duced by embedding the drug in a polylactic acid matrix (PLA) (*7*). A single intraperitoneal injection of DDP-PLA (100 mg/kg, in saline) cured the mice of *T. rhodesiense* (*8*). The amount of DDP released from the DDP-PLA matrix composite during the first week after the injection was less than the amount of drug administered for a single day during the 7-day regimen of 3 mg/kg-day. Consequently, the drug dose released from the composite was below the nephrotoxic levels for DDP.

Thus we have shown that DDP administered in conjunction with disulfiram rescue and hydration is an effective antitrypanosomal agent. Although the treatment was toxic to 3 of 40 animals (7.5 percent, Table 1), most of the animals were cured and their tissues did not harbor latent infectious organisms.

Trypanocidal effects of antibiotic and antineoplastic agents have been noted in several recent reports (*9*). We now add DDP, an antineoplastic agent commonly used in humans, to the list of trypanocidal agents with potential for application in vivo. The specific regimen that we used to ameliorate potential toxicity may prove useful in the treatment not only of infectious diseases but also of human malignancies.

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Small Nuclear RNA U2 Is Base-Paired to Heterogeneous Nuclear RNA

Abstract. *Eukaryotic cells contain a set of low molecular weight nuclear RNA's. One of the more abundant of these is termed U2 RNA. The possibility that U2 RNA is hydrogen-bonded to complementary sequences in other nuclear RNA's was investigated. Cultured human (HeLa) cells were treated with a psoralen derivative that cross-links RNA chains that are base-paired with one another. High molecular weight heterogeneous nuclear RNA was isolated under denaturing conditions, and the psoralen cross-links were reversed. Electrophoresis of the released RNA and hybridization with a human cloned U2 DNA probe revealed that U2 is hydrogen-bonded to complementary sequences in heterogeneous nuclear RNA in vivo. In contrast, U2 RNA is not base-paired with nucleolar RNA, which contains the precursors of ribosomal RNA. The results suggest that U2 RNA participates in messenger RNA processing in the nucleus.*

The small nuclear RNA's are abundant, metabolically stable low molecular weight RNA species that are present in all eukaryotes (*1*). Their functions are not understood. The first small nuclear RNA to be purified was the species termed U2 ("U" for uridylate-rich), which is 189 nucleotides long, and like most of the other small nuclear RNA's, carries an inverted trimethylguanosine "cap" at its 5′ end (*2*). We have investigated the possibility that U2 RNA is associated with high molecular weight nuclear RNA [heterogeneous nuclear RNA (hnRNA)] through intermolecular base-pairing of complementary se-

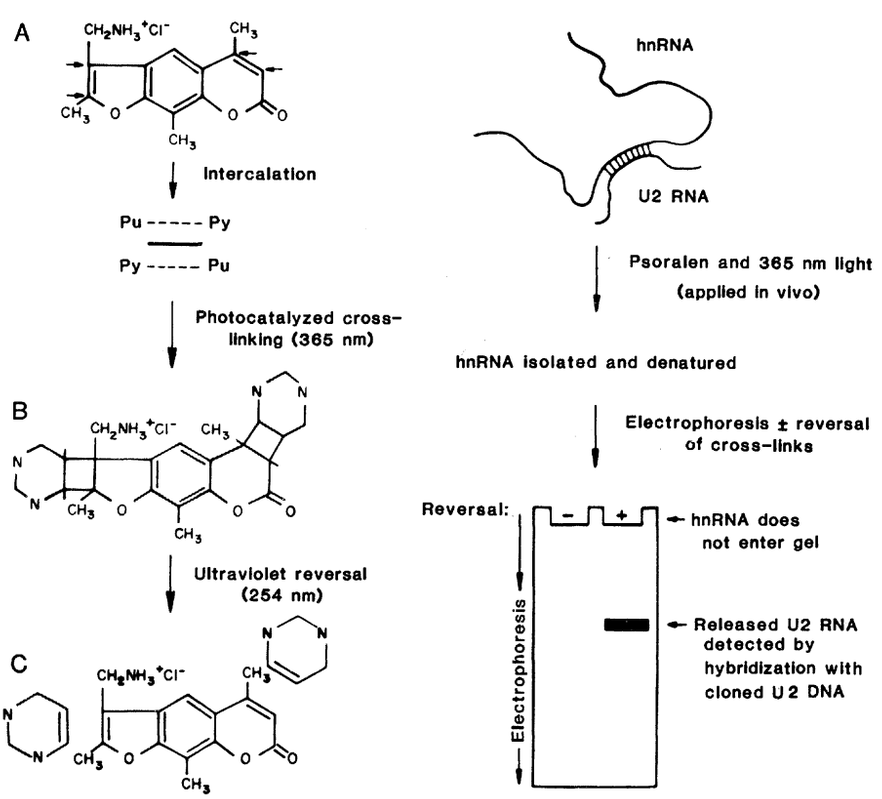

Fig. 1. Psoralen cross-linking and experimental strategy. (Left) Chemistry of cross-linking. 4′-Aminomethyl-4,5′,8-trimethylpsoralen (A) was synthesized from 4,5′,8-trimethylpsoralen as described (*3*, *5*). The arrows indicate carbon atoms in the pyrone and furan rings which, upon 365-nm irradiation, react with the carbons at positions 5 and 6 of pyrimidines to form cyclobutane bridges. In the case of diadducts, this constitutes a covalent, interstrand cross-link (B). The cross-links can be subsequently broken by irradiation at 254 nm (*7a*, *8*), as shown in (C). (Right) Base-pairing between small nuclear RNA U2 and high molecular weight hnRNA is detected by treating intact cells with 4′-aminomethyl-4,5′,8-trimethylpsoralen and 365-nm light. The hnRNA is then extracted and denatured, and equal amounts are subjected to electrophoresis with or without reversal of cross-links. The polyacrylamide concentration of the resolving gel is such that high molecular-weight RNA is excluded. Small RNA species released by reversal of psoralen cross-links are identified after electrophoresis by hybridization with cloned DNA specific for individual small nuclear RNA species.

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quences. This has been examined by use of a psoralen derivative, 4′-aminomethyl-4,5′,8-trimethylpsoralen (AMT), which is a bifunctional nucleic acid cross-linking reagent specific for base-paired regions (*3*). A particularly important feature of this reagent is its ability to penetrate intact cells. In fact, psoralens are used clinically in the management of hyperplastic skin diseases (*4*). This ability of psoralens to enter living cells permits probing base-pairing interactions of nuclear RNA in vivo (*5*, *6*).

Figure 1 (left) illustrates the mechanism of AMT cross-linking. AMT is intercalated in base-paired regions and, when subjected to irradiation at 365 nm, can form covalent interstrand cross-links between pyrimidines on opposite strands (*7*, *7a*). This part of the experiment is done in intact cells. After isolation of high molecular weight nuclear RNA, the cross-links can be reversed by irradiation at 254 nm (*7a*, *8*). This releases any small RNA's that had been base-paired to the high molecular weight RNA. The identity of such released RNA's can be determined by electrophoresis in a gel that resolves low molecular weight RNA's, followed by hybridization with cloned DNA probes that are specific for individual small nuclear RNA species (Fig. 1). Combined with an absence of such RNA's in the gel when the psoralen cross-links are not reversed before electrophoresis, such a result would demonstrate the existence of an in vivo base-pairing interaction between the small RNA and high molecular weight nuclear RNA. An important attribute of this technique is that any spurious RNA-RNA associations that might form during cell fractionation or subsequent RNA isolation and handling do not influence the results, because the method scores only RNA-RNA base-pairing that exists while the intact cells are being exposed to AMT at 365 nm.

As a hybridization probe for small nuclear RNA U2, we used a cloned fragment of human DNA containing sequences homologous with U2 RNA (*9*). To test the specificity of this DNA as a hybridization probe for U2 RNA, HeLa cell small nuclear RNA was subjected to electrophoresis, transferred to diazobenzyloxymethyl paper (*10*), and hybridized with U2 DNA that had been labeled with ^{32}P in vitro. Lane 1 of Fig. 2 shows unlabeled total small nuclear RNA that has been hybridized with U2 DNA; only U2 RNA hybridizes. This can be compared to lane 3, where total small nuclear RNA has been 3′-end-labeled in vitro (*11*) and run as markers. Lane 2 shows another hybridization analysis similar to that in lane 1, except that in this case the autoradiogram was deliberately overexposed. In addition to the major U2 RNA hybridization signal, three or more smaller RNA's also react with the U2 DNA probe. These might be cleavage products of U2 RNA or transcripts of truncated U2 pseudogenes (*9*, *12*).

The results of psoralen cross-linking are shown in Fig. 2, lanes 4 to 8. Lane 4 contains total HeLa small nuclear RNA and again shows the specificity of the DNA probe for U2 RNA. Lanes 5 and 6 contain RNA extracted from hnRNA-ribonucleoprotein particles (*13*) with (lane 5) or without (lane 6) reversal of the psoralen cross-links. In lane 5, an RNA species comigrating with U2 RNA hybridizes with the U2 DNA probe after cross-link reversal, whereas no hybridization is observed in this region of the gel without reversal of the cross-links (lane 6). In the latter case, hybridization is confined to the top of the gel, presumably representing U2 RNA molecules cross-linked to high molecular weight hnRNA and therefore unable to enter the low molecular weight RNA resolving gel.

The nuclear fractionation method used to isolate ribonucleoprotein particles containing hnRNA also yields nucleoli as a separate fraction (*13*, *14*). This nucleo-

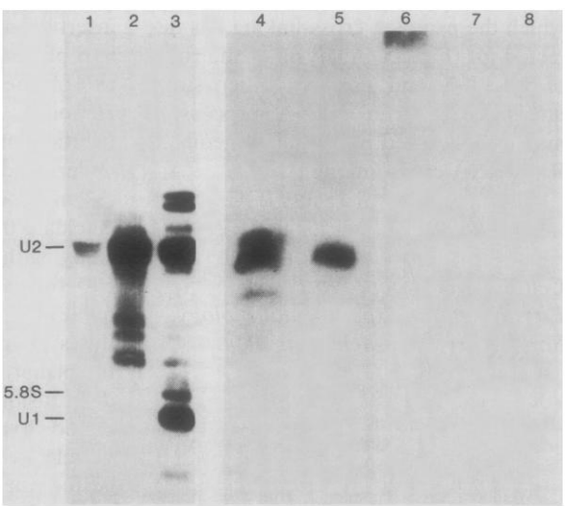

Fig. 2. (Lanes 1 to 3) Specificity of U2 DNA hybridization. Lanes 1 and 2 contained 25 μg of unlabeled HeLa cell total small nuclear RNA. Electrophoresis was in a 10 percent polyacrylamide gel containing 7*M* urea run at 150 V for 20 hours, at which time the xylene cyanol FF tracking dye had migrated 45 cm. The RNA was electrophoretically transferred to diazobenzyloxymethyl paper (*10*) and hybridized with cloned U2 DNA. The DNA was a 103-bp Sau3A I/Alu I fragment of the U2 DNA clone U2.7, subcloned in pBR322 (*9*). It was labeled by nick-translation to a specific activity of 5.4×10^7 trichloroacetic acid–precipitable counts per minute per microgram. (Lane 1) Hybridization with DNA probe (1.8×10^7 count/min) and autoradiography for 24 hours (−70°C; Kodak XR-5 film; DuPont Cronex Lightening-Plus intensifying screen). (Lane 2) Hybridization as in lane 1 but exposed longer. (Lane 3) Total HeLa small nuclear RNA markers labeled in vitro with [5′−^{32}P]cytidine-3′,5′-bisphosphate and T4 RNA ligase (*11*). (Lanes 4 to 8) Base-pairing between U2 RNA and hnRNA in vivo. HeLa cells (2×10^9) were harvested, washed (*5*), and resuspended in 9 ml of 0.15*M* NaCl–10 m*M* tris-HCl, *p*H 7.2, containing AMT (2 mg/ml). After 10 minutes at 22°C, the cell suspension was irradiated at 365 nm (flux, 6000 μW/cm^2) for 1.5 hours at 5°C (*5*). Nuclei were isolated and fractionated as described (*13*). The fraction containing hnRNA-ribonucleoprotein particles (*13*) was recovered and placed in a solution of 1 percent sodium dodecyl sulfate (SDS), 0.25*M* NaCl, and 20 m*M* EDTA (final concentrations). Proteinase K (200 μg/ml) was added and the sample was incubated at 37°C for 30 minutes. The nucleolar pellet fraction was resuspended in the mixture of SDS, NaCl, and EDTA and digested with Proteinase K similarly. The samples were extracted with one volume of a mixture of phenol, chloroform, and isoamyl alcohol (50:49.5:0.5 by volume), followed by further extractions with chloroform and isoamyl alcohol; the RNA was precipitated from the aqueous phase by the addition of ethanol to 67 percent (by volume). The RNA was collected by centrifugation and dissolved in 10 m*M* NaCl, 11.5 m*M* $MgCl_2$, 10 m*M* tris-HCl, *p*H 7.2, and digested with 100 μg of pancreatic deoxyribonuclease per milliliter for 15 minutes at 37°C. The RNA was extracted again with a phenol-chloroform mixture and collected by ethanol precipitation. Samples were subjected to electrophoresis in a preparative 10 percent polyacrylamide gel containing 98 percent formamide, and the tops of the gel lanes containing high molecular weight RNA were excised after electrophoresis. One-half of each gel slice was irradiated at 254 nm, while the other equivalent half-slice was not irradiated (*6*). These slices were then sealed into sample wells of a second gel with the use of hot agarose. This resolving gel was 10 percent polyacrylamide and contained 98 percent formamide. Electrophoresis was conducted at 120 to 150 V for 22 hours. The RNA was placed on diazobenzyloxymethyl paper and hybridized with cloned U2 DNA. The ^{32}P specific activity of the U2 DNA probe was 1.5×10^8 count/min-μg. The blot was hybridized with DNA probe (1.5×10^8 count/min) and autoradiographed for 24 hours at −70°C with an intensifying screen. (Lane 4) Unlabeled total small nuclear RNA; (lane 5) RNA from hnRNA-ribonucleoprotein particles, with reversal of AMT cross-links; (lane 6) same as lane 5 but without reversal; (lane 7) RNA from nucleolar fraction, with reversal of AMT cross-links; (lane 8) same as lane 7 but without reversal. The RNA samples in lanes 5 to 8 are from equivalent portions of the hnRNA-ribonucleoprotein and nucleolar fractions from the same culture of AMT cross-linked HeLa cells.

lar fraction is substantially enriched in ribosomal RNA precursors relative to hnRNA, and is also enriched in nucleolar small nuclear RNA's such as U3 (*6*). RNA from this nucleolar fraction is shown in lane 7 of Fig. 2 (cross-links reversed) and lane 8 (cross-links not reversed). The absence of hybridization with U2 DNA shows that U2 RNA is not base-paired with nucleolar RNA. This result and the fact that the RNA used in lanes 5 and 6 was isolated from well-characterized hnRNA-ribonucleoprotein particles (*13, 15*) indicate that U2 RNA is base-paired with hnRNA.

Because both U1 (*6*) and U2 RNA's are base-paired with hnRNA, we have considered the possibility that they might also interact with each other, leading to the formation of a base-paired U1-U2-hnRNA ternary complex. However, the fact that cloned U2 DNA does not hybridize with U1 RNA (Fig. 2, lanes 1, 2, and 4) and that cloned U1 DNA does not hybridize with U2 RNA (*6*) argues against extensive sequence complementarity (or homology) between these two RNA's. In addition, a computer-assisted search has revealed no thermodynamically stable complementarity between rat U1 and U2 RNA's (*16*). Both U1 and U2 RNA's have potential sequence complementarity with intron-exon borders in messenger RNA (mRNA) precursors (*17*), and it is possible that it is these sites which the psoralen cross-linking has detected (*6*) (Fig. 2). Analysis of the roles of U1, U2, and possibly other small nuclear RNA's in mRNA processing may be facilitated by psoralen-mediated RNA-RNA cross-linking.

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Osmotic Swelling of Phospholipid Vesicles Causes Them to Fuse with a Planar Phospholipid Bilayer Membrane

Abstract. *Fusion of phospholipid vesicles with planar bilayer membranes occurs if the vesicles that contact the planar membrane swell osmotically after the replacement in their medium of an impermeant solute by a permeant one. This finding directly demonstrates that osmotic swelling is a driving force for vesicle–planar membrane fusion. The method used to induce vesicle swelling and fusion may have relevance for biological systems.*

The fusion of two biological membranes is a ubiquitous phenomenon. It occurs in such diverse processes as neurotransmitter and hormone release (*1, 2*), insertion of integral membrane proteins into plasma membranes (*3, 4*), and raising of the fertilization membrane (*5*). Although many of the physiological functions mediated by fusion events are understood, the mechanism by which the fusion process itself occurs is not. To elucidate this mechanism, we have been studying the fusion of phospholipid vesicles to planar bilayer membranes as a model for the fusion process (*6–8*). In this system, an osmotic gradient across the planar membrane is required to produce fusion, with the vesicle-containing *cis* side hyperosmotic with respect to the vesicle-free *trans* side (*7*). It was suggested that Ca^{2+}, which greatly augments the rate of fusion, promotes the close association of vesicles to the planar membrane and that the osmotically induced water flow across the planar membrane and into the vesicles results in vesicle swelling and subsequent fusion (*7*). In this view it is not water flow across the planar membrane per se but rather the net movement of water into the vesicle that is required for fusion. This is crucial to the possible biological relevance of vesicle–planar membrane fusion, since in general an osmotic gradient does not exist across the plasma membrane of most animal cells. To substantiate that water flow across the planar membrane is not essential to the fusion mechanism, we performed experiments in which vesicle swelling occurs without water flow across the planar bilayer. We report that osmotic swelling of vesicles is the driving force for the fusion of vesicles to planar membranes.

Vesicles were formed with porin [a voltage-dependent, slightly cation-selective, channel-forming integral membrane protein isolated from the outer membrane of *Escherichia coli* (*9*)] reconstituted in their membranes. Fusion was assayed by monitoring the incorporation of this vesicle membrane marker into the voltage-clamped planar membrane. Entry of porin into the planar membrane occurs by vesicle–planar membrane fusion and not by its transfer, either directly or through the aqueous phase, from one membrane to the other (*6, 7, 10*).

Vesicles were added to the *cis* side of a planar membrane (Fig. 1A). Although, as reported previously (*7*), Ca^{2+} alone did not induce fusion, it did cause the close apposition of the vesicles to the planar membrane. This apposition was irreversible on a time scale of minutes (*10*). Thus, when the *cis* side was perfused, the vesicles in solution were washed out but the vesicles in close association with

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