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Queuine, a tRNA anticodon wobble base, maintains the proliferative and pluripotent potential of HL-60 cells in the presence of the differentiating agent 6-thioguanine

(c-*myc*/c-*fms*/phorbol 12-myristate 13-acetate)

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ABSTRACT **6-Thioguanine (6-TG)-induced differentiation of hypoxanthine phosphoribosyltransferase (IMP: pyrophosphate phosphoribosyltransferase, EC 2.4.2.8)-deficient HL-60 cells is characterized by 2 days of growth, after which morphological differentiation proceeds. Addition of the tRNA wobble base queuine, in the presence of 6-TG, maintains the proliferative capability of the cells. The ability of 6-TG to induce differentiation correlates with c-*myc* mRNA down-regulation, but queuine has no effect on this parameter. Treatment with 6-TG for 2–3 days commits HL-60 cells to granulocytic differentiation, and, once committed, these cells do not respond to the monocytic inducer phorbol 12-myristate 13-acetate. Nonetheless, when cells are treated with queuine and 6-TG, they maintain the promyelocytic morphology and are capable of being induced down the monocytic pathway by phorbol 12-myristate 13-acetate as indicated by stabilization of c-*fms* mRNA and cell adherence. In the absence of queuine, phorbol 12-myristate 13-acetate is incapable of inducing monocytic markers in the 6-TG-treated cells. The data presented indicate that 6-TG-induced differentiation of HL-60 cells is a tRNA-facilitated event and that the tRNA wobble base queuine is capable of maintaining both the proliferative and pluripotent potential of the cells.**

Human promyelocytic HL-60 cells offer a useful model for studying the interrelationship between maintenance of the cell proliferative state and terminal differentiation. HL-60 cells are pluripotent and can be induced to differentiate with a variety of chemically diverse reagents (1–3). These cells can be induced down the granulocytic pathway or the monocytic–macrophage pathway depending on the specific agent used. Dimethyl sulfoxide and retinoic acid induce granulocytic maturation of HL-60 cells. These compounds induce the rapid down-regulation of c-*myc* mRNA (4), which may be a requirement for the commitment to granulocytic differentiation (5). Phorbol 12-myristate 13-acetate (PMA) is a potent inducer of monocytic–macrophage differentiation in these cells (6, 7). PMA induction of monocytic–macrophage differentiation in HL-60 cells is characterized by increased cellular adherence and the stabilization of the c-*fms* mRNA coding for the colony-stimulating factor 1 (CSF-1) receptor (8).

Interestingly, HL-60 cell lines that are deficient in the purine salvage enzyme hypoxanthine phosphoribosyltransferase (HPRT; IMP: pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) can be induced to differentiate with purine antimetabolites without metabolic activation (3, 9). The antimetabolite 6-thioguanine (6-TG) is an especially effective inducer of granulocytic differentiation in HPRT-deficient HL-60 cells (3, 10, 11).

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A possible link between incorporation of 6-TG into the wobble position of specific tRNA isoacceptors and the induction of granulocytic differentiation in HL-60 cells has been suggested (12). The results indicated that 6-TG functions as a substrate for queuine tRNA-ribosyltransferase (tRNA-GRT; tRNA-guanine:queuine tRNA ribosyltransferase, EC 2.4.2.29) in HL-60 cells and is actively incorporated into the wobble position of the histidine tRNA isoacceptor. However, these results did not indicate whether it was 6-TG incorporation or hypomodification for queuine that was involved in cell differentiation.

The enzyme tRNA-GRT normally catalyzes the insertion of queuine {7-[(3*S*,4*R*,5*S*)-4,5-dihydro-2-cyclopent-1-en-3-ylaminomethyl]-7-deazaguanine} in the wobble position of histidine, tyrosine, asparagine, and aspartic acid tRNA isoacceptors (13, 14). This insertion of queuine into the wobble position of the tRNA occurs by a base-exchange mechanism whereby guanine is removed and queuine is inserted without breaking the phosphodiester linkage of the tRNA macromolecule (13, 15). A unique characteristic of tRNA-GRT is its ability to exchange guanine (or 6-TG) for guanine within the wobble position of tRNA isoacceptors normally containing queuine. However, queuine insertion is irreversible (see Fig. 1).

We demonstrate here that addition of the tRNA wobble base queuine prevents 6-TG-induced growth inhibition, thereby delaying commitment to granulocytic differentiation. Blocking of differentiation occurs only when the cells are in logarithmic growth. Once proliferation ceases due to the attainment of saturation density, commitment to differentiation begins. We observed no early effect of queuine on precommitment to differentiation as indicated by the down-regulation of c-*myc* mRNA, even though 6-TG effectively induced this down-regulation. However, queuine maintains the pluripotent potential of 6-TG-treated HL-60 cells as indicated by its ability to allow PMA-induced monocytic–macrophage differentiation.

MATERIALS AND METHODS

Cell Culture. HPRT-deficient HL-60 cells, provided by Linda F. Thompson (Scripps Clinic and Research Foundation, La Jolla, CA), were grown at 37°C in RPMI 1640 medium supplemented with 10% fetal bovine serum in a humidified atmosphere with 10% CO_2/90% air. Cells used for induction of differentiation were grown to late logarithmic phase and diluted to a concentration of 2×10^5 cells per ml. The cells were diluted into medium containing 400 μM 6-TG.

Abbreviations: 6-TG, 6-thioguanine; 8-AzaG, 8-azaguanine; tRNA-GRT, queuine tRNA-ribosyltransferase; HPRT, hypoxanthine phosphoribosyltransferase; PMA, phorbol 12-myristate 13-acetate; CSF, colony-stimulating factor.

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Queuine was purified from bovine amniotic fluid as described (15). Cells treated with queuine were pretreated 12 hr prior to the induction of differentiation at a final concentration of 1 μM. A final concentration of 1 μM queuine was added every 24 hr thereafter during the course of the experiments. Cellular adherence to the culture vessel was analyzed after the addition of PMA in RPMI 1640 medium at a final concentration of 0.2 μM for 24 hr (6, 7).

Cells stained for morphological differentiation were collected on glass slides by cytocentrifugation for 10 min at 600 rpm in a Shandon-Southern Cytospin. The cells were then stained with Wright–Giemsa stain.

Evaluation of purine analogs as inhibitors of [^{3}H]dihydroqueuine incorporation into tRNA in HL-60 cells was as described by Muralidhar *et al.* (16) and modified by Gibboney *et al.* (17).

RNA Isolation and Northern Blot Analysis. Total RNA was isolated by the guanidinium-isothiocyanate CsCl method (18). After denaturation, the RNA was fractionated by electrophoresis on a 1.2% agarose/2.2 M formaldeyde/Mops gel (19). The RNA was then transferred to Nytran membranes (Schleicher & Schuell) in 10× SSPE buffer (20× SSPE is 3.6 M NaCl/0.2 M sodium phosphate, pH 7.7/20 mM EDTA). The membrane was baked for 2 hr at 80°C, wetted with 5× SSPE, and prehybridized for 2 hr at 42°C in 50% formamide/5× Denhardt's solution (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone)/5× SSPE/0.1% SDS/200 μg of sonicated denatured herring testes DNA. The bound RNA was hybridized against ^{32}P-labeled DNA overnight at 42°C in the hybridization buffer described above.

After hybridization, the blot was washed in 1× SSPE/0.1% buffer at 60°C and exposed overnight to Kodak XAR-5 film at −70°C.

pSV2cMyc was used to probe for c-*myc* expression and pSM3 was used to probe for c-*fms* expression. Both probes were obtained from the American Type Culture Collection.

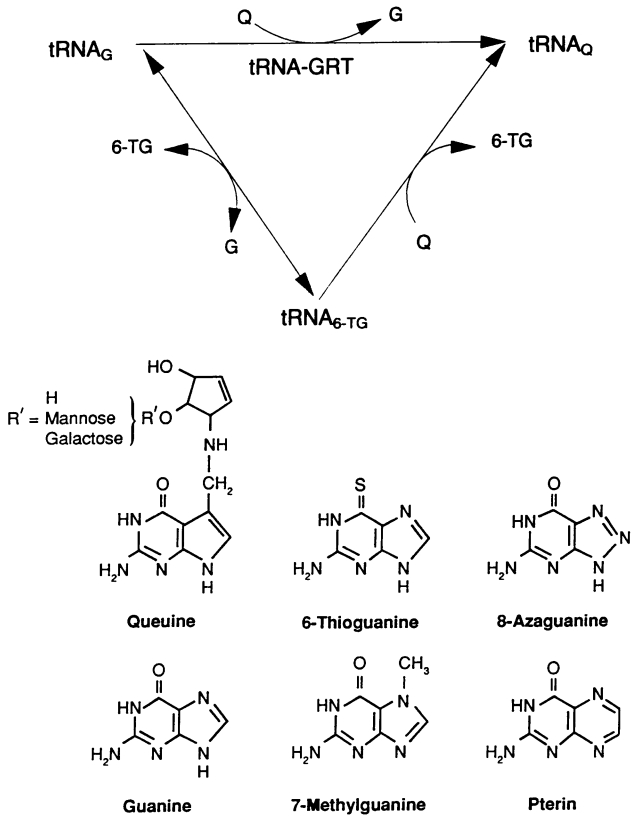

FIG. 1. General scheme of tRNA-GRT enzymatic activity and chemical structures of purine analogs that serve as substrates and/or inhibitors of the enzyme. Q, queuine. Double-headed arrows indicate reactions that occur bidirectionally. The nucleic acid base subscript indicates the base that is located in the wobble position of the tRNA anticodon. The R′ moiety on the cyclopentenyl ring is initially hydrogen when queuine is inserted into the wobble position. Queuine is then further modified in two tRNA isoacceptors, tyrosine and aspartic acid, to contain galactose and mannose, respectively.

RESULTS

Purine Analog Inhibition of tRNA-GRT. Two types of effects are known that modify the wobble position in queuine-containing tRNA isoacceptors; one in which the purine analog acts as a substrate for tRNA-GRT and the other in which the analog inhibits the ribosyltransferase activity but is not inserted into the tRNA macromolecule. We examined selected purine analogs (Fig. 1), at various concentrations, for their ability to inhibit incorporation of radiolabeled dihydroqueuine into HL-60 tRNA (Fig. 2). Guanine, 7-methylguanine, and pterin effectively inhibited incorporation into tRNA, leaving guanine in the wobble position of the tRNA. At a concentration extrapolated to 0.1 mM, these compounds inhibited radiolabel incorporation by 90%. 6-TG and 8-azaguanine (8-AzaG), substrates of tRNA-GRT, blocked incorporation by 70% and 90%, respectively, at the above concentration.

Purine Analog Effects on c-*myc* Down-Regulation. One common characteristic of HL-60 cell differentiation is the rapid down-regulation in c-*myc* transcripts. We examined the time course of 6-TG-induced down-regulation of c-*myc* mRNA in the HPRT-deficient HL-60 cell line. One hour after the addition of 6-TG, the level of the c-*myc* transcript had been reduced by >90% (Fig. 3*A*).

We next examined whether queuine hypomodification of the tRNA wobble position was sufficient to induce the down-regulation of c-*myc* mRNA. The various purine analogs known to alter queuine base modification (Fig. 2) were examined for their ability to induce c-*myc* down-regulation in HPRT-deficient HL-60 cells (Fig. 3*B*). 6-TG and 8-AzaG are two guanine analogs known to function as substrates, while the compounds 7-methylguanine and pterin are potent inhibitors of tRNA-GRT. Guanine functions as a substrate and is an inhibitor of queuine insertion. Of these compounds, only 6-TG and 8-AzaG inhibit logarithmic growth and are inducers

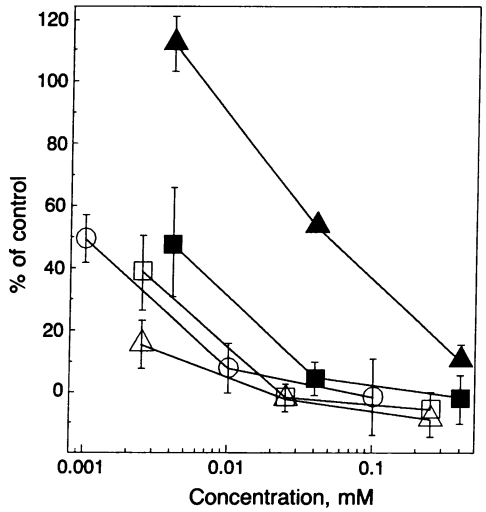

FIG. 2. Analog inhibition of [^{3}H]dihydroqueuine incorporation into tRNA by HPRT-deficient HL-60 cells. Cells were treated with various concentrations of 6-TG (▲), 8-AzaG (■), 7-MeG (△), pterin (□), or guanine (○). The mean of three independent experiments (±SD) is presented as the percentage incorporation of untreated control.

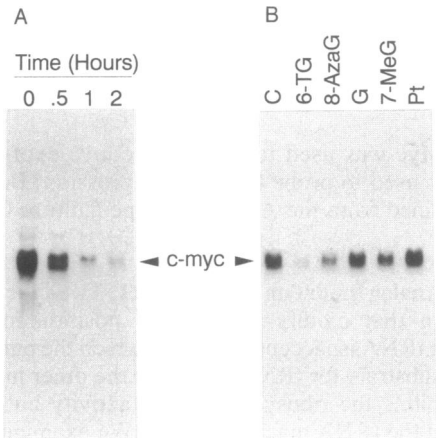

FIG. 3. Northern blot analyses of c-*myc* expression in HL-60 cells. (*A*) Time course of c-*myc* mRNA expression after treatment with 400 μM 6-TG. (*B*) Expression of c-*myc* mRNA after treatment with various purine analogs. Cells were treated for 4 hr, prior to the isolation of total mRNA, with the following analogs: C, control cells; 6-TG, 200 μM 6-TG; 8AzaG, 400 μM 8-AzaG; G, 100 μM guanine; 7-MeG, 250 μM 7-methylguanine; Pt, 250 μM pterin.

of granulocytic differentiation in HPRT-deficient HL-60 cells (data not shown). Northern blot analysis indicates that 6-TG and 8-AzaG were also the most effective analogs for inducing the down-regulation of c-*myc* mRNA. Guanine, 7-methylguanine, and pterin were not effective in down-regulating c-*myc* transcripts.

The possibility that queuine might be able to inhibit 6-TG-induced down-regulation of the c-*myc* message was then examined (Fig. 4). c-*myc* mRNA levels were monitored at various times after 6-TG induction of differentiation in the presence and absence of queuine. There was a rapid decrease in the level of c-*myc* transcript that was maintained over a 12-hr period. However, by 24 hr the level of c-*myc* mRNA was near control levels. The presence of queuine did not alter the effect of 6-TG on c-*myc* mRNA levels at any of the time points evaluated.

Queuine Prevents 6-TG-Induced Growth Inhibition. 6-TG inhibits proliferation of HPRT-deficient HL-60 cells ≈48 hr after initiation of granulocytic differentiation (Fig. 5). Daily addition of queuine blocked growth inhibition by 6-TG. The cells treated with a combination of 6-TG and queuine reached a saturation density near that of the control cells, while cells treated with 6-TG alone stopped proliferating after 48 hr.

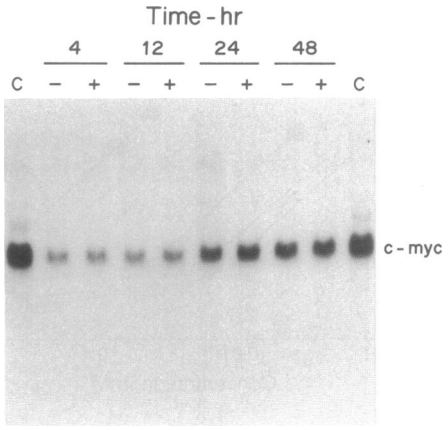

FIG. 4. Northern blot analysis of c-*myc* mRNA expression in HPRT-deficient HL-60 cells after treatment with 400 μM 6-TG in the presence or absence of 1 μM queuine. The presence or absence of queuine is indicated by the plus or minus sign, respectively. C, control.

Examination of morphological differentiation at various points along the growth curves in Fig. 5 indicates that addition of queuine delayed granulocytic differentiation until cell proliferation ceased (Fig. 6). At day 5, the cells treated with queuine were predominantly promyelocyte-like, whereas the cells treated with 6-TG alone were exhibiting characteristics of granulocytic morphology. By day 7, both the 6-TG-treated cells as well as the cells treated with 6-TG and queuine, exhibited characteristics of granulocytic differentiation. In the presence of 6-TG and queuine, once the late logarithmic or early stationary phase of cell growth was reached, the cells initiated morphological differentiation. Prior to this point, queuine blocked 6-TG-induced morphological differentiation.

Queuine Maintains the Pluripotence of HL-60 Cells in the Presence of 6-TG. HL-60 cells are pluripotent in that they can be induced down either the granulocytic or monocytic–macrophage pathway depending on the inducing agent. 6-TG is an effective inducer of granulocytic differentiation in these cells, while the phorbol ester PMA is a potent inducer of monocytic–macrophage differentiation. We monitored two characteristics of monocytic–macrophage differentiation induced by PMA; the first being the stabilization of the mRNA coding for the CSF receptor (c-*fms*) and the second being the ability of PMA to induce cellular adherence. When cells were treated with 6-TG for 2 hr in the presence or absence of queuine, PMA was unable to induce the stabilization of c-*fms* mRNA (Fig. 7*A*) and was not able to induce cellular adherence (data not shown). Because morphological differentiation by 6-TG was blocked by queuine over a 3- to 5-day period, we tested whether treatment of HPRT-deficient HL-60 cells with 6-TG for 3 days in the presence of queuine would maintain the ability to be stimulated to monocytic–macrophage differentiation by PMA. After 3 days of treatment with 6-TG in the presence or absence of queuine, PMA was added to the cells for 24 hr. In the absence of queuine, HL-60 cells treated for 3 days were not able to respond to PMA stimulation. However, the presence of queuine blocked granulocytic differentiation by 6-TG and thereby enabled PMA stabilization of c-*fms* (Fig. 7*B*) as well as PMA-induced cellular adherence (Fig. 7*C*). After continued treatment with PMA, the queuine-treated cells exhibited a morphology characteristic of monocytic–macrophage differentiation, whereas in the absence of queuine, the cells maintained a morphology characteristic of granulocytic differentiation (data not shown).

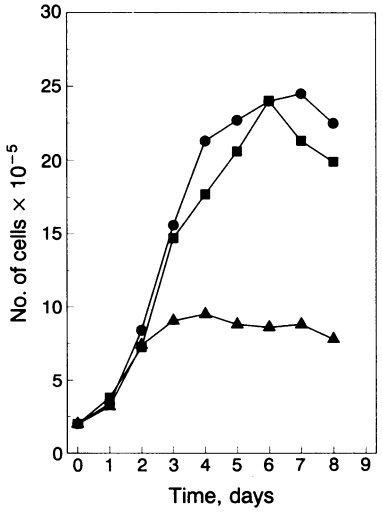

FIG. 5. Growth curves of HPRT-deficient HL-60 cells treated with 6-TG in the presence or absence of queuine. The cells were treated as follows: ●, untreated controls; ▲, 400 μM 6-TG; ■, 400 μM 6-TG plus 1 μM queuine. Queuine was added daily to a final concentration of 1 μM.

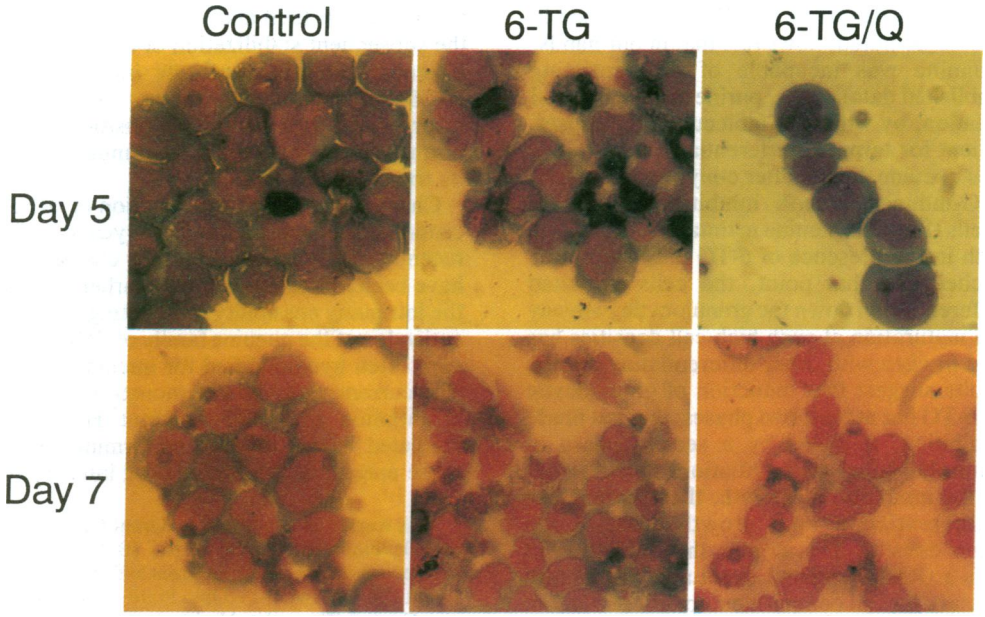

FIG. 6. Photomicrographs of HPRT-deficient HL-60 cells treated with 400 μM 6-TG in the presence or absence of 1 μM queuine (Q). Cells were seeded at 2×10^5 cells per ml. Cells treated with queuine were pretreated 12 hr before 6-TG treatment. Queuine was added every 24 hr thereafter at a final concentration of 1 μM. Cells were stained with Wright–Giemsa stain on days 5 and 7 after 6-TG treatment.

DISCUSSION

The observation that 6-TG is capable of being incorporated into the wobble position of queuine-containing tRNA isoacceptors suggested an unusual mechanism by which nucleic acid base analogs might induce cellular differentiation (12). Incorporation of 6-TG into the wobble position is related to the fact that tRNA-GRT actively exchanges guanine for guanine (14). 6-TG is capable of substituting in this exchange reaction. However, the base exchange of queuine for guanine in the wobble position is unidirectional and not reversible (14).

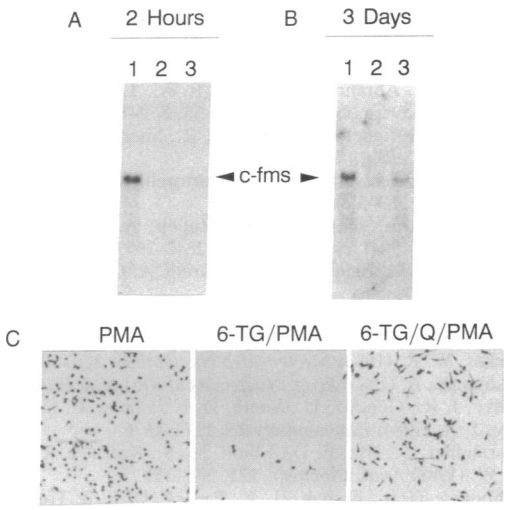

FIG. 7. (*A*) Northern blot analysis of c-*fms* mRNA expression after 2-hr treatment of HL-60 cells with 6-TG followed by treatment with PMA for 24 hr in the presence or absence of queuine. Lanes: 1, control HPRT-deficient HL-60 cells treated with 0.2 μM PMA for 24 hr; 2, cells treated 2 hr with 400 μM 6-TG followed by treatment with 0.2 μM PMA for 24 hr; 3, cells treated 2 hr with 400 μM 6-TG followed by treatment with 0.2 μM PMA for 24 hr in the presence of 1 μM queuine. (*B*) Northern blot analysis of c-*fms* mRNA expression after 3-day treatment of HL-60 cells with 6-TG followed by PMA for 24 hr in the continued presence or absence of queuine. Lanes: 1, control HPRT-deficient HL-60 cells treated with 0.2 μM PMA for 24 hr; 2, cells treated for 3 days with 400 μM 6-TG followed by treatment with 0.2 μM PMA for 24 hr; 3, cells treated for 3 days with 400 μM 6-TG followed by treatment with 0.2 μM PMA for 24 hr in the presence of 1 μM queuine added daily. (*C*) Photomicrographs of Wright–Giemsa-stained HPRT-deficient HL-60 cells treated for 3 days with 400 μM 6-TG followed by 0.2 μM PMA for 24 hr in the presence or absence of 1 μM queuine (Q). Queuine was added at 24-hr intervals over the course of the experiment.

A variety of queuine structural analogs (Fig. 1) known to serve as substrates and/or inhibitors of tRNA-GRT *in vitro* (20, 21) were evaluated for their ability to inhibit radiolabeled dihydroqueuine incorporation into tRNA in HPRT-deficient HL-60 cells (Fig. 2). All were effective inhibitors *in vivo*. However, while 6-TG and 8-AzaG function as substrates for tRNA-GRT, 7-methylguanine and pterin do not (20, 21). 6-TG and 8-AzaG also inhibit cell growth and induce granulocytic differentiation in HPRT-deficient HL-60 cells (12), whereas pterin and 7-methylguanine do not (data not shown). Taken together, these data indicate the likelihood that analog incorporation, not just queuine hypomodification, is involved in mediating terminal cell differentiation.

Published evidence suggests that down-regulation of c-*myc* expression, even transiently, is a requirement for, and in some cases sufficient for, granulocytic differentiation of HL-60 cells (5, 22, 23). c-*myc* mRNA down-regulation also occurred very rapidly in the presence of 6-TG (Fig. 3*A*). However, 6-TG did not inhibit overall RNA synthesis in HPRT-deficient HL-60 cells (data not shown), thus indicating that c-*myc* mRNA down-regulation is a specific response to 6-TG.

The fact that 6-TG and 8-AzaG, substrates for tRNA-GRT, induced c-*myc* down-regulation, while inhibitors of the enzyme that result in guanine being retained in the anticodon wobble position did not (Fig. 3*B*), indicated a possible role for tRNA in the c-*myc* mRNA changes. Therefore, c-*myc* mRNA expression was monitored for 48 hr after 6-TG treatment in the presence and absence of queuine. No effect of queuine was seen at any of the time points evaluated, and expression of c-*myc* mRNA returned to near control levels by 24 hr (Fig. 4).

Based on the inability of queuine to alter c-*myc* down-regulation, a non-tRNA-mediated mechanism must be considered. This conclusion is further supported by the data described by Schwartz and Eninger (24) whereby 9-ethyl-6-thioguanine (a purine analog not capable of acting as a

substrate for tRNA-GRT) induced c-*myc* down-regulation in HL-60 cells. It should be noted, however, that in our hands, 9-methyl-6-thioguanine was incapable of differentiating HL-60 cells (unpublished data). Thus, purine analog-induced c-*myc* down-regulation, by whatever molecular mechanism, may not be sufficient for terminal differentiation.

Although excess queuine did not alter c-*myc* expression, it did prevent 6-TG-induced growth inhibition in HPRT-deficient HL-60 cells (Fig. 5). Queuine maintained the cells in logarithmic growth in the presence of 6-TG until saturation density was reached. At that point, the cells exhibited morphological differentiation down the granulocytic pathway (Fig. 6). Terminal maturation at such high cell densities has not been observed *in vitro* with other differentiating agents.

The above results suggest that induction of granulocytic differentiation by 6-TG has at least two phases; the first phase being growth inhibition, followed by a second phase of functional and morphological differentiation. Queuine appears to prevent the inhibition of growth, thereby blocking morphological differentiation until growth ceases upon reaching saturation density. This argues that inhibition of growth by 6-TG is a tRNA-mediated event and that growth inhibition is necessary for full commitment to differentiation.

The ability of queuine to delay granulocytic differentiation offered the possibility that the HPRT-deficient HL-60 cells also maintained their pluripotent capability; a possibility we evaluated with the phorbol ester PMA. As already stated, HL-60 cells can be induced to differentiate down the granulocytic or monocytic–macrophage pathways, depending on the inducing agent used. PMA is a potent inducer of monocytic–macrophage differentiation in HL-60 cells (6, 7). This induction is characterized by cell adherence and the expression of monocytic–macrophage markers such as the CSF-1 receptor (c-*fms*). Studies have shown that PMA induces the cell-surface CSF-1 receptor by triggering the synthesis of a labile protein that stabilizes the c-*fms* mRNA coding for the receptor (8). The c-*fms* mRNA is synthesized constitutively but is rapidly turned over *in vivo* when the labile protein is absent.

We first examined the effect of 6-TG treatment in the presence and absence of queuine on the ability of PMA to induce the stabilization of c-*fms* mRNA (Fig. 7). In a 2-hr pretreatment with 6-TG, PMA failed to stabilize the c-*fms* message. Queuine also could not override the effect of 6-TG at this time (Fig. 7*A*). However, cells treated with 6-TG for 3 days in the presence of queuine were able to respond to PMA-induced stabilization of c-*fms* mRNA, whereas those in the absence of queuine were not (Fig. 7*B*).

The observations with c-*fms* stabilization paralleled the ability of PMA to induce cellular adherence of 6-TG-treated HL-60 cells. In a 2-hr pretreatment with 6-TG (± queuine), PMA was unable to induce cellular attachment to the substratum (data not shown). However, cells treated with 6-TG and queuine for 3 days responded to PMA-induced adherence while those without queuine did not (Fig. 7*C*). Therefore, it appears that queuine is, in fact, capable of blocking the commitment to granulocytic differentiation induced by 6-TG. The basis for the delay in acquiring protection by queuine remains to be investigated as does the basis for the rapid (2 hr) inhibition of PMA-induced differentiation by 6-TG.

Queuine's localization in the first position of the anticodon of four tRNA isoacceptors offers the possibility that modulation of translation is involved in the observed effects—i.e., incorporation of 6-TG into the wobble position would likely be disruptive to protein synthesis. The ability of 6-TG to block PMA stabilization of c-*fms* argues that this may be the case, since the protein synthesis inhibitor cyclohexamide yields an analogous response (8). Replacement of 6-TG by queuine might then allow synthesis of the labile protein and the subsequent stabilization of c-*fms*. However, 6-TG is not a general protein synthesis inhibitor in HPRT-deficient HL-60 cells (data not shown), so even though 6-TG is incorporated into the wobble position of tRNA isoacceptors normally containing queuine, it must be relatively specific in its translational effects.

Changes in queuine modification of tRNA have been associated with neoplasia for many years (13, 14), but it is only recently that functional roles for changes in this modification have been identified (25). Our earlier work with 6-TG offered the intriguing possibility of targeting therapeutic agents to the anticodon of the queuine family of tRNAs (12), tRNAs that are very often hypomodified for queuine in malignant cells (14). The current study now demonstrates that 6-TG-induced differentiation of HPRT-deficient HL-60 cells is a tRNA-mediated event, thereby confirming the potential of using these epigenetic targets for drug intervention.

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1. Bodner, A. J., Ting, R. C. & Gallo, R. C. (1981) *J. Natl. Cancer Inst.* **67**, 1025–1030.
2. Collins, S. J., Bodner, A., Ting, R. & Gallo, R. C. (1980) *Int. J. Cancer* **25**, 213–218.
3. Gusella, J. F. & Housman, D. (1976) *Cell* **8**, 263–269.
4. Westin, E. H., Wong-Staal, F., Gelmann, E. P., Dalla Favera, R., Papas, T. S., Lautenberger, J. A., Eva, A., Reddy, E. P., Tronick, S. R., Aaronson, S. A. & Gallo, R. C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2490–2494.
5. Freytag, S. O. (1988) *Mol. Cell. Biol.* **8**, 1614–1624.
6. Huberman, E. & Callahan, M. F. (1979) *Proc. Natl. Acad. Sci.* **76**, 1293–1297.
7. Pegoraro, L., Abrahm, J., Cooper, R. A., Levis, A., Lange, B., Meo, P. & Rovera, G. (1980) *Blood* **55**, 859–862.
8. Weber, B., Horiguchi, J., Luebbers, R., Sherman, M. & Kufe, D. (1989) *Mol. Cell. Biol.* **9**, 769–775.
9. Ishiguro, K., Schwartz, E. L. & Sartorelli, A. C. (1984) *J. Cell. Physiol.* **121**, 383–390.
10. Gallagher, R. E., Ferrari, A. C., Zulich, A. W., Yen, R. C. & Testa, J. R. (1984) *Cancer Res.* **44**, 2642–2653.
11. Schwartz, E. L., Blair, O. C. & Sartorelli, A. C. (1984) *Cancer Res.* **44**, 3907–3910.
12. Kretz, K. A., Katze, J. R. & Trewyn, R. W. (1987) *Mol. Cell. Biol.* **7**, 3613–3619.
13. Farkas, W. R. (1983) *Nucleosides Nucleotides* **2**, 1–20.
14. Nishimura, A. (1983) *Prog. Nucleic Acid Res. Mol. Biol.* **28**, 49–80.
15. Katze, J. R., Gündüz, U., Smith, D. L., Cheng, C. S. & McCloskey, J. A. (1984) *Biochemistry* **23**, 1171–1176.
16. Muralidhar, G., Utz, E. D., Elliott, M. S., Katze, J. R. & Trewyn, R. W. (1988) *Anal. Biochem.* **171**, 346–351.
17. Gibboney, D. S., French, B. T., Patrick, D. E. & Trewyn, R. W. (1989) *Cancer Chemother. Pharmacol.* **25**, 189–194.
18. Chirgwin, J. M., Przybyla, A. E., McDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
19. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
20. Farkas, W. R., Jacobson, K. B. & Katze, J. R. (1984) *Biochim. Biophys. Acta* **781**, 64–75.
21. Shindo-Okada, N., Okada, N., Ohgi, T., Goto, T. & Nishimura, S. (1980) *Biochemistry* **19**, 395–400.
22. Holt, J. T., Redner, R. L. & Nienhuis, A. W. (1988) *Mol. Cell. Biol.* **8**, 963–973.
23. Wickstrom, E. L., Bacon, T. A., Gonzalez, A., Freeman, D. L., Lyman, G. H. & Wickstrom, E. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1028–1032.
24. Schwartz, E. L. & Eninger, H. (1989) *Proc. Am. Assoc. Cancer Res.* **30**, 457 (abstr.).
25. Muralidhar, G., Ochieng, J. & Trewyn, R. W. (1989) *Cancer Res.* **49**, 7110–7114.