LARGE SPECIES DIFFERENCES IN THE PATTERN OF SNPI RNA WHICH CAN DISTINGUISH APE FROM HUMAN

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ABSTRACT

The snPI RNA species are a recently described set of molecules whose sizes range from 5S to 10S. They can be labeled in vitro in isolated nuclei and are apparently formed by an RNA polymerase I type of activity. However, in contrast to ribosomal precursor RNA, the usual polymerase I product, they are not found in the nucleolus but rather are located in the nucleoplasm.

The snPI RNAs have been found in all mammalian cell types studied. The spectrum seen in gel electrophoresis is unique to each animal species studied but is essentially the same in different cell types within a species. The differences in snPI patterns are quite large between even closely related species and are clearly distinguishable in gorilla and human cells.

KEY WORDS in vitro RNA synthesis · snPI RNA · mammalian cells · species-specificity

A new class of RNA molecules, designated small nuclear Polymerase I (snPI RNA), has been recently described in HeLa cells (1). Electropherograms show a complex spectrum of discrete molecules. These RNAs are tightly associated with the cell nucleus and are synthesized by an RNA polymerase that is resistant to high levels of α-amanitin and hence is designated as Type I. In contrast to ribosomal RNA precursor molecules, which are the principal product of a Type I polymerase and are found exclusively in the nucleolus (12), snPI RNAs are synthesized in the nucleoplasm, and their synthesis is not affected by low concentrations of actinomycin D (0.04 μg/ml). The snPI RNA molecules clearly differ in size, quantity, and location from previously described "small nuclear" (snRNA) RNA molecules (6, 9). Because the labeling of the snPI RNAs in vivo is largely obscured by the much greater amounts of heterogeneous nuclear RNA of similar size, the snPI RNAs have been studied in detail by labeling in vitro. Our initial report on snPI RNAs dealt only with their presence in the nuclei of HeLa cells (1). In this report, we compare the spectrum of snPI RNAs synthesized in a variety of mammalian cells, and show an unambiguous species-specificity of the spectrum of these RNA molecules.

MATERIALS AND METHODS

Cells

Mouse myeloma MOPC 315 cells were kindly provided by Dr. M. Gefter, Massachusetts Institute of Technology (MIT); human diploid fibroblasts by Dr. R. Hoffman, Massachusetts General Hospital, Boston; rat epithelial cells by Dr. R. Weinberg, Cancer Center, MIT; Friend erythroleukemia cells by Dr. D. Hausman, Cancer Center, MIT; 3T6 fibroblasts and BSC-1 cells by Dr. H. Green, MIT; and gorilla fibroblast cells by Dr. G. Todaro, National Institutes of Health. HeLa S3 cells were grown as described previously (1); human diploid fibroblasts, mouse 3T6 fibroblasts, mouse neuroblastoma cells, rat epithelial cells, and BSC-1 cells were grown in monolayer cultures with Dulbecco's medium supplemented with 10% fetal calf serum; gorilla fibroblast cells were grown similarly but with 20% fetal calf

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serum. Friend erythroleukemia cells, mouse L cells, and Chinese hamster ovary (CHO) cells were grown in suspension culture in Dulbecco's medium supplemented with 10% calf serum; mouse myeloma cells were grown similarly but with 15% calf serum.

Isolation of Nuclei and In Vitro RNA Synthesis

The cells were lysed either directly in suspension or from a monolayer by the addition of hypotonic buffer (1.6 mM $MgCl_2$, 6 mM KCl, 10 mM Tris (pH 8.0), 1 mM dithiothreitol [DTT]) in the presence of 0.5% Nonidet-P-40 (Shell Chemical Co., Houston, Tex.). Monolayer cells were scraped off the plates, and lysis was completed by vortexing them for 1 min. The nuclei were isolated by sedimentation at 800 rpm for three min. In the case of rat cells, mouse neuroblastoma cells, gorilla cells, and human diploid fibroblasts, an equal volume of suspension buffer (5 mM $Mg[Ac]_2$, 50 mM Tris (pH 8.0), 5 mM DTT, 1 mM EDTA, and 25% glycerol) was added to the nuclear suspension before centrifugation to facilitate subsequent resuspension of the nuclear pellet. The nuclei were labeled in the presence of three cold nucleoside triphosphates and radioactive UTP. The incubation conditions are described in detail in (1) and differ in some important details, such as buffer ion composition, nucleoside concentrations, and specific activities, from earlier studies (12). The nuclei were incubated at 25°C for 20 min as described by Marzluff et al. (4). The nuclei were collected by centrifugation, extracted with phenol-chloroform, and the RNA obtained was analyzed by slab gel electrophoresis as described in (1).

RESULTS

The snPI RNAs were first studied in the nuclei of HeLa cells. Some of their important characteristics are shown in the fluorographed gel electropherograms in Fig. 1. The molecules were labeled in vitro with HeLa cell nuclei derived from cells pretreated with low concentrations of actinomycin D (0.04 μg/ml), which suppresses labeling of ribosomal RNA during subsequent incubation in vitro. The labeling patterns obtained by using both low and high levels of α-amanitin are shown in Fig. 1. The gel in Fig. 1*A*, lane 2, shows the nuclear products synthesized in the presence of 0.5 μg/ml α-amanitin which is sufficient to completely inhibit the polymerase II-catalyzed synthesis of hnRNA but not polymerase III. Under these conditions, the majority of the label is incorporated into 5S and pre-tRNA but, in addition, a large number of labeled discrete bands appear in sizes ranging from 5S to 10S. 5S and pre-tRNA have previously been shown to be synthesized by polymerase III (10), which is fully active at this low concentration of amanitin (2, 7, 8). At high concentrations of α-amanitin (150 or 400 μg/ml), which are sufficient to inhibit polymerase III as well (lanes 3 and 4), the synthesis of 5S RNA and some minor larger molecules is nearly completely suppressed but the labeling of the larger RNA products designated snPI is unaffected. Thus, these molecules, which are found exclusively in the neoplasm, are labeled by an activity resistant to α-amanitin which is, in this sense, an enzyme with the properties of a type I RNA polymerase.

12 principal bands in the HeLa snPI spectrum have been designated by number in Fig. 1. Many more are detectable, depending on the preparation and exposure time of the fluorograph. Two-dimensional electrophoretic analysis also indicates a complex spectrum with numerous species. Only the most heavily labeled bands have been designated in the different preparations.

In contrast to the ribosomal precursor, which is the principal product of polymerase I and found exclusively in the nucleolus, the snPI RNAs are located in the nucleoplasm. This was first established by subnuclear fractionation (1). Another demonstration of the extranucleolar localization of these RNA molecules is afforded by the electron microscope autoradiogram shown in Fig. 1*B*. Cell nuclei selectively labeled with 150 μg/ml α-amanitin in vitro for snPI molecules show radioactivity exclusively in the nucleoplasm. This finding, together with the insensitivity of snPI RNA synthesis to conditions which inactivate ribosomal RNA formation, indicates that these RNA species are distinct from pre-ribosomal RNA.

The snPI molecules are found in all mammalian nuclei studied thus far, but show distinct electrophoretic patterns that reflect the animal species from which the cells originate. The comparisons shown here are made by labeling isolated nuclei from a variety of mammalian cell lines under conditions similar to those previously used in HeLa cells. These include pretreatment of cells with a low level of actinomycin D and the incubation of isolated nuclei in the presence of 150 μg/ml α-amanitin.

Fig. 2 shows the electrophoretic patterns of snPI RNA molecules labeled in vitro in nuclei from a variety of rodent cell lines. For each column, the relative positions of the major HeLa cell snPI RNA species (1-5, 7, 8), as seen on the same gels, are indicated. All the mouse (*Mus musculus*) cell lines used in these experiments

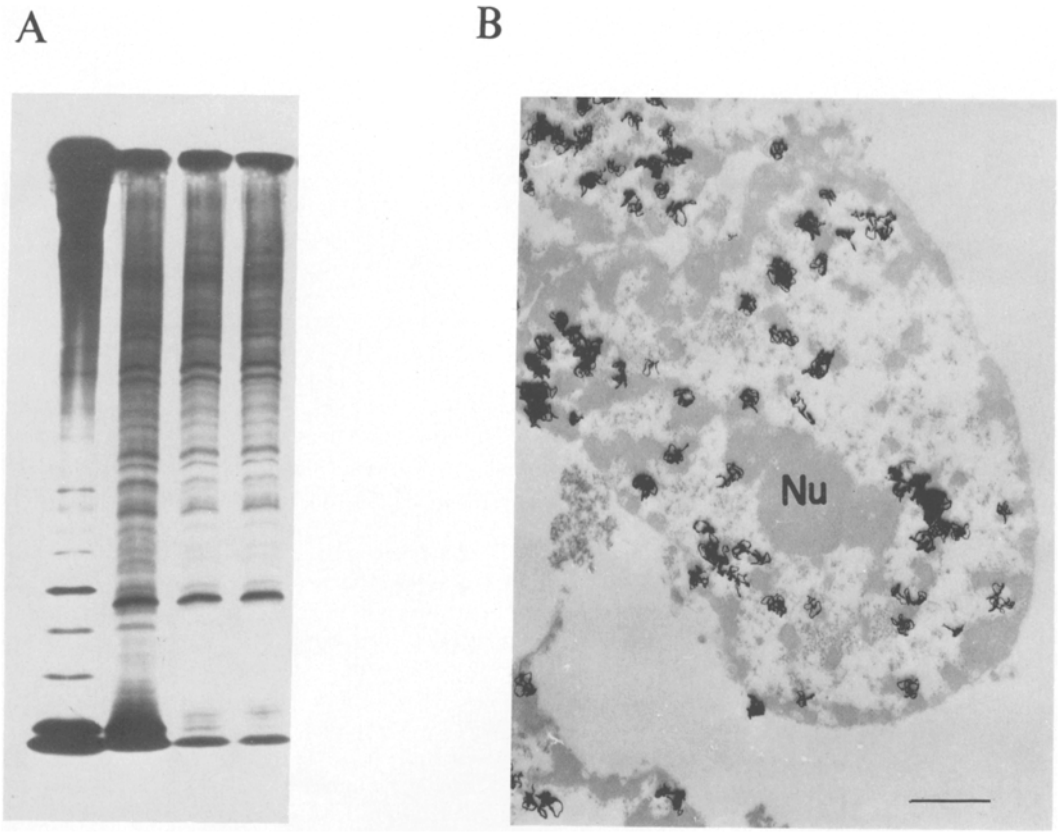

FIGURE 1 (*A*) Gel electropherogram of snPI RNA synthesized by HeLa cell nuclei in vitro. The cells were pretreated with low levels of actinomycin D and incubation was carried out at 25°C for 30 min with 0.5 μg/ml α-amanitin (lane 2), with 150 μg/ml α-amanitin (lane 3), and with 400 μg/ml α-amanitin (lane 4). Lane 1 shows HeLa cell low molecular weight RNA as a marker. Electrophoresis was done in a 4–10% acrylamide gradient gel for 3.5 h at 10 V/cm. Exposure of the gel to the film was carried out for 10 d. (*B*) Electron microscope autoradiogram of HeLa cell nuclei after snPI RNA synthesis in vitro. Nuclei were pretreated with low actinomycin and in vitro synthesis occurred in the presence of 200 μg/ml α-amanitin under standard conditions with 60 μCi [^{3}H]UTP/assay. The washed nuclei were prepared for electron microscope autoradiography by standard methods. Exposure was carried out for 22 wk. *Nu*, nucleolus. Bar, 1 μm. × 12,000.

show a basically similar pattern of three major snPI RNA products in the size range of 5.5S–6.5S, although obvious quantitative differences are seen and appear to be reproducible. Rat (*Rattus norvegicus*) nuclei, however, synthesize only two major products in the region of the principal mouse snPIs, whereas the pattern obtained from CHO (*Cricetulus griseus*) cell nuclei seems to almost completely lack these particular bands. In contrast, in the higher mol wt region of 8S–10S, where the majority of the HeLa cell snPI RNAs (No. 4-8) appear, mouse cell nuclei mainly show only diffuse products, and it is difficult to discern discrete bands. Rat and CHO cells, however, show a set of distinct bands in the 8S–10S region. While there is a resemblance to the distribution found in HeLa cells, rat snPI RNAs do not comigrate with HeLa cell bands. The snPI spectrum of hamster cells is also different. These results show that three rodent cell lines can be easily distinguished by the electrophoretic pattern of snPI RNA molecules. Two of these (rat and mouse) are muridea or modern mice while the third one (hamster) is more distant, belonging to the cricetidea or acient mice.

The ability of these measurements to distinguish closely related species is even more graphically demonstrated by a comparison of snPI RNA

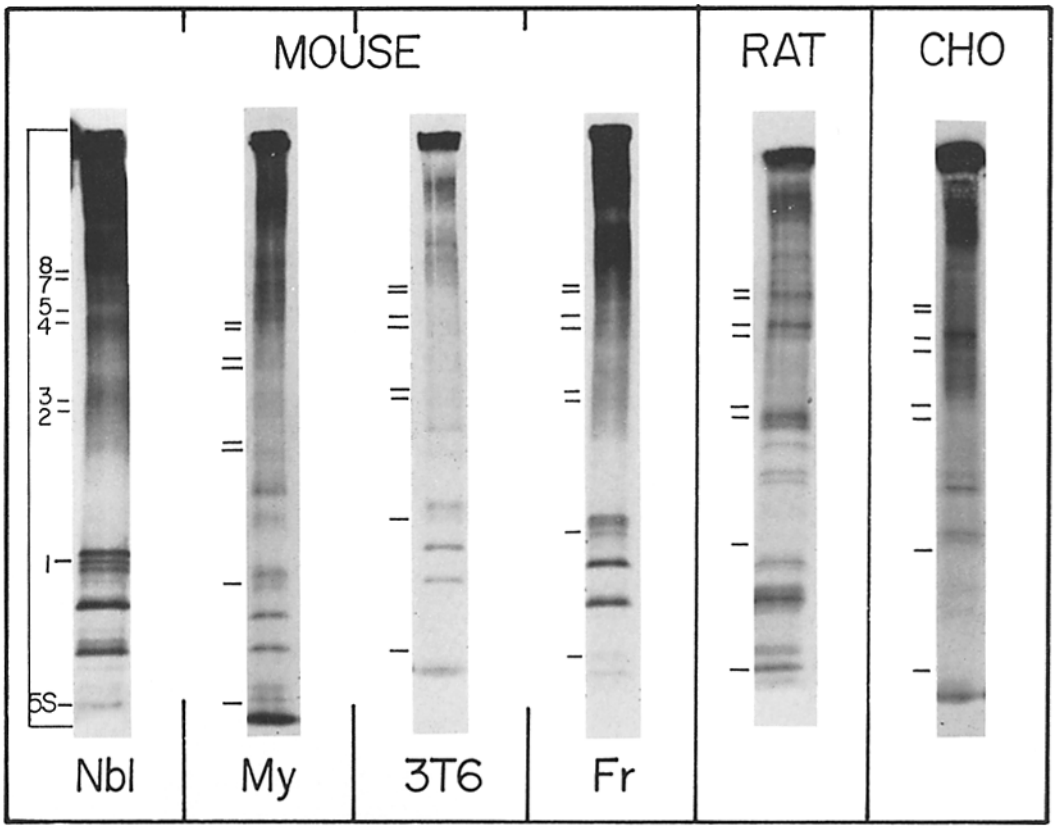

FIGURE 2 snPI RNAs synthesized by a variety of rodent cell lines in vitro. Isolation of the nuclei and incubation was performed as described in Materials and Methods. The cells were pretreated with 0.04 μg/ml actinomycin D and synthesis occurred in the presence of 150 μg/ml α-amanitin. The mouse cell lines used were: neuroblastoma (*Nbl*), myeloma (*My*), 3T6 fibroblasts (*3T6*), and Friend erythroleucemia (*Fr*) cells. The bars on each lane mark the position of HeLa cell 5S and snPI RNA bands No. 1, 2, 3, 4, 5, 7, and 8 (from bottom to top) as obtained from each individual gel.

molecules from different simian cell lines. One is an old world monkey, the African Green Monkey (*Ceropithecus aethiops*), which belongs to the group Cercopithecinae, while two are apes (Pongidae), the gorilla (*Gorilla gorilla*) and human (*Homo sapiens*), which are thought to have diverged about 5 million years ago. In this case, the spectrum differences are less obvious and the HeLa cell snPI RNA pattern from each individual gel is presented for a direct comparison. Fig. 3*A* shows an electropherogram of the nuclear products obtained in vitro from BSC cells, Fig. 3*B* from gorilla cells, and Fig. 3*C* from human fibroblasts, respectively. Although the overall pattern of the snPI RNAs from BSC-1 and gorilla cells seems to be quite similar to that of HeLa cells, differences are clearly detectable. BSC nuclei seem to have the major bands 1, 3, 4, and 5 in common with HeLa cells, but, for example, no equivalent for bands 2, 7, or 8 can be seen. In addition, BSC nuclei synthesize major products migrating between bands 1 and 2, and 3 and 4, respectively, in regions where the HeLa cells show either no products or only minor ones. The gorilla snPI pattern unfortunately appears rather weak, which is probably related to the extremely slow growth rate of these cells, the only ape line available to us at the time. However, clear differences with respect to the human pattern are to be seen again. Band 1 is missing completely, and two doublets that might correspond to human bands 2-3, and 4-5, respectively, migrate differently than the corresponding HeLa cell products. Another human cell line analyzed in these experiments, the human diploid fibroblast, shows, band-by-band, a pattern of snPI RNA molecules iden-

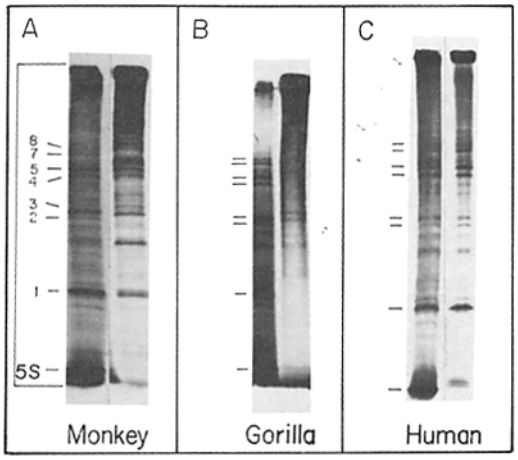

FIGURE 3 snPI RNAs synthesized by nuclei of simian cell lines in vitro. Fig. 3 shows a comparison of the snPI RNA patterns from HeLa cells (left) and BSC-1 cells (right) of (*A*) monkey cells, (*B*) gorilla cells, and (*C*) human diploid fibroblasts. As in Fig. 2, the position of 5S RNA and the major snPI RNA species from HeLa cells is marked by the bars.

tical to that of HeLa cells. This indicates that the aberrant karyotype, malignancy, and long culture history of HeLa cells have had little effect on the snPI pattern.

DISCUSSION

The newly described class of nuclear RNA molecules found initially in HeLa cells, the snPI RNA, is shown here to occur in a variety of mammalian cells, thus suggesting that these molecules may be a ubiquitous feature of vertebrate and, possibly, of all metazoan cells. The two major conclusions presented here are: (*a*) Nuclei of different animals, such as mouse, rat, and hamster, or monkey, ape, and man, synthesize a different spectrum of snPI RNA molecules. (*b*) Different types of cells from the same organism, such as mouse 3T6, neuroblastoma, Friend erythroleucemia, and myeloma cells, or HeLa and human fibroblast cells, synthesize the same spectrum of snPI RNA molecules, though with quantitative differences.

The snPI RNA spectrum shows a pronounced species-specificity that allows differentiation of even such evolutionarily related organisms as mouse and rat, or of even the very close species, gorilla and man. This latter comparison is especially interesting in view of the extensive studies of the biochemical relatedness of humans and apes (in particular, the chimpanzee). The results obtained by studying sequence homologies of proteins and DNA of these two organisms have indicated that the genetic distance between humans and chimpanzees is very small (3). They appear to be a good example of a general phenomenon in evolution. Morphological changes develop rapidly while biochemical differences develop very slowly (11). A recent report on the evolution of primate chromosomes suggests that the relation of gorilla and man is even closer than that of chimpanzee and man (5). The easily detectable snPI RNA mobility differences of gorilla and human cells, cells from two organisms which are otherwise probably nearly biochemically indistinguishable, suggest that the snPI RNAs are a particularly sensitive measure of species differences. They may evolve at a rate more comparable to that of morphological evolution.

The changes in RNA patterns reported here may, of course, not be related to speciation or morphological evolution in any causal way and could, in the extreme, simply reflect "genetic" drift in the absence of a strong selection pressure. Alternatively, the pronounced RNA spectrum specificity might be related to the chromosome changes which are the most obvious difference between cells of related species. In this regard, it is interesting to note that snPI RNAs are tightly associated with the nuclear matrix and chromatin and may play a role in determining higher-order chromatin organization. Of course, some unknown connection between nuclear RNA synthesis and higher-order cell behavior may be reflected in this result. Their relatively rapid evolutionary change might suggest a relation to the regulatory functions that determine whole organism structure. In this regard, it is interesting that snPI RNA molecules appear to be involved in hnRNA metabolism and are found, in part, to be hydrogen-bonded to hnRNA molecules.

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