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ARTICLES

Cell Transformation by c-Ha-*ras*^{Val12} Oncogene Is Accompanied by a Decrease in Histone H1° and an Increase in Nucleosomal Repeat Length

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Abstract The activated c-Ha-*ras*^{Val12} oncogene is often involved in the genesis of human malignancies. We show here that in c-Ha-*ras*^{Val12} oncogene-transformed mouse NIH 3T3 fibroblasts the copy number and expression level of the mutant *ras* oncogene correlates with the degree of chromatin decondensation, as assessed by micrococcal nuclease (MNase) and DNase I digestion. MNase and DNase I analyses further revealed that the nucleosomal repeat lengths were different in the normal and *ras* oncogene-transformed cells, 162.3 bp and 178.1 bp, respectively. These chromatin changes were accompanied by alterations in the content of histone H1°. Furthermore, using DNase I as a probe, we discovered that serum stimulation of normal and transformed cells, synchronized by serum starvation, induces rapid reversible changes in the structure of bulk chromatin that may be linked to transcriptional activation. Our data thus indicate that cell transformation by *ras* is associated with specific changes in chromatin structure that make it more vulnerable, and prone to additional mutations characteristic of cancer development in vivo.

Key words: c-Ha-*ras*^{Val12} oncogene, cell transformation, serum stimulation, chromatin structure, nucleosomal repeat length, histone H1°

The genes of *ras* family (c-Ki-*ras*, c-Ha-*ras*, and N-*ras*) have been reported to be involved in the genesis of a great number of human malignancies [Barbacid, 1987]. RAS proteins indirectly coupled to the receptor tyrosine kinases are now known to play a central role in the signal transduction pathway [for reviews, see Barbacid, 1987; McCormick, 1993]. The mutated and activated RAS proteins are known to generate a constitutive signal, which, at the transcriptional and/or post-transcriptional level, appears to trigger an aberrant increase in the accumulation of transcripts encoded by several genes [Barbacid, 1987; Sistonen et al., 1987, 1989; Wasylyk et al., 1988; Hölttä et al., 1988; Gutman and Wasylyk, 1991; Lloyd et al., 1991].

Recently, it has been found that expression of the active c-Ha-*ras*^{Val12} oncogene triggers reorganization of the chromatin structure [Laitinen et al., 1990]. Furthermore, expression of *ras* oncogenes is associated with chromosomal mutations, such as DNA rearrangements [Bremner and Balmain, 1990]. Conceivably, the point mutations, deletions, and amplifications of DNA segments observed during the multistep process of tumor progression may basically be derived from DNA perturbations at the chromatin level [for reviews, see Nowell, 1986; Hunter, 1991; Bishop, 1991]. Thus, it is important to look for the putative factors, such as oncoproteins [Laitinen et al., 1990; Gutman and Wasylyk, 1991; Drews et al., 1992], chromosomal proteins [Wolffe, 1991], and nucleosomal spacing [Leonardson and Levy, 1989; Pryciak et al., 1992], which might be responsible for the derangements in the propagation of chromatin and cancer development.

Of the chromosomal proteins, histone H1 appears to be an especially good candidate, as it has been implicated in the regulation of chromatin condensation and gene expression [Allan et al., 1980; Weintraub, 1984; Sun et al., 1990; Wolffe, 1991] and cell differentiation [Keppel et al., 1979; Tan et al., 1982; Rousseau et al., 1991]. Furthermore, Tan et al. [1982] showed

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that certain transformed cells display quantitative alterations in the amounts of H1 variants and suggested that they may be of significance for cell transformation.

The nucleosomal repeat length (NRL) that is equivalent to the average DNA content of the nucleosomal monomer [Compton et al., 1976; Noll and Kornberg, 1977; Sperling et al., 1980] is another potentially important parameter, as it reflects the structural organization of chromatin. Moreover, it has been reported to correlate with the transcriptional and/or replicational activity of chromatin [Annunziato and Seale, 1982; Moreno et al., 1986; D'Anna and Tobey, 1989]. Recent studies by Leonardson and Levy [1989] have, in addition, suggested that alterations in the NRL could be associated with tumour progression.

It is often difficult to obtain relevant data on chromatin structure in transformed cells as the cells studied have not been strictly related to each other and chromatin displays continuous transformation-independent changes, like cell cycle-dependent modifications [Annunziato and Seale, 1982; Moreno et al., 1986; D'Anna and Tobey, 1989; Laitinen et al., 1990; Holthuis et al., 1990]. Thus, we have used synchronized cells to study alterations in chromatin structure at the level of a defined transforming oncogene. We have recently shown that c-Ha-*ras*^{Val12} oncogene-transformed NIH 3T3 fibroblasts display more decondensed nucleosomal organization than the normal counterparts [Laitinen et al., 1990]. The present study was undertaken to examine structural elements (deoxyribonuclease-nuclease sensitivity, histone H1 content and NRL) involved in the chromatin structure. We report here that the copy number and expression level of activated c-Ha-*ras*^{Val12} oncogene in mouse 3T3 fibroblasts correlates with the degree of chromatin decondensation. We further show that in *ras*-transformed cells, the nucleosomal repeat length is increased, while the number of histone H1° variants is decreased. Moreover, we investigated how growth stimulation by serum affects the structure of bulk chromatin in normal and c-Ha-*ras*^{Val12} oncogene-transformed 3T3 cells.

MATERIALS AND METHODS

Cell Culture and Synchronization

Normal NIH 3T3 cells (N1), and c-Ha-*ras*^{Val12} oncogene-transformed NIH 3T3 cell lines, the fully transformed E4 cells, and partially transformed E2 cells [Sistonen et al., 1987; Hölttä et al., 1988] were grown in DMEM medium containing 10% fetal calf serum (FCS). The cells were synchronized by serum starvation in G_1 as described by Laitinen et al. [1990]. The DNA content of the cell nuclei stained with ethidium bromide was analyzed by flow cytometry (FACScan; Becton-Dickinson, San Jose, CA).

Immunoprecipitation of RAS Proteins

Exponentially growing cells were metabolically labeled with [^{35}S]methionine, and the RAS proteins immunoprecipitated with a rat monoclonal antibody against p21$^{c\text{-}Ha\text{-}RAS}$ (Y13-259) as described [Hölttä et al., 1988].

Isolation of Nuclei

Nuclei from 5×10^7 to 10^8 cells were isolated as described elsewhere [Moreno et al., 1986; Laitinen et al., 1990]. In brief, the cells lysed in 10 mM Tris–HCl; pH 7.4–10 mM NaCl–3 mM $MgCl_2$–0.5% NP-40 supplemented with 1 mM phenylmethylsulfonylfluoride (PMSF) were centrifuged at maximal speed in an Eppendorf centrifuge for 10 s and the pelleted nuclei washed and resuspended in 400–1,000 μl of the lysis buffer without NP-40.

Nuclease Digestions and Preparation of Supranucleosomes

For the micrococcal nuclease (MNase) and DNase I digestions the DNA content of isolated nuclei from each cell sample was carefully standardized by resuspending the isolated nuclei in RSB (10 mM Tris–HCl; pH 7.4–10 mM NaCl–3 mM $MgCl_2$) to a final absorbance of $A_{\lambda\,=\,260\,nm}$ = 10 or 20. The MNase (Boehringer Mannheim) digestion was carried out at +37°C as described elsewhere [Moreno et al., 1986; Laitinen et al., 1990]. In determining the nucleosomal repeat length (NRL) MNase digestion was also carried out at +4°C [Noll and Kornberg, 1977].

Supranucleosomes were prepared from nuclei ($A_{\lambda=60\,nm}$ = 20; V = 0.2 ml) by MNase digestions (using MNase at 0–100 μg/ml, depending on the enzyme batch) at +22°C for 20 min essentially as described by Weintraub [1984]. The resulting chromatin was loaded and analyzed on 2% agarose gels (20 × 20 × 0.6 cm).

The DNase I (Boehringer Mannheim) digestion was carried out in RSB buffer supplemented with 1 mM $MgCl_2$. The isolated nuclei were resuspended to a density of $A_{\lambda\,=\,260\,nm}$ = 20,

and the enzyme was added to a final concentration of 6–10 units/$A_{\lambda = 260\ nm}$.

Histone Extraction and Protein Electrophoresis

Total histones were extracted from nuclei by stepwise addition of concentrated (18.2 M) H_2SO_4 to a final concentration of 0.2 M with magnetic stirring at +4°C for 45 min essentially as described by D'Anna et al. [1982].

The isolated histones were fractionated by electrophoresis in 15% polyacrylamide slab gels [Laemmli, 1970], stained with Coomassie Blue, photographed and quantitated by densitometric scanning (Hewlett-Packard, ScanJet Plus, Greeley, CO; Scan Analysis, Biosoft, Cambridge, UK).

Isolation of DNA and Electrophoresis

DNA was isolated using the published protein salting-out method [Laitinen and Hölttä, 1994]. Aliquots of DNA (5–10 μg) were separated on 1.6–1.8% agarose gels [Sambrook et al., 1989]. DNA fragments were then visualized by ethidium bromide (EtBr) staining and UV illumination, and the gels were photographed.

Determination of the Nucleosomal Repeat Length of DNA

The photographs (positives) of the MNase and DNase I digestion patterns were scanned with a gray-scale scanner coupled to a computer (Apple Computer, USA; Hewlett-Packard, ScanJet Plus, Greeley, CO; Scan Analysis, Biosoft, Cambridge, UK). For NRL determinations, cells were taken from the same phases of the cell cycle and DNA samples with a similar level of digestion were analyzed to avoid the known variations in the NRL due to differences in cell cycling and the extent of MNase digestions [Annunziato and Seale, 1982; Moreno et al., 1986]. The average spacing between the nucleosomal DNA fragments was assessed by using a least-squares linear regression analysis as described elsewhere [Sperling et al., 1980; Annunziato and Seale, 1982; Smith et al., 1983].

RESULTS

We have previously reported that transformation of NIH 3T3 cells by c-Ha-*ras*^{Val12} oncogene is associated with decondensation of nucleosomal organization [Laitinen et al., 1990]. To study whether an increased c-Ha-*ras*^{Val12} expression strictly correlates with changes in the chromatin structure, we analyzed the chromatin structure in normal NIH 3T3 cells and in cells transfected with the c-Ha-*ras*^{Val12} oncogene in different copy numbers [Sistonen et al., 1987].

Copy Number and Expression Level of the c-Ha-*ras*^{Val12} Oncogene Correlates With Decondensation of the Nucleosomal Organization

Expression of the RAS proteins was quantitated by immunoprecipitation in normal NIH 3T3 cells (N1) and in two c-Ha-*ras*^{Val12} oncogene-transformed cell lines (E2 and E4) [Sistonen et al., 1987]. Figure 1 shows that the N1 cells express relatively low levels of the normal RAS protein, whereas the fully *ras* oncogene-transformed E4 cells display high expression of the mutated RAS. The morphologically partially transformed E2 cells appeared to express intermediate levels of the activated RAS protein.

To study the possible correlation between *ras* expression and the chromatin structure, we used micrococcal nuclease (MNase) as a probe for the nucleosomal organization of the bulk chromatin. Figure 2 depicts the MNase digestion patterns of the nuclei isolated from E2, E4, and N1 cells synchronized by serum stravation. As reported earlier [Laitinen et al., 1990], bulk chromatin of *ras* oncogene-transformed E4 cells exhibited a nucleosomal organization more sensitive to MNase than that of the normal N1

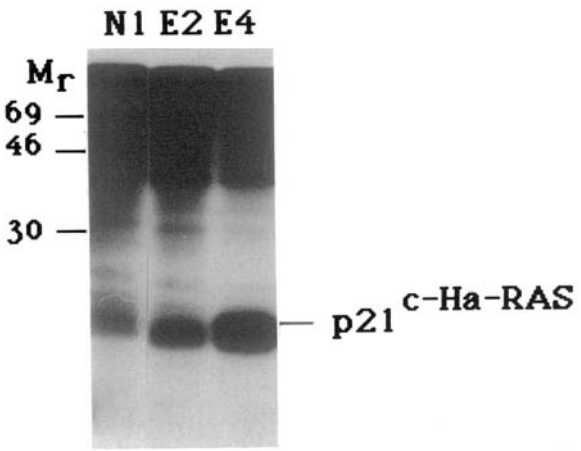

Fig. 1. Immunoprecipitation of RAS proteins from the normal and c-Ha-*ras*^{Val12} oncogene-transformed NIH 3T3 cells. Lysates of [^{35}S]methionine-labeled normal fibroblasts (N1), and c-Ha-*ras*^{Val12} oncogene-transformed cells (E2 and E4) were precipitated with the Y13-259 rat monoclonal antibody to RAS proteins, and the immunoprecipitates were analyzed in 15% polyacrylamide gels, followed by fluorography. M_r indicates [^{14}C]methionine-labeled relative molecular mass markers.

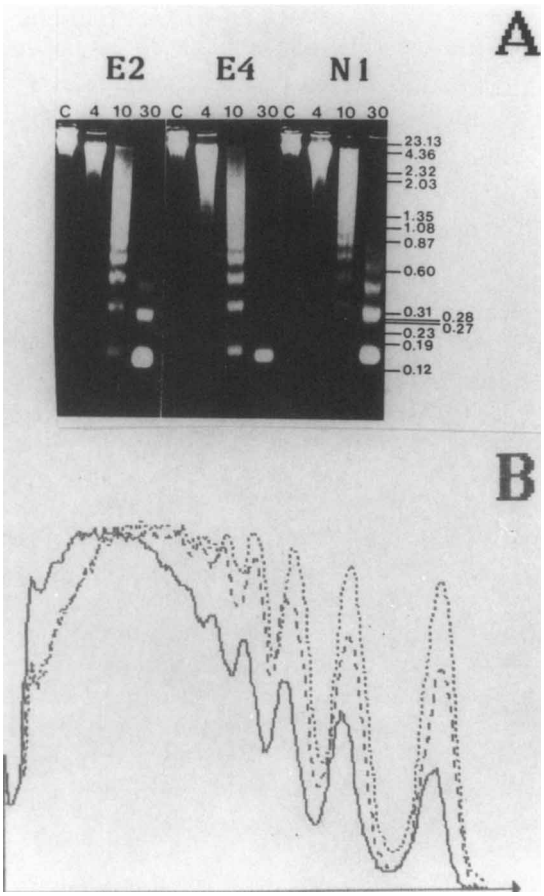

Fig. 2. Nucleosomal organization of the bulk chromatin in the normal and *ras*-transformed cells. **A:** Subconfluent cultures of the morphologically normal N1 cells and *ras*-transformed E2 and E4 cells were synchronized by serum starvation for 24 h and stimulated to reenter the growth cycle by the addition of 10% fetal calf serum. Nuclei were digested with micrococcal nuclease (7.5 units/$A_{\lambda=260nm}$), extracted for DNA and electrophoresed on 1.6% agarose gels. The numbers on top indicate digestion time with micrococcal nuclease in minutes. C indicates DNA from undigested nuclei. Molecular-weight markers (λ-phage and ϕK174 DNA digested with *HindIII or HaeIII*) are indicated on the right. **B:** Densitometric scans of the nucleosomal organizations (10-min digestion) shown in A. The solid and dotted lines indicate the nucleosomal organization of the N1 and E4 cells, respectively, and the dashed line indicates the nucleosomal organization of E2 cells. Migration is from left to right (arrow).

cells (Fig. 2). The nucleosomal organization of chromatin from the E2 cells displayed an intermediate accessibility to MNase as compared to the chromatin from the fully transformed E4 cells and normal N1 cells. These data show that the copy number and expression level of c-Ha-*ras*^{Val12} oncogene in mouse 3T3 cells correlates with the degree of chromatin decondensation. Since the chromatin structure of E4 cells was found to be significantly more decondensed than that of the E2 cells the E4 cell line was selected for all subsequent comparisons.

Expression of the c-Ha-*ras*^{Val12} Oncogene Is Accompanied by an Increase in Nucleosomal Repeat Length

Cell transformation has also been found to be associated with other structural changes in the chromatin, such as changes in the NRL [Leonardson and Levy, 1989]. To see whether cell transformation by the *ras* oncogene was associated with changes in NRL, we isolated nuclei from the synchronized cells and subjected them to MNase digestion analysis. DNA digests were electrophoresed in 1.6% gels, the resolved fragments stained with ethidium bromide (EtBr), photographed and then scanned with a densitometer. Irrespective of the nucleosome multimere used to carry out NRL determinations, normal N1 cells displayed a clearly shorter NRL than the *ras*-transformed E4 cells (Fig. 3). Standardization of the NRL calculations to the nucleosomal tetramer revealed that the NRL value of the N1 cells was 162.3 bp and that of the E4 cells 178.1 bp. Roughly similar values were obtained with DNase I (Table I).

DNase I also digested chromatin from the E4 cells faster than that of the N1 cells (Figs. 4, 5, 6B). For example, 8-min digestion of the nuclei from the quiescent E4 cells shows almost the

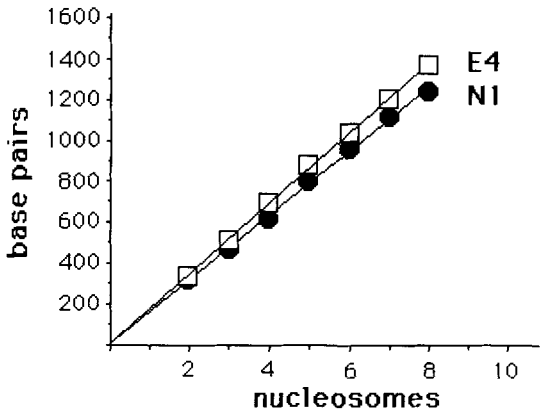

Fig. 3. Determination of the nucleosomal repeat length in normal and *ras*-transformed cells from computerized images of MNase digestion. The nucleosomal repeat length was established using a linear regression analysis. For determination of the nucleosomal repeat length in the N1 and E4 cells, the sizes of nucleosomal DNA (y-axis) were plotted as a function of the number of nucleosomes (x-axis). Filled circles and open squares represent N1 and E4 cells, respectively. The slopes are equal to the NRL. The data plotted here are typical values of one regression analysis. Data shown in Table I represent mean values of several analyses on the nucleosomal tetramer.

same degree of degradation as 20-min digestion of the corresponding N1 cell nuclei. Note that chromatin of the N1 nuclei was digested with a higher concentration of DNase I than chromatin of the E4 nuclei (Fig. 6B).

TABLE I. Nucleosomal Repeat Length in Normal (N1) and c-Ha-*ras* Oncogene-Transformed (E4) Mouse Fibroblasts*

	NRL	
Cell line	MNase (bp)	DNase I
N1	162.3 ± 2.7 (11)	167.2 ± 4.9 (7)
E4	178.1 ± 2.0 (7)	181.9 ± 5.1 (8)

*Values are given in base pairs ± mean standard deviation. Numbers in parentheses indicate the number of experiments. NRL calculations were standardized to the nucleosomal tetramer.

Serum Stimulation of the Resting Normal and *ras*-Transformed 3T3 Cells Triggers Rapid Modifications in the Structure of Bulk Chromatin

It is well documented that serum and growth factor stimulation of animal cells induce rapid and transient changes in the chromatin structure of early response genes that accompany their transcriptional activation [Chen and Allfrey, 1987; Feng and Villeponteau, 1990; Chen et al., 1990; Laitinen and Hölttä, 1994]. We wanted to study whether the bulk chromatin from the normal and fully *ras*-transformed E4 cells responds to serum stimulation. Since DNase I is a more sensitive probe for the chromatin structure than MNase, we used it in the analyses.

To obtain quiescent and synchronized cells, subconfluent cultures of the normal N1 and

Fig. 4. Deoxyribonuclease I digestion of nuclei from synchronized E4 and N1 cells before and after serum stimulation. E4 and N1 cells were synchronized by serum starvation and stimulated with 10% fetal calf serum; nuclei were isolated at 0, 1, and 3 h of stimulation (underlined numbers indicate the duration of serum stimulation). Nuclei were digested with DNase I (lanes 1–6) or MNase (lanes A–F), extracted for DNA, and electrophoresed on 1.6% agarose gels. **A:** Chromatin structure of E4 cells. Numbers 1 (control, undigested), 2 (4 min), 3 (6 min), 4 (8 min), 5 (10 min), and 6 (20 min) denote progressively advanced digestion with DNase I (6.5 units/$A_{\lambda=260nm}$). Letters A, C, E (10 min) and B, D, E (30 min) denote progressive MNase digestion (5 units/$A_{\lambda=260nm}$) of the nuclei at the indicated times of stimulation. M stands for the molecular mass marker (λ-phage and ϕK174 DNA digested with *Hind*III or *Hae*III). DN and MN stand for DNase I and MNase, respectively. Arrows indicate the positions of DNA from the nucleosome monomer. **B:** Chromatin structure of N1 cells. DNase I digestion was performed with 10 units/$A_{\lambda=260nm}$. Lane 6 of 1-hr stimulation contains no DNA. Symbols as in A.

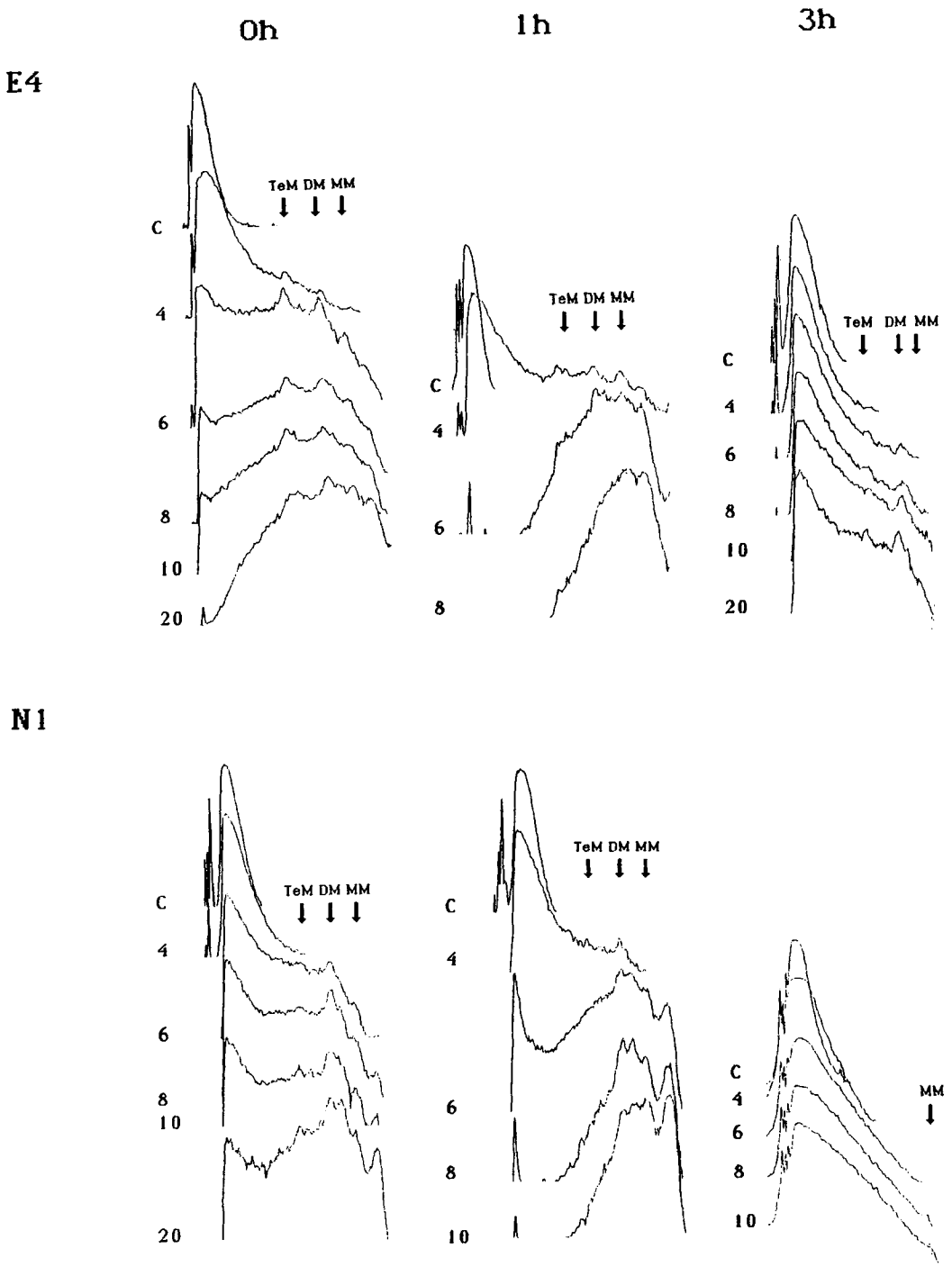

Fig. 5. Densitometric scans of the DNase I digestion patterns of nuclei from the resting and serum-stimulated E4 and N1 cells. Scans of DNase I digestion patterns shown in Figure 4 were generated from photographs (positives). The numbers on top indicate the duration of serum stimulation. 0 hr stands for quiescent cells. Numbers on left of scans indicate times of digestion. C indicates DNA from undigested nuclei. MM, nucleosome monomer; DM, dimer, TeM, tetramer. Notably, the nucleosome trimer is often missing as the digestion patterns generated by DNase I are dependent on the Mg^{2+}-ion concentration. Migration left to right.

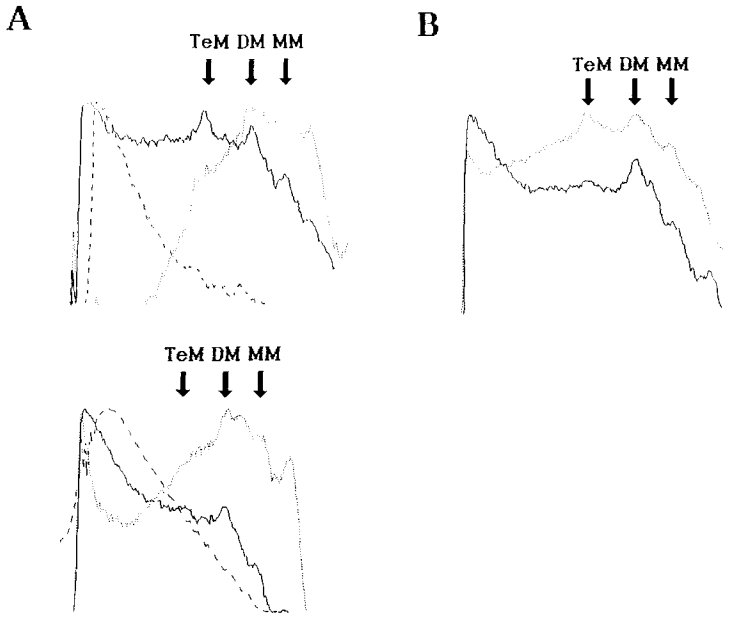

Fig. 6. Serum stimulation and expression of the *ras* oncogene alter the chromatin structure. Comparisons of the densitometric scans of the DNase I digestion patterns shown in lane 3 (6-min digestion) in Figure 4. MM, DM, and TeM as in Figure 5. Migration left to right. **A:** Digestion patterns of E4 and N1 cells are shown in the upper and lower panels, respectively. Solid and dotted lines indicate the chromatin structure of 0-h and 1-h cell nuclei, respectively, dashed line indicates the chromatin structure of 3-h nuclei. Note that the mobilities of DNA fragments (i.e., the origin) from 3-h nuclei differ from those of 0 h and 1 h nuclei as the DNA fragments from 3 h nuclei were analyzed on a separate gel. Electrophoresic mobility between the samples is comparable, however, as verified by migration of molecular mass markers. **B:** Densitometric scans of the structure of bulk chromatin from quiescent N1 and E4 cells. Scans represent 8-min digestion patterns shown in Figure 4. Solid and dotted lines indicate chromatin structure of N1 and E4 cells, respectively.

transformed E4 cells were starved for serum for 24 h. A part of the synchronized cells was then stimulated with 10% fetal calf serum for 1 and 3 h, and their nuclei were isolated for DNase I digestion. The resulting DNA digests were purified and electrophoresed on a 1.6% agarose gel and visualized by EtBr staining and ultraviolet (UV) illumination. As shown in Figure 4, digestion of bulk chromatin from both cells with DNase I produces a smeared nucleosomal ladder on the gel. In parallel with DNA fragments from the DNase I digests, we ran nucleosomal DNA obtained from the digestions with MNase to enable a direct comparison of the chromatin structure revealed by the two different nucleases. In both N1 and E4 cells, the chromatin was relatively DNase I resistant in the quiescent state, but it became highly sensitive after 1-h stimulation and again acquired a resistant configuration after 3 h of stimulation (Fig. 4). These changes are clearly evident from the densitometric scans (Fig. 5). A 20-min digestion of the nuclei from the quiescent E4 cells displays about the same degree of degradation as 6-min digestion of the nuclei from 1-h serum-stimulated E4 cells. By superimposing the densitometric scans of 6-min digestion shown in Figure 5, it can be seen that the N1 and E4 cells respond to serum stimulation (1 h) with a transient but marked change in the chromatin structure (Fig. 6A). On the basis of calculations of the ratios of low-molecular-mass DNA (smaller than tetranucleosomes) versus high-molecular-mass DNA (larger than pentanucleosomes), we estimate that the early serum stimulation (1-h) renders chromatin roughly 3–4 times more sensitive to DNase I than that in the quiescent cells (data not shown, see Fig. 6A). This is true for both cell types, indicating that cell transformation by the c-Ha-*ras*^{Val12} oncogene does not make chromatin refractory to growth stimulation. By contrast, the nucleosomal organization detected by MNase did not appear to undergo any appreciable modifications upon serum stimulation for up to 3 hr (Fig. 4A,B).

Decondensation of Chromatin in c-Ha-*ras*^{Val12} Oncogene-Transformed Cells Is Accompanied by a Decrease in Histone H1°

Next, we wished to analyze the higher-order chromatin structure of the N1 and E4 cells.

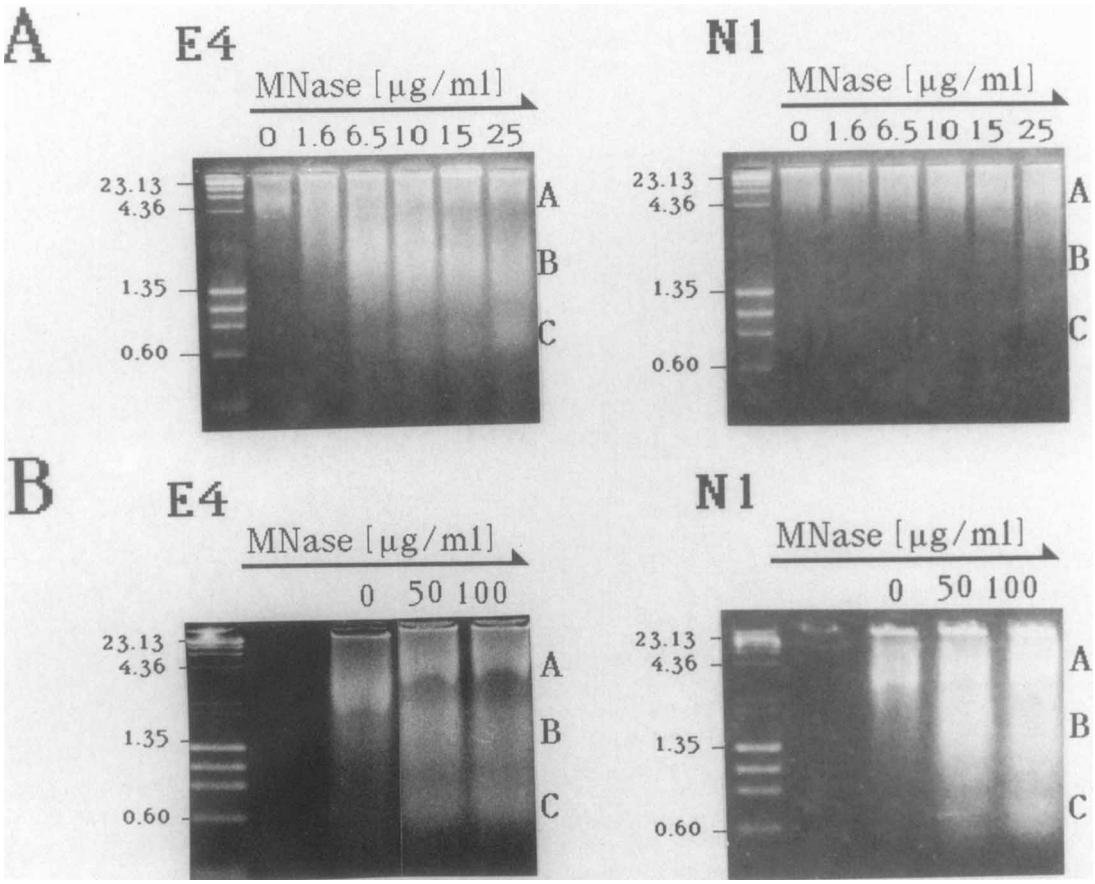

Fig. 7. Supranucleosomal organization of the bulk chromatin from the normal and *ras*-transformed cells. **A:** Chromatin was prepared from the nuclei of quiescent normal (N1) and *ras*-transformed (E4) cells, subjected to micrococcal nuclease (MNase) digestion and electrophoresed on 2% agarose gels. Supranucleosomal particles were visualized by ethidium bromide (EtBr) staining and UV illumination. **B:** Nuclei of the transformed (E4) and normal (N1) cells were digested in a separate experiment by using high concentrations of MNase. Concentrations of MNase are indicated on top. Letters A, B, and C indicate the positions of the supranucleosomal A, B, and C particles, respectively. Molecular-weight markers are indicated on left.

Eukaryotic chromatin appears to be organized into large chromatin DNA loop domains that contain both decondensed and condensed chromatin subdomains [Bonifer, 1991]. These loops and subdomains can be isolated as histone H1-dependent supranucleosomes by digesting nuclei mildly with MNase [Weintraub, 1984]. The supranucleosomes migrate in agarose gels as three distinct fractions, designated A, B, or C particles. The A particles contain condensed, inactive, and MNase-resistant chromatin (>20 kb DNA), and the B and C particles comprise decondensed and active genes sensitive to MNase [Weintraub, 1984]. It is evident from Figure 7 that the N1 and E4 cells have a similar pattern of supranucleosomal particles. However, comparisons of the digestion kinetics indicate that the B and C supranucleosomal particles are produced more rapidly in the E4 cells than in the N1 cells (Fig. 7A,B).

We then asked whether the chromatin decondensation and loosened supranucleosome structure of the c-Ha-*ras*^{Val12} oncogene-transformed cells could result from a decrease in histone H1, which is known to affect the packaging of DNA [Weintraub, 1984; Roche et al., 1985; Sun et al., 1990]. Thus, chromatin or sulfuric acid-extracted proteins from the nuclei [D'Anna and Prentice, 1983] of N1 and E4 cells were subjected to SDS–PAGE analysis. Three distinct protein bands corresponding to the H1 variants, H1B, H1A, and H1°, could be seen in both fractions from the normal N1 and transformed E4 cells (Fig. 8, data not shown). Since the different degrees of condensation could influence the acid extractability of histones, chromatin was used

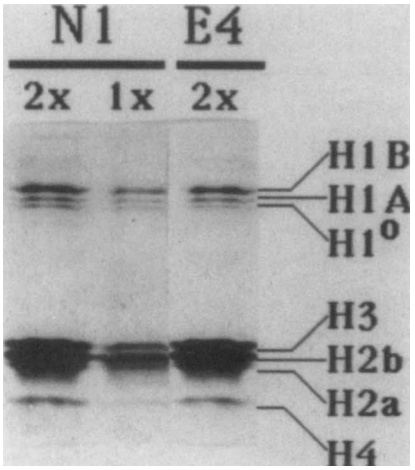

Fig. 8. Analysis of histone H1 proteins by SDS electrophoresis. Analysis of histone pattern of chromatin from the N1 and E4 cells. Chromosomal proteins (8–15 μg) were subjected to 15% polyacrylamide gel electrophoresis (SDS–PAGE) and stained with Coomassie Blue. Numbers indicate relative amounts of histone loaded on the gel.

TABLE II. Histone H1 and H1° Levels in Normal (N1) and c-Ha-*ras* Oncogene-Transformed (E4) Cells

	Relative (%) histone H1 content*		
Cell line	H1B	H1A	H1°
N1	44.2 (4.7)	29.7 (2.2)	26.1 (2.8)
E4	51.8 (3.6)	31.1 (1.9)	17.1 (2.7)

*Each ratio is an average of 8 (N1) or 9 (E4) observations, with the standard deviation shown in parenthesis. Amounts of histone H1 variants were standardized to the core histone H4.

for quantitative SDS–PAGE analyses (Fig. 8). From the data shown in Figure 8 one could infer that, in fully transformed E4 cells, the number of H1° molecules is decreased to about one-half of the H1° levels in the normal N1 cells. However, the densitometric scannings of 8–9 independent histone samples showed that the content of H1° is decreased in the E4 cells by a factor of 1.5 (to 69% of the H1° levels in normal cells) (Table II). Similar and comparable levels of histones were also obtained with acid extracted histones (data not shown). For quantification of the H1 histones, the amount of nucleosomal core histone H4 was considered as a reference.

DISCUSSION

Although it appears that cell transformation is associated with changes in the nuclear architecture, very little information is available on the chromatin structure in cells transformed with defined oncogenes [Laitinen et al., 1990]. In this work, we have used MNase and DNase I as probes for the structure of bulk chromatin in the normal and *ras* oncogene-transformed fibroblasts. Using MNase as a probe for the nucleosomal organization of chromatin, it was found that there is a clear correlation between the chromatin decondensation and the parameters of cell transformation by the c-Ha-*ras*^{Val12} oncogene in NIH 3T3 cells [Sistonen et al., 1987]. Similarly, chromatin of the fully transformed E4 cells is more sensitive to DNase I than that of the normal cells. Furthermore, analysis of nuclear protein composition showed that the decondensed chromatin is associated with a decrease in H1° and an increase in nucleosomal repeat length. It was also found that DNase I detects rapid structural modifications in the organization of bulk chromatin after the addition of serum in G_1-phase of the growth cycle.

In contrast to the early response genes [Chen and Allfrey, 1987; Feng and Villeponteau, 1990; Chen et al., 1990; Herschman, 1991; Laitinen and Hölttä, 1994], very little or almost nothing is known about the structure of bulk chromatin under serum stimulation of quiescent cells. Using DNase I as a probe, we found that the chromatin structures in normal and transformed cells display transient structural changes during serum stimulation. In both cells, the kinetics are particularly striking; the structure of bulk chromatin undergoes a rapid decondensation within 1 hr, followed by an extensive recondensation at 3-h stimulation. It has been shown that stimulation of serum-starved cells with serum and growth factors induce rapid long-range reorganization in the chromatin structure (extending several kilobases) and increase in the amount of transcripts of early response c-*myc*, c-*fos*, and *ornithine decarboxylase* (*odc*) genes [Chen and Allfrey, 1987; Feng and Villeponteau, 1990; Chen et al., 1990; Laitinen and Hölttä, 1994]. Therefore, it is possible that dynamic modifications in the structure of bulk chromatin are secondary to the burst of transcriptional activation of the known 50–100 early response genes [Chen and Allfrey, 1987; Feng and Villeponteau, 1990; Chen et al., 1990; Herschman, 1991]. This number of genes is, however, not likely to be sufficient for explaining the overall changes in bulk chromatin. Thus, another possibility is that serum stimulation is associated with a transient genome-wide open-

ing of chromatin, enabling binding of transcription factors at appropriate sites.

The increased MNase and DNase I sensitivities of chromatin of the E4 cells was also found to be accompanied by a change in (NRL). Similarly, Leonardson and Levy [1989] recently reported that the Friend leukemia tumor establishment is accompanied by an early period of transient reorganization of the nucleosomal repeat length. In this study, we found that the NRL value of E4 cells was 15 base pairs (bp) longer than that of normal N1 cells. Several results obtained from differentiation and cell synchronization studies have shown that similar changes in NRL of bulk chromatin (and specific genes) usually correlate with significant alterations in the accessibility of chromatin to MNase and with transcriptional and/or replicational activity [Annunziato and Seale, 1982; Smith et al., 1983; Yu and Smith, 1985; Moreno et al., 1986].

Several studies indicate that the MNase accessibility of chromatin correlates with the amount of histone H1 in the nucleosome fiber [Keppel et al., 1979; Allan 1980; Biard-Roche et al., 1982; Sun et al., 1990]. In addition, there appears to be an inverse correlation between the levels of the mammalian histone H1° or avian H5 variants and nuclease accessibility, transcriptional activity, and cell proliferation [Keppel et al., 1979; Pehrsson and Cole, 1980; Osborne and Chabanas, 1984; Roche et al., 1985; Sun et al., 1990; Rousseau et al., 1991]. In this work, we found that the amount of histone H1° was decreased in fully transformed E4 cells by a factor of 1.5, as compared to normal N1 cells. Since histone H1 binds to DNA cooperatively, even a decrease of this degree could explain the changes in chromatin structure seen in the c-Ha-*ras*^{Val12} oncogene-transformed cells. Thus, it is possible that the decrease in histone H1° could play a role in the chromatin changes of transformed cells by weakening the internucleosomal interactions and thereby destabilizing the organization of chromatin. In contrast to our results, Tan et al. [1982] showed that the ratio of H1A to H1B is significantly higher in rapidly growing neoplastic human HeLa cells than in the normal human fibroblasts. On the other hand, the transformed HeLa and other transformed cells were also found to contain little histone H1°, in agreement with our observations.

Our data show that several features known to be important for the self-propagatation of chromatin, such as nuclease inaccessibility and NRL, are perturbed in the *ras*-transformed E4 cells. Presumably, these changes in chromatin make also the *ras*-transformed cell more active in transcription. Although the origin of such changes and DNA rearrangements [Bremner and Balmain, 1990] remains unknown, these changes can be expected to facilitate or enhance the frequency of mutations (e.g., error-prone DNA replication, gene amplification, recombination errors), which are typical of tumor progression [Nowell, 1986; Hunter, 1991; Bishop, 1991; Marx, 1993]. In fact, in support of our speculation, Pryciak et al. [1992] recently showed that, in yeast TRP1ARS1 and SV40 minichromosomes, the retroviral DNAs integrate preferentially to exposed DNA on nucleosomes, near the nuclease-sensitive regions.

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