ethyl acetate, and the extracts were combined and evaporated to dryness under a jet of N_2. The residue was then redissolved in 0.5 ml methanol and analyzed by high-pressure liquid chromatography (*7*).

Both acetamide and HOA were detected in the urine of all five subjects taking metronidazole (Table 1). Neither metabolite was found in the urine of the control subjects. Thus these metabolites of metronidazole occur in the human as they do in the rat. Presumably, as in the rat (*6, 7*), they represent metabolites formed by the intestinal microflora.

Together, HOA and acetamide account for all the carbon and nitrogen atoms of metronidazole except for the nitrogen atoms in the nitro group. Approximately 1 to 2 percent of the daily 750-mg doses of metronidazole was recovered either as HOA or acetamide in patients, a recovery that is similar to that in the rat (*6, 7*). Because the yield of acetamide is approximately tenfold greater than that of HOA when metronidazole is either metabolized by bacteria (*6*) or reacted with xanthine oxidase (*8*), some acetamide must be lost by further mammalian metabolism (*6*), which implies that the patient is probably exposed to approximately tenfold more acetamide than is actually recovered in his or her urine.

Prolonged feeding of high doses of acetamide causes hepatocarcinoma in rats (*9*), and it is of interest that this is similar to one of the tumors reported in the rat after long-term feeding of metronidazole (*3*). Thus the finding of acetamide in the rat may provide insight into the mechanisms of metronidazole's carcinogenicity in the rodent. However, our finding of acetamide in the urine of human patients should not be interpreted as evidence that metronidazole is carcinogenic for humans. It has been known for nearly a decade that long-term feeding of maximally tolerated doses of metronidazole causes tumors in rats and mice (*3*), but two surveys of a limited number of patients treated with metronidazole suggest that the risk of this drug being a potent carcinogen in humans is probably small or negligible (*5*). Further studies of patients previously exposed to metronidazole may provide more accurate estimates of this risk.

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Two Novel Classes of Small Ribonucleoproteins Detected by Antibodies Associated with Lupus Erythematosus

Abstract. *The RNP and Sm antigens recognized by lupus erythematosus antibodies are located on discrete particles containing single small nuclear RNA's complexed with proteins. The antigens Ro and La are also on ribonucleoproteins. The small RNA's in ribonucleoproteins with Ro are discrete, like those associated with RNP and Sm; in contrast, ribonucleoproteins with La contain a striking highly banded spectrum of small RNA's from uninfected cells as well as virus-associated RNA from adenovirus-infected cells.*

Antibodies directed against a variety of nuclear and cytoplasmic constituents are characteristic of lupus erythematosus. The molecular identities of some of these antigens are known (for example, DNA), but four have simply been designated Sm, RNP, Ro (also called SS-A), and La (also called SS-B and Ha) (*1–6*). Defining the molecular nature of these antigens is one approach to the difficult problem of understanding why they so often become targets for immune responses in lupus erythematosus.

By examining immune precipitates of nuclear extracts prepared from ^{32}P-labeled mouse Ehrlich ascites cells, we established the molecular identity of RNP and Sm as small nuclear ribonucleoprotein particles, which we call snRNP's (*7*). Antigenically conserved for over 500 million years, snRNP's may play a role in messenger RNA biogenesis (*8*). Antibody to RNP precipitates the most abundant snRNP's, those containing the small nuclear RNA's (snRNA's) U1a (*9*) and U1b (*7*). Antibody to Sm precipitates these snRNP's and four others containing the snRNA's U2 (*10*), U4, U5, and U6. Both antibodies precipitate the same seven polypeptides with molecular weights of 12,000 to 32,000. At least some of these proteins are required for antigenicity, since purified U1a, U1b, U2, U4, U5, and U6 RNA's do not react with either antibody (*7*).

In the present study, we examined 29 serums (25 from patients with lupus erythematosus and four reference serums containing antibody to Sm, RNP, Ro, and La, respectively) for the ability to precipitate small RNA-protein complexes from total cell extracts of ^{32}P-labeled Ehrlich ascites cells (Fig. 1). Seven of the serums gave a pattern of small RNA's consistent with the presence of antibody to Sm (lane 1), and two with the presence of antibody to RNP (lane 2) (*7*). In contrast to these previously defined patterns, three serums precipitated the small RNA's Y1 and Y2 (lane 4), and a fourth serum precipitated Y1, Y2, and Y3 (lane 3). A single serum precipitated a highly banded spectrum of small RNA's (lane 5). Standard serums containing predominantly antibodies to Sm, RNP, Ro, or La yielded the same patterns as those seen in lanes 1, 2, 4, and 5, respectively. Because lane 3 is identical to lane 4, with the addition of Y3 in lane 3, we call this pattern Ro^b and that in lane 4 Ro^a; much darker exposures of lane 4 do not show Y3. Serums from three patients apparently contained combinations of antibodies; two of these precipitated a constellation of small RNA's that suggest the simultaneous presence of antibodies to Sm and Ro^a (lane 7), and the third contained antibodies to Sm, RNP, Ro^a, and La (lane 8). [Although the small RNA's precipitated by antibody to RNP are a subset of those precipitated by antibody to Sm, the intensity of U1a and U1b relative to U2 in lane 8 (compare to lane 1) indicates that antibodies to both RNP and Sm are present in this serum.] Finally, serums from

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eight patients did not contain antibodies to small ribonucleoproteins.

All the small RNA's in lanes 1 through 5 have been subjected to analysis by two-dimensional oligonucleotide mapping [performed as described in (*7*)] confirmed that they are discrete species and not degradation products of one another (*11*). Thus, although Y2 has the same electrophoretic mobility as U6, it is distinct from U6. The small RNA's in lanes 3 and 4 are cytoplasmic and are therefore designated by the Y prefix to distinguish them from the nuclear U series. The cytoplasmic location of the Ro antigen has been demonstrated (*3*). In addition, indirect immunofluorescence studies in which antibody to Ro was used on Vero (monkey) cells gave selective cytoplasmic staining (*12*). The highly banded spectrum of RNA's precipitated by antibody to La does not overlap the RNA's in lanes 1 to 4. Included in this class of ribonucleoproteins, which are apparently located mostly in the nucleus (*4, 12*), is a 4.5*S* RNA species previously described (*13*).

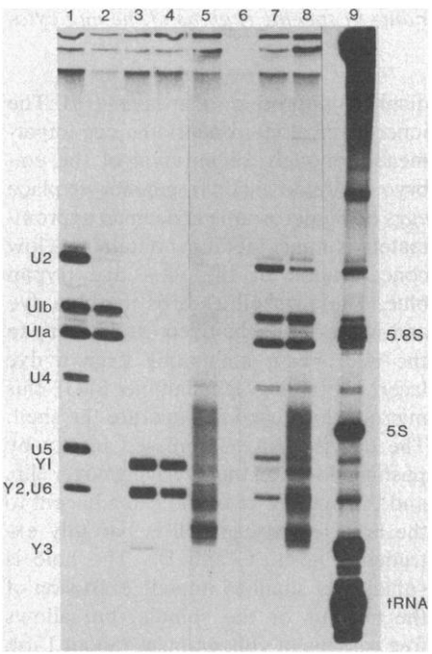

Fig. 1. Small RNA's from Ehrlich ascites cells precipitated by antibodies from patients with lupus erythematosus. Ehrlich ascites cells were labeled with $^{32}PO_4$ (*7*), and total cell extracts were prepared; 10^8 cells in 5 ml of 50 m*M* tris-HCl and 150 m*M* NaCl (*p*H 7.5, at 0°C) were ruptured by sonication for 15 seconds with a Branson sonifier at setting 2; the homogenate was centrifuged at 15,000*g* for 10 minutes to obtain a clear solution. RNA's were isolated from immune precipitates or extracts and were fractionated on a gel 400 mm long, 200 mm wide, and 0.5 mm thick, consisting of 10 percent polyacrylamide, 0.38 percent bisacrylamide, 7*M* urea, 1 m*M* EDTA, and 50 m*M* tris borate, at *p*H 8.3 (*7, 8*). Lane 9 shows small RNA's from a total Ehrlich ascites cell extract. Lanes 1 to 8 show small RNA's precipitated by immunoglobulin G isolated from serums (*7*). Lane 6 shows normal serum; the other lanes show serums containing antibodies to (lane 1) Sm, (lane 2) RNP, (lane 3) Ro^b, (lane 4) Ro^a, (lane 5) La, (lane 7) Sm and Ro^a, and (lane 8) Sm, RNP, Ro^a and La.

HeLa cells infected with adenovirus produce a small virus-associated RNA (VA RNA) that is encoded by the viral genome but is of unknown function (*15*). Small RNA's were extracted from HeLa cells before (lane 1) and 17 hours after (lane 2) infection with adenovirus (Fig. 2). The new multibanded small RNA present in the infected cells was identified as VA_I RNA (*16*) by two-dimensional oligonucleotide mapping [performed as in (*7*); not shown]. When extracts of normal and adenovirus-infected cells were reacted either with standard serum containing antibody to La or with serums from two patients that had antibodies to La (lanes 5 and 8 of Fig. 1), the small RNA's shown in lanes 3 and 4 of Fig. 2 were obtained. The highly banded pattern of RNA's in the uninfected HeLa cells (lane 3) is similar but not identical to that found by reacting antibody to La with extracts of mouse Ehrlich ascites cells (Fig. 1, lane 5). In lane 4, where infected HeLa cells were used, antibody to La precipitated VA RNA. Again proteins were required for antigenicity (not shown). Serum lacking antibodies to La did not precipitate the VA RNA–containing ribonucleoprotein.

Our simple but sensitive radioimmunoassay (*7*) has demonstrated that five antigens (Sm, RNP, Ro^a, Ro^b, and La) that are recognized by antibodies from patients with lupus erythematosus are actually distinct groups of small RNA-protein complexes. Three nonoverlapping classes (in terms of their RNA components) are represented: (i) the nuclear particles (snRNP's) recognized by antibodies to RNP or Sm, (ii) the cytoplasmic particles (scRNP's) recognized by the two forms of antibody to Ro, and (iii) the heterogeneous particles (probably nuclear) recognized by antibody to La. The last class of particles appear to share an antigenic determinant with at least one virus-induced RNA-protein complex. All of the above antigens are susceptible to trypsin or parahydromercuribenzoate, but only the RNP and La antigens are reported to be sensitive to ribonuclease (*2, 3, 5, 6*). Thorough characterization of the protein components of the Ro and La classes of small ribonucleoproteins awaits the construction of hybridomas specific for antibodies to Ro or to La; hybridomas specific for antibody to Sm have already demonstrated the presence of a single antigenic determinant on snRNP's containing U1, U2, U4, U5, or U6 RNA's (*17*).

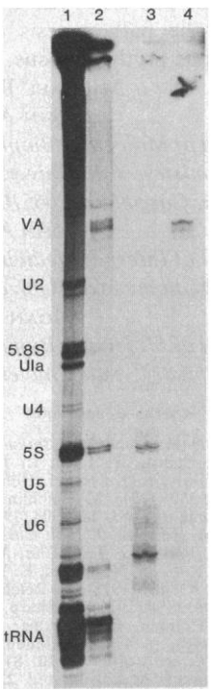

Fig. 2. Small RNA's precipitated by antibody to La from normal or adenovirus-infected HeLa cells. Small RNA's isolated from immune precipitates and extracts of ^{32}P-labeled uninfected or adenovirus-infected HeLa cells were fractionated as described in Fig. 1. HeLa cells were grown in low phosphate medium (*7*) for 24 hours and then infected by incubating concentrated cells (5×10^7 per milliliter) with 100 plaque-forming units of adenovirus 2 per cell for 1 hour. They were diluted 100-fold and labeled with $^{32}PO_4$ (1 mCi/100 ml) for 17 hours; cell extracts were prepared as described in Fig. 1. Lanes 1 and 2 show total small RNA's from extracts of HeLa and adenovirus-infected HeLa cells, respectively. Lanes 3 and 4 show small RNA's precipitated by antibody to La from extracts of HeLa and adenovirus-infected HeLa cells, respectively. The multiple bands labeled VA RNA all have the VA_I fingerprint (*15*) and are consistent with the known length heterogeneity of this RNA species (*16*); the precipitation of VA RNA is quantitative.

Biological roles for the Ro and La ribonucleoproteins have yet to be established. Their abundance (10^5 to 10^6 particles with each RNA per cell) and conservation in the mammalian species we have examined (mouse, human, and monkey) suggest an important function. With respect to RNA components of the La family, it has been suggested that the adenoviral-specified VA RNA participates in the splicing of late viral messengers (*18*), whereas other functions have been proposed for the 4.5*S* RNA synthesized by uninfected mouse cells (*13*, *19*). However, our finding that antibody to La recognizes ribonucleoprotein complexes containing VA RNA as well as many cellular RNA's hints that VA RNA may serve adenovirus in a manner similar to that of the cellular RNA's. It is also conceivable that this cross-reactivity is important in the pathogenesis of certain types of lupus erythematosus.

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Nematode Development After Removal of Egg Cytoplasm: Absence of Localized Unbound Determinants

Abstract. *Embryos of* Caenorhabditis elegans *develop into fertile adults after cell fragments, containing presumptive cytoplasm of somatic and germ line precursors, are extruded from uncleaved eggs or early blastomeres through laser-induced holes in the eggshells. This suggests that the determinate development of this worm is not dependent on the prelocalization of determinants in specific regions of the egg cytoplasm.*

The prelocalization of developmental determinants in the cytoplasm or cortex of fertilized eggs or early embryos is often proposed as a mechanism for differential cell determination, especially in organisms that display determinate cleavage patterns (*1*). Nematodes such as *Caenorhabditis elegans*, which is a favorable model for studying animal development (*2*), provide well-known examples of determinate cleavage (*3*). In *C. elegans*, asymmetrical early cleavages, invariant from individual to individual, produce a set of six precursor cells that are the founders of five somatic cell lines and one germ cell line (Fig. 1, A and B) (*4*). The precursor cells are irreversibly determined (*5-7*). Fate maps, describing which cytoplasmic regions of the uncleaved egg develop into the various precursor cells, have been constructed for several nematodes, including *C. elegans* (*6-9*). In each species the egg was found to be partitioned in a simple pattern of six parallel segments, one for each precursor. From these fate maps it has been hypothesized that the region of egg cytoplasm that each precursor receives is critical to its determination (*6*). We have tested this hypothesis in *C. elegans* by removing cell fragments from fertilized uncleaved eggs or from early blastomeres through holes in the eggshell made with a laser microbeam. Our results suggest that factors responsible for determinate development are not prelocalized in specific regions of the cytoplasm.

The embryo of *C. elegans* develops into a larval worm inside an impermeable eggshell. As in other nematodes, the eggshell consists of a rigid outer layer and a distinct lipoprotein inner layer (*10*). The inner layer alone renders the egg impermeable enough for survival of the embryo. To remove cell fragments we place eggs on a microscope slide in an approximately isotonic medium containing a low concentration of the vital dye trypan blue. The eggshell absorbs the blue dye and is thereby sensitized (at 590 nm) to the microbeam emanating from a dye laser containing Rhodamine 6 G; this microbeam is used to puncture the shell. The egg is then compressed briefly by pushing down on the overlying cover slip, and the cytoplasm of the cell adjacent to the hole in the eggshell is partially extruded (Fig. 1, C and D). The hole is sufficiently small to impede extrusion of the nucleus or the spindle, but allows free passage of yolk granules (about 1 μm in diameter). The granules can be seen to stream uniformly from all regions close to the hole. The cell then ligates at the constriction caused by the eggshell. Usually the resultant cell fragment becomes visibly detached from the egg.

Electron micrographs show that the cell fragment is surrounded by a plasma membrane. The extruded cytoplasm looks very similar to that in the corresponding region of an intact egg. It contains the usual organelles, for example, ribosomes, mitochondria, and yolk granules.

The medium used for extruding the fragments does not support the survival of the fragments or the exposed cells. Fragments degenerate soon after extrusion and take up the trypan blue present in the medium. Often an embryo from which a fragment is removed lyses

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