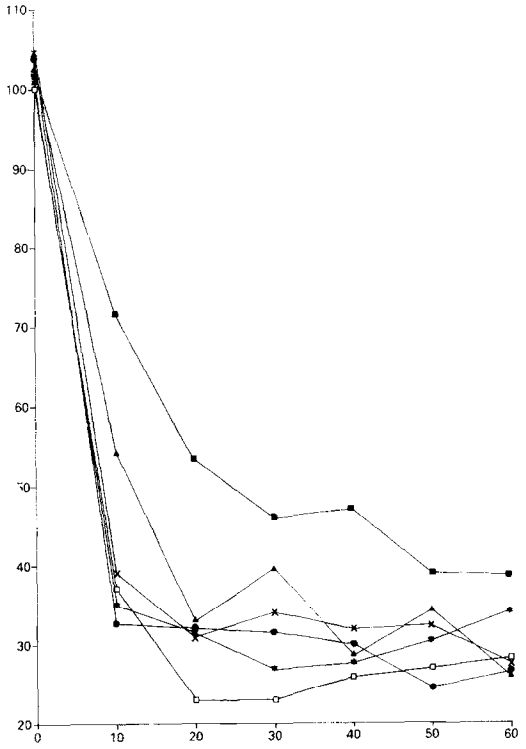

Fig. 5. Abscissa: time (min); *ordinate:* particle count/ml×10^{04}.

Cohesion of cells in buffers of different pH. pH 6.0 (□—□); pH 7.0 (▲—▲); pH 7.6 (×—×); pH 8.0 (●—●); pH 8.6 (*—*); pH 9.0 (■—■). pH 6.0 to 8.0 in M/60 phosphate buffer, pH 8.6 and 9.0 in M/60 Tris/HCl buffer.

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Integration of avian sarcoma virus specific DNA in mammalian chromatin

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Summary. Constitutive heterochromatin and euchromatin fractions from normal and avian sarcoma virus transformed cells of *Mus musculur* and *Microtus agrestis* were isolated in order to characterize the site of integration of the viral specific DNA sequences. The transformed mouse (BALB/c 3T3-B77) and *M. agrestis* (UMMA-RSV-21) cell lines, as well as a revertant clone of the *M. agrestis* (UMMA-RSV-R-4) were found to have integrated 1–2 viral copies per diploid genome. The number of viral copies was studied by the technique of DNA–DNA hybridization in solution, and in all cases the viral sequences were located in the euchromatin fraction.

In mammalian cells, the highly condensed constitutive heterochromatin of interphase nuclei contains the bulk of the repetitive DNA ($C_0t<5$) while the extended or dispersed euchromatin has most of the low repetitive (C_0t 5–100) and unique copy DNA [1, 2]. Transformation of these cells by avian sarcoma viruses (ASV) is known to introduce 1–2 copies of the viral genome into the host DNA [3]. In this work we report the localization of ASV copies in a specific chromatin fraction of two mammalian species.

Mouse cells (BALB/c-3T3) transformed with the B77 strain of ASV and *Microtus agrestis* fibroblasts (UMMA-50) transformed with the Schmidt-Ruppin (subgroup D) strain of Rous Sarcoma Virus (RSV) were used for these studies. In the mouse, pericentromeric heterochromatin contains most of the highly repetitive satellite DNA ($C_0t<10^{-3}$; ~10% of the genome) and can be isolated from the rest of the chromatin by sonication and differential centrifugation

Table 1. *Nuclear DNA content, percentage repetitive DNA and estimate of the number of viral copies per diploid genome present in total chromatin, euchromatin, intermediate chromatin and heterochromatin of BALB/c-3T3-B77* (A), *UMMA-R-21* (B) *and UMMA-RSV-R-4* (C) *cell lines*

BALB/c-3T3-B77 is a mouse cell line known to contain 1–2 specific DNA copies of avian sarcoma virus per diploid genome [3]. The UMMA-RSV-21 was obtained by infecting the UMMA-50 cell line [7] with the Schmidt-Ruppin strain of Rous sarcoma virus following the procedure of Toyoshima & Vogt [8]. The UMMA-RSV-R-4 is a revertant clone derived from the UMMA-RSV-21 on the fourth passage after transformation. This revertant clone has well-defined parallel orientation, is contact inhibited and does not grow in agar. The three cell lines studied do not produce virus detectable by focus formation on chick cells.

For the calculation of the number of viral copies present in each chromatin fraction, the computation of Gelb et al. [9] was used. Since the double-stranded products of the RSV polymerase represent a partial and heterogeneous product of the RSV genome, there is an inherent limitation placed upon the calculation of the exact number of copies present per diploid genome [3]

	Total chromatin	Euchromatin	Intermediate	Heterochromatin
A. *BALB/c 3T3-B77*				
% Total DNA	100	87.4	6.1	6.5
% Satellite DNA	9.8	5	18	52
No viral copies per diploid genome	1.1	0.78	0.04	0
B. *Transformed Microtus agrestis (UMMA-RSV-21)*				
% Total DNA	100	63.2	29.4	7.4
% Repetitive DNA ($C_0t<5$)	21	9	35	45
No viral copies per diploid genome	1.2	0.75	0.17	0
C. *Revertant Microtus agrestis (UMMA-RSV-R-4)*				
% Total DNA	100	62.8	28.7	8.5
% Repetitive DNA ($C_0t<5$)	20	8	37	47
No viral copies per diploid genome	1.6	0.75	0.17	0

[1]. In *Microtus agrestis*, highly repetitive DNA ($C_0t<10^{-3}$; ~5% of the genome) is also found in pericentromeric heterochromatin. In addition, the fast-intermediate repetitive DNA (C_0t 10^{-3}–5; ~15% of the genome) is largely located in the two giant sex chromosomes which are condensed in the interphase nucleus and represents the dispersed intercalary heterochromatin of other species [2]. The transformed mouse (BALB/c-3T3-B77) and *Microtus agrestis* (UMMA-RSV-21) cell lines and a revertant *Microtus agrestis* (UMMA-RSV-R-4) each contained 1–2 copies of viral DNA per diploid genome. When the DNA from the different chromatin fractions of both mammals was analysed, the viral DNA was found to be integrated in the euchromatin fraction.

Material and Methods

Clean nuclei were obtained by homogenizing the cells in an isotonic solution containing 0.3 M sucrose, 1.5 mM $MgCl_2$, 0.2 mM $CaCl_2$ and 0.01 M Tris-HCl, pH 7.2. The resulting nuclei were purified by centrifugation in heavy sucrose with divalent cations, washed in 0.01 M Tris-HCl, pH 7.2, 1.5 mM $MgCl_2$ and 0.2 mM $CaCl_2$, resuspended in 0.25 M sucrose and sonicated at 8 mA for 5 sec. Heterochromatin was collected by centrifugation at 3000 g for 30 min, intermediate chromatin (mixture of heterochromatin and euchromatin) at 18000 g for 60 min and the euchromatin precipitated with ethanol and spun at 8500 g for 20 min [4]. DNA was extracted following Marmur's [5] procedure and analysed by Cs_2SO_4-Ag^+ density gradient centrifugation in the case of the mouse DNA [6]. On the other hand, the content of repetitive DNA in each chromatin fraction from *Microtus agrestis* was measured by C_0t analysis [2] (table 1).

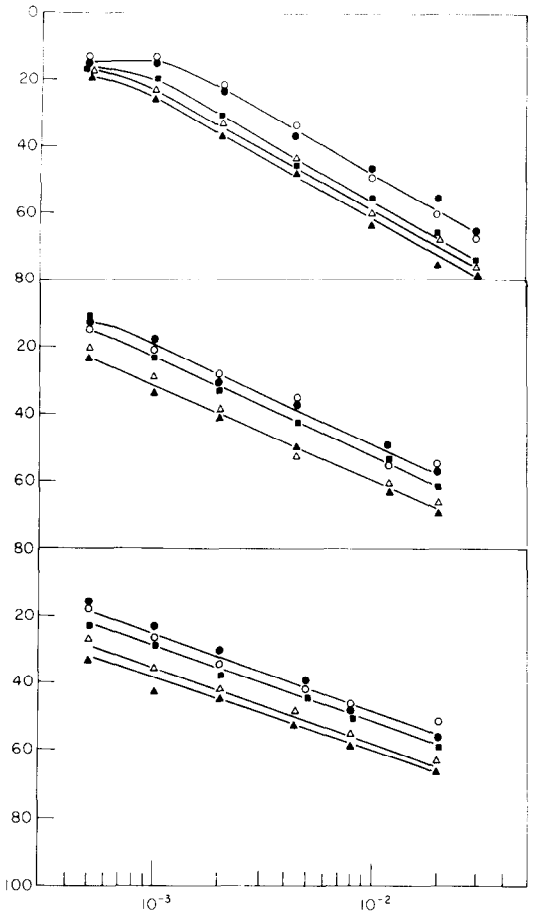

Fig. 1. Abscissa: C_0t (mole sec/l); *ordinate:* % ^{3}H-RSV DNA reassociated.

Measurement of RSV-specific DNA in (*a*) BALB/c-3T3-B77; (*b*) UMMA-RSV-21; and (*c*) UMMA-RSV-R-4 nuclear DNA, following the hybridization technique used by Varmus et al. [3]. ^{3}H-labelled double-stranded DNA (3 ng/ml of the slowly reassociating fraction of DNA synthesized in vitro by the RSV polymerase, representing at least 30% of the genome; $C_0t_1 \sim 1.1\times10^{-2}$; spec. act. 5×10^6 cpm/μg) was heat-denatured and incubated at 68°C in 0.40 M phosphate buffer with 4 mg/ml of unlabelled DNA from control calf (●), total nuclear DNA from transformed cells (▲), euchromatin DNA (△), intermediate chromatin DNA (■) and heterochromatin DNA (○). The cellular DNA was sheared to a 300–400 nucleotides size by limited depurination [10]. Aliquots were removed from the incubation mixture at several time points, diluted into 0.01 M phosphate buffer, and reassociation of labelled DNA was assayed by fractionation on hydroxyapatite. Results were plotted as a function of C_0t values computed for the labelled DNA polymerase product. Similar results were obtained in two separate experiments for each cell line.

Results

We detected ASV specific DNA in cells by reassociating ^{3}H-labelled viral DNA in the presence of unlabelled nuclear DNA [3]. Fig. 1*a*, *b* illustrates the reassociation curves obtained when ^{3}H-RSV–DNA was mixed with total DNA or DNA from heterochromatin, intermediate chromatin and euchromatin prepared from BALB/c-3T3-B77 or UMMA-RSV-21 cells, respectively. The total DNA and the euchromatin fraction increased the rate of reassociation of the ^{3}H-labelled viral DNA approx. 2 fold when the ratio of cellular DNA to ^{3}H-labelled viral DNA was 1.3×10^6. This increase indicates the presence of 1–2 viral copies per diploid mammalian genome (table 1). By contrast, DNA from the heterochromatin fraction did not increase the rate of reassociation of the ^{3}H-labelled viral DNA when compared with control calf thymus, BALB/c-3T3 or UMMA-50 DNA. Furthermore, the UMMA-RSV-R-4 subclone of *Microtus agrestis* retains the two copies of ASV-specific DNA per diploid genome subsequent to reversion to the nontransformed phenotype. As in the case for the transformed lines, the ASV-specific DNA sequences were found in the euchromatin fraction (fig. 1*c*, table 1).

We have shown in this report that avian sarcoma virus DNA can be integrated in the euchromatin fraction of *Mus musculus* and *Microtus agrestis* DNA. Examination of a larger number of clones of transformed and revertant cells will be needed to establish whether integration is a site-specific process, since only three integration events have been examined in these studies. It does appear, however, that constitutive heterochromatin and satellite DNA are not highly favored sites for integration.

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Uptake of proteins by red blood cells

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Summary. During hypotonic hemolysis red cells can take up ^{125}I-myoglobin and ^{125}I-immunoglobulin G. Cells which contain these proteins have distinctive cell morphology and are called gray ghosts. The association of protein with gray ghosts is fairly stable: these cells retain half of the proteins after 3 days. Passive diffusion of protein into the internal cell volume is the most plausible mechanism for uptake, and several lines of evidence indicate that the loaded proteins are freely diffusable within the red cells. Bacteriophage T4 is not taken up during hemolysis so uptake through large gaps in the red cell membrane with subsequent resealing seems unlikely. If an efficient procedure for fusing loaded gray ghosts to culture cells can be devised, it will be possible to introduce selected macromolecules into the cytoplasm of culture cells quite easily.

Red blood cells can be made to take up ions passively during hypotonic hemolysis. During this process hemoglobin is released, yet membrane integrity with respect to ionic permeation can be restored after hemolysis [1]. Seeman has shown that red cell ghosts will take up ferritin and colloidal gold during hypotonic hemolysis [2]. Uptake occurs only during a brief interval in the hemolytic process, again indicating a restoration of membrane integrity. More recently, Ihler et al. [3] have shown that human red blood cells will take up *E. coli* β-galactosidase during hypotonic hemolysis.

It has also been shown that in the presence of Sendai virus, mammalian red blood cells will fuse with themselves [4], and that avian red blood cells will fuse with tissue culture cells [5]. Conditions for the self-fusion of red blood cells without hemolysis have been carefully elucidated by Loyter and his colleagues [6–8].

If hypotonic hemolysis could be used to load red blood cells with macromolecules and Sendai virus cell fusion allowed the transfer of such new 'loads' to other cells, then an easy procedure for microinjection would be at hand. A report that small molecules can be transferred by such a procedure has recently been published [9]. It is probable that a procedure for the microinjection of macromolecules will be perfected within the near future. Toward this goal, I present an alternate method for loading proteins into red blood cells that utilizes small volumes and hence conserves protein during loading. This method also yields cells capable of fusion.

Methods and Materials

Blood was collected from the ear vein of rabbits into Ca^{2+}-Mg^{2+}-free Hanks solution containing i mg/ml EDTA. The red cells were washed 3 times in Ca^{2+}-Mg^{2+}-free Hanks solution containing 100 μg/ml penicillin and 100 μg/ml streptomycin. White blood cells were carefully removed during the washings, and the red cells were stored in Ca^{2+}-Mg^{2+}-free Hanks solution at 4°C until used.

Rabbit immunoglobulin G (IgG) was purified on DEAE-cellulose, measured spectrophotometrically and iodinated with carrier-free ^{125}I (New England Nuclear) as previously described [10]. Unbound ^{125}I was removed by gel filtration on a Sephadex G-25 column equilibrated with Hanks solution. ^{125}I-labeled IgG (^{125}I–IgG) was stored at −70°C at a concentration of 1.41 mg/ml until used. On the day of labeling the specific activity was 2×10^5 cpm/μg IgG.

Myoglobin (sheep skeletal muscle, type I; Sigma