Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 3257–3261, May 1985
Cell Biology

Defective macromolecule biosynthesis and cell-cycle progression in a mammalian cell starved for mevalonate

(restriction point)

MICHAEL SINENSKY AND JUDITH LOGEL

Eleanor Roosevelt Institute for Cancer Research, 4200 East Ninth Avenue, Box B129, Denver, CO 80262

Communicated by Theodore T. Puck, December 31, 1984

ABSTRACT **The isolation of a somatic cell mutant (Mev-1) with a block in one of the mevalonate-biosynthesizing enzymes (3-hydroxy-3-methylglutaryl-coenzyme A synthase, EC 4.1.3.5) has afforded us the opportunity to test and to extend the hypothesis that a product of mevalonate biosynthesis other than cholesterol is required for cellular proliferation. We present evidence here that both DNA synthesis and protein synthesis are inhibited in this mutant by mevalonate starvation, although RNA synthesis appears to be unaffected. The loss of DNA synthesis and the loss of protein synthesis in this mutant appear to be due to independent processes. DNA synthesis is reversibly inhibited by mevalonate starvation at a unique point in the cell cycle. Resumption of DNA synthesis after readdition of mevalonate exhibits a long lag; the peak of S-phase DNA synthesis occurs approximately 17 hr after mevalonate readdition, suggesting that mevalonate starvation puts cells into a quiescent (G_0) state owing to their failure to transit a restriction point. The loss of DNA biosynthesis in the Mev-1 cell is well correlated with the rate of turnover of mevalonate label of certain terpenylated polypeptides.**

The enzymes of mevalonate biosynthesis of mammalian cells in culture are highly regulated by exogenous cholesterol supplements (1–3). Extensive studies on the second and third steps of the cholesterol biosynthetic pathway, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase (EC 4.1.3.4) and HMG-CoA reductase (EC 1.1.1.8) have demonstrated that these enzymes can specifically undergo a 10- to 100-fold inhibition in various cell types by exogenous sterol supplements. However, inhibition of mevalonate biosynthesis in such cultured cells can result in a substantial loss of response of HMG-CoA reductase to exogenous sterol (4). This has been demonstrated unambiguously in a somatic cell mutant, Mev-1, which has no detectable HMG-CoA synthase activity (5). We have reported that incubation of Mev-1 with sterol regulators of HMG-CoA reductase produces only a minor inhibition of HMG-CoA reductase activity compared to the inhibition produced in wild-type cells (6). However, normal control of HMG-CoA reductase activity in Mev-1 can be restored by a combination of mevalonate and regulatory sterol. It has been demonstrated that HMG-CoA reductase activity is controlled at the level of enzyme biosynthesis by regulatory sterol (7, 8) so that, as expected in the Mev-1 cell, little or no inhibition of enzyme synthesis is produced by regulatory sterol under conditions in which synthesis in the wild-type cell decreases 90%. In the Mev-1 cell, normal control of HMG-CoA reductase synthesis by regulatory sterol also can be restored by treating these cells with a combination of sterol and low levels of mevalonate (6).

This "bivalent regulation" (9) of HMG-CoA reductase is reflected in Mev-1 in an apparent dual requirement for cholesterol and mevalonate for growth. Cholesterol can replace most, but not all, of the mevalonate requirement for growth (5). These studies on Mev-1 are quite comparable to studies performed with other mammalian cells treated with transition-state analog inhibitors (10) of HMG-CoA reductase in which there also is observed a nonsterol requirement for mevalonate for both growth (11–13) and regulation of HMG-CoA reductase activity (4). It has been suggested that the nonsterol requirement for mevalonate may reflect its role as a precursor of a compound necessary for DNA synthesis (11–13). Isopentenyladenine has been identified as a likely compound (14). It also has been pointed out that mevalonate can be incorporated, after conversion to a terpene, into a number of cellular polypeptides (15) and tRNA (16). In this report, we describe the effect of mevalonate starvation on macromolecular biosynthesis in the Mev-1 cell with particular regard to the kinetics of loss of macromolecular biosynthesis in mevalonate-starved cells and to the rate of disappearance of mevalonate-derived label from proteins and tRNA. Studies in which isopentenyladenine was tested for ability to reverse any of the effects of mevalonate starvation also will be discussed. Our results suggest that a deficiency in terpenylated proteins is the sole basis for the loss in DNA replication associated with mevalonate starvation.

MATERIALS AND METHODS

Chemicals and Radiochemicals. Mevalonic acid lactone, leupeptin, Nonidet P-40, phenylmethylsulfonyl fluoride, bovine liver tRNA, pepstatin, and RNase were obtained from Sigma. Proteinase K was obtained from Beckman. Radioactive mevalonate (tritiated, 2 mCi/mmol), uridine (tritiated, 10 Ci/mmol), thymidine (tritiated, 19.3 Ci/mmol), and methionine (^{35}S-labeled, 800 Ci/mmol) were from New England Nuclear (1 Ci = 37 GBq). Kyro EOB (nonionic detergent) was from Procter and Gamble, RNasin was from Promega Biotech, Madison, WI.

Cell Culture. The CHO-K1 (pro^-) cell line (17) and the Mev-1 (5, 6) mutant have been described. Cells were routinely grown in Ham's F-12 medium (18) supplemented with 8% neonatal calf serum. When mevalonate was used as a supplement, it was added to a final concentration of 50 μg/ml.

Measurement of the Rates of Cellular RNA, DNA, and Protein Synthesis. To measure the rate of cellular RNA synthesis, cells (2.5×10^5 per 60-mm dish) were incubated for 18 hr after inoculation (to permit recovery) and then pulse-labeled for 30 min at various times with [^{3}H]uridine (1 μCi/ml). The plates were washed two times with ice-cold phosphate-buffered saline, and then the cells were harvested by scraping with a rubber policeman into 1 ml of ice-cold phosphate-buffered saline. The suspension was homogenized by sonication, and a 0.2-ml aliquot was mixed with a 1-ml volume of 10% ice-cold CCl_3COOH. The CCl_3COOH-insoluble material was collected on a 0.45-μm Millipore filter and washed

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HMG-CoA synthase and reductase, 3-hydroxy-3-methyglutaryl-coenzyme A synthase and reductase.

extensively with cold 5% CCl_3COOH. The filter was then dried under a heat lamp, and the radioactivity incorporated was determined by liquid scintillation counting in Aqueous Counting Solution (Amersham). To correct for incorporation of [^{3}H]uridine into DNA, an identical aliquot was subjected to alkaline hydrolysis by overnight incubation at 37°C in 1 M KOH. The hydrolyzate was then neutralized, and the CCl_3COOH-insoluble residue determined as described above was used to measure and then correct for incorporation into DNA. Protein (19) was determined with a third aliquot.

To measure the rate of DNA synthesis, cells were pulsed as described above with 1 μCi/ml of [^{3}H]thymidine in medium F12 lacking hypoxanthine and thymidine. The CCl_3COOH-insoluble material was then determined as described for RNA.

To measure the rate of cellular protein synthesis, [^{35}S]-methionine (1 μCi/ml) incorporation into CCl_3COOH-insoluble material was determined as described for RNA synthesis except that a 60-min rather than a 30-min pulse was used.

Determination of [^{3}H]Mevalonate Incorporation into Polypeptides and tRNA. Cells (2×10^6 cells per 100-mm dish) were starved for mevalonate for 4 hr, followed by a 16-hr incubation with [^{3}H]mevalonate (10 μCi/ml) still in the absence of any unlabeled mevalonate supplementation. In our initial studies to obtain a complete analysis of derivatized polypeptides present, we harvested the cells in boiling buffer containing 3% $NaDodSO_4$ prior to $NaDodSO_4$/PAGE on 12.5% polyacrylamide. Similar qualitative and quantitative results could be obtained by harvesting cells by scraping with a rubber policeman into 50 mM Tris, pH 7.5/0.15 M NaCl/0.5 mM phenylmethylsulfonyl fluoride/0.1 μM pepstatin/5 mM EDTA, pelleting the cells in a clinical centrifuge, and extracting for 30 min with 150 μl of ice-cold buffer (10 mM Tris, pH 7.5/10 mM EDTA/0.1 M NaCl/50 mM NaF/50 μM leupeptin/1 mM phenylmethylsulfonyl fluoride/0.1 μM pepstatin) containing 0.25% nonionic detergent (Kyro EOB) prior to $NaDodSO_4$/PAGE. Lysates were cleared of insoluble material in an Eppendorf centrifuge. With minor modification, this harvest and lysis procedure was utilized for all studies shown in this report.

For $NaDodSO_4$/PAGE of polypeptides, these lysates were treated with RNase A (10 μg/ml) for 1 hr at 44°C in a final volume of 1 ml. The reaction was halted, and proteins were precipitated with ice-cold CCl_3COOH (final concentration, 10%). The proteins were collected by centrifugation and dissolved in $NaDodSO_4$ sample buffer as described (7). $NaDodSO_4$/PAGE was carried out as described by Laemmli (20) in 0.1% $NaDodSO_4$/Tris glycine buffer on a 12.5% acrylamide gel using a 5% (wt/vol) stacking gel.

For polyacrylamide gel analysis of tRNA, cells were harvested as described above and then lysed for 30 min in ice-cold 10 mM Tris, pH = 7.5/10 mM EDTA/0.1 M NaCl (TEN buffer) containing 0.5% Nonidet P-40 and 85 units of RNasin (an RNase inhibitor) per ml. The lysate was cleared of debris in an Eppendorf centrifuge and then extracted twice with redistilled phenol saturated with TEN buffer. The aqueous layer was adjusted to 0.25 M sodium acetate and then extracted twice with $CHCl_3$/isoamyl alcohol, 24:1 (vol/vol). The RNA in the aqueous layer was then precipitated by addition of 2.5 volumes of ice-cold ethanol, followed by incubation for 1 hr at −20°C. The precipitate was then collected by centrifugation, resuspended in 10 mM Tris, pH = 7.5/10 mM EDTA (TE buffer), and the RNA content of the sample was estimated from the OD_{280}/OD_{260}. This procedure was utilized to correct for procedural variation between samples. Ten micrograms of carrier mammalian tRNA was then added to the sample, and the RNA was precipitated with cold ethanol and taken up in TE buffer to which was added glycerol, Ficoll, and bromophenol blue, to final concentrations of 10%, 2.5%, and 0.025%, respectively. The samples were analyzed by electrophoresis on 10% acrylamide gels in 0.089 M Tris borate, pH 8.0/0.002 M EDTA (21).

All samples subjected to gel electrophoresis were visualized by fluorography at −80°C after impregnation of the gels with sodium salicylate.

A number of control experiments were performed to confirm identification of these derivatized macromolecules. Treatment of cells with cycloheximide (20 μg/ml) during the labeling period was not found to inhibit incorporation into any of the labeled species confirming the post-translational nature (15) of this process for the polypeptides. [Incorporation of [^{3}H]mevalonate into tRNA under the conditions used has been shown by others (16) to occur only by a specific post-transcriptional mechanism in cultured cells.] The material observed in total cell lysates and analyzed on gels was shown to be completely digestible by a combination of proteinase K (0.25 mg/ml) and RNase A (10 μg/ml) for 1 hr at 44°C. This material prepared for polypeptide analysis and purported to be free of tRNA was shown to be completely digestible by proteinase K in the presence of an RNase inhibitor (RNasin, 85 units/ml) but unaffected by RNase A in the presence of a cocktail of protease inhibitors (5 mM EDTA/50 μM leupeptin/1 mM phenylmethylsulfonyl fluoride/0.1 μM pepstatin). Enzymatically ^{32}P-end-labeled tRNA from *Escherichia coli* added to unlabeled cell extracts was shown to be completely hydrolyzed by RNase A under these same conditions. Likewise, material prepared for tRNA analysis and purported to be free of protein was shown to be insensitive to digestion by proteinase K but completely digested by RNase A.

Cell-Cycle Analysis by Flow Microfluorimetry. Cells growing as monolayers were harvested by treatment with trypsin, followed by removal of the cells from the dish (and neutralization of the trypsin) by aspiration in phosphate-buffered saline supplemented with 1% dialyzed fetal calf serum. The cells were resuspended and washed twice in phosphate-buffered saline and then fixed in 70% ethanol. The cells were then resuspended in phosphate-buffered saline and treated with RNase A (0.1 mg/ml) at 37°C for 1 hr. The cells then were stained with 10 μg of propidium iodide per ml of Ca^{2+}/Mg^{2+}-free phosphate-buffered saline and subjected to flow microfluorimetry in a FACS IV fluorescence-activated cell sorter (Becton Dickinson).

RESULTS

Effects of Mevalonate Starvation on Cellular Macromolecule Synthesis. The effect of mevalonate starvation on the rates of RNA, DNA, and protein synthesis in Mev-1 cells compared to CHO K1 wild-type cells is shown in Fig. 1. During the first 24 hr of mevalonate starvation, there was a minor effect on cellular RNA synthesis in Mev-1 compared to both DNA and protein synthesis, which were substantially inhibited by mevalonate starvation. After the respective lag periods, the half-life of DNA biosynthesis was observed to be 9 hr, whereas that of protein synthesis was 18 hr. DNA biosynthesis was undetectable after starvation for mevalonate for 24 hr. However, protein biosynthesis was not completely lost upon further mevalonate starvation (data not shown). The cells exhibited a level of 20% of the initial rate of protein synthesis after 48 hr of mevalonate starvation, at which time all cell viability was lost. When mevalonate supplements were restored to the culture medium of Mev-1 cells starved for mevalonate for 24 hr, DNA biosynthesis could be fully restored (Fig. 2), with the peak of DNA biosynthesis occurring 17 hr after the readdition of mevalonate supplements. This peak of DNA biosynthesis after mevalonate readdition is consistent with a synchronization of the cell culture produced by mevalonate starvation. We confirmed

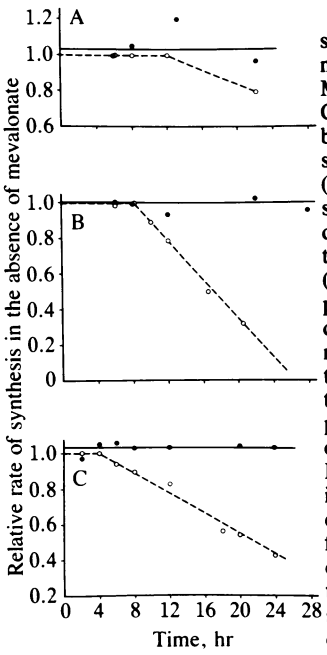

FIG. 1. Effect of mevalonate starvation on the rates of macromolecular biosynthesis in the Mev-1 cell. Mev-1 (o---o) and CHO-K1 (●—●) cells were incubated in normal culture medium supplemented with mevalonate (50 μg/ml) or in the absence of such supplements, and the rates of RNA synthesis (*A*), DNA synthesis (*B*), and protein synthesis (*C*) were determined at various periods of time. The relative rates of the synthesis of the various macromolecules are expressed as the ratio of the rate of synthesis in the absence of mevalonate supplements to the rate in the presence of mevalonate supplements. Both cell types were innoculated into culture medium supplemented with mevalonate and incubated for 18 hr prior to changing the medium to the experimental conditions at zero time. The results shown are the average of duplicate determinations.

this hypothesis by utilization of a fluorescence-activated cell sorter to perform cell cycle analysis on populations of Mev-1, first starved for mevalonate and then restored to medium supplemented with mevalonate. The entire population of such cells were in S phase 17 hr after mevalonate readdition (Fig. 3). This finding supports the hypothesis of other workers (11, 13) that mevalonate starvation can produce arrest of cells at a unique point in the cell cycle but, in contrast to these previous studies, demonstrates that there is a long lag between the time of mevalonate readdition and the restoration of cellular DNA replication.

The Rate of Disappearance of Mevalonate Label from Transfer RNA and Cellular Polypeptides. Earlier work suggesting a mevalonate requirement other than for cholesterol for cell growth has led to a search for macromolecules that are derivatized with terpene products of mevalonate metabolism. Two such candidates have been described: the post-transcriptionally modified transfer RNAs (16), which possess an isopentenyl substituent on the adenine adjacent to the 3′ end of the anticodon of tRNA species that recognize codons beginning with uridine (22); and what appear to be certain cellular polypeptides that are post-translationally modified by derivitization with terpenes (15). We found (see *Materials and Methods*) that the cellular content of these two classes of macromolecule could be determined by gel

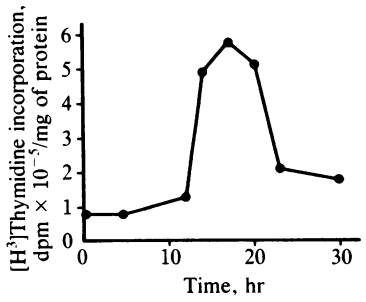

FIG. 2. Rate of DNA synthesis in Mev-1 after readdition of mevalonate (50 μg/ml) to cells starved for mevalonate for 24 hr. Cells (2.5×10^5 per 60-mm dish) were pulsed for 30 min with 1 mCi/ml of [^{3}H]thymidine in medium F-12 lacking hypoxanthine and thymidine. The rate of DNA synthesis was determined as the incorporation of [^{3}H]thymidine into CCl_3COOH-insoluble material as described. The results shown are the average of duplicate determinations.

electrophoresis. The polypeptides could be prepared free of contaminating RNA by treatment of cell lysates with RNase A in the presence of protease inhibitors, and the tRNA could be prepared free of proteins by phenol extraction. These two classes of macromolecules were labeled with tritiated mevalonate over a 16-hr period of incubation, and subsequently the cells were incubated for various periods of time in medium supplemented with 50 μg of unlabeled mevalonate per ml. Lysates could be prepared from such cells, and the labeled RNA and polypeptides were determined to assess the kinetics of disappearance of mevalonate label from these macromolecules. The labeled polypeptides of Mev-1 after RNase digestion showed a sharp band of 43,000 Da and a doublet between 23,000 and 25,000 Da as the three most prominent species observed. The disappearance of mevalonate label from all three of these polypeptides showed a half-life of 8.5 hr (Fig. 4). On the other hand, the population of isopentenylated tRNAs showed a half-life of 31 hr (Fig. 5). This latter result is consistent with half-lives that have been reported for bulk mammalian tRNA (23).

Failure of Isopentenyladenine to Affect Macromolecule Biosynthesis in Mev-1. Because it has been reported that isopentenyladenine can substitute for mevalonate in restoring DNA replication in cells treated with an inhibitor (compactin) of mevalonate biosynthesis, we examined the capacity of isopentenyladenine (10 and 20 μM) to substitute for mevalonate in Mev-1 cells. We tested the capacity of isopentenyladenine to reverse the loss of viability, DNA, and protein biosynthesis or to restore DNA biosynthesis in Mev-1 starved for mevalonate. In none of these experiments did we observe any effect whatever of isopentenyladenine on the processes under study. It also should be noted that cellular isopentenyladenine normally is produced by the degradation of isopentenylated tRNA (24). Therefore, intracellular levels of isopentenyladenine should remain constant until the isopentenylated tRNA is depleted. Since the half-life of degradation of the bulk isopentenylated tRNA appears to be 31 hr, whereas the half-life of loss of DNA synthesis is 8.5 hr, it does not seem likely that the loss in DNA biosynthesis can result from a deficit of cellular isopentenyladenine since it would not be expected that intracellular isopentenyladenine levels would be affected during an 8.5-hr starvation for mevalonate.

DISCUSSION

The data presented strongly suggest that neither a deficit in synthesis of isopentylated tRNA nor isopentenyladenine are responsible for the block to DNA synthesis observed in mevalonate-starved cells. There is, however, a close correlation between the half-life of the loss of DNA biosynthesis in mevalonate-starved cells and that of the terpenylated substituent of proteins that can be labeled post-translationally with mevalonate. These observations are consistent with the hypothesis that it is a deficit of function in such proteins after mevalonate starvation that is responsible for the block to DNA synthesis observed in such cells. However, the long lag observed between the readdition of mevalonate to Mev-1 cultures and initiation of DNA synthesis does not suggest a direct requirement for a mevalonate-derived product in DNA biosynthesis. Rather the long lag is reminiscent of protocols (25, 26), such as serum starvation, that reversibly arrest certain cell lines at a distinct point early in the G_1 phase of the cell cycle. This similarity leads us to speculate that the polypeptides that are observed to be derivatized with mevalonate play a role in the transition of cells between quiescence and proliferation. Further support for this hypothesis comes from the observation that inhibitors of mevalonate biosynthesis can block the cycling of mitogen-treated quiescent cells (12, 13). A comparably long time period between

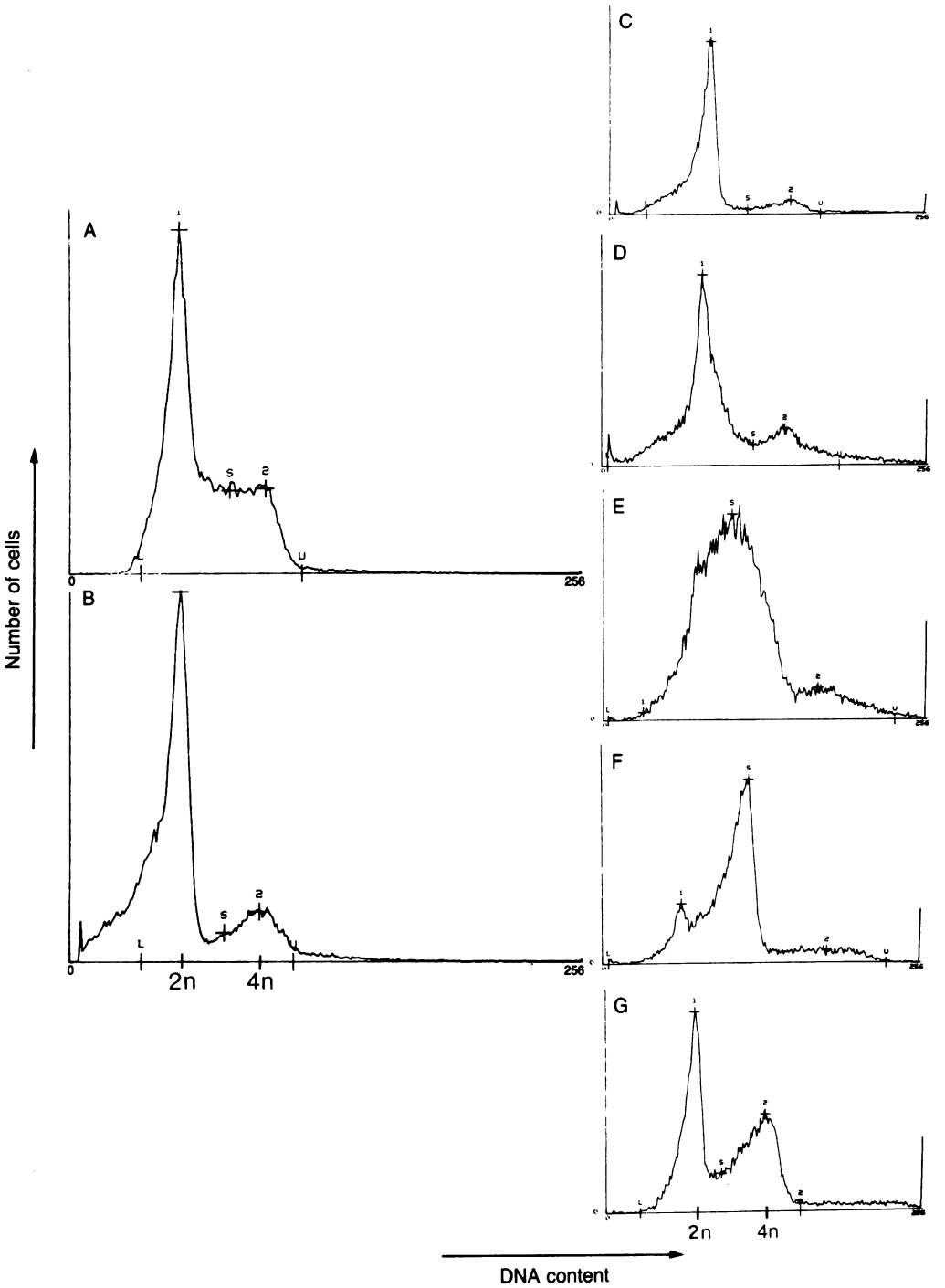

FIG. 3. Flow microfluorimetric cell-cycle analysis after readdition of mevalonate (50 μg/ml) to Mev-1 cells starved for mevalonate. (*A*) Randomly growing population of Mev-1 cells. (*B*) Mev-1 cells starved for mevalonate for 24 hr. Note the lack of S-phase cell population. Cells are arrested in G_1. Some 10% of wild-type CHO-K1 cells are tetraploid, accounting for the G_2 population. (*C–G*) Cell populations after mevalonate readdition for various periods of time: 11 hr (*C*), 14 hr (*D*), 17 hr (*E*), 20 hr (*F*), and 23 hr (*G*). 2n and 4n designate DNA content corresponding to diploid and tetraploid cells, respectively.

the point in the cell cycle at which mevalonate is required to the peak of S-phase DNA synthesis has been observed in one of these studies (13). Proteins required for the transition from a quiescent to the proliferative state define restriction points in the cell cycle (27) and have been referred to as "trigger" proteins (28), but thus far only indirect evidence for their existence has been presented (29–31). The data presented in this report suggest that one or more of the polypeptides labeled by mevalonate may be such a trigger protein.

The loss in protein synthesis observed after mevalonate starvation does not correlate well with the degradation rates of any of the macromolecules analyzed in this study. However, it should be noted that since multiple tRNAs can be isopentenylated, the turnover rate of the bulk tRNA so derivatized need not reflect the turnover rate of particular isopentenylated tRNAs, particularly if one of these is relatively scarce. Thus, it is possible that an abnormally labile isopentenylated tRNA is responsible for the loss in protein biosynthesis observed after mevalonate starvation. The impact of the loss of such a tRNA on overall rates of protein biosynthesis cannot be evaluated *a priori* because the effects observed would be a function of codon usage with regard to such a particular tRNA. A prior study assessing the role of isopentenylation in tRNA function has suggested that ribosomal binding of isopentenylated tRNAs is compromised by loss of this substituent and, hence, that polypeptide elongation is affected if these substituents are not present (32). Thus, it appears to be reasonable that the dysfunction observed in protein synthesis after mevalonate starvation could result from a loss of particular isopentenylated tRNAs. It should be noted that it has been well established that a block to protein synthesis will in turn produce a block to DNA synthesis (33, 34). However, in all instances studied in a synchronously growing cell population, the level of inhibition of

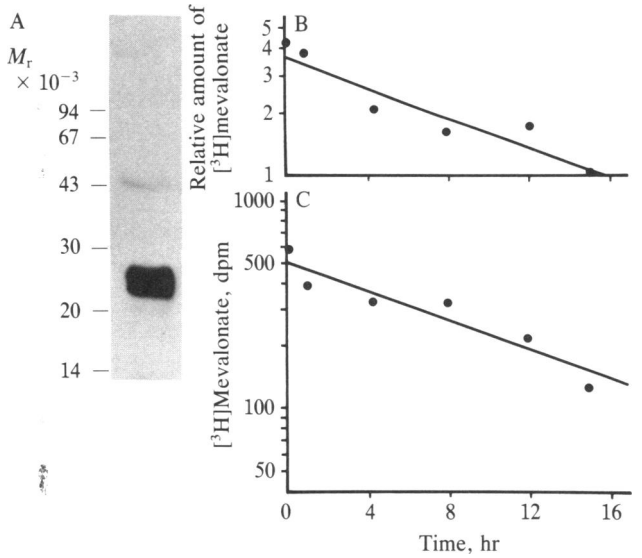

FIG. 4. The rate of disappearance of polypeptides of the Mev-1 cell labeled by [^{3}H]mevalonate. Polypeptides labeled by [^{3}H]mevalonate were prepared and fractionated by NaDodSO$_4$/PAGE as described. The results (*A*) show two major bands: a sharp single band corresponding to M_r 43,000 and a doublet of M_r 23,000–25,000. The radioactivity incorporated into the M_r 43,000 band was insufficient to permit quantitation by liquid scintillation, and so this material was quantitated by densitometric scanning (Helena Instruments, Beaumont, TX, Quickscan), whereas the material in the doublet was quantitated by liquid scintillation spectrometry. After [^{3}H]mevalonate labeling, the label was chased for various periods of time, as shown in medium supplemented with mevalonate, and the amount of the M_r 43,000 polypeptide (*B*) and the M_r 23,000–25,000 doublet (*C*) was determined. Identical half-lives were obtained for each half of the doublet as for their sum. The lines were fitted by the least-squares method.

the rate of protein biosynthesis has been proportionately reflected in the rate of DNA biosynthesis (33, 34). Therefore, it would be expected that if mevalonate starvation produced a loss in protein biosynthesis that was, in turn, responsible for the loss in DNA biosynthesis, there would be a similarity in the half-life of the two rates. Since this is clearly not the case, we conclude that the loss in DNA biosynthesis during the first 24 hr of mevalonate starvation is independent of the loss in protein biosynthesis.

The 8.5-hour half-life observed for the mevalonate-derivatized polypeptides is also consistent with a role for one or more of these polypeptides as a coregulator of HMG-CoA reductase biosynthesis with regulatory sterols. We have reported (6) that after a 16-hr mevalonate starvation, the response of HMG-CoA reductase synthesis in Mev-1 to regulatory sterols is reduced about 60%, which is comparable to the ≈70% decrease in these derivatized polypeptides that occurs in that period of time.

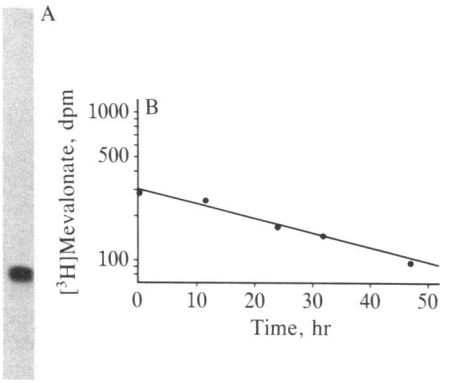

FIG. 5. Half-life of isopentenylated transfer RNAs of the Mev-1 cell. Cells (2×10^6 per 100-mm dish) were labeled with [^{3}H]mevalonate for 16 hr and then chased for various periods of time in medium supplemented with mevalonate (50 μg/ml). (*A*) Appearance of tritiated isopentenylated transfer RNA prepared from Mev-1 cells as described and visualized by flourography. (*B*) Amount of tritiated transfer RNA per 100-mm dish as a function of time of chase.

We thank Dr. John Lehman and David Fogelman for assistance in performance and interpretation of the flow microflourimetric cell-cycle analyses. These studies were supported by National Institutes of Health Grants HL 27877 and HD 02080. M.S. is an Established Investigator of the American Heart Association. This is contribution 531 from the Eleanor Roosevelt Institute.

1. Chang, T. Y. & Limanek, J. S. (1980) *J. Biol. Chem.* **255,** 7787–7795.
2. Schnitzer-Polokoff, R., Torget, R., Logel, J. & Sinensky, M. (1983) *Arch. Biochem. Biophys.* **227,** 71–80.
3. Rodwell, V. W., Nordstrom, J. L. & Mitschelen, J. J. (1976) *Adv. Lipid Res.* **14,** 1–74.
4. Brown, M. S., Foust, J. R., Goldstein, J. L., Kaneko, I. & Endo, A. (1978) *J. Biol. Chem.* **253,** 1121–1128.
5. Schnitzer-Polokoff, R., von Gunten, C., Logel, J., Torget, R. & Sinensky, M. (1982) *J. Biol. Chem.* **257,** 472–476.
6. Sinensky, M., Torget, R., Schnitzer-Polokoff, R. & Edwards, P. A. (1982) *J. Biol. Chem.* **257,** 7284–7286.
7. Sinensky, M., Torget, R. & Edwards, P. A. (1981) *J. Biol. Chem.* **256,** 11774–11779.
8. Faust, J. R., Luskey, K. L., Chin, D. J., Goldstein, J. L. & Brown, M. S. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5205–5209.
9. Brown, M. S. & Goldstein, J. L. (1980) *J. Lipid Res.* **21,** 505–517.
10. Alberts, A. W., Chen, J., Kuron, G., Hunt, V., Huff, J., Hoffman, C., Rothrock, J., Lopez, M., Joshua, H., Harris, E., Patchett, A., Monaghan, R., Currie, S., Stapley, E., Albers-Schonberg, G., Hensens, O., Hirshfield, J., Hoogsteen, K., Liesch, J. & Springer, J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3957–3961.
11. Quesney Huneeus, V., Wiley, M. H. & Siperstein, M. D. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5056–5060.
12. Habenicht, A. J. R., Glomset, J. A. & Ross, R. (1980) *J. Biol. Chem.* **255,** 5134–5140.
13. Fairbanks, K. P., Witte, L. D. & Goodman, D. S. (1984) *J. Biol. Chem.* **259,** 1546–1551.
14. Quesney Huneeus, V., Wiley, M. H. & Siperstein, M. D. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5842–5846.
15. Schmidt, R. A., Schneider, C. J. & Glomset, J. A. (1984) *J. Biol. Chem.* **259,** 10175–10180.
16. Faust, J. R., Brown, M. S. & Goldstein, J. L. (1980) *J. Biol. Chem.* **255,** 6546–6548.
17. Kao, F. T. & Puck, T. T. (1969) *J. Cell Physiol.* **74,** 245–258.
18. Ham, R. G. (1965) *Proc. Natl. Acad. Sci. USA* **53,** 288–293.
19. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
20. Laemmli, U. K. (1970) *Nature (London)* **277,** 680–685.
21. Peacock, A. C. & Dingman, C. W. (1967) *Biochemistry* **6,** 1818–1827.
22. McCloskey, J. A. & Nishimura, S. (1977) *Acc. Chem. Res.* **10,** 403–410.
23. Schlegel, R. A., Iverson, P. & Rechsteiner, M. (1978) *Nucleic Acids Res.* **5,** 3715–3729.
24. Hall, R. (1971) *The Modified Nucleosides in Nucleic Acids* (Columbia Univ. Press, Irvington-on-Hudson, NY), p. 339.
25. Pardee, A. B., Dubrow, R., Hamlin, J. L. & Kletzien, R. F. (1978) *Annu. Rev. Biochem.* **47,** 718–720.
26. Scott, R. E., Florine, D. L., Willie, J. J., Jr., & Yun, K. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 845–849.
27. Pardee, A. B. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1286–1290.
28. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. & Watson, J. D. (1983) *Molecular Biology of the Cell* (Garland, New York), p. 617.
29. Rossow, P. W., Riddle, V. G. H. & Pardee, A. B. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4446–4450.
30. Tyson, J., Garcia-Herdugo, G. & Sachsenmaier, W. (1979) *Exp. Cell Res.* **119,** 87–98.
31. Croy, R. G. & Pardee, A. B. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4699–4703.
32. Gefter, M. & Russell, R. L. (1969) *J. Mol. Biol.* **39,** 145.
33. Stimac, E., Housman, D. & Huberman, J. A. (1977) *J. Mol. Biol.* **115,** 485–511.
34. Brooks, R. F. (1977) *Cell* **12,** 311–317.