57 film using an orange filter.

C) Ultraviolet shadowing of 1.% (w/v) agarose gel with 6% (w/v) formaldehyde, containing 50 µg of each of the Δ^9-THC-treated and control samples of total cellular RNAs from HeLa cells. The gel was placed on a cellulose-fluorescent thin layer chromatography plate and illuminated from above by shortwave ultmviolet ligkt. The gel was photographed with polaroid type 57 film using an orange filter.
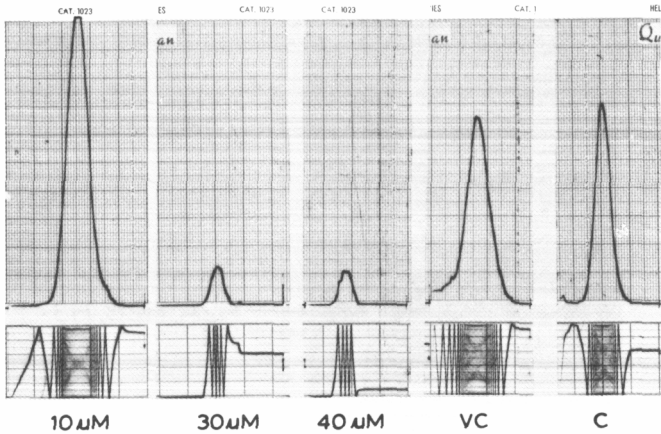

FIGURE 1D

D) Densitometric scan of autoradiographic hybridization signals obtained when 50 µg of electrophoretically fractionated nitrocellulose immobilized total cellular RNAs from HeLa cells treated with varying concentrations of Δ^9-THC were hybridized to a cloned human DNA sequence (pFF435) encoding H2A, H2B, and H3 histones. The top portion of the scan meusures the absorbance of the signal which is determined electronically within the densitometer based on the measured optical density. The lower portion is the Zig-Zag time base integrator and is used to quantitate the area under the curve and thus, the concentration of the sample.
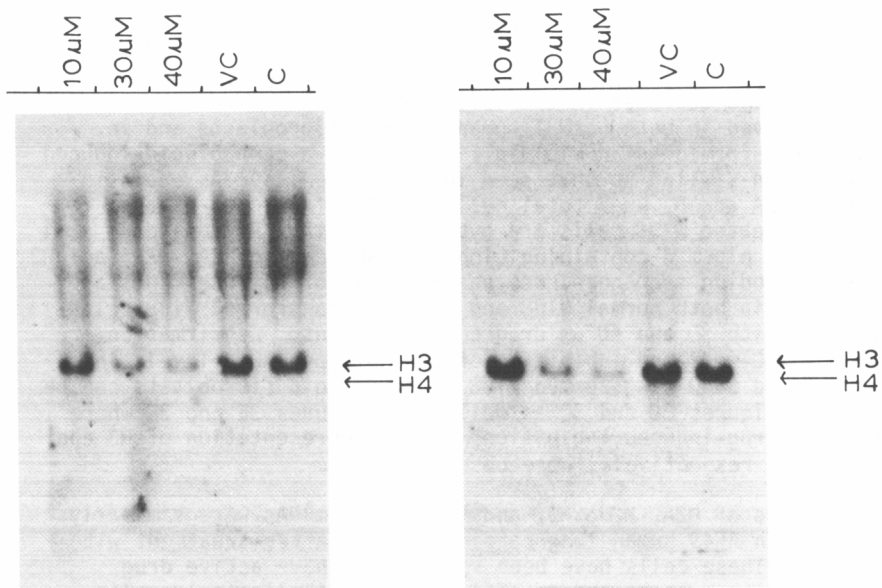
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FIGURE 2A

FIGURE 2B

Effects of varying concentrations (10 μM, 30 μM, 40 μM, VC-vehicle treated control and C-control) of Δ^9-THC on the representation of mRNAs for histones H3 and H4. The signals shown were obtained when 5 0 μg of electrophoretically fractionated, nitrocellulose-immobilized total cellular HeLa cell RNAs were hybridized to cloned hwnan DNA sequences encoding: A) H3 histone (pF0422) and B) H4 histone (pF0108A).

noted that because equivalent amounts of RNA from control and drug-treated cells were analyzed, the data in figure 1A reflect a dose-dependent, Δ^9-THC-mediated inhibition in the relative representation of three core histone mRNA species. A dose-dependent inhibition of the absolute amounts of H2A, H2B, and H3 histone mRNA/cell, with pronounced inhibition evident at 30 and 40 uM drug concentrations, was also observed when equivalent aliquots (by volume) of RNA extracts from equivalent numbers of control and Δ^9-THC-treated cells were similarly analyzed (see figure 1D). The data in figure 2 are results from experiments in which total cellular RNAs from control and Δ^9-THC-treated exponentially growing HeLa S3 cells were analyzed by hybriaization with cloned genomic H3 (pF0 422) (figure 2A) or H4 (pF0 108A) (figure 2B) histone sequences. Consistent with the results shown in figure 1, a greater than 80% inhibition in the representation of H3 and H4 histone mRNAs was observed following treatment with 30 and 40 μM drug concentrations.

The influence of Δ^9-THC on the levels of histone mRNAs was then
studied in normal human diploid cells (WI38 human diploid fibro-
blasts) and in SV40-transformed WI38 cells. A dose-dependent,
drug-induced decrease in the levels of all four core nistone mRNAs
was observed in both normal human diploid fibroblasts and in
SV40-transformed human diploid fibroblasts--a cannabinoid-induced
inhibition similar to that seen in HeLa S3 cells. As shown in
figures 3A and B, when total cellular RNAs from control and
Ag-THC-treated WI38 cells are hybriaized with [32p]-labeled
pFF435, a plasmid containing cloned human genomic H2A, H2B, and H3
histone coding sequences, decreased levels of histone mRNAs are
observed in both normal WI38 and in SV40-transformed WI38 cells
treated with 30 and 40 µM drug concentrations. Confirmation of
the Δ^9-THC-mediated inhibition of core histone mRNA levels in
normal and SV40-transformed WI38 human diploid fibroblasts can be
seen in figures 3C and 3D as well as in figures 3E and 3F where
similar drug-induced inhibitions in the representation of H3 and
H4 mRNAs, respectively, were observed.

The levels of H2A, H2B, H3, and H4 histone mRNAs were similarly
assayed in A549 human lung carcinoma cells after treatment with
A^g-THC. These cells have been reported to have active drug
metabolizing systems and to efficiently metabolize polycyclic
hydrocarbon-containing carcinogens. A pronounced decrease in the
inhibitory effect of $\Delta^{.9}$-THC on the representation of core
histone mRNAs was observed in A549 cells compared With HeLa S3
cells and WI38 cells (normal and SV40-transformed). It is
unlikely that the reduced sensitivity of A549 cells to cannabinoid
treatment is attributable to changes in drug uptake. Tne
intracellular levels of Δ^9-THC in SV40-transformed WI38 cells
and in A549 cells, when monitored by intracellular incorporation
of ^{3}H-Δ^9-THC (table 4) do not reflect the differences seen in
histone mRNA levels (figures 3 and 4).

TABLE 4

Cellular Uptake and Subcellular
Distribution of ^{3}H-Δ^9-THC

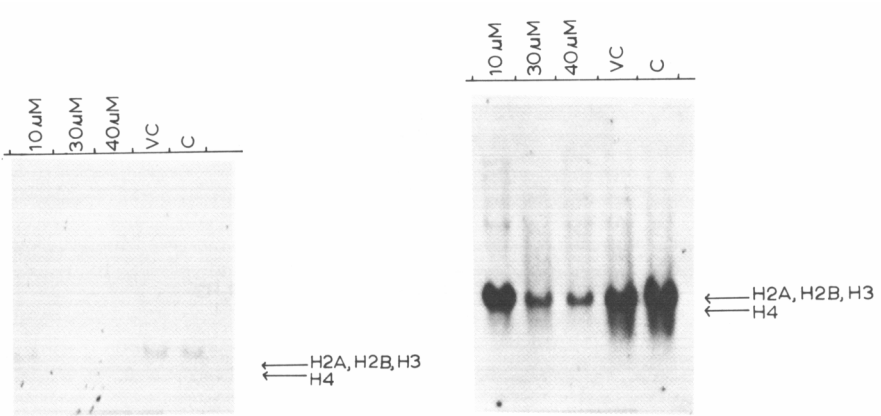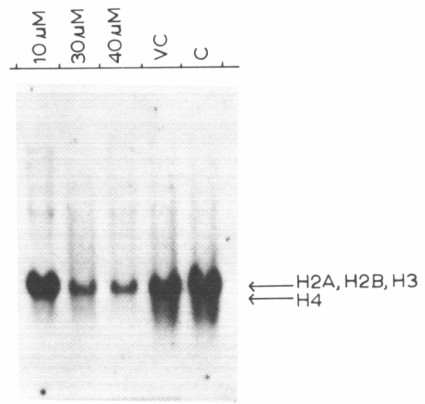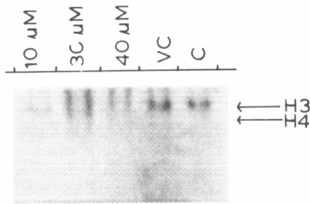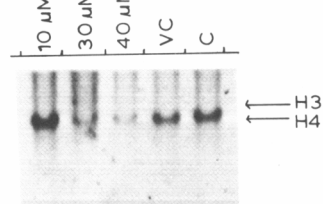| cell type	cpm/10^7 cells	% nucleus	% cytoplasm
SV-40-WI-38	1.2×10^5	32.6%	67.4%
A549	1.3×10^5	37.9%	62.1%

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FIGURE 3A
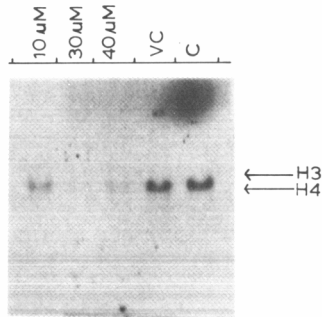
FIGURE 3B
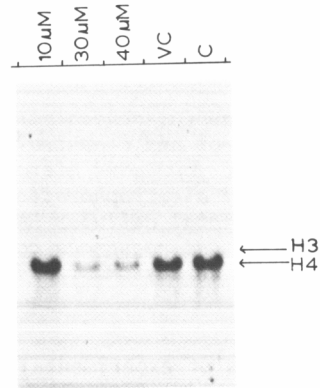
FIGURE 3C

FIGURE 3D

FIGURE 3E

FIGURE 3F

Effects of varying concentrations (10 µM, 30 µM, 40 µM, VC-vehicle treated control and C-control) of Δ^9-THC on the representation of mRNAs for the four core histones. The Signals shown were obtained when 50 µg of electrophoretically fractionated nitrocellulose-immobilized total cellular RNAs were hybridized to cloned human DNA sequences. A) WI38 and B) SV40-WI38 total cellular RNA hybridized to a DNA probe (pFF435) encoding H2A, H2B and H3 histones; C) WI38 and D) SV40-WI38 total cellular RNA hybridized to a DNA probe (pFO422) encoding H3 histone; E) WI38 and F) SV40-WI38 total cellular RNA hybridized to a DNA probe (pF0108A) encoding H4 histone.
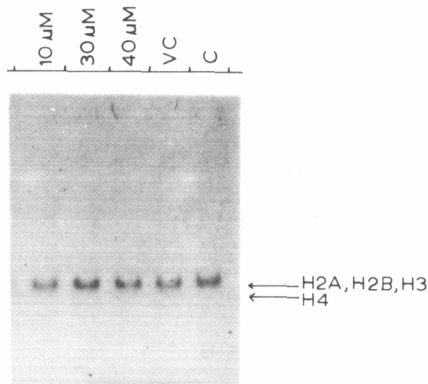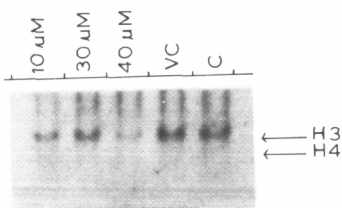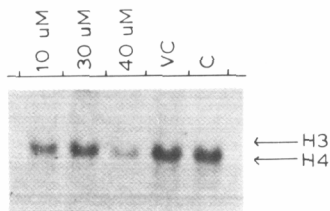
FIGURE 4A

FIGURE 4B

FIGURE 4C

Effects of varying concentrations (10 µM, 30 µM, 40 µM, VC-vehicle treated control and C-control) of Δ^9-THC on the representation of mRNAs for the four core histones. The signals shown were obtained when 50 µg of electrophoretically fractionated, nitrocellulose-immobilized total cellular RNAs from A549 cells were hybridized to cloned human DNA sequences coding for: A) H2A, H2B, and H3 histones (pFF435); B) H3 histone (pFF422); C) H4 histone (pF0108A).

Several lines of experimental evidence suggest that the Δ^9-THC-induced reductions in histone mRNA levels we have observed in normal and transformed human cells are not merely a reflection of a general, nonspecific cannabinoid-induced inhibition in RNA synthesis. As reported previously, the cannabinoid-induced inhibition of ^{3}H-uridine incorporation into total cellular RNAs largely reflects a drug-induced influence on the intracellular nucleotide precursor pool rather than an effect on cellular RNA metabolism (Mon et al. 1981a,b). The absence of a significant quantitative effect of psychoactive and nonpsychoactive cannabinoids on levels of nuclear (Mon et al. 1981a,b) or chromatin (Mon et al. 1981a,b) transcription in vitro further suggests that these drugs do not interfere with the general levels or rates of cellular RNA synthesis.

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The inability of Δ^9-THC, at concentrations between 10 and 40 μM, to modify the levels of ribosomal RNAs provides more direct evidence for some extent of specificity to the cannabinoid-mediated decrease in histone mRNA levels. In all experiments reported in this paper, the representation of 18S and 28S ribosomal RNAs was monitored in control, and in Δ^9-THC-treated, cells by staining gels with ethidium bromide and by ultraviolet shadowing. A typical example of a gel showing the levels of the major ribosomal RNAs in control and in drug-treated cells is shown in figures 1B and 1C. Additionally, when electrophoretically fractionated cellular RNAs from control and Δ^9-THC-treated cells were hybridized with ^{32}P-labeled cloned human 18S (LS-2) and 28s (LS-6) ribosomal RNA coding sequences, a dose-dependent decrease in the representation of these RNAs was not observed. Figures 5A and 5B show no change in the levels of 28S ribosomal RNAs from Δ^9-THC-treated HeLa and SV40-transformed WI38 cells in the same RNA samples where greater than 80% reduction was observed for the representation of core histone mRNAs in treated cells. Figure 5C shows unchanged levels of 18S ribosomal RNA in these same cells following hybridization with ^{32}P-labeled human 18s ribosomal DNA.

A long-standing question has been whether cannabinoids influence the expression of specific genetic sequences. While cannabinoid-induced effects on cell structure and function, coupled with cannabinoid-mediated modification in macromolecular biosynthesis, are consistent with such a contention, direct experimental evidence for an effect of cannabinoids on expression of specific genes has to date not been reported. In this paper we present data which indicate that treatment of exponentially growing normal diploid and transformed human cells with Ag-THC results in a dose-dependent decrease in the representation of histone mRNAs, with a decreased sensitivity of cells with highly developed drug metabolizing systems. This cannabinoid-mediated reduction of cellular histone mRNA levels does not simply reflect a general decrease in cellular mRNA levels or in cellular RNA metabolism. We also present data indicating that the levels of ribosomal RNAs are not altered by the concentrations of Δ^9-THC used in our studies, and we have reported previously that general levels of _in vitro_ and _in vivo_ RNA synthesis are not quantitatively affected by either psychoactive or nonpsychoactive cannabinoids.

While our results clearly indicate that Δ^9-THC preferentially inhibits expression of histone genes, the levels at which regulation is perturbed and the biological implications of this cannabinoid-mediated effect remain to be resolved. The reduction in cellular levels of histone mRNAs after cannabinoid treatment may be attributable to alterations in mRNA stability, tran- scription, or processing of histone transcripts. Additionally, drug-induced structural modifications in the histone genes and in their flanking regulatory sequences should also be considered within this context. By analogy with other moderately reiterated eukaryotic sequences which have been shown to unaergo structural modifications in conjunction with phenotypic changes, cannabinoid- induced effects on the structural features of human histone genes
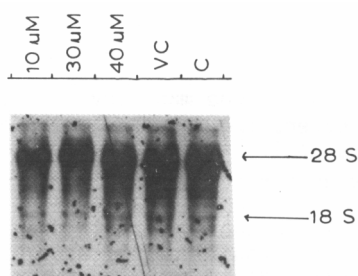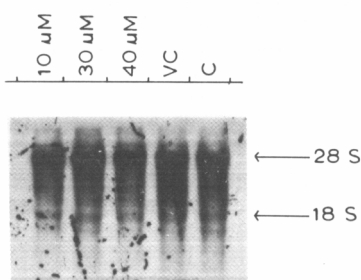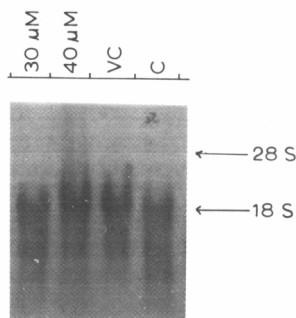
FIGURE 5A

FIGURE 5B

FIGURE 5C

Effects of varying concentrations (10 μM, 30 μM, 40 μM, VC-vehicle treated control and C-control) of Δ9-THC on the representation of 28S and 18S ribosomal RNAs. The signals shown were obtained when 10 μg of electrophoretically fractionated, nitrocellulose-immobilized total cellular RNAs were hybridized to cloned human DNA sequences. A) HeLa and B) SV40-WI38 total cellular RNA hybridized to a DNA probe (LS-6) encoding 28S RNA; C) HeLa total cellular RNA hybridized to a DNA probe (LS-2) encoding 18S RNA.

could be a possibility. The extent to which the expression of specific genetic sequences other than histone sequences is affected by cannabinoids is also an open-ended question--one which is particularly important because the organization and regulation of the moderately reiterated human histone genes differ con- siderably from those of the more complex spliced single copy genes.

From a biological standpoint the selective effect of Δ9--THC on expression of histone genes may be understandable. Expression of histone genes has been shown to be temporally and functionally coupled with DNA replication (Stein et al. 1979; Stein and Borun 1972; Wu and Bonner 1981), and cannabinoids have been shown to bring about a dose-dependent inhibition in cell proliferation (Blevins and Regan 1976; Carchman et al. 1976a,b; Desoize et al. 1979; End et al. 1977; Green et al. 1983; Nahas et al. 1974a,b, 1977; Lemberger 1973; McClean and Zimmerman 1976; Mon et al. 1978, 1981a,b; Nahas and Desoize 1974; Nahas and Paton 1979; White et

al. 1976; Zimmerman and McClean 1973; Zimmerman and Zimmerman 1976; Zimmerman et al. 1979). In fact, in the normal and transformed cell lines we have examined, the extent to which histone mRNA levels are affected by Δ^9-THC is paralleled by the extent to which proliferative activity is affected by cannabinoids. It remains to be determined whether expression of other genetic sequences, whose expression is prerequisite for DNA replication or mitotic division, are preferentially inhibited by cannabinoids. Equally important is whether the cannabinoid-mediated modifications in cellular histone mRNA levels are attributable to a direct effect on the histone genes or the transcripts, or alternatively, whether the effects of cannabinoids on histone gene expression are indirect, e.g., acting initially on other genetic sequences or cellular macromolecules.

III.   Approaches to Defining Effects of Cannabinoids on Specific
       Genes

Cannabinoid-induced modifications in cell structure and function have been well-documented as have a series of physiological effects resulting from such drug-induced cellular changes. Two pivotal biological processes which have been shown to be dramatically influenced by cannabinoids are endocrine function and cell proliferation--both of which have been reviewed in this monograph. Moreover, these are not unrelated processes since in many cases proliferation is responsive to hormonal control. Understanding the manner in which drug-induced alterations in gene expression are brought about should provide insight into the molecular basis of cannabinoid-related modifications in cellular function.

Since cannabinoid-induced alterations in gene expression can result from changes in the organization of genetic sequences and/or in the manner in which genetic information is transcribed and processed, a critical and systematic evaluation of the influence of cannabinoids on the structure and expression of specific genetic sequences, particularly in human cells, should be a high priority. Understanding cannabinoid-induced effects on human gene organization and expression is prerequisite to evaluating possible short-term, long-term, and heritable disorders that may arise from the use of these drugs either therapeutically or as abused substances. Of equal importance, despite the history of fragmentary and often controversial reports of cannabinoid-induced modification in genome-related phenomena (e.g., chromosomal changes, alterations in RNA synthesis, etc.), we are now in a position to address these issues directly and definitively. Availability of a series of cloned human genes permits evaluation of drug-related effects on specific genes, on defined regions of genes, and on transcription and processing of genetic information. Examples of ways in which cloned genetic sequences can be utilized as high resolution probes for the identification and quantitation of several specific human gene transcripts were presented in the previous section of this chapter.

It will be instructive to focus efforts where possible on human studies; for example, drug-induced effects on the organization of specific genetic sequences or regions thereof can be performed using DNA from only 20 ml of blood. Thereby the opportunity is available to determine the effects of cannabinoias on the genomes of subjects participating in endocrine function and behavior studies. A number of normal and tumor-derived human cell lines are available and should be utilized to complement such an approach. By combining both intact organism and cell culture approaches, it is possible to draw on the physiological reality of the organism and the biochemical simplicity of isolated cells. It would also be appropriate to concentrate efforts on evaluating drug-induced effects on a limited series of genetic sequences, those related to proliferation and endocrine function, where phenotypic effects are well understood and the information obtained can be integrated with other ongoing investigations.

In summary, we have attempted to discuss a series of high resolution approaches and procedures which can provide important information regarding the influence of cannabinoids on genome structure and function. Examples of the applications of several of these approaches and procedures have been presented in an attempt to document the feasibility of their implementation. We are highly optimistic that in the next few years our understanding of the genetic effects of cannabinoids at the cellular and molecular level will be significantly enhanced. These same approaches can be implemented for assaying the influence of unfractionated marijuana extracts, psychoactive and nonpsychoactive components of marijuana, natural and synthetic cannabinoids, and cannabinoid metabolites as well as other abused substances, individually or in conjunction with cannabinoids.

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