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The chromatin structure of Rous sarcoma proviruses is changed by factors that act in *trans* in cell hybrids

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In several lines of Rous sarcoma virus (RSV)-transformed rat cells the proviruses are in a configuration typical of active eukaryotic genes. They are sensitive to pancreatic DNase I, with sites hypersensitive to nuclease near the 5′ end of the genome, they are close to the nuclear 'cage' and they show a low level of cytosine methylation in CpG doublets. In contrast, in phenotypically untransformed hybrids between these cells and uninfected rat or mouse cells, RSV inactivity is associated with hypermethylation of the provirus, reduced DNase I sensitivity (in two out of three examples) and, where examined, relative remoteness from the nuclear cage. These changes in proviral configuration, which occur rarely in spontaneous reversion of transformed cells, can thus be induced at high frequency and stability in cell hybrids by *trans*-acting influences of the uninfected parents.

Key words: retrovirus transformation/cell hybrids/gene expression/chromatin structure

Introduction

Gene expression in eukaryotes is regulated at two levels. At one level DNA tracts flanking (and possibly within) the coding sequences of genes determine the precision and rate of transcription. In some cases proteins are known to interact with such DNA elements to alter the amplitude of RNA polymerase activity. The other level of regulation, however, exerts an overriding influence; transcription only occurs if genes are 'primed' by mechanisms perceived as changes in chromatin structure. Expression of retroviral genomes within their host cells is apparently controlled in similar fashion. Proviral promoter and enhancer elements are the primary determinants of transcription but expression of integrated proviruses can be varied, probably by mechanisms similar to those regulating the activity of other eukaryotic genes. It is important to understand this variable expression for two reasons: (i) it has implications for the design and use of retrovirus vectors, (ii) it provides a manipulable model for studying the crucial but poorly understood temporal and spatial control of eukaryote gene activity.

We have examined the expression of Rous sarcoma virus (RSV) in cultured Rat-1 cells, using transformation by the RSV *src* gene to monitor viral gene activity. Only a minority of Rat-1 cells that habour RSV genomes are transformed, but clonal analyses of cell lineages show that transformed segregants can arise from normal cells and can in turn give rise to morphologically normal revertant offspring (Boettiger, 1974; Turek and Oppermann, 1980; Wyke and Quade, 1980; Chiswell *et al.*, 1982a). The frequency of phenotypic modulation varies from lineage to lineage, probably because of RSV integration at different locations in the cell genome (Wyke and Quade, 1980; Varmus *et al.*, 1981; Dyson *et al.*, 1982; J.A.Wyke unpublished data; H.E.Varmus, personal communication). Phenotypically normal and phenotypically transformed sibling clones usually show no detectable differences in the restriction map of their integrated provirus(es) but differ markedly in viral RNA levels (Chiswell *et al.*, 1982a). Concomitant differences in proviral chromatin structure are detected as alterations in nuclease sensitivity (Chiswell *et al.*, 1982b; Nicolas *et al.*, 1983; D.A.F.Gillespie, unpublished data), cytosine methylation (Chiswell *et al.*, 1982b; Searle *et al.*, 1984) and proximity of the proviruses to the 'nuclear cage' (Cook *et al.*, 1982). Thus modulation of provirus expression is accompanied by the alterations in chromatin organisation that are considered important in regulating the activity of other eukaryotic genes.

To obtain some insight on the mechanisms regulating the differences between normal and transformed cells we studied the phenotype of hybrids between uninfected Rat-1 derivatives or mouse 3T3 cells and various RSV-transformed Rat-1 clones. Certain RSV-transformed clones containing a single integrated provirus almost always formed phenotypically normal hybrids, whereas other clones, also bearing a single provirus, uniformly yielded hybrids of transformed morphology. Morphological variations were accompanied by marked differences in virus expression: virus-specific RNA and levels of the $pp60^{src}$ protein were unaltered in transformed hybrids but undetectable in those that were normal (Dyson *et al.*, 1982). We now show by three criteria (sensitivity to pancreatic DNase, cytosine methylation and proximity to the nuclear cage), that the RSV proviruses in the morphologically normal hybrids lost the chromatin structure characteristic of the transformed parent. Thus, factors contributed by a normal cell and acting in *trans* can lead to stable changes in chromatin structure in transformed cells.

Results

Proviral sensitivity to DNase in parental cells and hybrids

Retroviral genes are among the many that may have been shown to have increased sensitivity to DNase I when primed for expression (Panet and Cedar, 1977; Groudine *et al.*, 1978, 1981; Chiswell *et al.*, 1982b; Feinstein *et al.*, 1982). We previously found that in the RSV-infected Rat-1 clone A11 the active proviral region showed not only a greater overall sensitivity to nuclease than the inactive region in morphologically normal cell revertants, but it also displayed hypersensitive sites, some of which disappeared on reversion (Chiswell *et al.*, 1982b). Without undertaking such detailed analysis with the clones studied here, it is clear that comparable phenomena exist (Figures 1–3).

Figure 1, panel A, shows the effect of partial DNase diges-

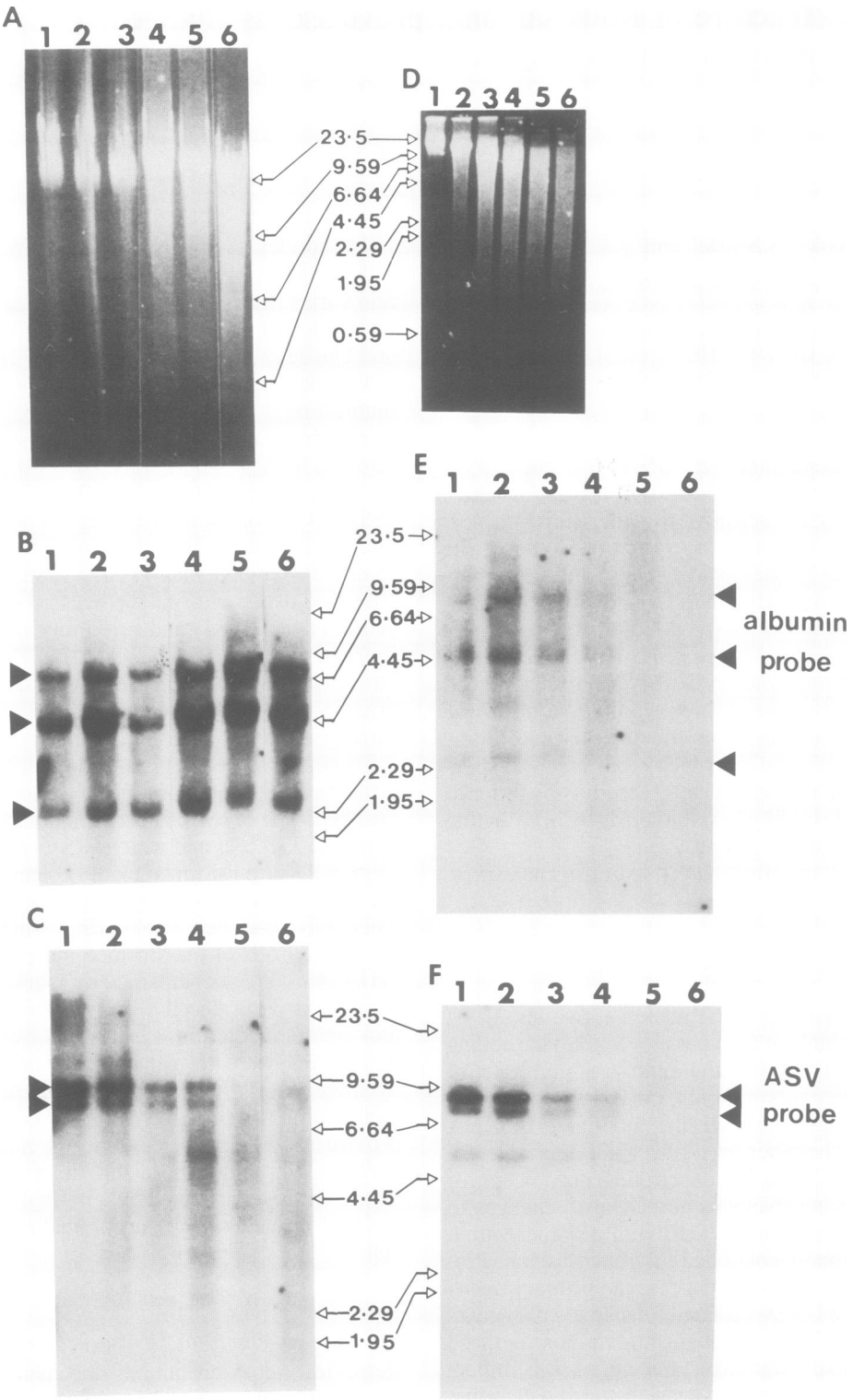

Fig. 1. DNase I sensitivity of chromatin from the transformed line B2*LA*8 and a morphologically normal hybrid between B2*LA*8 and TK3. **Panels A, B, C. Lane 1**, undigested and **lanes 2–6**, increasing digestion of B2*LA*8 nuclei with DNase I, the DNA separated by electrophoresis on 1% agarose. **Panel A** shows total DNA stained with ethidium bromide, the other panels show the same DNA digested with *Hind*III and hybridized with the rat serum albumin probe, pRSA-8 (**panel B**) or the RSV probe, pSRA-2 (**panel C**). Solid arrowheads indicate the three albumin-specific and two RSV-specific fragments. *Hind*III cleaves twice close together in the RSV genome, generating two detectable virus-host junction fragments (see diagram in Figure 3); the presence of only two virus-specific bands is thus *prima facie* evidence for a single integrated provirus in this clone. **Panels D, E, F** are equivalent to **A, B** and **C** but with DNA from a morphologically normal B2*LA*8/TK3 hybrid. **Panel E** is the same filter as that shown in **panel F**, reprobed with pRSA-8 after removing the pSRA-2 probe. The fragment of ~5 kb in **panel F** is seen in normal cells (not shown) and represents mouse c-*src* sequences. Mol. wt. markers, in kilobases (kb) are indicated by small open arrows.

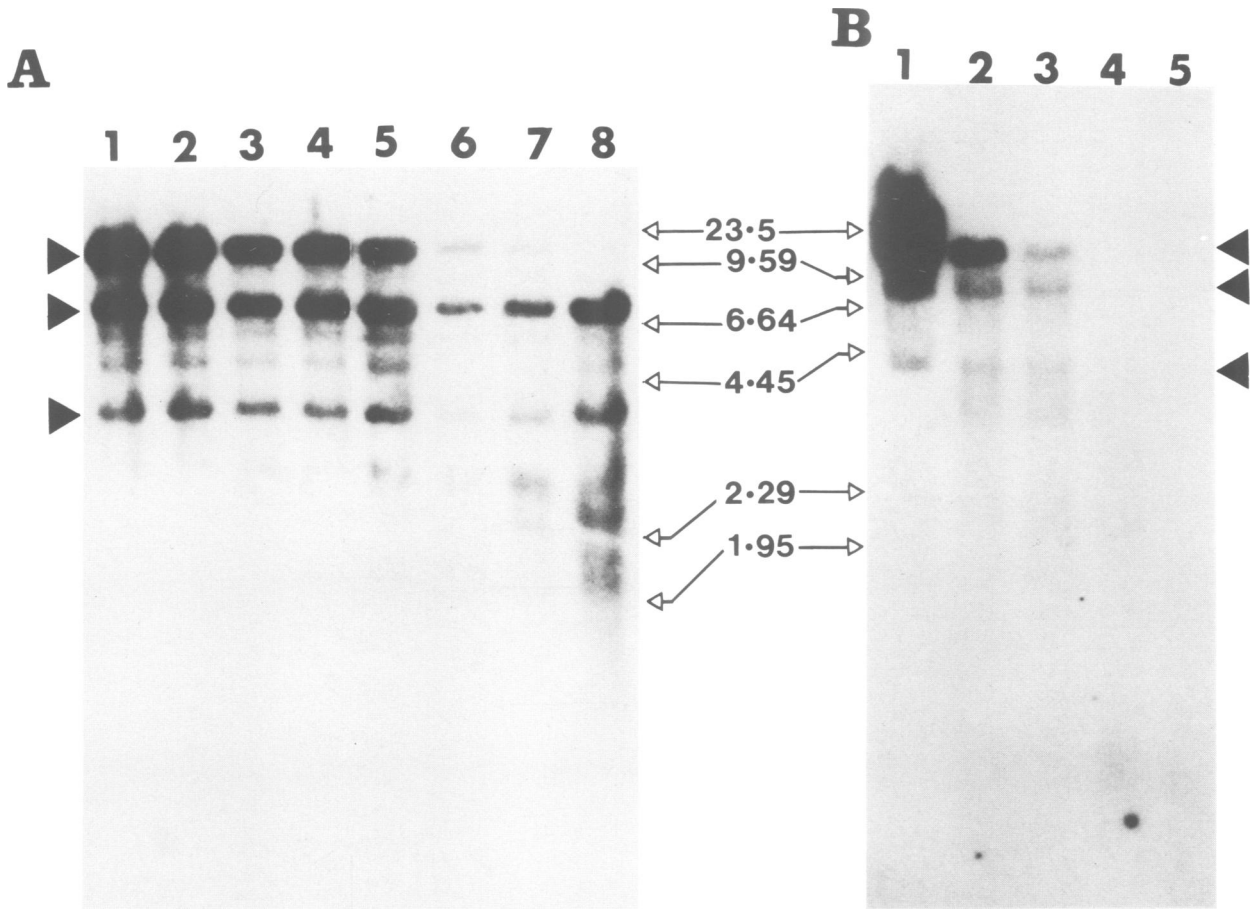

Fig. 2. DNase I sensitivity of chromatin from the transformed line B2*LA*10 and a morphologically normal hybrid between B2*LA*10 and TK3. In each panel **lane 1** is undigested, with increasing DNase I digestion from left to right. **Panel A**, B2*LA*10; **panel B**, B2*LA*10/TK3 hybrid, both treated as in Figure 1 and probed with pSRA-2. Solid arrowheads show virus-specific fragments.

tion of nuclei of the transformed cell B2*LA*8. In panels B and C this DNA is then digested to completion with *Hind*III, electrophoresed and hybridised with either an albumin probe or a probe for the whole RSV genome. Albumin, an inactive gene in these cells (Cook *et al.*, 1982), is clearly more resistant to nuclease than the proviral sequences (compare lanes 2 and 6 in panels B and C). However, when DNA from a phenotypically normal hybrid between B2*LA*8 and the mouse 3T3 line, TK3, is digested by DNase I to a much greater degree (Figure 1, panel D), the albumin and RSV sequences both show a similar level of resistance to nuclease (Figure 1, panels E, F) suggesting that the provirus is now less sensitive.

The provirus in the transformed line A23, like that in B2*LA*8, is sensitive to DNase I when compared with albumin sequences and this sensitivity also decreases in a morphologically normal hybrid between A23 and the Rat-1 derivative, B2E3 (data not shown). However, a further transformed clone, B2*LA*10, is more complex. *Hind*III digestion generates three major fragments of different intensity (Figure 2, panel A, lane 1; Dyson *et al.*, 1982), suggesting the presence of at least two integrated proviruses, one or more of which may be defective and lacking proviral *Hind*III sites. The larger proviral fragment of >10 kb is far more sensitive to DNase I digestion than either of the two smaller fragments (Figure 2, panel A). Viral sequences in this large fragment may thus be the only ones active in B2*LA*10, a possibility strengthened by the finding that in one of five morphologically normal hybrids between B2*LA*10 and TK3 this larger fragment has been lost (see Figure 2 of Dyson *et al.*, 1982). DNase I digestion of a normal hybrid retaining all three proviral fragments (Figure 2, panel B) shows, in contrast to the findings with A23 and B2*LA*8 hybrids, that the >10 kb band of B2*LA*10 retains nuclease sensitivity. Although it disappears at the same level of digestion as the smaller fragments its relative reduction in intensity upon nuclease treatment is far greater (compare the intensities of the >10 kb and 4 kb fragments in lanes 1 and 3 of Figure 2, panel B). The massive over-representation of the >10 kb fragment in the hybrid is, in fact, surprising but probably reflects chromosomal instability in hybrid cells, again enforcing the likelihood that the smaller bands are in different location(s) to the >10 kb fragment.

Tracks in Figure 1, panel C that have been treated with nuclease show indistinct, but apparently discrete, hybridizing fragments of 5 kb or less (there is no evidence for comparable fragments in the hybrid cells shown in Figure 1, panel F). The presence of these fragments suggests the existence of sites that are hypersensitive to DNase I and this is confirmed by gentler digestion and hybridization with a probe, pgag, recognizing only the 5′ of the two viral *Hind*III restriction enzyme fragments. With both A23 and B2*LA*8 ladders of discrete fragments are seen (Figure 3, panels A and B). Available data do not allow us to map these fragments unambiguously, but the sizes of both the probe and the 5′ *Hind*III virus-host junction fragments in each clone (Figure 3, lanes 1) mean that at least some of these smaller fragments must

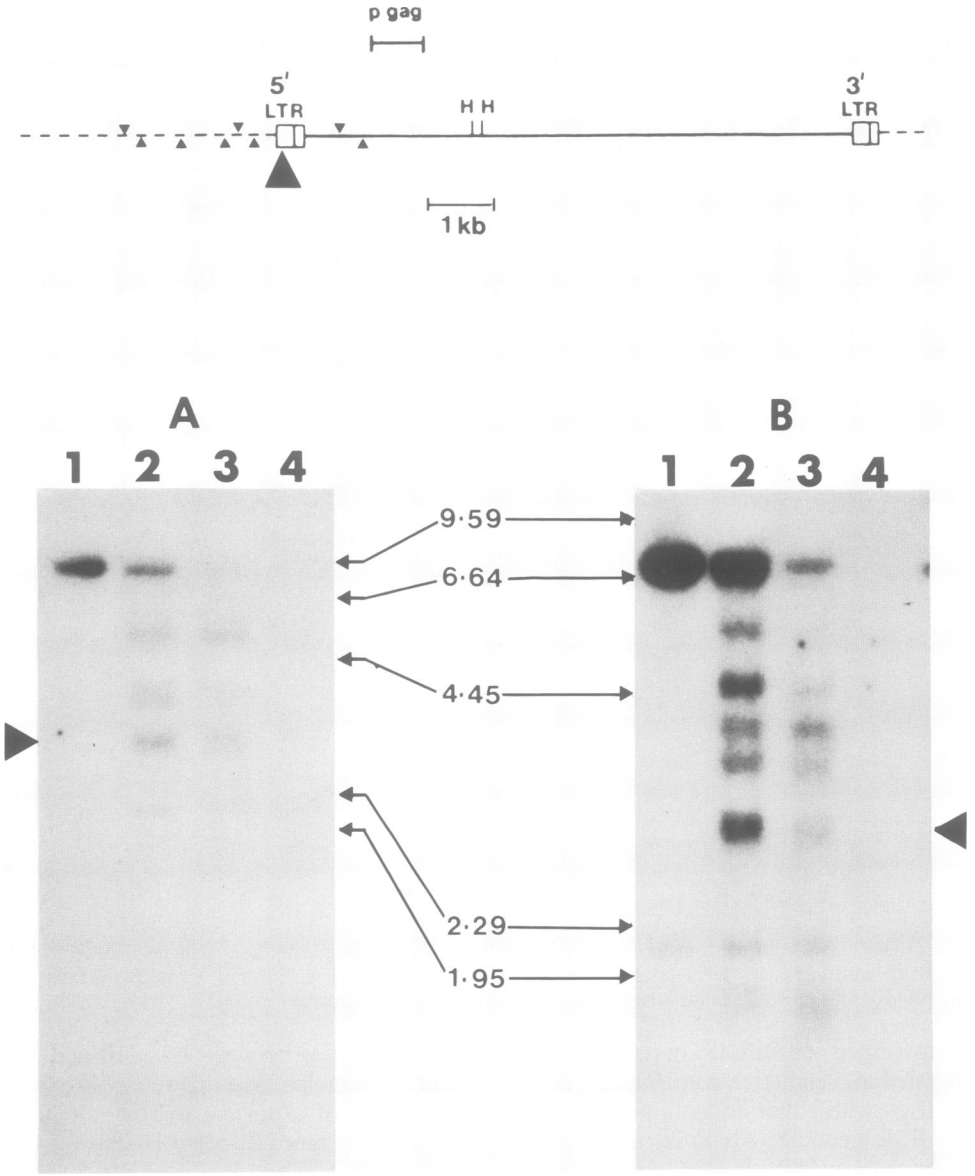

Fig. 3. Detection of chromatin sites hypersensitive to DNase I in the vicinity of A23 and B2*LA*8 proviruses. The diagram shows the position of the two *Hind*III sites within the provirus (H) and the large arrow the probable position of the major hypersensitive site in the 5′ LTR of these two proviruses and in that of A11 (see text). Small arrows above the line are the possible locations of other DNase I hypersensitive sites in A23 as detected in **panel A**. Small arrows below the line are equivalent sites in B2*LA*8 as detected in **panel B** (assuming that the 3′ end of each fragment is the 5′ viral *Hind*III site). The location of the pgag probe is shown. **Panels A and B: lane 1** undigested, **lanes 2** and **3** increasing mild DNase I digestion of nuclei from A23 and B2*LA*8 respectively, followed by restriction with *Hind*III, electrophoresis in 1% agarose and probing with pgag. Open arrows indicate a major 2.9-kb fragment. **Lane 4** in **panel A** shows a more extensive DNase digestion showing near disappearance of all fragments. **Lane 4** of **panel B** is normal Rat-1 DNA not digested with DNase I.

be overlapping rather than contiguous.

The only fragment of the same size in both cell clones is an obvious band of 2.9 kb (indicated by an open arrow in Figure 3, panels A and B) and it is interesting that this is also the size of a major fragment generated by mild digestion of DNA of the transformed clone A11 (Chiswell *et al.*, 1982b). If we make the reasonable assumption that the 3′ end of this fragment is the left hand viral *Hind*III site at nucleotide 2740 (Figure 3, diagram) then its 5′ end is in the U3 region of the viral LTR. Thus, all three cell clones studied probably share a nuclease hypersensitive region located near the elements controlling virus transcription. The variable sizes of the larger fragments show that there are also nuclease hypersensitive sites 5′ to the proviruses but in locations that differ from clone to clone (Figure 3, diagram).

Proximity of proviral sequences to the nuclear cage

We have previously shown that active proviral sequences in nucleoid preparations of RSV-infected Rat-1 cells are far more resistant to detachment by restriction endonucleases than are inactive sequences in sibling revertant clones (Cook *et al.*, 1982). Our interpretation of these observations was that proviral sequences became remote from the nuclear cage (matrix) when they were not being transcribed, and recent work with other regulated genes has supported this view (Robinson *et al.*, 1982; Ciejek *et al.*, 1983). This phenomenon is also seen in cell hybrids (Table I). Sequences of the single proviruses in the parental RSV-transformed clones A23 and B31 are far

Table I.

	% DNA remaining[a]	Relative enrichment of sequences[b]	
		Albumin probe (pRSA-8)	ASV probe (pSRA-2)
Parent			
A23	6.0%	0.5x	3.0x
B31	5.0%	0.6x	3.9x
Non-transformed hybrid			
A23/B2E3	6.0%		0.6x
	2.0%		0.7x
Transformed hybrid			
B31/B2E3	9.0%		3.0x
	1.4%		7.8x

[a]The percentage of DNA remaining associated with the nuclear cage after limited digestion of nucleoids with *Eco*RI.
[b]The relative enrichment of sequences detected by the respective probe in remaining DNA compared to their level in undigested DNA (quantitated by microdensitometry).

less readily detached from the nuclear cage than are inactive albumin sequences. However, in a morphologically normal hybrid between A23 and B2E3, the A23 proviral sequences become readily detachable. In contrast, B31 forms transformed hybrids with B2E3, in which proviral expression persists (Dyson *et al.*, 1982) and in one of these the proviral sequences remain resistant to detachment by restriction endonuclease.

Cytosine methylation in parental cells and hybrids

Hypomethylation of cytosine residues, mainly in the doublet CpG, is a frequent but not invariable occurrence in and around many active eukaryote genes (reviewed by Bird, 1984; Trautner, 1984). One in 16 CpG doublets exists in the tetranucleotide CCGG where, if the cytosine of the doublet is methylated, the restriction endonuclease *Msp*I will cut, but its isoschizomer *Hpa*II will not. Comparisons of DNA digestions with *Msp*I and *Hpa*II thus provide a frequently-used method of sampling DNA methylation. Viral fragments generated by *Msp*I digestion and detectable by a probe, pSRA-2, that recognises the whole RSV genome, vary in size from ~200 bp to 840 bp (Searle *et al.*, 1984). Such fragments are seen in the transformed line A23 when digested with either *Msp*I or *Hpa*II (Figure 4, panel A, lanes 1 and 2). The higher mol. wt. bands seen after *Msp*I and *Hpa*II digestion are also apparent in uninfected cell DNA (Figure 4, panel A, lanes 5 and 6) and presumably represent c-*src* sequences. An A23-B2E3 hybrid clone shows increased CpG methylation at CCGG residues as evidenced by the reduced intensity in *Hpa*II digests of the fragments of <800 bp and the appearance of many larger bands (Figure 4, panel A, lane 4). Novel fragments of ~700 and 1700 bp in *Msp*I-digested hybrid cell DNA (Figure 4, panel A, lane 3) suggest that some CpC residues in CCGG sites are also methylated, rendering them insensitive to *Msp*I cleavage.

In contrast, the provirus in both B31 and its transformed hybrid with B2E3 shows low levels of CCGG methylation (Figure 4, panel B). However, the results with B2*LA*8, B2*LA*10 and their normal hybrids with TK3 (Figure 4, panels C and D) resemble the findings with A23 and its hybrid. Both transformed lines show some methylation of CCGG sites and, as expected, this is most obvious in B2*LA*10 where some proviral sequences are probably inactive (Figure 4, panel D, lanes 1 and 2). The hybrids, however, show far more extensive methylation of proviral sequences, with marked decreases in intensity of lower mol. wt. fragments. Thus, proviral hypermethylation is not an invariable feature of cell hybrids but its presence correlates with lack of detectable proviral transcription (Dyson *et al.*, 1982).

Discussion

Three separate RSV-transformed Rat-1 clones studied here (A23, B2*LA*8 and B2*LA*10) form morphologically normal hybrids when fused to either normal mouse (TK3) or normal Rat-1 (B2E3) cells (Dyson *et al.*, 1982). The proviral chromatin in the transformed parents has characteristics typical of active genes as judged by nuclease sensitivity, the presence of hypersensitive sites, proximity to the nuclear cage and hypomethylation. Our consensus finding is that on fusion the chromatin structure of the same integrated proviruses alters; they become less sensitive to DNase, remote from the nuclear cage and hypermethylated (Table II). The exception to this rule is the hybrid between B2*LA*10 and TK3 in which the DNase sensitivity of proviral chromatin is apparently retained in a phenotypically non-transformed hybrid, even although the genome becomes more highly methylated. This is the only example we have found in RSV-infected rat cells of a dissociation between hypermethylation and decreased nuclease sensitivity. Unfortunately, the complexity of integrated proviruses in B2*LA*10 makes it difficult further to investigate and interpret this finding.

Numerous studies suggest that changes in chromatin structure are a prerequisite for gene activity. However, such changes are not themselves sufficient, but seem to 'prime' chromatin regions making them responsive to additional regulatory elements that positively control transcription. Many investigations, for example, on chick globin genes (Weintraub *et al.*, 1981) or *Drosophila* heat-shock loci (Keene *et al.*, 1981) suggest that once a gene acquires an active chromatin conformation in a cell lineage it retains this feature, whether or not transcription is maintained (the B2*LA*10/TK3 hybrid studied here resembles that situation as far as nuclease sensitivity is concerned). However, it is possible that the very features of high constitutive or inducible expression that render certain genes amenable to analysis may also reflect their unusual regulation. Indeed, in at least some developmental lineages, mechanisms must exist not only to activate genes but also to reduce stably the ability to express certain genes. At its most dramatic, this is seen in inactivation of one X-chromosome (reviewed by Shapiro and Mohandas, 1982; Wolf and Migeon, 1982) and in the efficient incorporation of integrated viral genomes in inactive chromatin configurations in embryonic cells (Stuhlmann *et al.*, 1981; Jähner *et al.*, 1982). More subtly, in rigorously defined clonal lineages of RSV-infected Rat-1 cells an active chromatin configuration was acquired and subsequently an inactive structure was reinstated (Chiswell *et al.*. 1982b). However, clones differ in their propensity for modulation of this kind (Wyke and Quade, 1980; Varmus *et al.*, 1981). This may be because RSV can integrate at many cell locations and its subsequent regulation may reflect a sampling of the control mechanisms operating at these different locations.

These considerations point to elements that function in *cis* to regulate viral gene expression, and the low frequency of

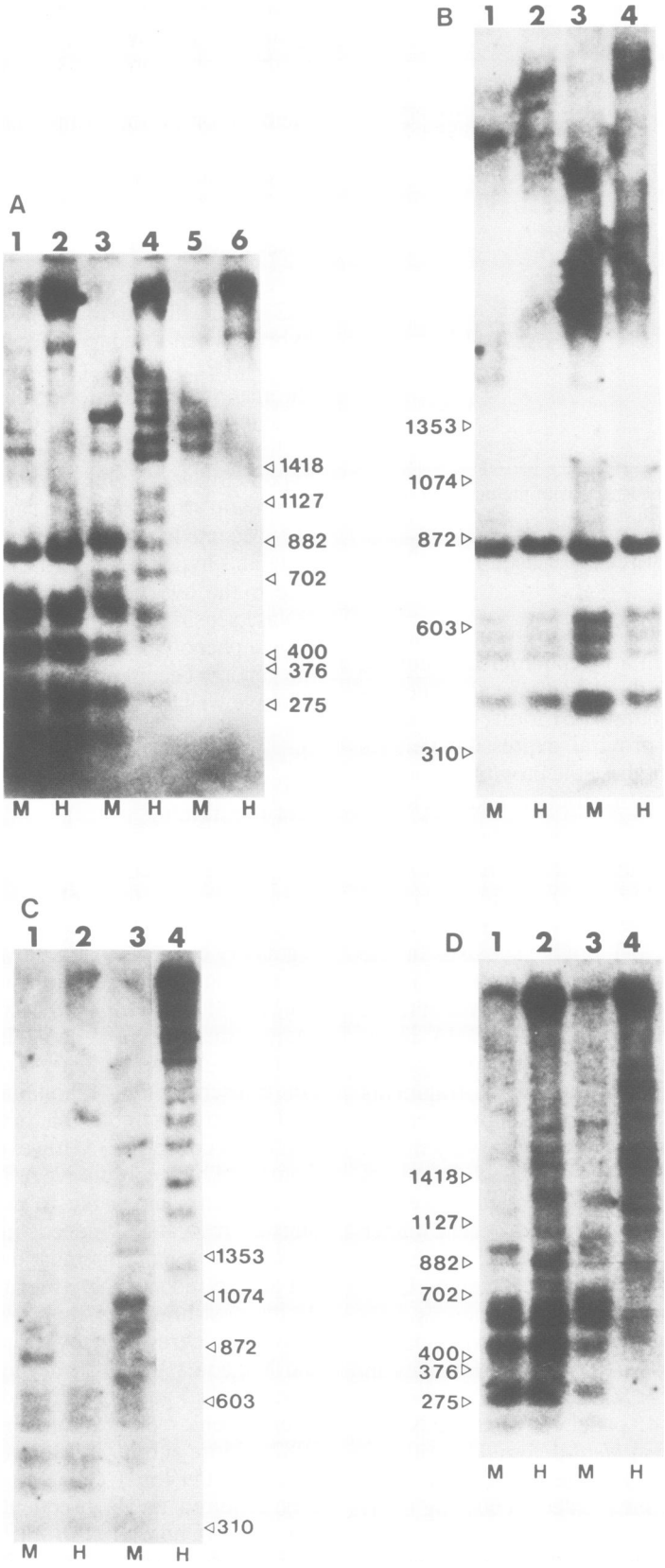

Fig. 4. Patterns of proviral DNA methylation in transformed parental cells and normal hybrids as revealed by paired digestions with either *Msp*I or *Hpa*II. All digested samples were electrophoresed and probed with nick-translated pSRA-2 that recognises all proviral sequences and (depending on stringency) cellular *src* sequences. **Panel A, lanes 1** and **2**, A23; **lanes 3** and **4**, A23/B2E3 hybrid; **lanes 5** and **6**, a hybrid between uninfected Rat-1 and B2E3. **Panel B, lanes 1** and **2**, B31; **lanes 3** and **4**, B31/B2E3 hybrid. **Panel C, lanes 1** and **2**, B2*LA*8; **lanes 3** and **4**, B2*LA*8/TK3 hybrid. **Panel D, lanes 1** and **2**, B2*LA*10; **lanes 3** and **4**, B2*LA*10/TK3 hybrid. In all panels odd numbered lanes show *Msp*I digestions, even numbered lanes *Hpa*II digestions, identified by letters (M and H) at the bottom of each panel. Arrows indicate markers, with their sizes in base pairs.

Table II. Summarised properties of proviral chromatin in parental and hybrid clones

	Sensitivity to DNase I	Proximity to nuclear 'cage'	CCGG methylation
A23	Sensitive	Close	Low
A23/B2E3[a]	Resistant	Distant	Increased
B2*LA*8	Sensitive	ND[c]	Low
B2*LA*8/TK3[a]	Resistant	ND	Increased
B2*LA*10	Partly sensitive	ND	Partial
B2*LA*10/TK3[a]	Apparently unchanged	ND	Increased
B31	Sensitive	Close	Low
B31/B2E3[b]	ND	Close	Low

[a]Morphologically normal hybrids.
[b]Morphologically transformed hybrid.
[c]ND = not done.

spontaneous modulation does not eliminate a possible role for events such as mutation or DNA rearrangement. However, our most significant conclusion from the present results is that a normal cell factor in the hybrids acts in *trans* to reimpose an inactive chromatin conformation around the integrated RSV provirus. The constancy of this event with given parental combinations and its wide variation between combinations (Dyson *et al.*, 1982) seem to rule out strictly mutational mechanisms and point to a factor that operates in a site-specific manner as a negative regulator of gene activity. We cannot say whether the observed changes in chromatin configuration are a cause or consequence of reduced transcription, but presumably the postulated factor must in some way interact with chromatin in the vicinity of the integrated provirus to generate a regulatory locus that acts on adjacent genes.

A few additional points bear on the nature of this regulation. Firstly, it is easy to envisage how cells might repress genes in a hybrid partner different to themselves, as in hybrids between transformed rat and normal mouse cells (e.g., B2*LA*8/TK3). However, B2E3 is a subclone of Rat-1 so, unless it has radically changed, it is harder to explain how proviral chromatin structure is altered on hybridization with a sibling to give the A23/B2E3 hybrid. One possibility, given the low frequency with which RSV transforms rat cells, is that transformation is permitted when a mutation alters the activity of a regulatory factor, fusion to a normal cell restoring the *status quo*. Another possibility is that regulation is very dose dependent, since autosomal dosage affects X-inactivation (Migeon *et al.*, 1979). We are now trying to distinguish these alternatives. A second important question is the identity and mode of action of the regulatory factor. It has been suggested that the DNA-associated proteins HMG14 and HMG17 play a role in activated chromatin (Weisbrod, 1982) so interaction with these is one conceivable mechanism for a negative regulatory element. Since we find no evidence for a role for either HMG14 or HMG17 in regulation of spontaneous modulation of provirus expression in rat cells (Nicolas *et al.*, 1983) we feel it unlikely that they will prove important in provirus regulation in hybrids. However, other factors that act as positive regulators of chromatin structure or gene activity have been described (Bogenhagen *et al.*, 1982; Emerson and Felsenfeld, 1984) and interference with such elements must be considered. Indeed, although it is generally thought that eukaryotic gene expression is mediated mainly by positive regulators, in whose absence genes are inactive (see, for example, Alberts *et al.*, 1983), negative regulation is harder to detect and its role has yet to be assessed. Thus, our demonstration of site-specific *trans*-acting imposition of inactive chromatin structure is likely to be of general significance. It also has implications for some topical concepts.

Firstly, retrovirus long terminal repeats efficiently direct activity of introduced genes (Gorman *et al.*, 1982; Mitsialis *et al.*, 1983) and are popular candidate vectors for gene transfer (Williams *et al.*, 1984). However, for long-term regulated gene expression under retroviral control, we must understand the site-specific effects on virus expression pointed out here and elsewhere (Wyke and Quade, 1980; Jaenisch *et al.*, 1981; Feinstein *et al.*, 1982).

Secondly, much current work on the molecular basis of cancer concentrates on the possible role of cellular proto-oncogenes (c-*onc*) and how this is affected by their involvement in DNA rearrangements such as translocation. A popular concept is that translocation may place c-*onc* genes under powerful positive transcriptional control, but Leder *et al.* (1983) suggest that translocations may, in fact, serve to remove these genes from a location where they are subject to negative regulation. The phenomena we describe here seems a clear example of such site-specific negative regulation. Furthermore, although the effects of oncogene activity are phenotypically dominant, it has been suggested that mutations to regulatory genes that suppress oncogene activity may be important in naturally occurring neoplasia (Comings, 1973). RSV integrations at random cell sites may serve as sensors to detect the effects of such regulatory loci (Wyke *et al.*, 1984) and it would thus be extremely interesting to isolate and characterise the gene(s) in normal cells that suppress RSV activity in these hybrids. We are now trying to do this.

Materials and methods

Cells

The parental normal and RSV-transformed cells, the production of cell hybrids and their properties have been described previously (Wyke and Quade, 1980; Varmus *et al.*, 1981; Chiswell *et al.*, 1982a; Dyson *et al.*, 1982). In brief, the uninfected parents in the hybrids were either TK3 (a Swiss/3T3 line lacking thymidine kinase) or B2E3 (a ouabain-resistant subclone of the thioguanine-resistant derivative of Rat-1, 208F). The transformed parents were the ASV-transformed Rat-1 clones B31 (which gave transformed hybrids with B2E3), A23 (which gave mainly normal hybrids with B2E3) and the ASV-transformed B2E3 clones B2*LA*8 and B2*LA*10 [each of which gave mainly normal hybrids with TK3 but transformed hybrids with Rat-1 (Dyson *et al.*, 1982)]. A single representative hybrid clone from each parental combination was used in all experiments.

Analytical procedures

Sensitivity to DNase I (pancreatic DNase EC 3.1.4.5) was examined as described previously (Chiswell *et al.*, 1982b). Nuclei ($0.5-1 \times 10^7$) were digested with $1-100$ μg of DNase I (Sigma) in 1 ml volumes at 37°C for 5 min. DNA was then purified, its size distribution assessed in ethidium bromide stained agarose gels and it was then digested to completion with the restriction enzyme *Hind*III. Detachment mapping of DNA in nucleoids was performed as described by Cook *et al.* (1982). DNA purification, restriction enzyme digestion, gel electrophoresis, transfer and filter hybridization followed standard techniques as used in this laboratory (Wyke and Quade, 1980; Chiswell *et al.*, 1982a, 1982b; Dyson *et al.*, 1982). The probes used were pSRA-2 (DeLorbe *et al.*, 1980; representing the whole RSV genome), pgag (Chiswell *et al.*, 1982b; a *Bam*HI subclone of pSRA-2 from nucleotide 532 to 1916 and representing the viral *gag* gene) and pRSA-8 (Sargent *et al.*, 1979; representing rat serum albumin).

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