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Characterization by human autoantibody of a nuclear antigen related to the cell cycle

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Using a serum from a patient with an autoimmune disease, we have recently described a novel 55 000-dalton antigen (p55) in the nucleus of several animal cells including human ones. This antigen, designated PSL, was not related to the previously defined antigens recognized by sera from patients with systemic rheumatic diseases (Sm, n-RNP, SS-B, Scl-70). We have now found that p55 is associated with chromatin structures as it is released from the nucleus of mink cell fibroblasts by saline + DNase treatments. Analysis by sucrose gradient centrifugation of the nuclear material released in these conditions indicated that p55 co-migrated with core histones. Meanwhile, p55 was absent from the residual nuclear matrices (achromatinic nuclei). Localization of p55 in synchronized cells was performed by indirect immunofluorescence and immunoprecipitation. P55 appeared to accumulate in the nucleus during the S phase. Finally, it was not recognized by an anti-SV40 tumor serum that specifically precipitated the protein p53, which has been recently related to cell proliferation. Thus, PSL and p53, although apparently not antigenically related, appear to be implicated in the same step of the cell cycle.

Key words: autoantibodies/cell cycle/chromatin/nuclear antigen/p53

Introduction

Circulating autoantibodies occurring in various human autoimmune disorders are of great interest for defining different nuclear antigens (for a review, see Tan, 1982). They are convenient tools for characterizing the corresponding molecular structures (Lerner *et al.*, 1979) and for elucidating the basic functions of these structures in cell metabolism (De Robertis *et al.*, 1982; Yang *et al.*, 1981; Lenk *et al.*, 1982). For this purpose, the most extensively studied anti-nuclear antibodies are found in systemic rheumatic diseases. In addition to antibodies to DNA, they correspond to Sm, n-RNP, SS-B (La) and Ro antigens. Recently, Sm and n-RNP antigens have been described as components of small nuclear ribonucleoproteins (snRNPs) (Lerner *et al.*, 1979; MacGillivray *et al.*, 1982). These particles contain U1, U2, U4, U5 and U6 small nuclear RNAs (Liautard *et al.*, 1981; Zieve and Penman, 1981). They are generally assumed to have a role in the biogenesis of mRNA (Yang *et al.*, 1981; Lerner *et al.*, 1980). On the basis of the complementarity between sequences at the 5′ end of U1 snRNA and those at the splice junctions in heterogeneous nuclear RNA (hnRNA), it has been suggested that U1-containing snRNP would align the ends of introns for splicing (Yang *et al.*, 1981; Rogers and Wall, 1980). The functions of the other nonhistone nuclear antigens specifically recognized by autoantibodies is presently unknown (Tan, 1982).

While screening sera from patients with various autoimmune disorders, we found one that detected, in nuclear extracts from several animal species, a major, if not unique, polypeptide with an apparent mol. wt. of 55 000 daltons (Barque *et al.*, 1982). The presence of this polypeptide was detected in non-infected cells as well as in retrovirus-infected cells and also in Friend tumor cells.

Here we report further characterization of this antigen, designated PSL; we show that it accumulates in the nucleus during the S phase and that it follows the fate of chromatin-containing structures. Although similar properties have been attributed to a family of p53 proteins involved in proliferation processes (Klein, 1982), PSL is antigenically distinguishable from p53.

Results

Nuclear localization of PSL antigen

Characterization of PSL antigen has been reported previously (Barque *et al.*, 1982). Briefly, sera from three individual patients with thrombocytopenic disorders were found to react in Ouchterlony immunodiffusion tests with saline rabbit thymus nuclear extracts, giving three lines with a pattern of apparent complete identity and intersecting reference with Sm, RNP, SS-B and Scl-70 lines. When immunoprecipitation tests were performed with amino acid-labeled nuclear extracts of mouse and mink fibroblasts and Friend tumor cells, one major band running at 55 K was found with one of the three sera, designated as serum 1 (for example, see Figure 3, lane R). Other bands were occasionally observed but their relative intensities were low and fluctuant compared with that of the 55 K protein (p55), and they were sometimes revealed by normal human serum, while p55 was not. For these reasons, material recognized by serum 1 is designated as PSL or p55 throughout this paper. To localise PSL more precisely, two approaches were attempted.

Indirect immunofluorescence microscopy. Coverslips of mink CCL cells were prepared and incubated with normal human serum, serum 1, anti-Sm and anti-RNP sera. While no fluorescent staining was apparent with normal serum (Figure 1D), a speckled pattern was seen with serum 1, (Figure 1A and B), that apparently was not different from those obtained with anti-Sm serum (Figure 1C) or anti-RNP serum (not shown). However, closer examination revealed that the cells were unevenly stained by serum 1, some of them being almost unstained, while others fluoresced intensely (Figure 1A and B, see arrows). This was in contrast to the uniform staining of the nuclei of cells reacted with anti-Sm serum. On the other hand, no fluorescence was detected in the cytoplasm of cells stained with serum 1 or with anti-Sm and anti-RNP sera.

Immunofluorescence studies were performed with cells which had been previously incubated with non-ionic detergent Nonidet P40 (NP-40) to remove cytoplasm, and

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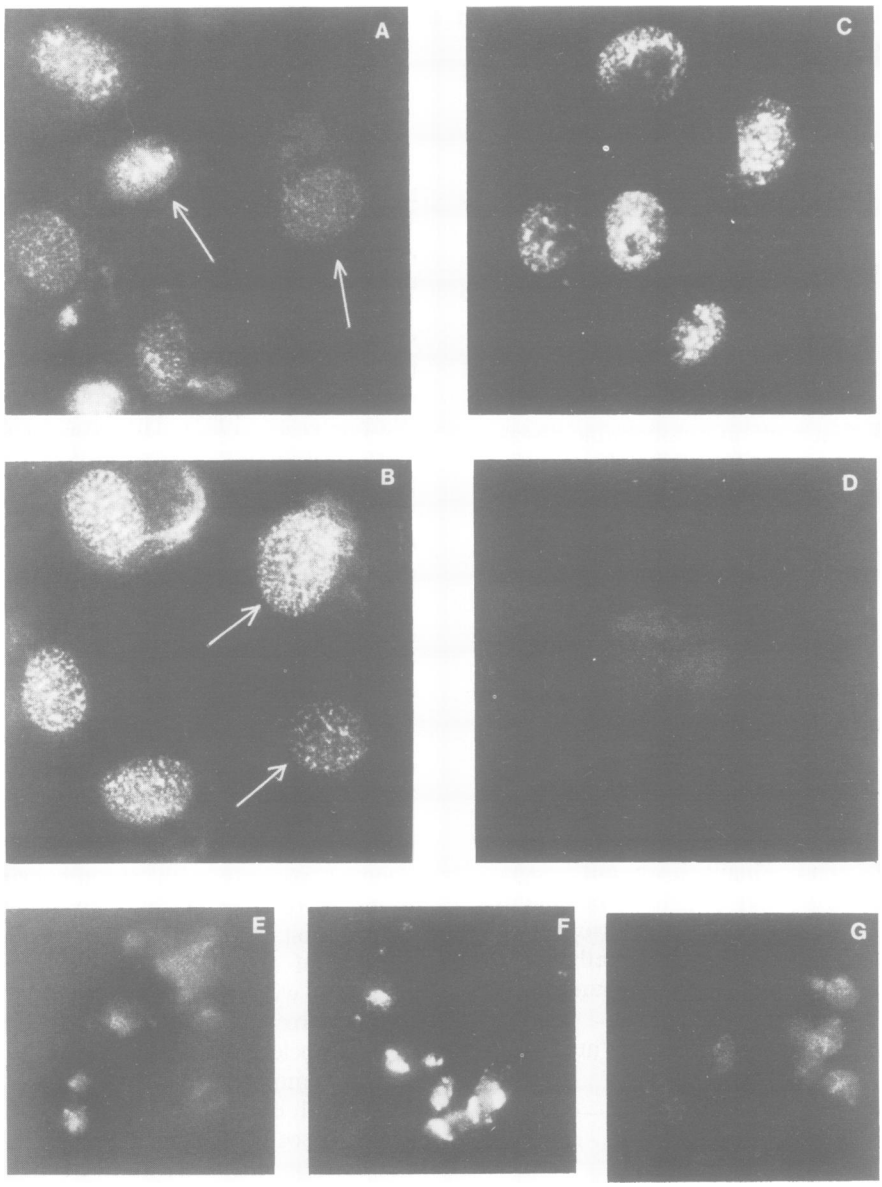

Fig. 1. Fluorescence photomicrographs of serum 1 (**A**, **B** and **G**), anti-Sm (**C** and **F**) and normal human serum (**D** and **E**) staining of CCL cells (**A**, **B**, **C** and **D**) or of achromatinic nucleic (**E**, **F** and **G**).

subsequently treated with 0.35 M NaCl and DNase (see Materials and methods). These conditions have been shown to leave nuclear matrices attached to the coverslip through residual cytoskeleton elements (Vogelstein and Hunt, 1982). This treatment almost completely abolished fluorescence following staining with serum 1 (Figure 1G) but did not reduce anti-Sm related fluorescence (Figure 1F). This indicates that PSL antigen is released from nuclei by treatments known to expel chromatin-containing structures.

Immunoprecipitation. We used the method described by Miller *et al.* (1978) for purifying nuclei devoid of chromatin (achromatinic nuclei or AN). Detergent-purified nuclei of CCL cells, labelled for 24 h with tritiated leucine and valine, were treated with 0.35 M NaCl and then DNase. The mixture was fractionated by sedimentation through a sucrose gradient (see Figure 2). As expected, the majority of u.v.$_{254}$-absorbing material was located in the top fractions of the gradient, while a thin band was found against the 72% sucrose cushion at the bottom of the tube. Under the microscope, material in this band was made up of empty structures similar to those described previously (Miller *et al.*, 1978) (not shown). Moreover, >95% of a [^{3}H]uridine pulse label for 15 min in the presence of actinomycin (0.04 μg/ml) was recovered in this fraction, indicating that nascent hnRNA was still attached to organized structures. On the other hand, the great majority of [^{3}H]thymidine radioactivity incorporated by CCL cells in a 24 h label was found at the top of the gradient. Taken together, these results indicated that the achromatinic nuclei were relatively intact nuclear matrices.

Immunoprecipitation of fractions collected across the sucrose gradient was performed with serum 1 using equal amounts of radioactivity for every sample (Figure 3). A faint band at the position of 55 K was found in achromatinic nuclei (lane AN). In contrast, serum 1 precipitated p55 in fractions 6–10 with a maximum intensity centered around fractions 7–8. The protein composition in the same fractions (Figure 4) showed the presence of several bands in the 12 000–14 000 dalton range, while no trace of this material

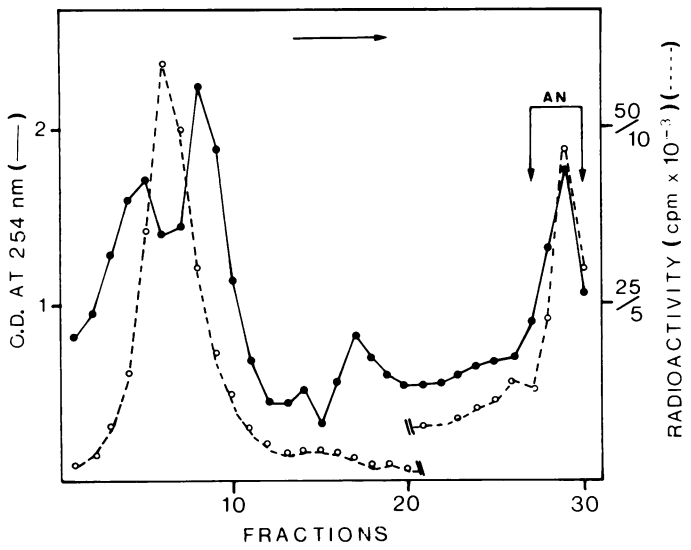

Fig. 2. Sucrose gradient centrifugation of nuclei of CCL cells treated with 0.35 M NaCl + DNase. Cells were labelled for 24 h with [^{3}H]leucine and [^{3}H]valine. AN: achromatinic nuclei.

was detected in achromatinic nuclei. These bands co-migrated with purified histones (not shown). Furthermore, u.v. spectra of the fractions indicated that nucleic acid-containing structures were preferentially located in fractions 6–10.

We concluded that PSL antigen was released from achromatinic nuclei and migrated in fractions containing most if not all the histone cores. These data suggest that p55 may be associated with chromatin.

Relationship of PSL antigen to cell cycle

The fact that p55 was found to co-sediment with chromatin fractions and that, in contrast to Sm or n-RNP antigens (unpublished data), it was rapidly labelled during 1 h periods of cell incubation with labelled amino acids, led us to look for a relationship between the presence of p55 in the nucleus and the cell cycle. To study this point, cultures of CCL cells were synchronized by a thymidine double-block, which arrests cell growth at the very end of G1 (Galavazi *et al.*, 1966), or by deprivation of fetal calf serum which maintains cells in a G0/G1 quiescent stage (Lerner and Hodge, 1971). Synchronized growth was resumed when thymidine was removed or when fetal calf serum was added to the culture medium. In our hands, both techniques gave essentially the same results,

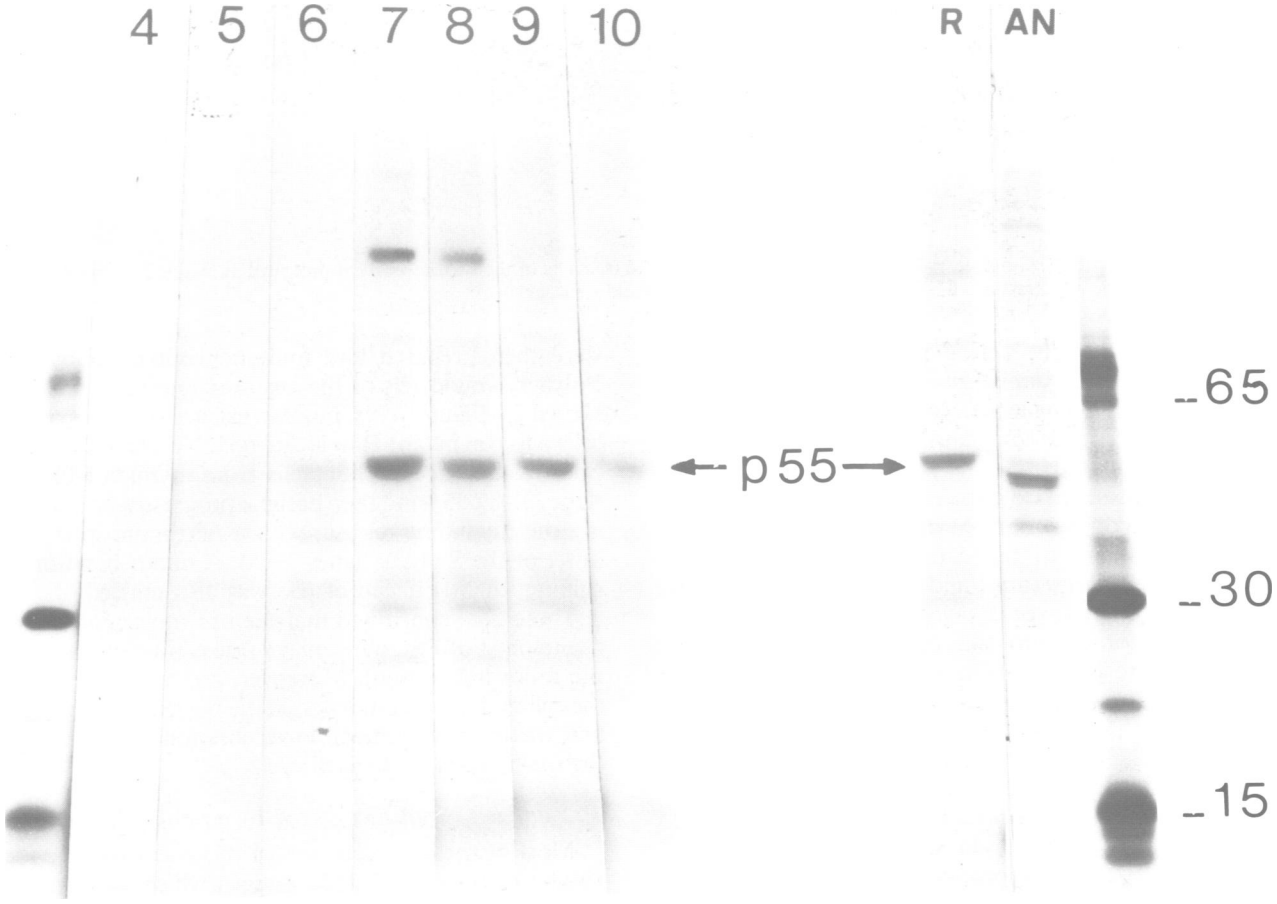

Fig. 3. Immunoprecipitations of the fractions of the sucrose gradient presented in Figure 2. Samples corresponding to every fraction were pretreated with normal human serum. Supernatants were incubated with serum 1 and processed as described in Materials and methods. **Lanes 4–10**: fractions 4–10 of the gradient. **Lane AN**: fractions 27–30 of the gradient were pooled and sonicated before pretreatment with normal human serum. **Lane R**: total nuclear extract immunoprecipitated with serum 1 to provide a p55 marker. Mol. wt. calibration was performed with murine leukemia retrovirus (MuLV) proteins (left and right lanes). Bands running ahead of p55 were also found in normal human serum precipitate (see Barque *et al.*, 1982). Similarly, the band with a mol. wt. of 53 K precipitated by serum 1 in achromatinic nuclei **(lane AN)** does not correspond to p53 (see the last section and Figure 7B). It is not precipitated by Tu-17 serum and serum 1. This latter serum has some reactivity to p53 (Figures 6 and 7A). In fact this 53-K band is a major polypeptide of achromatinic nuclei (Figure 4, lane AN).

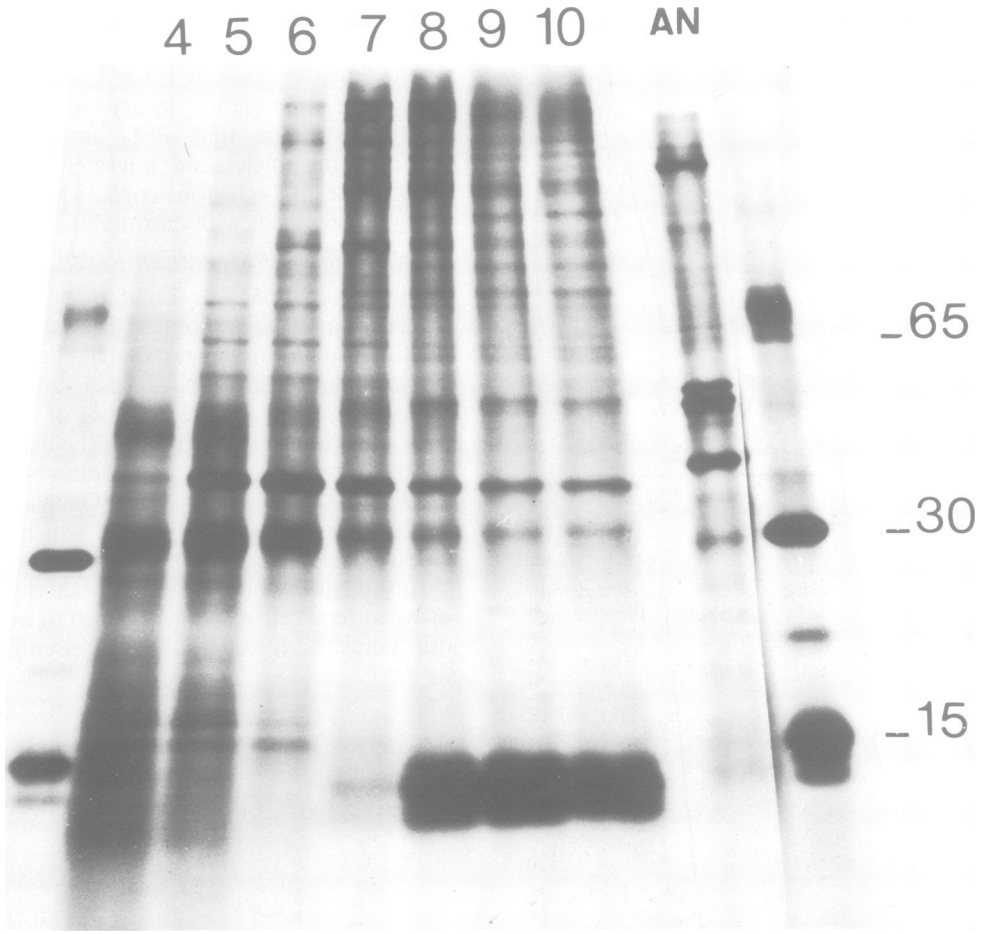

Fig. 4. Autoradiography of the SDS-polyacrylamide gel of the proteins from the fractions of the sucrose gradient presented in Figure 2. **Lanes 4–10**: fractions 4–10 of the gradient. **Lane AN**: fractions 27–30 of the gradient were pooled and sonicated.

with the exception that the period preceding S phase was longer in the case of serum-deprived cells than in serum-replenished cells. For convenience, we used serum deprivation for immunofluorescence studies and a thymidine block for studying biosynthetic steps.

Figure 5 illustrates an experiment with CCL cells synchronized by serum deprivation. At different times following addition of calf serum to the cultures, cell smears were prepared, incubated with serum 1 and stained as described. In parallel, DNA synthesis was assayed by performing [^{3}H]-thymidine pulses for 15 min and counting TCA-insoluble radioactivity (Figure 5). The S phase started at the 8th hour and reached a maximum around the 12th hour. Fluorescence was absent during the whole period preceding the S phase (Figure 5A). Then, during S phase, all examined nuclei presented a speckled fluorescence pattern that reached its highest intensity during the peak of DNA synthesis (Figure 5C), and decreased slowly during the G2/M phase. Fluorescence had almost disappeared in cells at the 12th hour following mitosis (Figure 5D).

To ascertain the size of the molecule bearing the PSL antigen observed in the previous experiment, cell cultures were synchronized by thymidine block. When thymidine was removed, maximum [^{3}H]thymidine incorporation took place at the 6th hour following the release of the block (Figure 6). Cells were labelled with [^{3}H]leucine and valine for 2 h periods (see Figure 6). Nuclear extracts corresponding to each period were then prepared and immunoprecipitated by serum 1. Polyacrylamide gels of the resulting immune precipitates are shown in Figure 6. A nuclear extract of non-synchronized cells was run in lane B to locate p55. No band at the p55 position was detected in the nuclei from thymidine-blocked cells (lane 1). A p55 band then became progressively visible following the thymidine release, with a maximum intensity at the onset of the S phase (lanes 3–5). A minor band of material running slightly ahead of p55 was also noticed.

These data confirmed that the p55 molecular species gives rise to most of PSL antigenicity (the extra band is discussed in the following section). Moreover, p55 was closely related to the cell cycle; it accumulated in the nucleus during the S phase and was present in much lower amounts, or even absent, at the other stages of the cell cycle.

P55 antigen is not recognized by an anti-p53 serum

Most of the characteristics of p55 reported here were very similar to those of the p53 antigen which was first found in SV40-transformed cells and various cell lines transformed by oncogenic viruses and chemical carcinogens (for a review, see Klein, 1982). P53 was initially considered to be a transformation protein, but recent evidence suggests rather that it is implicated in the regulation of cell proliferation (Milner and Milner, 1981). Moreover, microinjection into cells of antibodies to p53 transiently inhibits serum-stimulated DNA synthesis (Mercer *et al.*, 1982), suggesting that p53 is crucial for

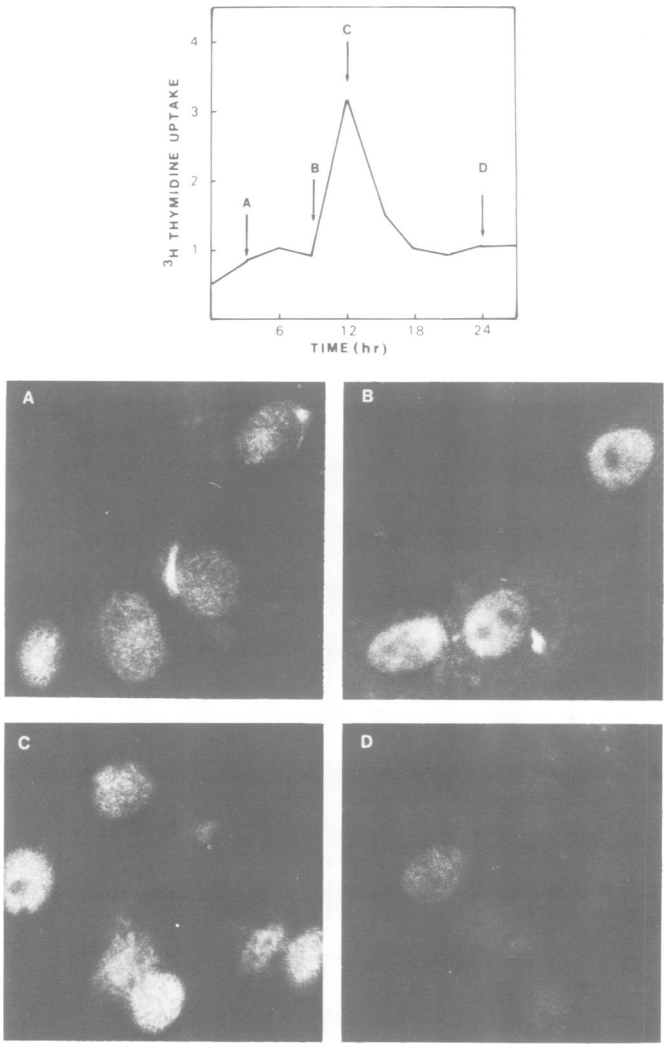

Fig. 5. Fluorescence photomicrography of serum 1 staining of synchronized CCL cells at different phases of the cell cycle: G1**(A)**, G1/S**(B)**, S**(C)** and late G1 **(D)**. Cell samples were examined at the times indicated by arrows in the graph illustrating DNA synthesis.

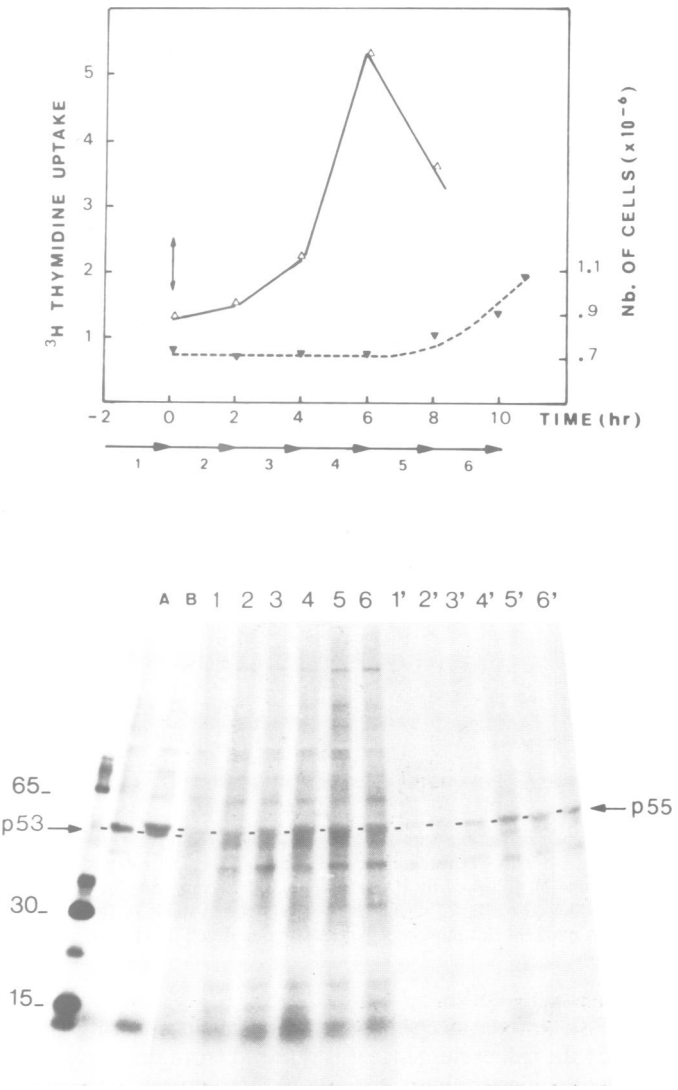

Fig. 6. Appearance of p55 in the nucleus of thymidine-double-blocked Raji cells. Incorporation of [^{3}H]thymidine (▼——▼) and mitotic index (▽- - -▽) in Raji cells. Cell cultures (corresponding to samples 1–6 in the graph) were labelled for 2 h with [^{3}H]leucine and [^{3}H]valine. Notice that sample 1 was labelled before double-block release (↓). Nuclear extracts were prepared and pretreated with normal human serum before treatment with serum 1 **(lanes 1–6)**. Otherwise, nuclear extracts were treated with Tu-17 serum and resulting supernatants were precipitated with serum 1 **(lanes 1′–6′)**. In a parallel experiment, nuclear extracts of non-synchronized Raji cells were labelled for 2 h, pretreated with normal human serum and the resulting supernatants were precipitated with Tu-17-serum **(lane A)** or serum 1 **(lane B)**.

cell transition from quiescence to growth. It was possible, therefore, that the p55 recognized by our serum 1 might be p53 itself, or a close relative. This possibility was tested using a hamster anti-SV40 tumor serum containing antibodies specific for p53 (serum Tu-17, a generous gift of P. and E.May). When a tritiated amino acid-labeled nuclear extract of Raji cells selected for their moderately high content of p53 (Benchimol *et al.*, 1982) was immunoprecipitated with Tu-17 serum, a unique band with an apparent mol. wt. averaging 53 000 was found (Figure 6, lane A). This band did not coincide with that of p55 but with the minor species precipitated by serum 1 (Figure 6, lane B). In addition, when nuclear extracts of synchronized Raji cells were sequentially treated with Tu-17 serum and serum 1, the band corresponding to p53 disappeared, while that of p55 was conserved (Figure 6, lanes 1′–6′).

To confirm the validity of these results, a Raji cell nuclear extract was sequentially treated with a monoclonal antibody to human p53 (PAb421, Harlow *et al.*, 1981, a generous gift of L.V.Crawford) and serum 1 (Figure 7A). In agreement with the above results, no 55 K band (lane 1 and 2) was detected by the monoclonal antibody, which has a stronger affinity toward human p53. On the other hand, serum 1 again precipitated p55 and p53 (lane 4) as in the experiment described in Figure 6 (lane B). A similar experiment was performed with mink CCL cells (Figure 7B) using hamster Tu-17 serum and serum 1. In both cases, the 55 K was detected only by serum 1, whatever the sequence of immunoprecipitation. No p53 was precipitated by Tu-17 serum, indicating its absence in these cells. From these data, p55 (PSL) and p53 appear not to be identical proteins.

Discussion

The screening of human autoimmune sera led us to identify a new nuclear antigen. We discuss more particularly two

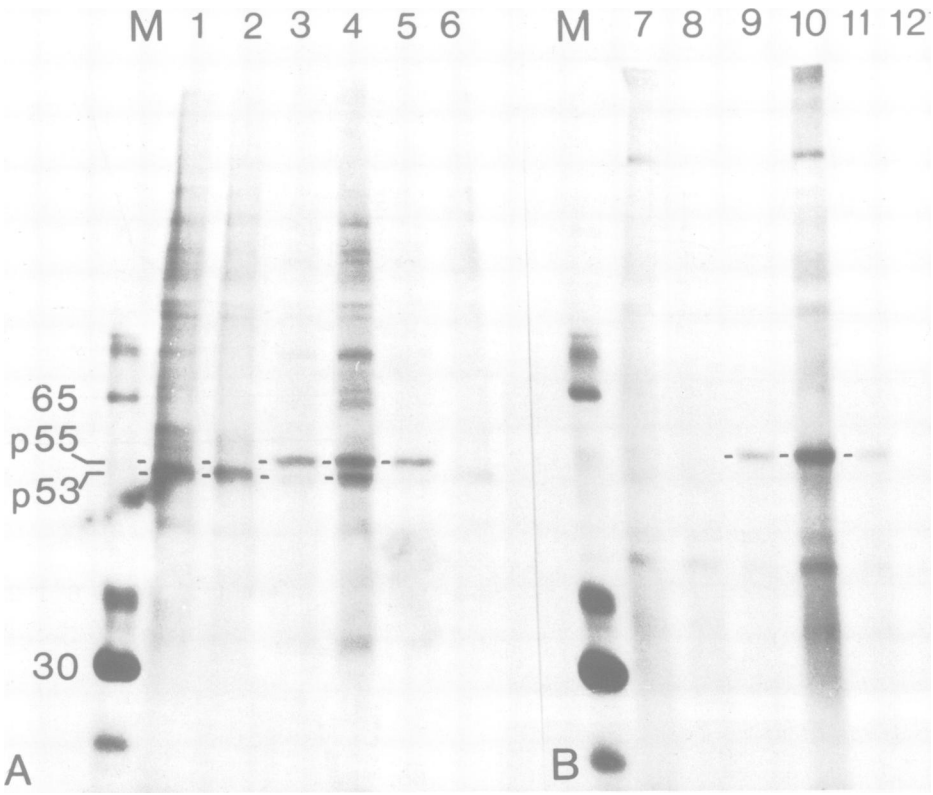

Fig. 7. Cascade immunoprecipitation of Raji cell **(A)** and mink cell **(B)** nuclear extracts with PAb421 and serum 1 **(A)** or Tu-17 and serum 1 **(B)**. Cells were labelled for 2 h with [^{3}H]leucine and [^{3}H]valine. The nuclear extracts were pretreated with normal human serum and treated sequentially as follows: **(A): (a)** PAb421 **(lane 1)**, followed by PAb421 **(lane 2)** or serum **(lane 3)**. **(b)** serum 1 **(lane 4)**, followed by serum 1 **(lane 5)** or PAb421 **(lane 6)**. **(B): (a)** Tu-17 serum **(lane 7)**, followed by Tu-17 serum **(lane 8)** or serum 1 **(lane 9)**. **(b)** serum 1 **(lane 10)**, followed by serum 1 **(lane 11)** or Tu-17 serum **(lane 12)**. **Lanes M**: Mol. wt. calibration markers. Immune precipitates were analyzed by electrophoresis in 10% polyacrylamide slab gels.

aspects of our work.

(a) PSL antigen accumulates in the nucleus during the S phase of the cell cycle. Since it is released from the nucleus by treatments destroying chromatin structures, we can reasonably conclude that it is associated with chromatin. Furthermore, we verified that PSL does not exist in a soluble form in nuclear extracts but sediments at the density of chromatin in metrizamide gradients (unpublished results). In that respect, PSL might correspond to the 54 000 protein tightly bound to DNA, described by Werner *et al.* (1981), although no data have been provided by these authors about its cell cycle-related metabolism. More interestingly, a recent paper described the cell cycle-dependent changes in the electrophoretic pattern of chromatin-associated nonhistone proteins from HeLa cells (Adolph and Phelps, 1982). One of these, with a mol. wt. of 54 K, appears to be very similar to PSL in terms of mol. wt., metabolic turnover and chromatin association.

The eventual presence of PSL in the cytoplasm could not be ascertained. Immunofluorescence assays and metabolic labeling experiments gave negative results. However, this cannot reflect the actual situation since PSL must be synthesized just before it penetrates the nucleus at the very beginning of the S phase. Alternatively, PSL might be present in the cytoplasm but in a polypeptide form not recognized by serum 1.

(b) A second important aspect concerns the possible relationship between PSL and p53. Both PSL and p53 are cell-cycle related, they appear to be conserved among animal species and have affinity towards chromatin. Furthermore, the presence of p53 in the nucleus has been documented in numerous instances. Nevertheless, it is evident that normal cells contain very low amounts of p53 compared with transformed cells, which possess large quantities of p53 associated with other proteins such as T or EBNA antigens, two molecules which bind efficiently to DNA (Lane and Crawford, 1979; Luka *et al.*, 1980).

Obvious differences between p53 and PSL are their size and the non-reactivity of PSL toward a hamster anti-p53 serum and a monoclonal antibody to human p53. Moreover, we always detected appreciable quantities of PSL in the nuclei of cultured normal mouse and mink cells, while we were unable to characterize p53 in the same cells, in agreement with other findings (Benchimol *et al.*, 1982; Dippold *et al.*, 1981). Human Raji cells were found to contain both p53 and PSL. This latter cell line has been described as a moderate producer of p53 (Benchimol *et al.*, 1982).

Together these facts suggest two alternative hypotheses. (i) Hypothesis 1: the two molecules are different forms of the same antigenic moiety which are differentially recognized by antibodies. PSL might be the nuclear form of the antigen, as it is found in all cells screened so far, whatever their normal or malignant state. p53 might move to the nucleus in particular circumstances including cell transformation and/or association with nuclear antigen (EBNA, T antigen). In that case, the inability or PAb421 monoclonal antibody to recognize PSL might be explained by the loss of an epitope or by a particular conformation or both. It has been shown that DNA synthesis in normal mouse cells is inhibited by microinjection into the nucleus of an anti-p53 monoclonal antibody

(Mercer *et al.*, 1982). However, p53 could not be detected in these cells by immunofluorescence. This paradox could be explained by our model, since p53 would be complexed by the monoclonal antibody when it enters the nucleus, blocking its conversion into PSL. (ii) Hypothesis 2: p53 and PSL might be two distinct molecules which play a role at the same step of the cell cycle. This would explain the similarities in their metabolism.

Our negative results with anti-p53 antibodies in mink and mouse cells are more likely to be relevant to the second hypothesis. It should be recalled that our human serum 1 can discriminate between 53 K and 55 K species when they are both present in the same cell.

Materials and methods

Cell and culture conditions

Mink cells (CCL) were grown as monolayers in MacCoy Medium supplemented with 10% fetal calf serum and 5 mM glutamine. Human lymphoblastic (B type) Raji cells were grown in stationary suspension in RPMI medium containing 10% fetal calf serum and 5 mM glutamine.

Cell labelling

Cell cultures were labelled for appropriate times with 25 μCi/ml [^{3}H]leucine and 25 μCi/ml [^{3}H]valine (sp. act. 50 Ci/mmol and 43 Ci/mmol, respectively) in leucine and valine free Minimum Essential Medium supplemented with 10% non-dialyzed fetal calf serum. DNA synthesis was estimated by adding 10 μCi/ml [^{3}H]thymidine (sp. act. 48 Ci/mmol) to MacCoy Medium or RPMI Medium. Trichloroacetic acid insoluble radioactivity was recovered on 0.22 μm nitrocellulose filters.

Preparation of nuclei

Appropriate amounts of cells (10^7-10^8 cells) were rapidly washed twice in cold buffer A (10 mM Tris HCl, pH 7.5, 10 mM NaCl, 2 mM $MgCl_2$) containing 0.5 mM phenylmethylsulfonylfluoride (PMSF). They were suspended in the same buffer (20 volumes) and a solution of 5% Triton X-100 or NP-40 was added to a 0.5% final concentration. After 5 min incubation on ice, the cell suspension was homogenized by pipeting, and centrifuged at 2000 r.p.m. for 5 min. Nuclear pellets were washed in buffer A and finally suspended in buffer B (10 mM Tris-HCl, pH 8.5, 100 mM NaCl, 1 mM $MgCl_2$) for sonication, or in buffer C (10 mM Tris HCl, pH 7.4, 500 mM NaCl, 5 mM $MgCl_2$) + DNase I (500 μg/ml) for high salt DNase treatment.

To obtain achromatinic nuclei (AN), pellets of purified nuclei were suspended in D buffer (100 mM NaCl, 5 mM $MgCl_2$, 25 mM Tris-HCl, pH 7.5) with 0.5 mM PMSF. Then, the NaCl concentration was raised to 0.35 M. DNase (500 μg/ml) was added and the mixture was incubated for 2 h at room temperature, centrifuged 10 min at 1000 *g* and the resulting supernatant was layered onto a 30–68% sucrose gradient in E buffer (350 mM NaCl, 5 mM $MgCl_2$, 25 mM Tris-HCl, pH 7.5) with 0.5 mM PMSF. A 72% sucrose cushion was placed at the bottom of the tube before forming the gradient. Centrifugation in a SW-41 Beckman rotor was for 18 h at 36 000 r.p.m. at 4°C. Gradients were fractionated with an ISCO device. U.v.$_{254}$ absorbance and radioactivity were determined.

Immune precipitation and polyacrylamide gels analysis

10^6 to 5 x 10^6 c.p.m. of [^{3}H]amino acid-labeled material were put into Eppendorf 1.5 ml plastic tubes and diluted with buffer B to a final volume of 0.5 ml. Appropriate sera (2–5 μl) were added and the tubes were incubated 2 h at 4°C. Then, 100 μl of a 10% *Staphylococcus aureus* suspension (Cowan I strain) were added and incubation was continued for one more hour. The complexes were harvested by centrifugation and treated as reported (Barque *et al.*, 1981).

Immunoprecipitation of nuclear extracts with PAb421 monoclonal antibody (generously provided by L.Crawford) were performed under the same conditions, with 25 μl of culture supernatant from cells producing the antibody. 50 μl of *S. aureus* suspension were used to precipitate the immune complexes.

Immune precipitates were analyzed by electrophoresis in 8–20% polyacrylamide slab gels and the gels were processed for fluorography (Barque *et al.*, 1981).

Synchronization of the cells

Two methods of cell synchronization were used. CCL cells were synchronized by serum deprivation. In a typical experiment, equal amounts of cells were seeded in Falcon flasks (containing 20 ml of MacCoy Medium supplemented with 10% fetal calf serum, 5 mM glutamine). When subconfluent monolayers were formed, culture medium was removed and replaced by serum-free medium. The cells were maintained in this medium for 3 days. Then, 10% fetal calf serum was added to the culture. Thereafter, every 2 h, a flask was labeled with [^{3}H]thymidine for 15 min. An aliquot was taken off to estimate the DNA synthesis. The rest was used for preparing cell smears.

In the second method, synchronized Raji cells were obtained with a double block of thymidine. Thymidine was added to a suspension culture of Raji cells at a final concentration of 4 mM. Afte 16 h, the block was released by washing and resuspending the cells in normal medium, and after a further 6 h incubation, the cells were submitted to a second block for 16 h. Releasing the culture from the second block gave a population of cells synchronized at the G_1/S boundary. Incorporation of [^{3}H]thymidine into DNA and the number of cells were measured every 2 h to determine the phases of the cell cycle.

Immunofluorescent assay

Anti-nuclear antibodies were revealed by the indirect immunofluorescent technique on CCL and Raji cells. The cell spreads were fixed in acetone for 10 min at room temperature. The conjugate used was fluorescein isothiocyanate (FITC)-labeled sheep anti-Cohn fraction II (Institut Pasteur). This conjugate was diluted 1/20 in phosphate-buffered saline. Stained slides were observed at a magnification of x400.

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Note added in proof

While this work was submitted, we learnt from Dr G.Klein, that 'p53s' are actually composed of two different classes of molecules which can be discriminated by immunospecific reactions (Luka *et al.*, 1983, *Proc. Natl. Acad. Sci. USA*, **80**, 1199-1203). Our PSL antigen is likely to correspond to the $p53_E$ found by these authors in Epstein-Barr virus-transformed Raji cells.