Cancer Chemother Pharmacol (1989) 25: 37–44

Cancer Chemotherapy and Pharmacology

Effects of 5-fluorouracil on cytotoxicity and RNA metabolism in human colonic carcinoma cells

D. A. Greenhalgh* and J. H. Parish

Department of Biochemistry, University of Leeds, Leeds LS2 9JT, UK

Summary. The cytotoxicity of 5-fluorouracil (5-FU) is due in part to the incorporation of the base into RNA molecules. We assessed the cytotoxicity of 5-FU in human colonic carcinoma HT-29 cells and examined mRNA activity (measured by protein biosynthesis in vivo and in vitro) and the maturation of rRNA precursors as two possible modes of action of 5-FU. The rRNA processing pathways were studied using rDNA sequences as probes in blot hybridisation protocols and were specific for both the precursors and mature rRNA species of the maturation pathways. The conclusion from the studies was that although differences in mRNA activity were detected in vivo and in vitro, the significance of these changes are as yet unknown. In contrast, the effects on the pre-rRNA processing pathways proved to be highly significant cytotoxic consequences of 5-FU administration. We discuss the implications of this finding for an understanding of the mode of action of the drug and for the future monitoring of tumour sensitivity to 5-FU.

Introduction

5-Fluorouracil is used alone and in combination with other drugs as a chemotherapeutic agent in the treatment of solid tumours. Its mode of action has been extensively studied, and three mechanisms have been proposed: (1) inhibition of thymidylate synthase [13], (2) generation of DNA lesions as a result of incorporation into DNA [19, 20, 25, 28] and (3) cytotoxic consequences of incorporation into newly synthesized RNA molecules.

The first mechanism is supported by the observation that metabolic activation of 5-FU leads to a variety of derivatives, including 5-fluoro-2'-deoxyuridine-5'-monophosphate, a potent inhibitor of thymidylate synthase. In cells relying on de novo synthesis of thymine nucleotides, the inhibition is tantamount to inhibition of DNA synthesis. Mechanism 2 is believed to arise from the erroneous repair of DNA involving uracil-DNA glycosylase. The incorporation of 5-FU into RNA via the 5-fluorouridine-5'-triphosphate analogue (mechanism 3) may have several consequences. These include disruption of mRNA activity, which could potentially mediate phenotypic reversion [5, 27] and also cause mis-processing of RNA precursors, e.g. pre-rRNAs. The mode(s) of action of 5-FU on rRNA are unclear, although it is known that 5-FU affects the processing of pre-rRNA as judged by the incorporation of radiolabelled molecules into RNA precursors in vivo [7, 14, 15, 30, 33].

Human rRNA is composed of four species of molecules (5S, 5.8S, 18S and 28S), of which the last three are derived from a common 45S precursor in the nucleolus. Although there are a limited number of nuclease cleavage sites in 45S RNA, there are several alternative processing pathways that differ in the order in which the cuts are made [12]. The cleavage sites and the maturation pathways (excluding details of nucleoside modification) are summarised in Fig. 1.

The extent of 5-FU inhibition of rRNA processing is particularly important for two reasons: a rapid analysis of mis-processing could lead to a chemical pathological approach to the monitoring of the effectiveness of the drug in clinical cases. In addition, 5-FU is almost unique among antineoplastic agents in having RNA maturation as a metabolic target; therefore, a molecular understanding of the process might lead to a new generation of chemotherapeutic strategies. In this paper we assess effects of the drug on mRNA activity and developed a nucleic acid hybridisation method that enables the measurement of steady-state concentrations of rRNA-related sequences. This method does not rely on the more conventional in vivo isotope-incorporation assays, which are influenced by nucleotide pool sizes.

Materials and methods

Drugs and chemicals. Proteinase K and RNAse-free DNAse were obtained from Boehringer-Mannheim (Lewes, UK). Non-ionic detergent Nonidet (NP-40) and polyethylene glycol (PEG) 6000 were obtained from BDH (Poole, UK). The cell-culture reagents were purchased from Gibco (Paisley, UK), and other drugs and chemicals were obtained from Sigma Chemical Company (Poole, UK).

Cell culture. Human colonic tumour cell line HT-29 [9] was maintained at 37° C in an atmosphere comprising 5% CO_2: 95% air in Dulbecco's modified Eagle's minimal essential medium supplemented with 10% (v/v) foetal calf serum

* *Present address:* Department of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
Offprint requests to: J. H. Parish

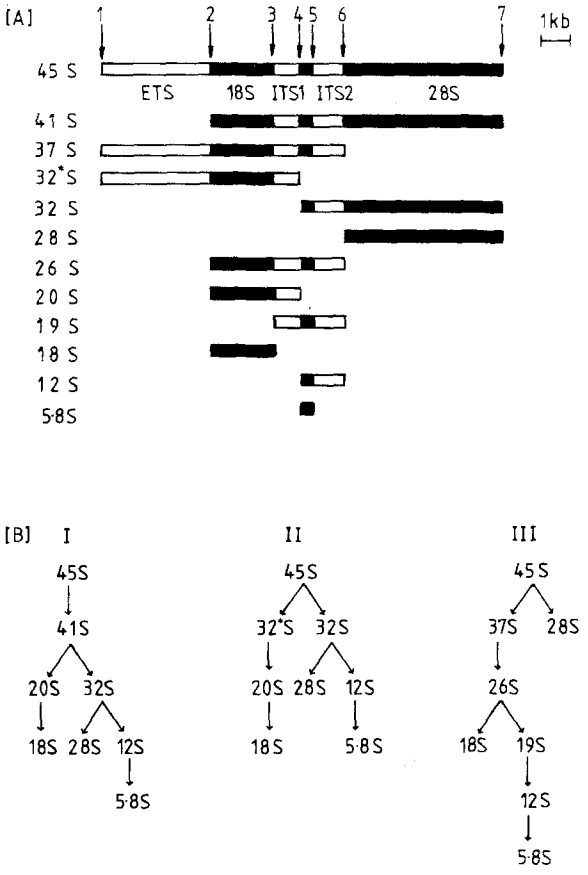

Fig. 1. Schematic representation of the rDNA transcriptional unit and potential pre-rRNA processing pathways. **A** rDNA transcriptional unit. Possible processing sites (1–7) and the sizes of the resultant RNA molecules are indicated. **B** rRNA maturation pathways deduced by Bowman et al. [2]

(FCS), 0.3% (v/v) $NaHCO_3$, 1 m*M* sodium pyruvate and 50 μg/ml gentamycin. Sub-confluent stock cultures were passaged after incubation with trypsin-EDTA and confirmed to be free of mycoplasmas by staining with Hoechst 33258 stain [6].

Cytotoxicity determination. Cell viability was assessed by a modification of the method of Bullen et al. [3]. After trypsinisation, the concentration of viable cells was determined using 0.25% (w/v) trypan blue dye in a counting chamber. Microtitre tissue-culture plates were seeded with 200 μl stock solution containing 1,000 viable cells/ml. Following incubation (2 days) and washing in PBS, drugs in complete medium were added. Each drug was added to six wells, and a further six untreated wells served as controls. The cells were washed with PBS and incubated in complete medium for 96 h. After washing, 100 μl solution containing 3.0 μCi/ml [^{3}H]-thymidine in medium supplemented with 0.5% (v/v) FCS was added and incubated for 18 h. The cells were washed, trypsin-EDTA was added and the cells were harvested on Whatman G/FC glass-fibre paper in an Ilacon cell-harvester manifold. Each disc was washed (twice for 30 s) with distilled water (2 l/min) and the radioactivity was determined by liquid scintillation counting. The values represent the mean of six counts for each drug and are expressed as a percentage of the mean control.

RNA isolation. mRNA was isolated from polysomal RNA as previously described [1]. Both nuclear and cytoplasmic RNAs were isolated from their resultant respective subcellular fractions. Cells grown on a 150-mm tissue-culture dish were harvested, washed in PBS and resuspended in lysis buffer containing 0.15 *M* NaCl, 10 m*M* TRIS-HCl (pH 7.8), 5 m*M* $MgCl_2$ and 0.65% (v/v) NP-40. After incubation on ice for 10 min, the mixture was vortexed for 10 s and the nuclei were pelleted by centrifugation at 500 *g* for 2 min at 4° C. The cytoplasmic fraction was removed and the nuclei were washed in fresh lysis buffer before reharvesting. The cytoplasmic fraction was centrifuged (10,000 *g*; 4° C; 10 min) and the supernatant was used to isolate cytoplasmic RNA.

To each 5 ml supernatant we added 5.5 g urea, 0.24 ml 0.5 *M* EDTA (pH 7.8), 0.69 ml 5 *M* NaCl and 2.4 ml 10% (w/v) sodium dodecyl sulphate (SDS) and the solution was extracted with phenol/chloroform/isoamyl alcohol (25:24:1 by vol.). The RNA was precipitated from the aqueous phase with ethanol and resuspended in water. Nuclear rRNA was isolated by a modification of the method of Vaessen et al. [32]. Nuclei were lysed by forcing the suspension through a 21-gauge needle in 3 *M* LiCl/6 *M* urea and incubated on ice for 16 h. The nucleic acids were pelleted by centrifugation at 12,000 *g* for 30 min at 4° C, washed with fresh LiCl/urea and rewashed with 70% (v/v) ethanol. The pellet was then resuspended in a solution containing 10 m*M* TRIS (pH 7.8), 5 m*M* EDTA, 0.5% (w/v) SDS and 200 μg/ml Proteinase K.

After a 2-h incubation at 37° C, the solution was phenol extracted and the nucleic acids were precipitated with ethanol. The nucleic acid pellet was resuspended in a buffer containing 5 m*M* $MgSO_4$, 0.1 *M* NaOAc (pH 5.0) and RNAse-free DNAse (20 units). The solution was incubated at 25° C until no longer viscous and extracted with the phenol mix. The RNAs were ethanol-precipitated, harvested by centrifugation and resuspended in 3 *M* NaOAc (pH 5.5). After incubation on ice for 2 h, the RNA was harvested by centrifugation and resuspended in sterile distilled water. The RNA was reprecipitated with ethanol, harvested, washed with 70% (v/v) ethanol and, finally, resuspended in sterile distilled water.

Blot hybridisation analysis. A 7-μg sample of RNA was fractionated by agarose gel electrophoresis using formaldehyde as a denaturant. The running buffer contained 20 m*M* morpholinopropane-sulphonic acid, 5 m*M* NaOAc and 1 m*M* EDTA (pH 7.0); other details of electrophoresis and subsequent transfer to nitrocellulose filters followed standard methods [22]. The filter was prehybridised [31] and then hybridised at 42° C for 24 h in fresh prehybridisation buffer containing 6% (w/v) PEG 6000 and a heat-denatured, [^{32}P]-labelled DNA probe prepared by the hexamer labelling method [8] for 24 h. The filter was washed twice for 30 min in 0.2X SSC, 0.1% (w/v) SDS at 42° C, followed by a 30-min wash in 0.1X SSC, 0.1% (w/v) SDS at 65° C, and exposed to X-ray film at −70° C using a DuPont Lightning Plus intensifying screen. For certain experiments, the blots were scanned using an LKB UltroScan XL scanning densitometer.

For slot blots, DNA (200 ng) was suspended in 50 μl distilled water and denatured for 10 min at 100° C, 50 μl 1 *M* NaOH was added and the solution was incubated at room temperature for 20 min. The samples were placed on

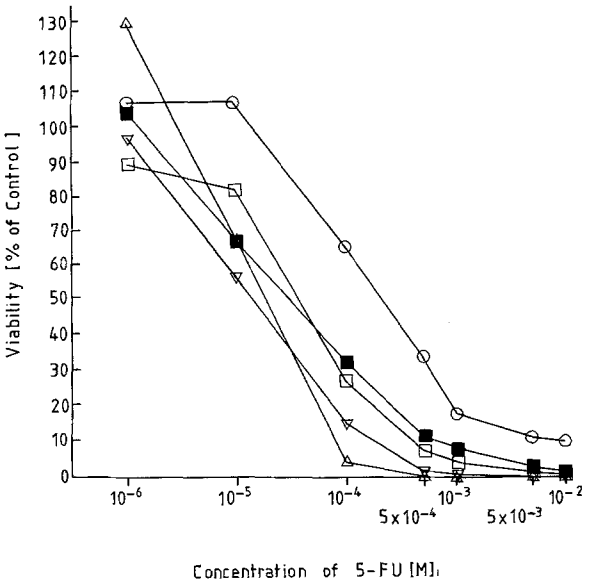

Fig. 2. Viability of HT-29 cells following exposure to 5-FU. The effects of different concentrations of 5-FU incubated for 3 (○——○), 6 (■——■), 12 (□——□), 24 (▽——▽), and 48 h (△——△) on cell viability determined as described in *Materials and methods.* Each value is the mean of 12 determinations and in every case the SEM was < 10% of the mean value

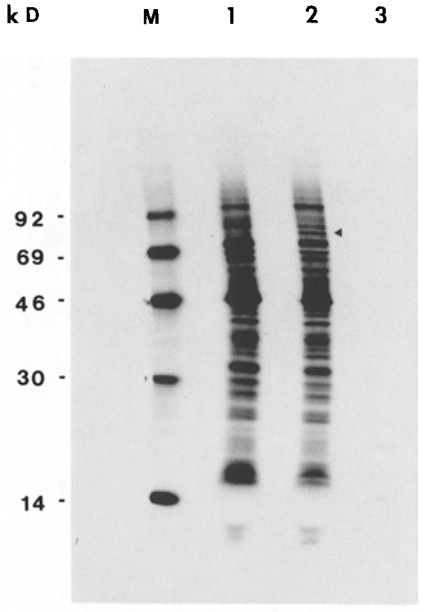

Fig. 3. One-dimensional analysis of [^{35}S]-labelled polypeptides synthesized in vitro. The mRNAs were translated in a rabbit reticulocyte lysate cell-free protein-synthesizing system in the presence of [^{35}S]-methionine. The resultant polypeptides were analysed using discontinuous 10%–15% (w/v) SDS-PAGE and detected by fluorography. *Lane 1,* polypeptides synthesized in vitro from untreated cells; *lane 2,* polypeptides synthesized in vitro from cells treated for 6 h with 100 μ*M* 5-FU; *lane 3,* endogenous translation products of the cell-free system; *lane M,* molecular weight markers (kDa)

ice and 400 μl neutralisation buffer containing 1.5 *M* NaCl, 0.15 *M* sodium citrate, 0.25 *M* TRIS-HCl (pH 8.0) and 0.25 *M* HCl was added. The samples were quickly bound to nitrocellulose wetted in 2X SSC in a slot-blot manifold and the filters were baked at 80° C for 2.5 h. The filters were prehybridised for 6 h at 65° C in buffer containing 6X SSC, 1X Denhardt's solution [22], 0.1% (w/v) SDS and 100 mg/ml heat-denatured salmon-sperm DNA, hybridised in fresh prehybridisation buffer containing [^{32}P]-labelled DNA probe at 65° C for 24 h and washed four times at 65° C in 0.1X SSC and 0.1% (w/v) SDS. The filter was subjected to autoradiography as before.

Polypeptide synthesis and analysis. Protein biosynthesis in vitro was carried out in a rabbit reticulocyte lysate cell-free system [26] containing HT-29 mRNA and [^{35}S]-labelled methionine. The newly synthesized polypeptides were separated by discontinuous 10%–15% (w/v) SDS-polyacrylamide gel electrophoresis (SDS-PAGE) [18] and two-dimensional isoelectric focussing (IEF/SDS-PAGE) [23]. Samples were prepared for IEF/SDS-PAGE according to Burland et al. [4] using the buffer system of Zurfluh and Guilfoyle [35]. Fluorography was carried out as previously described [18], and films were exposed at −70° C before photographic processing. Cells were metabolically labelled for 3 h in methionine-free medium supplemented with 30 μCi/ml [^{35}S]-methionine at 37° C. One aliquot was sonicated in lysis buffer [23] and another, in SDS-PAGE sample buffer [17]. Electrophoresis was carried out as described above.

Results

Effect of 5-FU on viability

The effect of 5-FU on the viability of the HT-29 cell line was dependent on both the concentration and exposure time of the drug. At 3 h exposure, toxic effects of 5-FU are apparent only at concentrations in excess of 10 μ*M*. For longer exposures, concentrations as low as 1 μ*M* are toxic (Fig. 2).

Disruption of RNA metabolism with 5-FU

When the polypeptides synthesized in vitro were fractionated by SDS-PAGE, a novel band with an apparent mol. wt. of 78 kDa appeared after 5-FU treatment (Fig. 3). Further analysis using two-dimensional gel electrophores is suggested that a polypeptide doublet (mol. wt. 29 kDa, pI 6.4) and a single polypeptide (mol.wt. 32 kDa, pI 5.3) present in the untreated cells did not appear following drug treatment (Fig. 4).

When the metabolically labelled polypeptides were separated using SDS-PAGE, no major differences were detected in the profiles derived from the untreated and 5-FU-treated cells (Fig. 5). However, high-resolution analysis using IEF/SDS-PAGE resolved several differences (marked on Fig. 6) between the untreated and 5-FU-treated cells. These included down-regulated polypeptides in a constellation of sizes (range, 44–70 kDa; pI range, 5.2–6.0) and a 54-kDa polypeptide with a pI of 6.9. Several of the polypeptides observed in these regions in control cells were not detected after drug treatment. Several up-regulated polypeptides, including those in the ranges 69–63 kDa, pI 5.9; 33 kDa, pI 6.9; 24-19 kDa, pI 5.3; 23-21 kDa, pI 4.8 and 21 kDa, pI 5.5, were also observed in the metabolically labelled cells after 5-FU treatment.

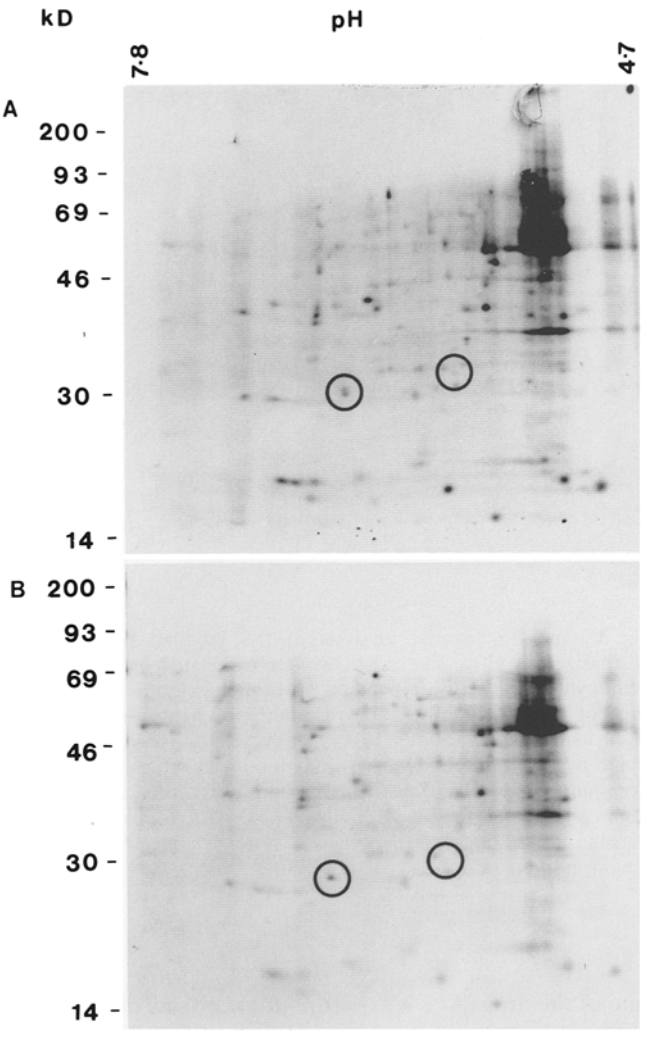

Fig. 4. Two-dimensional analysis of [^{35}S]-labelled polypeptides synthesized in vitro. Translations in vitro were carried out in rabbit reticulocyte lysate cell-free protein-synthesizing systems in the presence of [^{35}S]-methionine. The resultant polypeptides were fractionated by two-dimensional IEF/SDS-PAGE and detected by fluorography. The mol. wt. of protein standards are indicated. The mRNAs were extracted from **A** untreated cells, and **B** cells that had been incubated with 100 μM 5-FU for 6 h

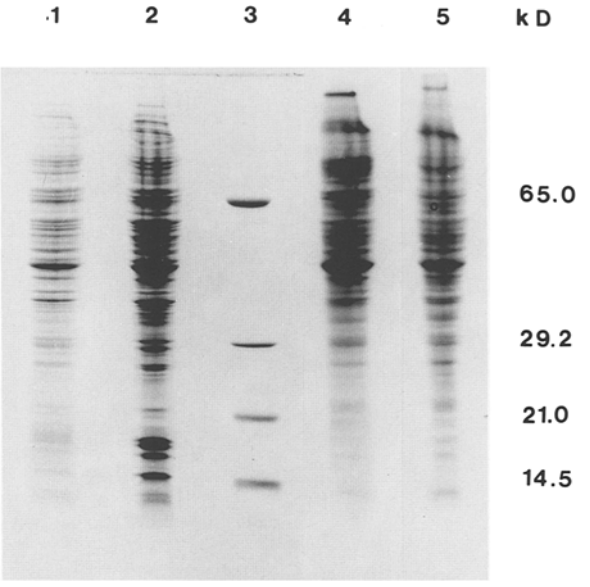

Fig. 5. One-dimensional analysis of [^{35}S]-labelled polypeptides synthesized in vivo. Metabolically labelled polypeptides were fractionated by discontinuous 10%–15% (w/v) SDS-PAGE. *Lanes 1 and 2* contain Coomassie blue-stained polypeptide profiles of untreated cells and cells that had been incubated for 6 h with 100 μM 5-FU, respectively. *Lane 3* contains protein standards with mol. wt. as indicated (kDa); *lanes 4 and 5* are fluorographs of lanes 1 and 2, respectively

Effect of 5-FU on rRNA metabolism

A novel approach was used to investigate the effect of 5-FU on intracellular rRNA processing. Blot hybridisations were applied to analyse the rRNA transcripts using rDNA subclones derived from the rDNA transcriptional unit (Fig. 7). B/SX and B/XE were derived from the clone pHr B/SE; B/XX, from the clone pHr B/SS; and A/EB and A/BB, from the clone pHr A. These were used to analyse the rRNA derived from both the nuclear and cytoplasmic fractions of HT-29 cells. Since the human rDNA transcriptional unit has not been completely sequenced, putative sequence homologies between the NTS and ITS regions may occur. In effect, the rDNA probes may also detect non-specific rRNA precursors. Homologies between the subclones were investigated and, although the labelled probes hybridised to their homologous sequences, no cross-hybridisations due to repeated sequences within the ETS and ITS regions were detected (Fig. 8), even in highly overexposed autoradiographs (data not shown).

The rRNA profiles obtained after hybridisation of the nuclear and cytoplasmic transcripts with the DNA probes mentioned above were in agreement with previous studies [2]. Under these conditions, no cross-species hybridisations between the rRNA-rDNA molecules were possible. Since several rRNA species produced by alternative maturation pathways comigrate during electrophoresis, different molecules of the same size are denoted either S or *S. B/XX should have hybridised to the 45S rRNA precursor but it also hybridised to 32S rRNA and 28S precursors (Fig. 9 A). These precursors are consistent with the molecules generated during pathways I, II and III, respectively, in Fig. 11. Hence, more than one rRNA processing pathway was identified in the HT-29 cell line.

The nuclear and cytoplasmic rRNA isolated from cells incubated for 6 h in the presence of different concentrations of 5-FU was blot-hybridised with probe B/XX followed by B/XE (homologous to pre-rRNA and mature rRNA transcripts, respectively). The probe B/XX revealed that a 6-h exposure of 5-FU disrupted pre-rRNA processing at concentrations exceeding 50 μM and, as the concentration increased, a pre-rRNA species in the region of approximately 36S appeared and became more prominent (Fig. 9 A). As predicted, the probe did not detect cytoplasmic molecules (even numbered tracks in Fig. 9 A) and its also demonstrated that no nuclear rRNA processing intermediates were exported from the nucleus into the cytoplasm. When the same nitrocellulose filter was probed with B/XE, the nuclear profile revealed that increasing concentrations of 5-FU induced a significant decrease in the amounts of 20S and 18S RNA (Fig. 9 B). These data

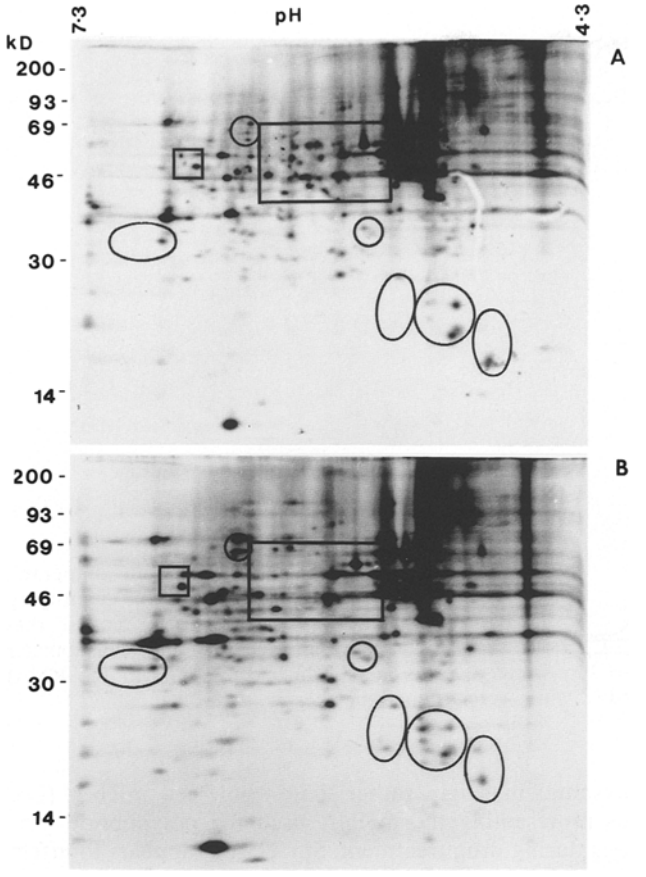

Fig. 6. Two-dimensional analysis of [^{35}S]-labelled polypeptides synthesized in vivo. Metabolically labelled polypeptides were fractionated by two-dimensional IEF/SDS-PAGE and detected by fluorography (mol. wt. of protein standards are indicated). Polypeptides believed to be down-regulated by 5-FU treatment are marked with *boxes*, whereas those believed to be up-regulated are marked with *circles*. The polypeptides synthesized in vivo were extracted from **A** untreated cells, and **B** cells that had been incubated for 6 h in 100 μ*M* 5-FU

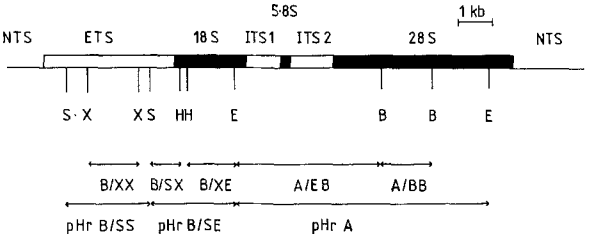

Fig. 7. Simplified restriction map of the rDNA transcriptional unit, including the regions of rDNA used in this work [21]. *Vertical lines* identify restriction sites B Bam H1, E Eco R1, S Sal 1, H Xba 1, and X Xho 1. NTS, ETS, and ITS correspond to the non-transcribed and external and internal transcribed spacers, respectively

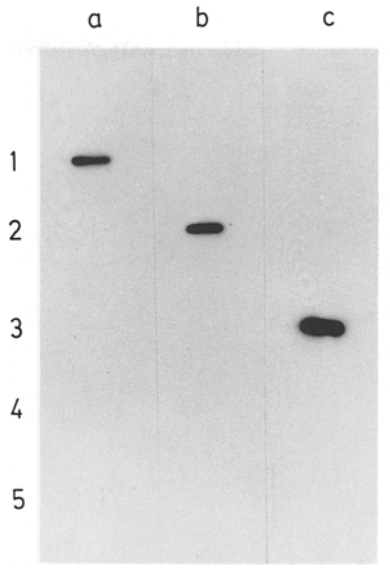

Fig. 8. Cross-hybridisation analysis of the sequences derived from the rDNA transcriptional unit. rDNA from regions A/EB (*1*), A/BB (*2*), B/XX (*3*), B/SX (*4*), and B/XE (*5*) were immobilised on nitrocellulose. The filters were blot-hybridised with [^{32}P]-labelled A/EB (*a*), A/BB (*b*), and B/XX (*c*) and the radiolabel was detected by autoradiography

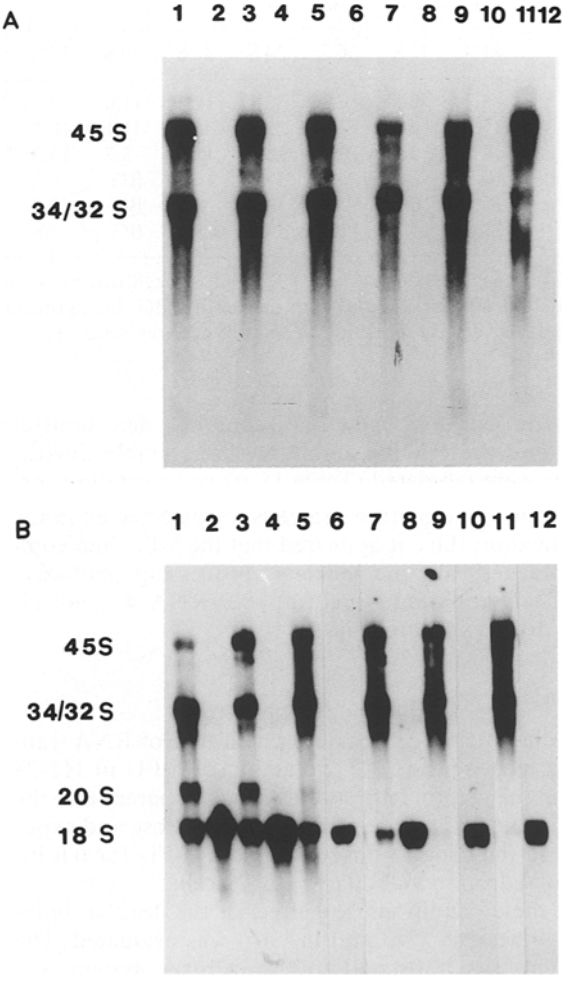

Fig. 9. Blot-hybridsation analysis of pre-rRNA processing during 5-FU treatment. RNA was purified from the nuclear and cytoplasmic fractions of the HT-29 cells and the fractionated RNA was transferred to nitrocellulose and blot-hybridised with probe B/XX **(A)**. The filter was stripped and then hybridised with the probe B/XE **(B)**. *Lanes 1, 3, 5, 7, 9, and 11* contain nuclear RNAs isolated from untreated cells and cells treated for 6 h with 10, 50, 100, 500, and 1000 μ*M* 5-FU, respectively. *Lanes 2, 4, 6, 8, 10, and 12*, respectively, correspond to the cytoplasmic RNAs isolated after these treatments

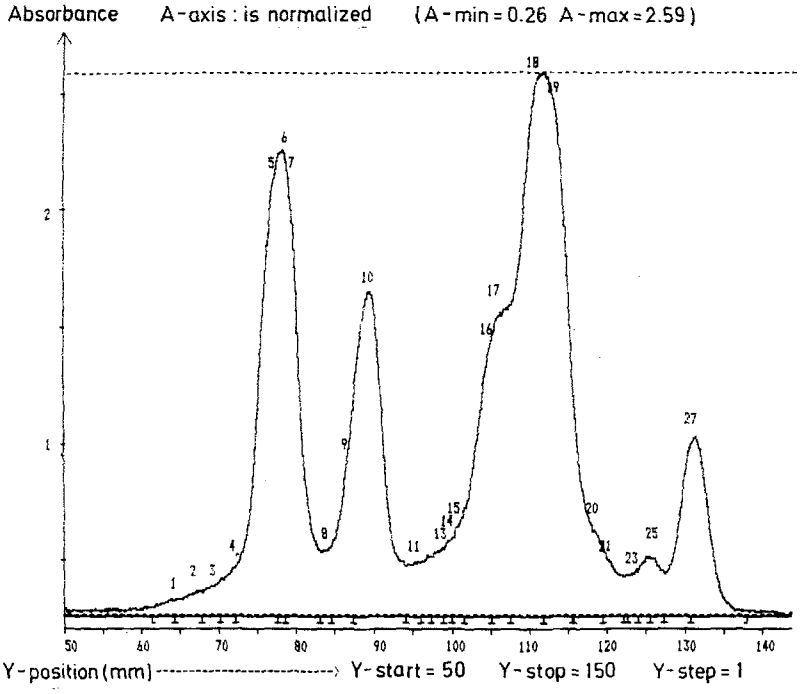

Fig. 10. Example of a scan of an autograph (Fig. 9B, *lane 1*). In using such data for quantifying RNA complementary to the probe, "peaks" that were artefactually resolved by the densitometer (e.g., *5, 6, and 7*, which correspond to 18S RNA) were grouped together

Table 1. Effect of increasing concentrations of 5-FU on the accumulation of rRNA species and precursors in nuclear extracts of *HT-29 cells*

5-FU concentration (μM)	Integrated peak size (%)						
	45 S	41 S	36 S	34 S	32 S	20 S	18 S
0	3.7	0.8	BG	30.1	12.1	11.0	22.7
10	15.9	4.4	BG	23.9	6.9	11.1	10.5
50	14.5	3.5	BG	38.7	11.8	3.7	13.5
100	19.5	10.1	6.9	39.5	16.2	BG	4.7
500	15.7	10.6	5.4	33.2	25.5	BG	2.1
1,000	21.6	14.3	11.7	30.2	18.3	BG	0.6

The data (from Fig. 9B) do not total 100% across each row because we did not subtract background film darkening. BG, background (i.e., no peak was discernible and the absorbance was $<0.5\%$)

were quantified by using a scanning densitometer (Fig. 10), and the relative amounts of detectable nuclear transcripts were tabulated (Table 1). At concentrations exceeding 0.5 mM, no mature 18S rRNA was detected in the nuclear fraction; thus, it appeared that the 5-FU had completely blocked all the nuclear processing pathways (Fig. 9). The cytoplasmic (mature) 18S rRNA was not affected by 5-FU exposure (Fig. 9).

Discussion

These studies attempted to assess which type of RNA transcript was most sensitive to the action of 5-FU in HT-29 cells. From the cytotoxicity studies it was apparent that the toxic effect of 5-FU was related to both the dose and exposure time to the drug; a dose of 100 μM 5-FU for 6 h induced a 70% drop in viability in HT-29 cells.

Using these conditions, the effect of the drug on polypeptide synthesis in vivo and in vitro was evaluated. The mRNA that was translated in the cell-free system was polysomal in origin rather than whole-cell mRNA [11], thus representing the mRNA used for polypeptide synthesis during drug treatment. Since 5-FU appears to affect the translation of mRNA both in vivo and in vitro, our results do not agree with the concept that the drug does not induce mis-translational events during polypeptide biosynthesis [10]. In previous studies, analysis of the polypeptides synthesized in vitro revealed that the mRNA was degraded, making the conclusions drawn from such work tenuous. Significant differences were observed when the two-dimensional patterns of polypeptides synthesized in vivo were compared with those synthesized in vitro, suggesting the existence of co- and post-translational modifications in vivo. By combining two-dimensional gel electrophoresis and protein sequencing [16], we are currently investigating whether 5-FU down-regulates the same polypeptides synthesized in vivo and in vitro. From the data it should be possible to evaluate whether 5-FU affects the synthesis of particular target polypeptides or whether the effect is non-specific on all cellular mRNA transcripts.

rRNA is the most abundant form of cellular RNA and has a rapid rate of synthesis (rapidly growing mammalian cells can produce 5,000 primary pre-rRNA molecules per minute [11]). It is therefore probable that a large quantity of 5-FU would be incorporated into newly synthesized rRNA transcripts, consequently inducing an observable disruption of pre-rRNA processing. In the presence of 5-FU, differences in rRNA metabolism have been observed using radiolabel-incorporation assays in vivo [7, 14, 15, 30, 33]. The results demonstrated a retention of label in the 45S precursor but are limited by the resolution of the gels and slicing methods. Another disadvantage of this method is that a pyrimidine xenobiotic such as 5-FU can in principle alter sizes of precursor pools, including those of the labelled compound [13].

The present experiments demonstrate that the processing of rRNA occurs by several pathways in the HT-29 cell line. These alternative pathways could not be identified by

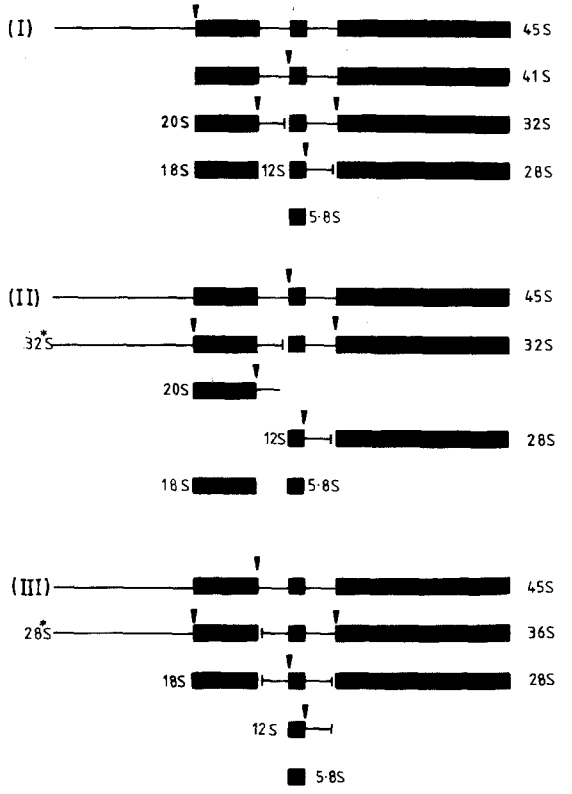

Fig. 11. Putative pre-rRNA processing pathways in HT-29 cells

previous in vivo labelling experiments. The presence of 5-FU was shown to induce changes in pre-RNA processing, detectable by blot hybridisations, while having no apparent effects on mature cytoplasmic rRNA. 5-FU apparently adversely affects rRNA precursor processing using the major maturation pathways by its incorporation into newly synthesized transcripts (Fig. 11, pathways I and II). Minor processing pathways also appear to become more significant in cells treated with 5-FU (Fig. 11, pathway III).

The results are consistent with effects previously observed on dihydrofolate reductase mRNA processing [34], where nuclear RNA processing was inhibited but steady-state levels of cytoplasmic mRNA were unaffected by 5-FU treatment. Such a common effect may be caused by a disruption of the small nuclear ribonucleoprotein "U particles", which are believed to be an integral requirement for both pre-rRNA and hnRNA processing [24]. Current studies are under way to determine the 5-FU-sensitive nuclease sites in pre-rRNA. We are also investigating whether the use of molecular adjuvants that are believed to enhance 5-FU incorporation into RNA [29] would also cause an increase in the disruption of rRNA precursor processing. These data could provide significant information for assessing whether nuclear RNA processing is a significant site of action of 5-FU.

Acknowledgements. We are grateful to B. E. H. Maden for providing the rDNA clones pHr A and pHr B/SE, G. N. Wilson for the clone pHr B/SS, and P. Higgins for valuable advice and help with the polypeptide biosynthesis experiments. D. A. G. was an SERC Research Student.

References

1. Bowles DJ, Hogg J, Small HE (1989) The effects of elicitor treatment on translatable mRNAs of carrot cells in suspension culture. Physiol Mol Plant Pathol 34: 463–470
2. Bowman LH, Rabin B, Schlessinger D (1981) Multiple ribosomal RNA cleavage pathways in mammalian cells. Nucleic Acids Res 9: 4951
3. Bullen BR, Russell CW, Giles GR (1978) A comparison of the chemosensitivity of murine tumours in vivo and in cell culture. Clin Oncol 4: 265
4. Burland TG, Gull K, Schedl T, Boston RS, Dove WF (1983) Cell type-dependent expression of tubulins in Physarum. J Cell Biol 97: 1852
5. Champe SP, Benzer S (1962) Reversal of mutant phenotypes by 5-fluorouracil: an approach to nucleotide sequences in messenger RNA. Proc Natl Acad Sci USA 48: 532
6. Chen TR (1977) In situ detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. Exp Cell Res 104: 255
7. Cohen MB, Glazer RI (1985) Cytotoxicity and the inhibition of ribosomal RNA processing in human colon carcinoma cells. Mol Pharmacol 27: 308
8. Feinberg AP, Vogelstein B (1984) A technique for radiolabelling DNA restriction endonuclease fragments to a high specific activity. Anal Biochem 137: 266
9. Fogh J, Trempe G (1975) New tumour cell lines. In: Human tumour cell lines in vitro. Plenum, New York, p 115
10. Glazer RI, Hartman KD (1983) In vitro translation of messenger RNA following exposure of human colon carcinoma cells in culture to 5-fluorouracil and 5-fluorouridine. Mol Pharmacol 23: 540
11. Hadjiolov AA (1985) Regulation. In: The nucleolus and ribosome biogenesis. Springer, New York, p 161
12. Hadjiolov AA, Hadjiolova KV (1979) The effect of 5-fluoropyrimidines on the processing of ribonucleic acids in liver. In: Antimetabolites in biochemistry, biology and medicine. Pergamon, New York, p 77
13. Heidelberger C, Danenberg PV, Moran RG (1983) Fluorinated pyrimidines and their nucleosides. Adv Enzymol 54: 57
14. Herrick D, Kufe DW (1984) Lethality associated with incorporation of 5-fluorouracil into ribosomal RNA. Mol Pharmacol 26: 135
15. Kanamaru R, Kakuta H, Sato T, Ishioka C, Wakui A (1986) The inhibitory effects of 5-fluorouracil on the metabolism of preribosomal and ribosomal RNA in L-1210 cells in vitro. Cancer Chemother Pharmacol 17: 43
16. Kennedy TE, Wager-Smith K, Barzilai A, Kandel Er, Sweatt JD (1988) Sequencing of proteins from acrylamide gels. Nature 227: 499
17. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680
18. Laskey RA, Mills AD (1975) Quantitative film detection of [3H] and [^{14}C] in polyacrylamide gels by fluorography. Eur J Biochem 56: 335
19. Lonn U, Lonn S (1986) DNA lesions in human neoplastic cells and cytotoxicity of 5-fluoropyrimidines. Cancer Res 46: 3866
20. Lonn U, Lonn S (1988) Increased growth inhibition and DNA lesions in human colon adenocarcinoma cells treated with methotrexate or 5-fluoro-deoxyuridine followed by calmodulin inhibitors. Cancer Res 48: 3319
21. Maden BEH, Dent CL, Farrell T, Garde J, McCallum FS, Wakeman J (1987) Clones of human ribosomal DNA containing the complete 18S and 28S genes. Biochem J 246: 519
22. Maniatis T, Fritsch EF, Sambrook J (1982) Molcular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
23. O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250: 4007

24. Parker KA, Steitz JA (1987) Structural analyses of the human U3 ribonucleoprotein particle reveal a conserved sequence available for base pairing with pre-rRNA. Mol Cell Biol 7: 2899
25. Parker WB, Kennedy KA, Klubes P (1987) Dissociation of 5-fluorouracil-induced DNA fragmentation from either its incorporation into DNA or its cytotoxicity in murine T-lymphoma (S-49). Cancer Res 47: 979
26. Pelham RB, Jackson RJ (1976) An efficient mRNA-dependent translation system from reticulocyte lysates. Eur J Biochem 67: 247
27. Rosen B, Rothman F, Weigert MG (1969) Miscoding caused by 5-fluorouracil. J Mol Biol 44: 363
28. Sawyer RC, Stolfi RL, Martin DS, Spiegelman S (1984) Incorporation of 5-fluorouracil in murine bone marrow DNA in vivo. Cancer Res 44: 1847
29. Spiegelman S, Sawyer R, Nayak R, Ritzi E, Stolfi R, Martin D (1980) Improving the anti-tumor activity of 5-fluorouracil by increasing its incorporation into RNA via metabolic modulation. Proc Natl Acad Sci USA 77: 4966
30. Takimoto CH, Tan YY, Cadman EC, Armstrong RD (1987) Correlation between ribosomal RNA production and RNA-directed fluoropyrimidine cytotoxicity. Biochem Pharmacol 36: 19
31. Thomas PS (1980) Hybridisation of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA 77: 5201
32. Vaessen RTMJ, Houweling A, Eb AJ van der (1987) Posttranscriptional control of class 1 MHC mRNA expression in adenovirus 12-transformed cells. Science 235: 1486
33. Wilkinson Ds, Pitot HC (1973) Inhibition of ribosomal ribonucleic acid maturation in Novikoff hepatoma cells by 5-fluorouracil and 5-fluorouridine. J Biol Chem 248: 63
34. Will CL, Dolnick BJ (1986) 5-Fluorouracil augmentation of dihydrofolate reductase gene transcripts containing intervening sequences in methotrexate resistant KB cells. Mol Pharmacol 29: 643
35. Zurfluh LL, Guilfoyle TJ (1982) Auxin- and ethylene-induced changes in the population of translatable messenger RNA in basal sections and intact soybean hypocotyl. Plant Physiol 69: 338

Received 23 August 1988/Accepted 12 May 1989