Amplification of the UMP Synthase Gene and Enzyme Overproduction in Pyrazofurin-resistant Rat Hepatoma Cells

MOLECULAR CLONING OF A cDNA FOR UMP SYNTHASE*

(Received for publication, July 25, 1983)

John J. Kanalas‡ and D. Parker Suttle

From the Audie L. Murphy Memorial Veterans' Hospital and Division of Hematology, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas 78284

The levels of UMP synthase protein and mRNA are increased in rat hepatoma cells that have acquired resistance to pyrazofurin, a potent inhibitor of pyrimidine biosynthesis. A cDNA plasmid library was prepared from partially purified poly(A)+ mRNA isolated from the resistant cell line. Recombinant plasmids with inserts complementary to UMP synthase mRNA were selected by differential hybridization with cDNA prepared from wild type and resistant cell mRNA and analysis of hybrid-selected mRNA by in vitro translation reactions. One plasmid, pUMPS-2, contains a 850-base pair insert and was used to analyze UMP synthase gene sequences in the wild type and resistant cell lines. Blot hybridization of restricted genomic DNA demonstrated amplification of the UMP synthase gene in the resistant cells. The number of UMP synthase genes is increased 15-fold as determined by a modified dot hybridization procedure. Previous studies have shown that the resistant cells have a 16-fold increase in UMP synthase mRNA but a 40-fold increase in synthase activity (Suttle, D. P. (1983) J. Biol. Chem. 258, 7707-7713). To further investigate this discrepancy between the amount of increase in DNA and mRNA versus the increase in enzyme activity, we have determined the relative rate of synthesis and degradation of UMP synthase. The rate of synthesis was 13-fold faster in the resistant cells. The degradation rate was not significantly different between the two cell lines. These data indicate that gene amplification is the major factor contributing to the enzyme overproduction in the pyrazofurin-resistant cells.

In recent years, evidence has become available to substantiate the hypothesis that gene amplification is a reasonably common occurrence in cells. Given the proper drugs or enzyme inhibitors to impose selective pressure on cells, one can select a particular cell that is resistant to the inhibitors because it has an increased number of genes coding for the enzyme or protein in question (see Schimke, 1982, for examples and review). The increased number of genes results in increased enzyme levels that cannot be totally inhibited at a particular selective drug level. Resistant cell lines with gene amplifications provide an opportunity to characterize the mechanisms by which cells can control gene expression through increasing the quantity of specific segments of their genome. The resistant cells also encode proteins, mRNAs, and genes that are in very low abundance in normal cells to be effectively characterized.

We have been studying rodent cell lines that are resistant to the drug pyrazofurin due to increased UMP synthase activity. UMP synthase is a bifunctional enzyme containing the two activities orotate phosphoribosyltransferase and orotidin-5'-phosphate decarboxylase in a single polypeptide chain (McCland et al., 1980). These activities catalyze the conversion of orotic acid to orotidine 5'-monophosphate and orotidine 5'-monophosphate to UMP, the last steps in the de novo biosynthesis of UMP. Pyrazofurin monophosphate, produced by the action of adenosine kinase on pyrazofurin (Dix et al., 1979; Suttle et al., 1981), is a potent competitive inhibitor of the decarboxylase activity of UMP synthase (Gutowski et al. 1975; Dix et al., 1979). In pyrazofurin-resistant rat hepatoma cells, UMP synthase activity is increased up to 45-fold. The level of UMP synthase mRNA is also increased in the resistant cells, but not proportionally to the increase in enzyme activity (16-fold increase in mRNA, 40-fold increase in activity) (Suttle, 1983). In this report, we describe the selection of a recombinant cDNA plasmid specific for UMP synthase. Hybridization experiments show gene amplification as the major factor causing the increased enzyme level in the pyrazofurin-resistant cells. However, the extent of gene amplification does not completely account for the higher UMP synthase levels in the resistant cells, and other possible mechanisms for increasing specific enzyme activity levels are explored.

EXPERIMENTAL PROCEDURES

Cell Lines—Cells of the rapidly growing, poorly differentiated Morris rat hepatoma line 3924A were cultured as the wild type. They were routinely grown in McCoy's 5A media supplemented with 10% dialyzed fetal bovine serum, penicillin (100 units/ml), and streptomycin sulfate (100 µg/ml) (Gibco) in a 5% CO2, 95% air atmosphere. Resistant cells, 3P39-9, were selected in two stepwise increases of pyrazofurin and were maintained in media as above with the addition of 50 µM pyrazofurin (Suttle, 1983). Pyrazofurin was kindly supplied by Dr. Richard W. Dyke, Eli Lilly and Co., Indianapolis, IN.

Preparation of Poly(A)+ mRNA—Poly(A)+ mRNA from both 3924A and 3P39-9 cell lines was isolated as previously described (Suttle, 1983). To enrich for UMP synthase specific mRNA, the poly(A)+ mRNA was fractionated on a 10-30% sucrose gradient in an SW 41 rotor for 16 h at 35,000 rpm. Fractions were assayed by in vitro translation and analysis on SDS-polyacrylamide gels.

Construction and Screening of a 3P39-9 Resistant Cell cDNA Library

by guest on March 24, 2020http://www.jbc.org/ Downloaded from

* This work was supported by the Veterans' Administration Research Service. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by a Veterans' Administration Merit Review Award to Dr. J. J. Hutton.

† The abbreviations used are: SDS, sodium dodecyl sulfate; PIPES, 1,4-piperazineethanesulfonic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.


Gene Amplification in Pyrazofurin-resistant Cells

---

**Differential Hybridization and Hybrid-selected mRNA Translation** — The selection of colonies by differential hybridization was a modification of previous procedures (St. John and Davis, 1979; Wahl et al., 1979). The colonies grown on duplicate nitrocellulose filters were lysed and the DNA bound by laying the filters sequentially for 5 min each on Whatman 3MM papers saturated with 10% SDS; 0.5 M NaOH, 1.5 M NaCl; 0.5 M Tris-HCl, pH 8.0, 1.5 M NaCl; and 2 × SSPE (SSPE = 0.18 M NaCl, 20 mM NaH2PO4, pH 7.4, 1 mM EDTA) (Maniatis et al., 1982). The filters were air-dried and baked for 2 h at 80 °C in a vacuum oven. The baked filters were soaked in 300 μl of LB media containing 25 μg/ml of tetracycline and 15% glycerol in 96-well microtiter plates. The cells were grown overnight at 37 °C after which duplicate nitrocellulose filters were prepared using a replica plating device. The master plates were used for the hybridization.

**Relative Rate of Synthesis and Degradation** — For determination of the relative rate of synthesis, two 100-mm plates at 50% confluency on 10 150-mm culture dishes. The cells were washed on 10 mM Tris, pH 7.4, 1 mM EDTA by gentle mixing overnight. Ribonuclease A was added (10 μg/ml) and the solution incubated 1 h at 37 °C. The solution was made 0.1% SDS, 50 μg/ml of proteinase K were added, and incubation continued at 37 °C for 1 h. The DNA was extracted and precipitated as above and dissolved in 1 ml of 10 mM Tris, pH 7.4, 1 mM EDTA. The procedure yields approximately 1 mg of DNA greater than 25 kilo base pairs in length.

---

For determination of the degradation rate, a modification of the procedure of Padgett et al. (1979) was followed. Multiple 150 mm plates at 20% confluence were labeled as described above. After the labeling period, the cells were washed twice with phosphate-buffered saline, and lysed by sonication. The cell lysate was centrifuged for 6 min in a Beckman Airfuge at 150,000 × g. Aliquots of the supernatant solution were taken for determination of total trichloroacetic acid precipitable counts. The amount of UMP synthase synthesis was determined by immune precipitation and analysis on SDS-polyacrylamide gels as described (Suttle, 1983).

---

**RESULTS**

**Construction and Identification of Recombinant Plasmids Containing UMP Synthase Sequences** — In the pyrazofurin-resistant rat hepatoma cells, 3P39-9, UMP synthase mRNA is increased approximately 16-fold over synthease mRNA lev-

---

**Gene Amplification in Pyrazofurin-resistant Cells**
els in the wild type cells (Suttle, 1983). To further enrich for UMP synthase mRNA, poly(A)\(^+\) mRNA from resistant cells was fractionated on a sucrose gradient. The fraction with the greatest enrichment of UMP synthase mRNA as determined by in vitro translation assays was used as the template for cDNA production. Following second strand synthesis, digestion with S1 nuclease, and addition of poly(dC) tails, the cDNA was annealed with poly(dG)-tailed pBR322. The recombinant plasmids were used to transform E. coli K12 RR1 with an efficiency of \(5 \times 10^4\) transformants/\(\mu g\) of double-stranded cDNA. Over 2000 colonies were isolated and screened by differential hybridization of duplicate nitrocellulose filters using a modification of the procedure of St. John and Davis (1979).

Two independent differential hybridization screenings were conducted on separate sets of duplicate filters. One set of filters was screened using cDNA probes prepared from the equivalent sucrose gradient fractions of wild type and resistant cell mRNA enriched for UMP synthase mRNA. The other set was screened using cDNA probes prepared from total poly(A)\(^+\) mRNA of wild type and resistant cells. Fifty-eight colonies were selected as showing differential signals in one or both of the screenings. These colonies were replica-plated for a third time and rescreened using cDNA prepared from a second pair of equivalent UMP synthase-enriched sucrose gradient fractions of wild type and resistant cell mRNA. Five colonies gave distinct differential signals in this screen and were analyzed further by mRNA dot hybridization and hybrid-selected translation.

Plasmid DNA from the five selected colonies was isolated and labeled by nick translation. The DNA was then tested for hybridization to poly(A)\(^+\) mRNA from wild type and resistant cells bound to nitrocellulose according to the procedure of Thomas (1980). DNA from four of the five clones (pUMPS-2–pUMPS-5) showed increased binding to resistant cell mRNA (data not shown). These four plasmids were checked by hybrid-selected mRNA translation to confirm the presence of UMP synthase sequences. Fig. 1 shows the results of two of the selections. The plasmid DNA specifically selects an mRNA that upon translation gives a product indistinguishable from UMP synthase when analyzed by SDS-polyacrylamide gel electrophoresis with or without prior immune precipitation with UMP synthase-specific antiserum. No other hybrid-selected translation protein besides UMP synthase is detectable when in vitro translation products are analyzed without immune precipitation. The use of plasmids containing inserted ribosomal or calcitonin sequences in the hybrid selection procedure did not produce a detectable band with or without immune precipitation of the translation products. The immune precipitated bands smaller than full length UMP synthase probably result from proteolysis or incomplete translation in the in vitro reticulocyte lysate system. These same bands are seen with longer exposure times in translation products of unselected poly(A)\(^+\) mRNA from the overproducing 3P39-9 cells. As shown in lanes B and G, no similar bands are found when calcitonin-selected mRNA is used to program the translation reaction or when no exogenous mRNA is added. The other two plasmids were analyzed in equivalent experiments and identical results were obtained. Plasmid DNA was digested with PstI to determine the size of the inserted sequences. The length of the inserts ranged from about 350 base pairs for pUMPS-3 to 850 base pairs for pUMPS-2.

Analysis of Cell DNA for Amplification of the UMP Synthase Gene—We have previously determined by both analysis of in vitro translation products and by dot hybridization with labeled pUMPS-2 DNA that UMP synthase mRNA levels are increased 16-fold in pyrazofurin-resistant cells. The increase in UMP synthase mRNA could be explained by a decreased turnover rate for the mRNA or by increased synthesis of the mRNA. Increased synthesis could result from an increased rate of transcription of the UMP synthase gene, an increase in the rate of mRNA processing, or an increase in the number of copies of the gene. We have used nick-translated pUMPS-2 DNA as a probe to characterize the UMP synthase gene in wild type and resistant cells and to determine if amplification of the gene has occurred. High molecular weight DNA from both cell lines was digested with EcoRI and

![Figure 1](http://www.jbc.org/)  
SDS-gel electrophoresis of in vitro translation products from hybrid-selected mRNA. Poly(A)\(^+\) mRNA from 3P39-9 cells was mixed with plasmid DNA bound to nitrocellulose filters. The mRNA that hybridized to the bound DNA was eluted and translated in a rabbit reticulocyte lysate system. The products were electrophoresed on 8.5% SDS-polyacrylamide gels and the \(^{35}S\)-labeled proteins visualized by fluorography. Proteins in lanes B–G were immunoprecipitated with antibody to UMP synthase. Lanes H–J show total translation products. A, molecular weight standards; B and J, mRNA selected with calcitonin-specific plasmid DNA; C and I, mRNA selected by pUMPS-2; D and H, mRNA selected by pUMPS-3; E, total, unselected poly(A)\(^+\) mRNA from 3P39-9 cells; F, total, unselected poly(A)\(^+\) mRNA from 3924A cells; G, no mRNA added to system. The arrow marks the position of UMP synthase.

1850  
Gene Amplification in Pyrazofurin-resistant Cells
BamHI and the fragments analyzed by the method of Southern (1975) (Fig. 2). For EcoRI-cut DNA, hybridization to a fragment of approximately 6.7 kilobase pairs is greatly increased in the resistant cells. For BamHI-digested DNA, there is a similar amplification of a 8.0-kilobase pair fragment. Only a single UMP synthase-specific fragment is detected in the restriction digests. After exposure of the autoradiograph for 7 days, no additional fragments were visible. To estimate the extent of amplification, the areas of nitrocellulose filters containing the UMP synthase-specific fragment were cut out from Southern blots and the counts bound to the filter determined by scintillation counting. In two digests, there was a 16-fold and an 18-fold increase in bound counts in the resistant cell DNA.

A more precise determination of the extent of UMP synthase gene amplification was achieved through a modified dot blot procedure. Fig. 3 shows the amount of hybridization versus the amount of DNA bound to the filter. A comparison of the slopes of these lines (1094 and 78) indicates the number of synthase gene copies to be increased 14-fold in the resistant cells. For BamHI-digested DNA, there is an 18-fold increase in mRNA levels.

Relative Rate of in Vivo Synthesis and Degradation for UMP Synthase—Although there appears to be an equivalent increase in the resistant cells of UMP synthase gene copies and UMP synthase mRNA (14-fold and 16-fold, respectively), this increase does not correlate with the increase in UMP synthase activity (40-fold). This variance might be explained by an alteration in the resistant cells resulting in either an increased rate of synthesis or a decreased rate of degradation for UMP synthase protein. The relative rate of synthesis of UMP synthase was determined by labelling the cells for 2 h in culture with [3H]leucine. The cells were harvested, lysed, and immunoprecipitated with antibody against UMP synthase. The immunoprecipitates were run on an SDS-polyacrylamide gel, and that portion of the gel containing UMP synthase was cut out and counted. Taking into account the differences in total trichloroacetic acid-precipitable protein, the resistant cells exhibit a 13-fold increase in the relative rate of synthesis of UMP synthase (Table I). This result correlates reasonably well with the increase in mRNA levels and indicates no alteration in the resistant cells of the specific synthesis rate of UMP synthase/unit of mRNA. The amount of UMP synthase in the cell lines was also determined after a 72-h labeling period to calculate the steady state level of enzyme. The ratio of counts in UMP synthase relative to total trichloroacetic acid-precipitable counts was increased 14-fold in the resistant cells.

The relative rate of degradation was calculated by harvesting replica plates of cells at various times following a 4-h pulse labeling and determining the amount of label remaining in UMP synthase protein. There was no significant difference in the degradation rate of UMP synthase in the wild type and resistant cells (Fig. 4). The rate of degradation in the resistant cells was not altered by the presence of 50 µM pyrazofurin in the media.

Examination of the Resistant Cells for the Presence of Double Minute Chromosomes—The increased level of UMP synthase enzyme in the resistant cells is stable if the cells are maintained at 50 µM pyrazofurin. If the selective pressure of the pyrazofurin is removed, the cells will gradually lose these elevated enzyme levels (Suttle, 1983; Suttle and Stark, 1979). This phenomenon has been reported for methotrexate-resist-
Both wild type and resistant cells were examined using Giemsa staining. The solid lines indicate UMP synthase from either wild type (C) or resistant (G) cells. The dotted line represents trichloroacetic acid-precipitable counts for both wild type (○) and resistant (■) cells. All lines were determined by linear regression analysis with correlation coefficients of 0.96 for total protein, 0.98 for wild type UMP synthase, and 0.96 for resistant UMP synthase.

**DISCUSSION**

Several studies have reported the selection of cultured cell lines resistant to high doses of specific inhibitors because of an increased level of the target enzyme. Methotrexate-resistant rodent cells have increased levels of the enzyme dihydrofolate reductase (Hakala et al., 1961; Littlefield, 1969; Biedler et al., 1980; Alt et al., 1976). The resistant cells have a coordinate increase in the level of dihydrofolate reductase mRNA and gene copies (Kellems et al., 1976; Chang and Littlefield, 1976; Alt et al., 1978; Nunberg et al., 1978; Melera et al., 1980). Similarly, in N-phosphoacetyl-L-aspartate-resistant hamster cells, there is a large increase in the pyrimidine biosynthesis enzyme CAD (the multifunctional protein containing the enzymatic activities carbamyl-P synthetase, aspartate transcarbamylase, and dihydroorotatase) (Kempe et al., 1976; Coleman et al., 1977). The resistant cells have amplification of mRNA and DNA coding for CAD at levels greater than or equal to the increase in the amount of enzyme (Padgett et al., 1979; Wahl et al., 1979). Cells that overproduce adenosine deaminase to extremely high levels have been developed using deoxycoformycin in different selection procedures for rat hepatoma (Hunt and Hoffee, 1983a) and mouse cells (Yeung et al., 1983). In the rat hepatoma cells, the level of adenosine deaminase mRNA is increased coordinately with the enzyme, and this overproduction of the mRNA is the result of gene amplification (Hunt and Hoffee, 1983b). Gene amplification is also reported to fully account for the increased deaminase in the overproducing mouse lines (Yeung et al., 1983). In contrast to these examples, a canavanine-resistant human cell line exhibits increased levels of argininosuccinate synthetase enzyme and mRNA (Su et al., 1981a), but the corresponding gene is not amplified (Su et al., 1981b). Cadmium-resistant mouse cells have a 6-fold amplification of the metallothionein I gene, but mRNA levels are about 14-fold greater than normal (Beach and Palmiter, 1981). This result may indicate an increase in mRNA stability in the cadmium-resistant cells. Chinese hamster ovary cells selected for resistance to compactin have a 500-fold increase in 3-hydroxy-3-methylglutaryl coenzyme A reductase (Chin et al., 1982). These cells exhibit only a 15-fold increase in the reductase gene but have a greater increase in mRNA levels. This suggests that the rate of transcription of the amplified gene may be increased (Luskew et al., 1983). These various studies demonstrate that a variety of mechanisms may interact and contribute to the selective overproduction of a particular enzyme. Gene amplification is a major factor in all but one of these systems; however, it does not account in and of itself for the total overproduction of enzyme in two of these resistant cell lines. These examples may represent different types or combination of mechanisms resulting in enzyme overproduction.

Pyrazofurin-resistant rat hepatoma cells are a case in point. UMP synthase activity is approximately 40-fold higher in the resistant cells relative to wild type cells. Quantitation of mRNA levels by both in vitro translation and dot hybridization with specific probe has shown UMP synthase mRNA to be increased only 16-fold (Suttle, 1983). Quantitation of the relative UMP synthase gene copy number by hybridization of the cloned UMP synthase cDNA in Southern blot analysis and in a DNA dot blot procedure has demonstrated a 14-fold increase in the resistant cells. The level of UMP synthase mRNA is increased coordinately with the increase in synthase DNA.

Although gene amplification would constitute the primary mechanism for the overproduction of UMP synthase, it appears another mechanism may also contribute to the total accumulation of synthase enzyme in the resistant cells. One mechanism that would yield still higher levels of enzyme is a higher efficiency of in vivo translation for the synthase mRNA in the resistant cells. This would result in a rate of in vivo synthesis greater than the 16-fold that results from increased mRNA levels alone. However, analysis of the in vivo synthesis rates in the cell lines showed only a 12-13-fold increase, slightly lower than the expected value if mRNA translation for UMP synthase proceeds at equal efficiency in the wild type and resistant cells.

A second possibility for increasing the steady state level of UMP synthase in the resistant cells would be a decrease in the degradation rate of the protein. A small increase in the turnover time of UMP synthase in the resistant cells combined with the 13-fold increase in synthesis rate could increase the protein levels to 40-fold over wild type. However, our experiments determining the degradation rate of UMP synthase in the two cell lines indicate no appreciable difference in the half-life of the enzyme between wild type and resistant cells.

UMP synthase is found in at least two isozyme forms in mouse ascites cells (McCland et al., 1980) and in the rat hepatoma cells used in this study. One possible explanation for the discrepancy between the increased synthase activity and the increase in mRNA and DNA would be the presence of two UMP synthase isozymes of differing specific activities coded for by different genes. Amplification of the gene for the
isoenzyme of higher specific activity in the resistant cells would lead to the apparent disproportionate increase in enzyme activity compared to wild type cells. The possibility of more than one gene coding for an enzyme with UMP synthase activity has also been proposed by Krooth et al. (1974). These investigators found increases in the level of orotidine-5'-monophosphate decarboxylase activity when normal and UMP synthase-deficient human cells were grown in the presence of oxipurinol or barbituric acid. They hypothesized that the incremental decarboxylase activity might stem from a genetic locus other than the one that generates the basal level of activity. Further studies of the wild type and amplified UMP synthase gene will help to clarify the possibilities.

Double minute chromosomes have been found in many drug-resistant cell lines and in various tumor cells (Schimke, 1982, for examples). The presence of the double minutes is associated with amplification of the gene for the target enzyme in methotrexate-resistant cells of unstable phenotype (Kaufman et al., 1979). We have been unable to detect double minute chromosomes in Giemsa-stained metaphase spreads of the pyrazofurin-resistant cells. It may be the amplified DNA is located on extrachromosomal elements that are too small to be recognizable as double minutes. Drug resistance, enzyme overproduction, and gene amplification have also been correlated with the presence of homogeneously staining regions in the Giemsa banding pattern (Schimke, 1982). These homogeneously staining regions may be associated with either an unstable drug-resistant phenotype (Biedler and Spengler, 1976; Biedler et al., 1980) or a stable phenotype (Dolnick et al., 1979; Wahl et al., 1982). The wild type 3924A rat hepatoma cells used in this study contain at least one homogeneously staining region of unknown origin as well as several chromosome rearrangements and marker chromosomes (Olah and Weber, 1979). This makes it very difficult to detect new or altered homogeneously staining regions in the resistant cells using only Giemsa banding techniques. Experiments are in progress to determine the location of the amplified DNA containing the UMP synthase gene by in situ hybridization.

The UMP synthase-specific recombinant cDNA we have selected from the pyrazofurin-resistant rat hepatoma cells will hybridize with both hamster and human sequences. In contrast to the cells that overproduce UMP synthase, Chinese hamster ovary cells have been selected that have a deficiency of UMP synthase activity (Patterson, 1980). Fibroblast cell lines are also available from patients with the autosomal recessive disease orotic aciduria that have severe deficiencies in UMP synthase (Pinsky and Krooth, 1967). The specific probes now available will enable the study of not only UMP synthase gene structure and expression in normal and pyrazofurin-resistant cells but also the characterization of alterations that result in deficiency of UMP synthase.

Acknowledgments—We thank John H. Blodgett for expert technical assistance. Dr. J. W. Jacobs provided encouragement through many helpful discussions and also made available plasmid DNA containing a cDNA insert for calcitonin. We also thank Dr. Nan Clare for preparation of the metaphase spreads.

REFERENCES

Alt, P. W., Kellems, R. E., and Schimke, R. T. (1976) J. Biol. Chem. 251, 3963-3974

Biedler, J. L., and Spengler, B. A. (1976) (Wash. D. C.) Science 191, 180-187

Biedler, J. L., Meleria, P. W., and Spengler, B. A. (1960) Cancer Genet. Cytogenet. 2, 47-60

Blin, N., and Stafford, D. W. (1976) Nature (London) 263, 266

Chang, S. E., and Littlefield, J. W. (1976) Cell 7, 391-396

Chin, D. J., Laskey, K. L., Anderson, B. G. W., Feust, J. R., Goldstein, J. L., and Brown, D. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 3710-3714

Coleman, F. S., Sutcliffe, D. P., and Stark, G. R. (1977) J. Biol. Chem. 252, 6379-6385

Dix, D. E., Leiman, C., Jakubowski, A., Moyer, J. D., and J. Cell. Biochem. 15, 343-347

Dolnick, B. J., Benencon, R. J., Bertino, J. R., Nunberg, J. H., and Schimke, R. T. (1979) J. Cell Biol. 83, 384-402

Gutowski, G. E., Sweetman, M. K., Delong, D. C., Hamlin, R. L., and Gerzon, K. (1975) Ann. N. Y. Acad. Sci. 255, 544-556

Hakala, M. T., Zakrzewski, S. F., and Nichol, C. A. (1981) J. Biol. Chem. 256, 932-938

Hunt, S. W., and Hoffer, P. A. (1984) J. Biol. Chem. 258, 41-44

Kafatos, F. C., Jones, C. W., and Efstratiadis, A. (1979) Nature (London) 274, 154-155

Kaufman, R. J., Brown, P. C., and Schimke, R. T. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 5669-5673

Kellems, R. E., Alt, P. W., and Schimke, R. T. (1976) J. Biol. Chem. 251, 697-699

Kemp, T. D., Seyer, E. A., Breust, M., and Stark, G. R. (1976) Cell 9, 541-550

Krooth, R. K., Lam, G. F. M., and Chen, S. Y. (1974) Cell 1, 55-57

Littlefield, J. W. (1980) Proc. Natl. Acad. Sci. U. S. A. 82, 95-96

Laskey, R. L., Feust, J. R., Chin, D. J., Brown, M. S., and Goldstein, J. L. (1983) J. Biol. Chem. 258, 8462-8468

Mandel, M., and Higa, J. A. (1970) J. Mol. Biol. 53, 154

Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Masters, J., Keeler, B., Gay, H., and Attardi, G. (1982) Mol. Cell. Biol. 2, 458-477

McCandd, R. W., Black, M. J., Livingstone, L. R., and Jones, M. E. (1980) Biochemistry 19, 3851-3859

Melia, F. W., Lewis, J. A., Biedler, J. L., and Heuson, C. (1980) J. Biol. Chem. 255, 7924-7930

Nunberg, J. H., Kaufman, R. J., Schimke, R. T., Uhrig, A., and Chaisan, L. A. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 5503-5508

Olah, E., and Weber, G. (1979) J. Cell. Biol. 39, 1388-1391

Padgett, R. A., Wahl, G. M., Coleman, P. F., and Stark, G. R. (1979) J. Biol. Chem. 254, 974-980

Piek, R., Vela, B., Felsenfeld, A., Ramanathan, L., Ferrini, U., Appella, E., and Seidman, J. G. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 2523-2527

Patterson, D. R. (1970) Semin. Cell Biol. 6, 101-114

Pinsky, L., and Krooth, R. S. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 925-932

Ricciardi, R. P., Miller, J. S., and Roberts, B. E. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4677-4681

Rigby, P. W. J., Dieckmann, M., Rhodes, C., and Berg, P. T. (1977) J. Mol. Biol. 113, 237-251

Schimke, R. T. (1982) Gene Amplification, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Southern, E. M. (1970) J. Mol. Biol. 39, 503-517

St. John, T. P., and Devia, R. W. (1979) Cell 14, 443-452

St. J., T., Beaudet, A. L., and O'Brien, W. E. (1981) Biochemistry 20, 2560-2566

St. J., T., Bock, H.-G., O'Brien, W. E., and Beaudet, A. L. (1981b) J. Biol. Chem. 256, 11806-11821

Suttle, D. P. (1980) J. Biol. Chem. 255, 7707-7711

Suttle, D. P., and Stark, G. R. (1979) J. Biol. Chem. 254, 4602-4607

Suttle, D. P., Hartkocder, R. J., and Jackson, R. C. (1981) Eur. J. Cancer 17, 43-51

Thomas, P. S. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5901-5906

Wahl, G. M., Padgett, R. A., and Stark, G. R. (1979) J. Biol. Chem. 254, 8679-8690

Yeung, C.-Y., Ingolia, D. R., Bocobis, C., Dunbar, B. S., Riser, M. J., Siciliano, M. J., and Kellems, R. E. (1982) J. Biol. Chem. 258, 8338-8345
Amplification of the UMP synthase gene and enzyme overproduction in pyrazofurin-resistant rat hepatoma cells. Molecular cloning of a cDNA for UMP synthase.
J J Kanalas and D P Suttle

J. Biol. Chem. 1984, 259:1848-1853.

Access the most updated version of this article at http://www.jbc.org/content/259/3/1848

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/259/3/1848.full.html#ref-list-1