The messenger RNA species that code for two extra-embryonic endodermal cytoskeletal proteins (Endo A and Endo B) have been identified. Endo A and Endo B messenger RNA species are differentially expressed. They are absent, or at basal levels, in undifferentiated embryonal carcinoma cells (F9.22 cell line) and relatively abundant in parietal endoderm (PFHR9 cell line) or in embryonal carcinoma cells that have been induced, by retinoic acid, to differentiate to parietal endoderm. Endo A and Endo B messenger RNA can be detected in F9.22 cells 48 to 72 h after exposure to retinoic acid, which is coincident with the expression of Endo A and Endo B proteins. The size of Endo A and Endo B messenger RNA has been determined by denaturing methyl mercury hydroxide agarose gels to be 2.0 ± 0.1 and 1.5 ± 0.2 kilobases, respectively.

Recently, two new cytoskeletal proteins, Endo A and Endo B, have been identified and isolated (1). Both proteins can be detected in extra-embryonic endodermal cell lines derived either from murine teratocarcinomas or from mouse blastocysts, but are not found in EC cells, the stem cell of the teratocarcinoma, or in a variety of other differentiated cell types. The amino acid composition, cellular immunofluorescent localization, and protein solubility properties of Endo A and Endo B proteins resemble other intermediate filament proteins (1). However, based on their presence in a very limited number of cell lines, size, immunological cross-reactivity, and partial peptide maps, Endo A and Endo B proteins are distinct from vimentin, desmin, glial fibrillary acid protein, neurofilament protein, keratins, and tubulin (1).

When malignant EC cells are exposed to physiological concentrations (10⁻¹⁰ to 10⁻⁷ M) of retinoic acid, they differentiate to benign presumptive parietal endoderm and express Endo A and Endo B proteins (1), along with other functions characteristic of parietal endoderm (2-5). Endo A and Endo B proteins are of interest because they make excellent markers of tissue-specific, differentiated functions which appear when embryonal carcinoma cells are induced to differentiate. Thus, they are useful in evaluating developmental control during cellular differentiation. In addition, because of their physical association with filaments, they are potentially involved in cell shape and tissue morphogenesis.

This paper describes the identification and sizing of the messenger RNA species that encode Endo A and Endo B proteins. Also, the time of appearance of Endo A and Endo B messenger RNA, after treatment of EC cells with retinoic acid, has been determined.

EXPERIMENTAL PROCEDURES AND RESULTS

The experiments presented in this investigation have identified two messenger RNA molecules which are absent, or at basal levels, in undifferentiated EC cells and are relatively abundant in parietal endoderm cells. Immunoprecipitation analysis of in vitro translation products, followed by SDS-polyacrylamide gel electrophoresis, provides evidence that these messenger RNA molecules code for the Endo A and Endo B cytoskeletal proteins. The differentiation-dependent regulation of Endo A and Endo B messenger RNA is demonstrated by their appearance in F9.22 EC cells which have been induced to differentiate to parietal endoderm by treatment with retinoic acid.

The in vitro differentiation of certain murine embryonal carcinoma cell lines appears to reflect normal early embryonic development (19, 20). Strickland and Mahdavi (2) have shown that other embryonal carcinoma cell lines, which spontaneously differentiate infrequently, respond to low concentrations of retinoic acid by differentiating to cells resembling extra-embryonic endoderm. The differentiated cells which appear have altered cellular morphology (2); synthesize and secrete plasminogen activator (2), laminin (1, 4), and type IV collagen (2, 21); and synthesize the two recently described cytoskeletal proteins, Endo A and Endo B (1). All these characteristics are consistent with the interpretation that the differentiated cells that arise in response to retinoic acid are parietal endoderm.

Increased levels of Endo A and Endo B messenger RNA have been detected in F9.22 EC cultures approximately 48 to 72 h after exposure to retinoic acid.
72 h after exposure to retinoic acid. This corresponds well with the earliest time of detection of increased Endo A and Endo B protein synthesis (1). Also, other parietal endoderm characteristic functions (secretion of laminin and plasminogen activator) are expressed 2 days after exposure to retinoic acid.

The Endo A and Endo B messenger RNA species are 2.0 ± 0.1 and 1.5 ± 0.2 kilobases, respectively. Based on the molecular weights of Endo A protein (Mr = 55,000) and Endo B protein (Mr = 50,000), it was calculated that the Endo A messenger RNA has approximately 500 bases which are non-coding and the Endo B messenger RNA has approximately 140 bases which are non-coding. This variation in the size of the noncoding regions has also been observed for messenger RNA species that code for desmin (22) and keratin (23) intermediate filament proteins.

REFERENCES

Differential Expression of Endo A and Endo B mRNA

IDENTIFICATION OF mRNA SPECIES IN DIFFERENTIATED DERIVATIVES OF MOUSE ENDODERMAL EMBRYONIC CARCINOMA CELLS

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EXPERIMENTAL PROCEDURES

Cells

The FS-22 cell line is an EC cell line subcloned from the FS cell line (6,7). The FS-22 cell line spontaneously differentiates at very low frequencies in vivo and in vitro. PFRAD was isolated from differentiated cultures of the plating-EC cell line, FS-22, and was obtained from Dr. Albert C. Gishmann (8). We consider PFRAD a partial endodermal cell line because of the active synthesis and secretion of laminin and plakoglobin in complex with actin filaments and desmosomes, and lack of extensional carcinoma cell surface antigens (9,10). Cells were cultured in Ham's F12 modified Eagle's medium supplemented with porcine (10,11) and bovine (2,3) growth factor (2,4).

Retinyl Alcohol Induction

FS-22 cells were seeded at 10^5 cells per 90 mm roller bottle. Retinyl alcohol was added to all cultures at a concentration of 1.75 x 10^-5 M for 24 hours. Retinyl alcohol was removed and most cell lines were harvested by trypsinization, after 12, 24, 48 and 72 hours exposure to retinyl alcohol. Cells were pelleted by centrifugation and frozen immediately in liquid nitrogen.

Immunofluorescence

The method for immunofluorescence staining of basement membrane proteins laminin has been described (12). Staining of FS-22 and PFRAD membranes for laminin revealed similar levels of laminin immunostaining, indicating that PFRAD is undifferentiated (12). Immunofluorescence staining of FS-22 cells was performed as described by Sertin and Bredt (12).

Polyadenylated Messenger RNA Isolation

Isolation of total RNA from frozen cell pellets was performed by the guanidine thiocyanate and cesium chloride method as described by Sertin et al. (12). Polyadenylated messenger RNA was isolated from total RNA by oligo-oligo-dT chromatography (13).

In Vivo Translation

Lysates of rabbit reticulocytes were prepared from rabbits made acute by antilymphocytic serum (14). Polyadenylated messenger RNA was translated in microsomal nucleated translatable reticulocyte lysates by the method of Pelham and Jackson (15).

Immunoprecipitation

Proteins were completely denatured by heating on ice for 15 min. in 5X SDS buffer (50 mm NaCl, 2.5 mm EDTA, 100 mm glycerol, 0.01% bromphenol blue, and 4% SDS) and then electrophoresis was performed on a 24.5% SDS polyacrylamide gel. The gels were fixed in 40% methanol, 10% acetic acid for 30 min. and then stained with Coomassie blue. The gels were destained in 7.5% acetic acid, 20% methanol. The protein bands were cut out and subjected to electrophoresis. The protein bands were detected by the addition of Coomassie blue to the gel. The bands were then extracted with ethanol, dried, and counted in a gamma counter.

RESULTS

Identification of Polyadenylated mRNA Species for Endo A, Endo B and Vimentin

Polyadenylated RNA was isolated from FS-22 EC (15) and PFRAD (16) undifferentiated (vimentin) cell lines as described in Methods. Direct immunofluorescence staining of cells for the basement membrane protein laminin was used to measure the frequency of cells expressing vimentin and other markers. Cellular preparations used in the electrophoresis of polyadenylated messenger RNA from Fu-22 EC cell line were prepared from a single cell suspension (2,7). The frequency of cells expressing vimentin and other markers was determined by differential centrifugation. The frequency of vimentin positive cells was determined by direct immunofluorescence and by differential centrifugation. The results of the centrifugation procedure were compared with the frequency of cells expressing vimentin and other markers. The frequency of vimentin positive cells was determined by differential centrifugation and by direct immunofluorescence. The frequency of vimentin positive cells was determined by differential centrifugation and by direct immunofluorescence.

Retinyl Alcohol Induction of FS-22

Polyadenylated messenger RNA was extracted from FS-22 EC cell line which had been exposed to 10^-5 retinyl alcohol for 12, 24, 48 and 12 hours. At 12 hours greater than 50% of the cells expressed laminin as detected by indirect immunofluorescence. Expression of laminin (LSA) in treated EC (6,13) cells was determined by indirect immunofluorescence. Expression of laminin (LSA) in treated EC (6,13) cells was determined by indirect immunofluorescence. Expression of laminin (LSA) in treated EC (6,13) cells was determined by indirect immunofluorescence.
Differential Expression of Endo A and Endo B mRNA

Previous studies [1] have shown that increased amounts of Endo A and Endo B proteins can be immunoprecipitated from F9.22 cells extract 48 hours after exposure to retinoic acid. In addition, mRNA levels of Endo A and plasminogen activator [2] appear 48 hours after the addition of retinoic acid. These data are consistent with the coordinate expression of Endo A and Endo B messenger RNA and proteins 48 to 72 hours after F9.22 cells are treated with retinoic acid. Changes in the pharmaceutical and biochemical characteristics of differentiated peripheral mouse liver appear [2].

Size Determination of Endo A and Endo B Messenger RNA

F9.22 messenger RNA was electroeluted in denaturing gels containing sodium dodecyl sulfate. Figure 1 shows that a new bands immunoprecipitated from F9.22 cell extracts. Figure 1 shows that the mobility of Endo A and Endo B messenger RNA with the mobilities of mouse 18S and 28S rRNA (and RNA) and the mobility of Endo A was estimated to be 2.5 S. 6.1 kDa and Endo B was estimated to be 1.5 S 0.2 kDa (Figure 1). F9.22 messenger RNA and molecular weight markers were electroeluted in the same denaturing gel for this analysis (gel not shown).

Figure 1: Mobility of Endo A and Endo B was compared to that of 18S and 28S rRNA (6.1 kDa and 145,000 mol. wt.) [4]. The figure shows a semilogarithmic plot of molecular weight as a function of mobility.

Figure 2: Size determination of Endo A and Endo B messenger RNA. (A) Photograph of etched film autoradiograms of RNA stained 18S and 28S rRNA (180 kDa) and micrococcal nuclease digestion 48 kDa and 3000 Da. (B) Lane 1, 15% SDS-polyacrylamide gel; lane 2, 7.5% SDS-polyacrylamide gel. (C) Lane 1, 15% SDS-polyacrylamide gel; lane 2, 7.5% SDS-polyacrylamide gel. (D) Lane 1, 15% SDS-polyacrylamide gel; lane 2, 7.5% SDS-polyacrylamide gel. (E) Lane 1, 15% SDS-polyacrylamide gel; lane 2, 7.5% SDS-polyacrylamide gel.