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Improved method to prepare RNA-free DNA from mammalian cells

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Abstract

To isolate DNA for nucleoside analog incorporation studies, many investigators use RNase A to remove RNA from total cellular nucleic acid. We observed persistence of ribonucleotides from RNA in nucleic acid samples treated with RNase A alone. Although incubation of [5-^{3}H]uridine-labeled nucleic acid with 50 μg/ml RNase A decreased tritium by 97%, HPLC analysis of the resulting DNA preparation digested to nucleosides revealed high levels of ribonucleosides. Increasing RNase A 10-fold (500 μg/ml) effected only a 1.7-fold reduction in ribonucleosides. Overall, the level of ribonucleosides was one-fourth that of the deoxynucleosides, primarily due to the high levels of guanosine. It was hypothesized that the ribonucleosides originated from guanosine-rich tracts of RNA since RNase A cuts preferentially 3′ to pyrimidine monophosphates and to some extent after AMP. The addition of 0.05 μg/ml RNase T1, which preferentially cleaves RNA 3′ to GMP, decreased total ribonucleosides by nearly 20-fold. In conclusion, we have developed a rapid method which removes greater then 99% of cellular RNA from nucleic acid extracts and a reversed-phase HPLC procedure that detects RNA contamination more sensitively than [5-^{3}H]uridine labeling. These methods are useful for the determination of analog incorporation into DNA, especially for agents which incorporate into both DNA and RNA. © 1997 Elsevier Science B.V.

Keywords: DNA; RNA

1. Introduction

Nucleoside and nucleobase analog antimetabolites are an important group of chemotherapeutic agents which have contributed significantly to the curative and palliative treatment of patients with hematopoietic malignancies and solid tumors [1–3]. This class of drugs includes compounds such as cytosine arabinoside, which is the single most effective agent for acute myelocytic leukemia [2,4], fludarabine which is used currently in patients with chronic lymphocytic leukemia [5], and thioguanine which is effective in treating childhood leukemias [1]. Antimetabolites also have demonstrated activity in solid tumors. Fluorouracil is included as standard therapy for the treatment of colorectal carcinoma [6,7], and a new agent, gemcitabine, has shown good activity in several solid tumor types [8]. Although these nucleoside and nucleobase analogs are metabolized through many different pathways, they share the common property of eliciting cytotoxicity by interfering with DNA synthesis. This can occur through several mechanisms, including alteration of DNA precursor levels, inhibition of replicative DNA polymerases or incorporation into DNA. DNA incorporation appears to be the key lesion leading to cell death for several analogs, including cytosine arabinoside [9], thioguanine [10] and gemcitabine [11]. Conse-

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quently, investigators are interested in quantitating the extent and location of nucleotide analog incorporation into DNA, as well as evaluating the importance of DNA incorporation to the cytotoxic mechanism of newly developed analogs.

For studies analyzing the relationship between nucleotide analog incorporation in DNA and antitumor activity, DNA preparations must be substantially free of RNA to ensure that the analog has been incorporated into DNA and not RNA. This is particularly important for agents such as thioguanine [10] and gemcitabine [12] which have been shown to incorporate into both DNA and RNA. Based on chemical differences between DNA and RNA, there are several straightforward techniques to separate these two species of nucleic acids. For example, cesium sulfate or cesium chloride density gradient centrifugation can isolate DNA from RNA based on their different buoyant densities [9,13]. A more rapid and commonly used technique selectively degrades RNA in the nucleic acid fraction by the addition of RNase A. However, HPLC analysis of nucleic acid digests in this laboratory have demonstrated that degradation of RNA with RNase A is incomplete, leaving as much as 10% of the undegraded RNA in the DNA fraction. Clearly, for analogs that incorporate into both RNA and DNA, this level of RNA contamination in a DNA preparation is unacceptably high. Here we report an improved technique to remove greater than 99% percent of total RNA with a combination of RNase A and RNase T1 and a reversed-phase HPLC procedure to sensitively measure the purity of the resulting DNA preparation. These methods are useful for studies on nucleotide analog incorporation into DNA, especially for agents which incorporate into both DNA and RNA.

2. Experimental

2.1. Chemicals

[Methyl-^{3}H]thymidine (64 Ci/mmol) was purchased from ICN Biomedicals (Irvine, CA, USA). [5-^{3}H]Uridine (20.0 Ci/mmol) and [2,8-^{3}H]2′-deoxyadenosine (29.9 Ci/mmol) were purchased from Moravek Biochemicals (Brea, CA, USA). Other nucleoside and nucleotide standards and EHNA [14] were obtained from Sigma (St. Louis, MO, USA). HPLC-grade ammonium acetate was purchased from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals were of the highest purity available.

2.2. Enzymes

RNase A, EC 3.1.27.5 (50 units/mg); spleen phosphodiesterease, EC 3.1.16.1 (2 U/mg); alkaline phosphatase from calf intestine, EC 3.1.3.1 (2000 U/mg); proteinase K, EC 3.4.21.14 (20 units/mg); and micrococcal nuclease, EC 3.1.31.1 (15 000 units/mg) were obtained from Boehringer Mannheim (Indianapolis, IN, USA). Micrococcal nuclease (100–200 μmol/mg protein) was also obtained from Sigma. RNase T1, EC 3.1.27.3 (4618 U/μg) was obtained from Gibco-BRL (Grand Island, NY, USA).

2.3. Cell culture condition

The C6BSTK rat glioma cell line was a gift from Dr. Xandra O. Breakefield, Harvard Medical School. C6BSTK cells lack mammalian thymidine kinase and stably express the herpes simplex virus thymidine kinase gene [15]. C6BSTK cells were maintained in exponential growth in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum plus 100 U/ml penicillin, and 100 μg/ml streptomycin.

2.4. Radiolabeling of nucleic acid

Between 5×10^6 and 1×10^7 C6BSTK cells in exponential growth were incubated with [methyl-^{3}H]thymidine (2 μCi/ml medium) or [5-^{3}H]uridine (3–5 μCi/ml medium). Subsequently, the tritium-containing medium was removed and the cells were rinsed twice with PBS, trypsinized (0.25%) and resuspended in fresh medium. An aliquot of the well-dispersed cell dispersion was removed for estimation of cell number using an electronic particle counter (Coulter Electronics, Hialeah, FL, USA). The rest of the cell suspension was spun down at 250 *g* at 4°C for 5 min and the supernatant was removed from the cells. The cells were resuspended in 4°C PBS and spun down at 250 *g* at 4°C for 5 min. The PBS was removed and the cell pellet was stored at −70°C.

2.5. Isolation of nucleic acid

Nucleic acid was isolated from tritium-labeled cell pellets using standard techniques as previously described [16,17]. Briefly, each cell pellet was suspended in 500 μl 4°C Hirt lysis buffer (10 m*M* Tris, 1 m*M* EDTA, 0.01% SDS, pH 8.0), sheared by five to 10 passages through an 18-gauge needle and treated with 0.05 mg/ml proteinase K (20 units/mg) for 2 h at 37°C. Nucleic acid was then isolated by extraction with phenol (pH 7.9)–chloroform–isoamyl alcohol (25:24:1), followed by reextraction with chloroform–isoamyl alcohol (24:1) and then precipitated twice with two volumes 95% ethanol with a final concentration of 70 m*M* NaCl and 3.3 m*M* $MgCl_2$.

2.6. Removal of RNA from nucleic acid

The RNA and DNA fraction isolated as described above was pelleted, air-dried, and dissolved in 200–500 μl TE buffer (10 m*M* Tris, 1 m*M* EDTA, pH 8.0). A 200-μl aliquot containing 20–60 μg nucleic acid was treated with 50–5000 μg/ml pancreatic RNase A (50 units/mg, Boehringer Mannheim) alone or with 0.05–5.00 μg/ml RNase T1 (4618 U/μg, Gibco-BRL) and incubated at 37°C for 1 or 2 h. The DNA was precipitated twice with ethanol to ensure no carryover of digested RNA fragments. The DNA pellet was then resuspended in the appropriate digestion buffer, and an aliquot was removed for estimation of DNA concentration determined by absorbance at 260 nm.

2.7. Digestion of DNA to 3′-monophosphate nucleotides and terminal nucleosides

The purified DNA was digested to 3′-monophosphate nucleotides and nucleosides using a modification of previously published techniques [18–20]. Five to 25 μg DNA were dissolved in 200 μl 1 m*M* Tris, pH 9.5, and incubated with 5 m*M* $CaCl_2$ and 0.4 μg micrococcal nuclease (100–200 units/mg protein (Sigma) or 15 000 units/mg (Boehringer Mannheim)) for 1 h at 37°C. Then 0.4 μg micrococcal nuclease was added again and incubated for an additional h at 37°C. Next, the pH was adjusted to 6.5, 1 m*M* EDTA was added and the mixture was incubated with 50 μg of spleen phosphodiesterase (2 U/mg, Boehringer Mannheim) for 1 h at 37°C. This step was repeated two more times so that a total of 150 μg of spleen phosphodiesterase was added in three consecutive additions of 50 μg and incubated for 3 h total.

2.8. Degradation of nucleotides to nucleosides

The 3′-monophosphate nucleotides were degraded to their corresponding nucleosides with calf intestinal alkaline phosphatase (CIAP) as described [16]. The DNA was digested to nucleotides as described above except that 1 m*M* EDTA was omitted from the protocol. Then, 1 m*M* $ZnCl_2$ and 1 m*M* $MgCl_2$ were added and the pH was adjusted to 8.3. The mixture was incubated with 20 units of CIAP (2000 U/mg, Boehringer Mannheim) and 10 μ*M* EHNA for 1 h at 37°C.

2.9. Reversed-phase HPLC separation of nucleotides and nucleosides

The 3′-monophosphate nucleotides and nucleosides were analyzed using a HPLC system obtained from Waters (Milford, MA, USA) composed of two Model 501 pumps, a U6K injector module and a Model 996 photodiode array detector. The system was controlled by Millenium 2010 software operated on a Dell pentium computer equipped with a System Interface Module. Before injection, every sample (volume = 100–200 μl) was spun at 11 750 *g* for 5 min to remove particulate matter and the pH was adjusted to approximately 5.4 (to match elution buffers pH). Each sample was loaded on a Partisphere C18 column (123×4.6 mm I.D.) purchased from Whatman (Hillsboro, OR, USA) with 5 μm particle size and pore diameter of 85 Å. Samples were eluted at room temperature with 0.1 *M* ammonium acetate, pH 5.4, isocratically for 8 min followed by a linear gradient which added methanol–0.1 *M* ammonium acetate (pH 5.4) at a rate of 0.9% per min for 24 min. One-minute fractions were collected and assayed for radioactivity. Nucleotides and nucleosides were identified based on their ultraviolet spectrum over the range of 200–355 nm and elution with authentic standards.

Nucleotides and nucleosides in DNA digests were quantified by comparison of their peak areas with that of a known amount of the appropriate standard at wavelengths of 254 and 281 nm. The limit of detection was 0.01–0.05 nmol and the linearity range exceeded 0.01–50 nmol.

3. Results

3.1. Measurement of radiolabeled DNA and RNA after RNase A treatment

In order to quantitate the nucleic acid extraction efficiency and purity of the DNA, cells were incubated with [5-^{3}H]uridine or [methyl-^{3}H]thymidine for 10–30 min to label the RNA and DNA, respectively. Total nucleic acid was isolated with phenol/chloroform–isoamyl alcohol extraction and treated with 50 μg/ml RNase A. Following this protocol, nearly 90% of the [methyl-^{3}H]thymidine was recovered while 97% percent of the [5-^{3}H]uridine was removed. The low amount of [5-^{3}H]uridine in the final ethanol precipitation wash (0.14% of total) and in the DNA pellet (3.44% of total) indicated that ribonucleotides resulting from RNA digestion and free intracellular ribonucleotides were adequately removed (Table 1). These results suggested that the RNA was digested effectively and separated from the DNA preparation.

Table 1
Recovery of [methyl-^{3}H]thymidine or [5-^{3}H]uridine radioactivity in nucleic acid after RNase A treatment

	Percent total radioactivity	
	[Methyl-^{3}H]thymidine	[5-^{3}H]Uridine
Ethanol precipitation 1		
Supernatant	4.88±1.93	93.00±1.04
Ethanol wash	2.65±0.46	0.93±0.21
Ethanol precipitation 2		
Supernatant	2.36±0.77	2.49±1.51
Ethanol wash	1.58±0.59	0.14±0.04
DNA pellet	88.54±2.04	3.44±0.81

Nucleic acid labeled with [methyl-^{3}H]Thd or [5-^{3}H]Urd was extracted from C6BSTK cells with phenol/chloroform and precipitated twice with ethanol. At this time radioactivity measured in [methyl-^{3}H]Thd- and [5-^{3}H]Urd-labeled pellets was 11 160±756 and 3871±642 CPM/10^6 cells, respectively. Nucleic acid samples were then resuspended and treated with 50 μg/ml RNase A and DNA was precipitated twice with ethanol. Aliquots of the ethanol precipitation supernatants, ethanol washes and the final DNA pellet were assayed for radioactivity. Results are the means±SE of triplicate determinations.

3.2. Reversed-phase HPLC detection of RNA contamination in DNA preparations

The [methyl-^{3}H]thymidine-labeled DNA isolated by this procedure was digested to 3′-monophosphate nucleotides and terminal nucleosides with micrococcal nuclease and spleen phosphodiesterase as described in Section 2.7. Reversed-phase HPLC analysis showed that the DNA was digested primarily to the four internally incorporated 3′-monophosphate deoxynucleotides and that 97% of the [methyl-^{3}H]-thymidine radioactivity coeluted with thymidine 3′-monophosphate (Fig. 1A,B). The DNA digestion appeared to be complete since 98% of the radioactivity injected onto the column was recovered. Trace amounts of all four deoxynucleosides were also observed and 3% of the [methyl-^{3}H]thymidine radioactivity coeluted with thymidine. While the presence of low levels of deoxynucleosides would be expected from digestion of DNA isolated from exponentially growing cells, approximately two-thirds of the deoxynucleosides observed resulted from the dephosphorylation of nucleotides by contaminating phosphatase activity in the micrococcal nuclease stock solution. Contaminating enzyme activities were variable between micrococcal nuclease preparations from different suppliers, and the preparation (from Boehringer Mannheim) shown in Fig. 1 had the lowest amount of phosphatase activity.

In addition to the four 3′-monophosphate deoxynucleotides, four additional peaks were observed which corresponded to the 3′-monophosphates of CMP, AMP, UMP and GMP based on ultraviolet spectra and coelution times with authentic standards (Fig. 1A). The identification of the compounds was further confirmed when the ribonucleotides were converted with alkaline phosphatase treatment to their corresponding nucleosides, which allowed greater resolution by HPLC of the ribo and deoxy compounds (Fig. 2A). The level of ribonucleosides was one-fourth that of the deoxynucleosides (ratio of total ribonucleosides to total deoxynucleosides was

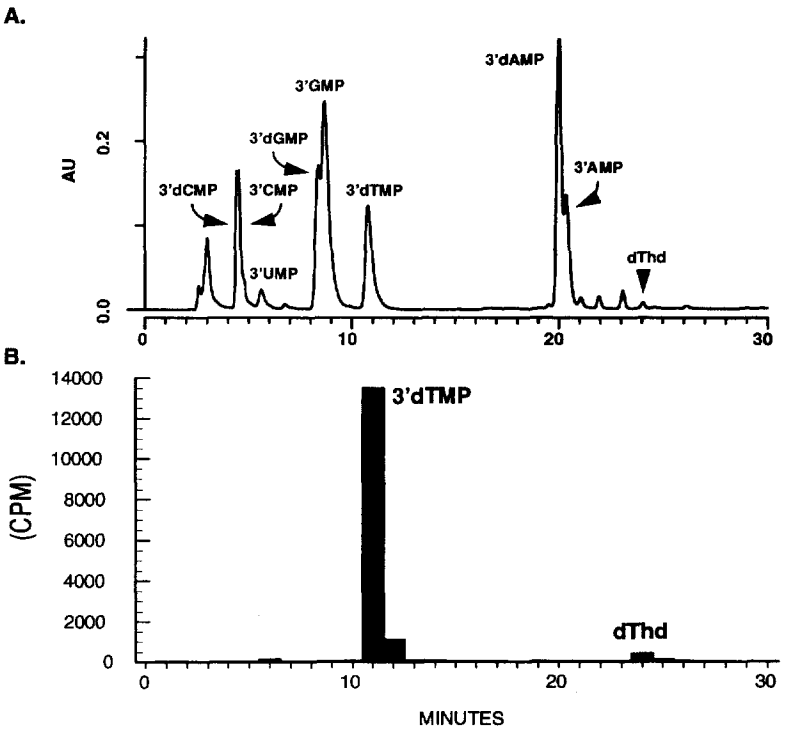

Fig. 1. Reversed-phase HPLC analysis of 3′-monophosphate nucleotides derived from [methyl-^{3}H]thymidine-labeled nucleic acid. Nucleic acid preparations were treated with 50 μg/ml RNase A and digested to nucleotides with micrococcal nuclease and spleen phosphodiesterase as described in Section 2. The chromatogram represents approximately 2 μg DNA at wavelength 254 nm.

0.248 as shown in Table 2). Of the ribonucleosides detected, guanosine levels were highest with a ratio of guanosine to deoxyguanosine of 0.683 (Table 2). The presence of inosine and deoxyinosine in Fig. 2A most likely resulted from deamination of adenosine and deoxyadenosine. This problem appeared to be due to contaminating adenosine deaminase activity in one of the enzyme preparations and was resolved in subsequent experiments by addition of the potent adenosine deaminase inhibitor, EHNA [14], to the digestion protocol.

The ribonucleotides detected in the DNA digest clearly coeluted with authentic 3′-monophosphates, and their elution times were distinctly different from the corresponding 5′-monophosphates (Fig. 1A). Therefore, the presence of the ribonucleotides was not due to the inadequate removal of free intracellular 5′-monophosphate ribonucleotides from the nucleic acid preparation. The appearance of high levels of guanosine 3′-monophosphate in the DNA digest suggested that the 3′-monophosphate ribonucleotides were not due to carryover of nucleotides from RNase A digestion. In that case, it would be expected that all four 3′-monophosphate ribonucleotides would appear in the DNA digest in roughly equal proportions. The 3′-monophosphate ribonucleotides most likely were derived from contamination of the DNA pellet with oligoribonucleotides which were subsequently digested by micrococcal nuclease and spleen phosphodiesterase.

Therefore, the more probable explanation for the presence of ribonucleotides in the DNA digest was incomplete degradation of RNA by 50 μg/ml RNase A. Consequently, we analyzed the effect of higher RNase A concentrations on ribonucleoside levels in nucleic acid preparations degraded to nucleosides (Table 2). A 10-fold increase in RNase A (500 μg/ml) reduced the ratio of total ribonucleosides to deoxynucleosides by less than 2-fold and consequently high levels of cytidine, adenosine and par-

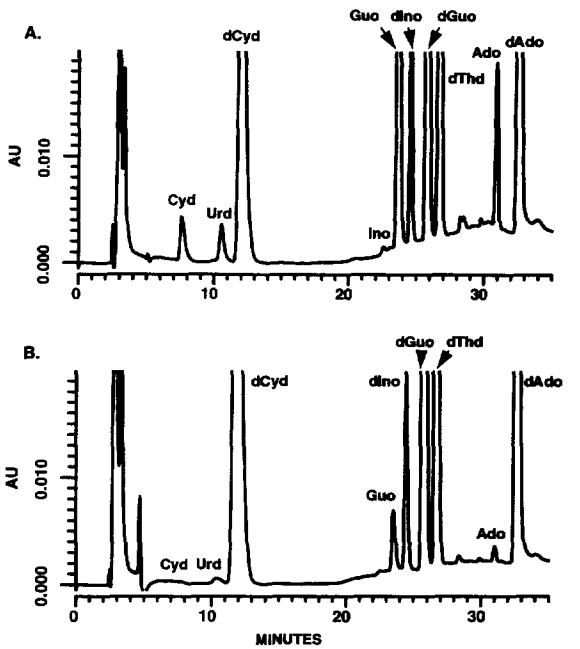

Fig. 2. Reversed-phase HPLC analysis of nucleosides derived from nucleic acid extracts treated with 500 μg/ml RNase A alone (A) and 500 μg/ml RNase A plus 0.05 μg/ml RNase T1 (B). Nucleic acid samples were degraded to nucleosides with micrococcal nuclease, spleen phosphodiesterase and alkaline phosphatase as described in Section 2. Each chromatogram represents approximately 3 μg DNA at wavelength 254 nm.

ticularly guanosine remained in the preparation. We did not observe any further decrease in ribonucleoside levels with an additional 10-fold increase in RNase A to 5000 μg/ml (data are from a single trial and not shown).

3.3. RNase T1 and RNase A removal of RNA contamination in DNA extracts

Since guanosine levels were particularly high in the nucleic acid digests, we hypothesized that the majority of the ribonucleotides derived from guanosine monophosphate-rich tracts of RNA that were resistant to RNase A degradation. RNase A may not be able to completely digest these regions since it is known to preferentially cleave RNA 3′ to pyrimidine monophosphate residues (UMP and CMP). [21,22] Cleavage of adenosine monophosphate diester bonds can also occur with RNase A although considerably less readily [23]. Consequently, RNase T1, a ribonuclease which preferentially cleaves RNA 3′ to guanosine monophosphate residues, was added to the protocol [21,24]. We analyzed the effect of various concentrations of RNase A and RNase T1 on ribonucleoside levels in nucleic acid digests. The combination of 500 μg/ml RNase A and 0.05 μg/ml RNase T1 resulted in a substantial decrease in total ribonucleoside levels as shown in Fig. 2 and Table 2. This treatment reduced the ratio of guanosine/deoxyguanosine 30-fold as compared to treatment with 50 μg/ml RNase A alone (Table 2). Only trace levels of the other three ribonucleosides were observed with ratios of ribonucleosides/deoxynucleosides no higher then 0.005 (Table 2). Increasing the amount of RNase T1 up to 5 μg/ml did not decrease ribonucleoside levels further. These findings were not limited to the C6BSTK cell line, since the addition of RNase T1 to the RNase A digestion protocol also substantially

Table 2
Ratio of ribonucleosides to deoxynucleosides in nucleic acid preparations treated with varying concentrations of RNase A and RNase T1 and subsequently digested to nucleosides

RNase A (μg/ml)	RNase T1 (μg/ml)	Adenosine/ deoxyadenosine	Uridine/ thymidine	Guanosine/ deoxyguanosine	Cytidine/ deoxycytidine	Total ribonucleosides/ deoxynucleosides
50	0	0.123±0.001	0.044±0.001	0.683±0.146	0.170±0.004	0.248±0.012
500	0	0.046±0.020	0.025±0.007	0.500±0.226	0.105±0.064	0.150±0.071
500	0.05	0.004±0.001	0.004±0.001	0.023±0.004	0.005±0.002	0.008±0.001
500	0.5	0.010±0.011	0.005±0.004	0.033±0.018	0.010±0.005	0.013±0.011
500	5	0.012±0.011	0.012±0.011	0.027±0.016	0.009±0.011	0.016±0.013

After RNase treatment, nucleic acid extracts were degraded to nucleosides with micrococcal nuclease, spleen phosphodiesterase and alkaline phosphatase. Ribonucleosides and deoxynucleosides were then separated by reversed-phase HPLC. Values represent the mean±S.D. of duplicates.

reduced RNA contamination observed in DNA isolated from U-251 human glioma cells (data not shown).

4. Discussion

To isolate DNA many investigators use RNase A alone to enzymatically remove RNA from nucleic acid preparations extracted from cultured cells. The results presented here demonstrated that degradation of RNA with RNase A was incomplete. Consequently, utilizing reversed-phase HPLC, we observed high levels of ribonucleotides in nucleic acid preparations digested with micrococcal nuclease and spleen phosphodiesterase. This level of RNA contamination is unsatisfactory for the study of nucleoside analog incorporation into DNA because many analogs such as thioguanine [10] are anabolized to metabolites that can be incorporated into both RNA and DNA. Also, contamination of a radiolabeled analog with low levels of a RNA precursor could result in substantial RNA labeling. For instance, we observed that treatment of U251 cells with [^{3}H]deoxyadenosine resulted in the cellular accumulation of over 30-fold higher levels of adenosine triphosphate compared to deoxyadenosine triphosphate, due to the presence of $<1.2\%$ [^{3}H]adenine. Therefore, to accurately quantitate analog incorporation into DNA, it is necessary to develop techniques that result in more complete separation of RNA from DNA.

While the specificities of RNase A for cleaving at pyrimidine residues has been known for some time [21,22,25], investigators have routinely used only RNase A to degrade RNA in nucleoside analog DNA incorporation studies, including work on gemcitabine [12], cytosine arabinoside [26,27], adenine arabinoside [20], chlorodeoxyadenosine [28], fluorouracil [29,30] and bromodeoxyuridine [17]. In addition, degradation of RNA using RNase A alone is considered a standard protocol for many molecular biology applications [13,16]. Based on typical ratios of RNA to DNA in mammalian cells ranging from 1.6 to 5, the data presented here indicated that as much as 10% of cellular RNA was resistant to RNase A cleavage. It appeared that the RNase A-resistant fragments of RNA were rich in guanosine monophosphate residues. These regions would be difficult for RNase A to degrade since it cleaves RNA primarily after pyrimidine monophosphate residues and to some extent after adenosine monophosphate residues [21–23]. Consequently, the addition of RNase T1, which preferentially cleaves RNA 3′ to guanosine monophosphate [21,24], to the RNase A digestion protocol resulted in the removal of greater than 99% of RNA from subsequent DNA digests.

Based on our results in two cell lines, it is likely that incomplete eradication of RNA by RNase A digestion alone is a frequent problem, but one that is not apparent by the commonly used method of [^{3}H]Urd labeling to track RNA. As shown here, [^{3}H]Urd labeling was inadequate to detect substantial purine ribonucleotide contamination in DNA preparations. A few isolated reports also indicate that removal of RNA was incomplete with RNase A treatment alone [31,32]. In the preparation of bacterial DNA plasmids, Skingle et al. reported substantial RNA fragment contamination after RNase A treatment [32]. Although the DNA plasmids appeared pure on standard 1% agarose electrophoresis minigels, Superose 6 chromatography revealed RNA levels as high as 96% of total nucleic acid [32]. Another group used a Sephacryl S-300 gel permeation technique to remove RNase A-resistant oligoribonucleotides from DNA to study the level of DNA methylation in cultured *Dances carota* plant cells [31]. While this method resulted in the preparation of highly purified DNA, small oligodeoxynucleotides may have been lost. This is undesirable in the study of nucleoside analog DNA incorporation, because fraudulent analogs may become incorporated into small fragments of DNA.

Other commonly used methods for separating RNA from DNA include cesium sulfate density gradient centrifugation which results in high-purity DNA [9,13]. However, the formation and analysis of gradients is time consuming, particularly for large numbers of samples. In comparison, the method described here is rapid and allows the investigator to assay many samples simultaneously.

Another significant finding described in this manuscript is that HPLC analysis more sensitively detects RNA contamination in DNA isolation protocols than [5-^{3}H]uridine labeling. While we observed that RNase A treatment of [5-^{3}H]uridine-labeled nucleic

acid eliminated 97% of radioactivity, HPLC analysis of the resulting DNA sample digested to nucleotides revealed high levels of all ribonucleotides, especially guanosine 3′-monophosphate. It appeared that the ribonucleotides derived from contaminating RNA fragments that escaped detection by the [5-^{3}H]uridine label method because of the large amount of unlabeled uridine monophosphate in the RNA. Therefore, the HPLC method we describe here which measures all four residues of RNA at levels as low as 10–50 pmol, provided a more sensitive determination of DNA purity.

In conclusion, our data show that a substantial percentage of RNA in cultured cell extracts is resistant to RNase A digestion. Consequently, we have developed an improved method, using a combination of RNase A and RNase T1, to remove RNA from nucleic acid isolated from mammalian cells. HPLC analysis of nucleic acid digests showed that this procedure degraded greater than 99% of cellular RNA, resulting in DNA preparations more suitable for studies on nucleoside analog incorporation into DNA.

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