Nakanishi, Y., Sekimizu, K., Mizuno, D., & Natori, S. (1978) *FEBS Lett. 93*, 357.
Natori, S. (1972) *J. Biochem. (Tokyo) 72*, 1291.
Natori, S., Takeuchi, K., Takahashi, K., & Mizuno, D. (1973) *J. Biochem. (Tokyo) 73*, 879.
Natori, S., Takeuchi, K., & Mizuno, D. (1974) *J. Biochem. (Tokyo) 76*, 263.
Natori, S., Takeuchi, K., & Mizuno, D. (1975) *J. Biochem. (Tokyo) 77*, 1319.
Revie, D., & Dahmus, M. E. (1979) *Biochemistry 18*, 1813.
Roeder, R. G. (1976) in *RNA Polymerase* (Losick, R., & Chamberlin, M., Eds.) p 285, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Segawa, K., Yamaguchi, N., & Oda, K. (1977) *J. Virol. 22*, 679.
Seifart, K. H., Juhasz, P. P., & Benecke, B. J. (1973) *Eur. J. Biochem. 33*, 181.
Sekimizu, K., Kobayashi, N., Mizuno, D., & Natori, S. (1976) *Biochemistry 15*, 5064.
Sekimizu, K., Nakanishi, Y., Mizuno, D., & Natori, S. (1979a) *Biochemistry 18*, 1582.
Sekimizu, K., Mizuno, D., & Natori, S. (1979b) *Exp. Cell. Res. 124*, 63.
Spindler, S. R. (1979) *Biochemistry 18*, 4042.
Stein, H., & Hausen, P. (1970) *Cold Spring Harbor Symp. Quant. Biol. 35*, 709.
Sugden, B., & Keller, W. (1973) *J. Biol. Chem. 248*, 3777.
Swandogo, M., Sentenac, A., & Fromageot, P. (1980) *J. Biol. Chem. 255*, 12.
Ueno, K., Sekimizu, K., Mizuno, D., & Natori, S. (1979) *Nature (London) 277*, 145.
Vesterberg, O. (1972) *Biochim. Biophys. Acta 257*, 11.
Weil, P. A., Luse, D. S., Segall, J., & Roeder, R. G. (1979) *Cell (Cambridge, Mass). 18*, 469.

Sequential Translocation of Two Phenobarbital-Induced Polysomal Messenger Ribonucleic Acids from the Nuclear Envelope to the Endoplasmic Reticulum†

Frank J. Gonzalez and Charles B. Kasper*

ABSTRACT: Quantitation of rat liver messenger ribonucleic acids (mRNAs) coding for epoxide hydratase and NADPH–cytochrome *c* oxidoreductase was accomplished with a rabbit reticulocyte protein synthesizing system in conjunction with immunoprecipitation with monospecific antibodies. Translatable levels of both mRNAs were found associated with nuclear envelope bound, rough endoplasmic reticulum bound, and rapidly sedimenting endoplasmic reticulum bound polysomes. Soon after a single dose of phenobarbital, translatable levels of these mRNAs rose in all three membrane fractions; however, the rates of increase were markedly different. Elevated levels of these mRNAs first appeared in the nuclear envelope bound polysomes and then in the polysomes bound to the rough endoplasmic reticulum. Maximal levels that were approximately 3- and 5-fold above the control level for oxidoreductase and epoxide hydratase mRNAs, respectively, were reached for both membrane systems 4–6 h after administration of the drug. These levels then declined concomitantly with an increase in the translatable levels of these mRNAs associated with rapidly sedimenting endoplasmic reticulum, which peaked and stabilized at a level approximately 5-fold above the control value 14–20 h after administration of phenobarbital. Increased in vivo synthesis of nuclear envelope epoxide hydratase and oxidoreductase paralleled closely the levels of their mRNAs associated with the rough endoplasmic reticulum but did not correlate with the rapid increase in their nuclear envelope mRNA levels. The increased synthesis in vivo of these enzymes in the microsomal membrane, however, paralleled closely the increased levels of their respective polysomal mRNAs associated with rapidly sedimenting endoplasmic reticulum. These data strongly indicate that newly synthesized phenobarbital-induced mRNAs first become associated with the nuclear envelope and the adjacent rough endoplasmic reticulum. Polysomes containing these mRNAs are then translocated via the rough endoplasmic reticulum to a region of the endoplasmic reticulum morphologically characterized by closely positioned parallel arrays of rough-surfaced membrane, where the messages are stabilized.

The nuclear envelope is composed of an inner and outer membrane, joined via the pore complex structure. A unique structural element of the envelope is the pore complex lamina, a lipid-free subfraction composed primarily of three polypeptides (Dwyer & Blobel, 1976). Topological interrelationships exist between two of these polypeptides, and two show extensive sequence homology (Lam & Kasper, 1979). The physicochemical and biochemical features of the nuclear envelope have been reviewed (Kay & Johnston, 1973; Franke & Scheer, 1974; Kasper, 1974b; Wunderlich et al., 1976; Maul, 1977; Harris, 1978; Zbarski, 1979); however, much remains to be learned about its biogenesis and its role in nucleocytoplasmic transport. In these regards, the phosphorylation of a pore complex lamina associated protein by an endogenous nuclear envelope protein kinase has been described (Lam & Kasper, 1978) which may play a role in RNA transport (Agutter et al., 1979) as well as in the polymerization and depolymerization of the nuclear envelope during mitosis (Gerace & Blobel, 1980).

Biosynthesis and regulation of enzymes associated with the nuclear envelope are poorly understood. It is interesting to

† From the McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706. *Received August 15, 1980.* This work was supported by Grants CA-23076, CA-17300, and CA-07175 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

0006-2960/81/0420-2292$01.25/0 © 1981 American Chemical Society

note, however, that the outer leaflet of the nuclear envelope is continuous with the endoplasmic reticulum, and many enzymatic activities are common to both membrane systems. Among these are cytochrome P-450, NADH–cytochrome b_5 reductase, cytochrome b_5, UDPglucuronosyltransferase, NADPH–cytochrome *c* (P-450) oxidoreductase, epoxide hydratase, and cytochrome P-448 (Kashnig & Kasper, 1969; Kasper, 1971; Fry & Wishart, 1976; Sikstrom et al., 1976; Fahl et al., 1978; Mukhtar et al., 1979). In addition, the latter three enzymes isolated from the endoplasmic reticulum are immunologically cross-reactive with their counterparts in the nuclear envelope; this indicates that both intracellular membranes contain the same molecular entity (Zimmerman & Kasper, 1978; Thomas et al., 1979). Of significance is the finding that the epoxide hydratase, NADPH–cytochrome *c* oxidoreductase, and aryl hydrocarbon hydroxylase activities associated with the endoplasmic reticulum are induced by chronic phenobarbital administration, but their corresponding nuclear envelope activities are not (Kasper, 1971; Khandwala & Kasper, 1973; Fahl et al., 1978; Pezzuto et al., 1978). The aryl hydrocarbon hydroxylase activity of the nuclear envelope and endoplasmic reticulum, however, is induced by 3-methylcholanthrene (Khandwala & Kasper, 1973; Fahl et al., 1978; Pezzuto et al., 1978). In an effort to understand this differential induction phenomenon and further enhance our understanding of membrane biogenesis, an in-depth study of the mechanism of phenobarbital induction was undertaken. Of particular interest are the intracellular sites of synthesis and the mechanism of insertion of the intrinsic membrane proteins associated with both the nuclear envelope and the endoplasmic reticulum; therefore, emphasis is directed toward these phenomena and their role in the phenobarbital induction process. Recently, we found that two phenobarbital-inducible enzymes, NADPH–cytochrome *c* oxidoreductase and epoxide hydratase, are synthesized by endoplasmic reticulum bound polysomes in forms not detectably larger than the native enzymes, whereas cytochrome b_5, a protein that is not induced under these conditions, is synthesized on free polysomes (Gonzalez & Kasper, 1980a,b). It was also found that phenobarbital administration causes an accumulation of epoxide hydratase and oxidoreductase messenger ribonucleic acids (mRNAs) and that this accumulation may result from an increase in transcriptional and/or posttranscriptional nuclear events (Gonzalez & Kasper, 1980a,b).

Since nuclei and isolated nuclear envelopes are studded with ribosomal particles (Blobel & Potter, 1966b; Bornens & Courvalin, 1978), the question arises as to whether these polysomes are responsible for the synthesis of nuclear envelope associated enzymes and are under different control than their endoplasmic reticulum counterparts with respect to phenobarbital induction. In this report, the synthesis in vitro of epoxide hydratase and oxidoreductase from nuclear envelope bound polysomal RNA is described, and translatable levels of the mRNA coding for these enzymes are examined at various times after a single dose of phenobarbital is administered. In addition, analysis of induced translatable levels of epoxide hydratase and oxidoreductase mRNAs from two discrete cellular fractions of endoplasmic reticulum bound polysomes is performed, with serum albumin used as a non-induced control.

Materials and Methods

Treatment of animals (Gonzalez & Kasper, 1980a), purification of NADPH–cytochrome *c* oxidoreductase (Zimmerman & Kasper, 1978) and epoxide hydratase (Gonzalez & Kasper, 1980b), and preparation of monospecific γ globulin have been described (Masters et al., 1971). Techniques used in the in vitro synthesis, immunoprecipitation, and quantitation of each protein have also been described in detail (Gonzalez & Kasper, 1980a). Measurements of the specific radioactivity of oxidoreductase after a 40-min pulse of [^{3}H]leucine were carried out exactly as outlined previously (Zimmerman & Kasper, 1978; Gonzalez & Kasper, 1980a). Epoxide hydratase was solubilized from pulse-labeled microsomes by treating microsomes with 1% Triton X-100 and 1% deoxycholate, and the unsolubilized protein was sedimented at 200000*g* for 2 h before immunoprecipitation was carried out. Radial immunodiffusion (Mancini et al., 1965; Zimmerman & Kasper, 1978) of epoxide hydratase was performed in the presence of 1% NP-40 (BDH Chemicals Ltd., Poole, England). Nuclear envelope used for synthesis studies in vivo was isolated by the method of Bornens & Courvalin (1978).

Isolation of Polysomes. Free polysomes and polysomes tightly membrane bound to the rough endoplasmic reticulum of rat liver were prepared from postmitochondrial supernatant as described previously (Gonzalez & Kasper, 1980a). Rapidly sedimenting endoplasmic reticulum bound polysomes were isolated by a modification of a procedure described by Ramsey & Steele (1976). The initial centrifugation was reduced from 100000*g* for 12 min to 20000*g* for 10 min, so that only large membranous structures were sedimented. In addition, 0.5 mg of heparin/mL was included after membrane-bound polysomes were liberated by Triton X-100 treatment, and the residual unsolubilized material was removed by centrifugation. The membrane-released polysomes were sedimented through a 2 M sucrose cushion (Ramsey & Steele, 1976).

Nuclear envelope bound polysomes were liberated from highly purified nuclei (Blobel & Potter, 1966b; Kasper, 1974a) by a procedure similar to that outlined by Whittle et al. (1968) and Sadowski & Howden (1968). Yeast RNA (2 mg/mL) and 5 mM 2-mercaptoethanol were added to the homogenizing medium. Purified nuclei from 90 g of liver (wet weight) were washed with 30 mL of 50% high-speed supernatant (Kruppa & Sabatini, 1977), a source of ribonuclease inhibitor (Blobel & Potter, 1966a). The nuclei were then resuspended in 15 mL of high-speed supernatant, and heparin (10 mg/mL) was added to give a final concentration of 0.5 mg/mL. The suspension was immediately diluted with a 20% stock solution to contain 2% Triton X-100 (w/v), and the membrane-denuded nuclei were sedimented by centrifugation at 1000*g* for 10 min. The supernatant was made 250 mM in KCl, and the polysomes were sedimented through a 2 M sucrose cushion as previously described (Gonzalez & Kasper, 1980a). RNA was extracted from polysomes with a chloroform–phenol emulsion (Perry et al., 1972) and treated as previously described (Gonzalez & Kasper, 1980a). Concentrations were determined spectrophotometrically; 1.0 absorbance unit at 260 nm was considered equal to 45 μg of RNA. Typical yields of purified RNA were 9.4, 218, and 848 μg/g of liver for nuclear envelope bound, rough endoplasmic reticulum bound, and rapidly sedimenting endoplasmic reticulum bound polysomes, respectively. Yields of RNA from control and induced preparations were not statistically different. Our yields of nuclear envelope bound polysomal RNA were similar to those reported by Whittle et al. (1966). Poly(adenylic acid) [poly(A)] RNA was isolated by use of oligo(deoxythymidine)–cellulose [oligo(dT)–cellulose], as described by Aviv & Leder (1972), with the following modification: RNA was first heated in doubly distilled H_2O at 60 °C for 5 min and quickly chilled in a 0 °C ice bath before a 5-fold concentrate of the high-salt buffer was added. Sedimentation

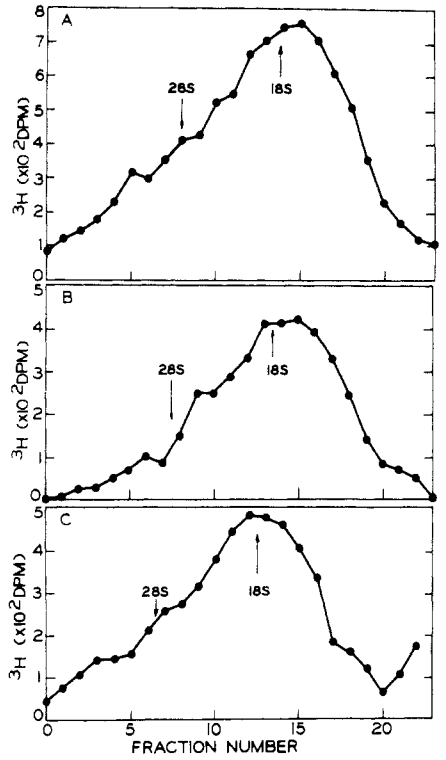


FIGURE 1: Size distribution of poly(A) mRNA isolated from (A) nuclear envelope bound, (B) rough endoplasmic reticulum bound, and (C) free polysomal poly(A) mRNA. Polysomes were isolated from rats that had been injected intraperitoneally with 100 μCi of [5-^{3}H]orotic acid 3 h prior to sacrifice. Poly(A) mRNA isolated by oligo(dT)–cellulose chromatography was sedimented through a 15–30% sucrose gradient as described under Materials and Methods, and 0.5-mL fractions were analyzed for radioactivity. Ribosomal RNA was run concurrently to mark the positions of the 18S and 28S ribosomal RNA peaks on the gradients.

analysis of poly(A) mRNA was performed as outlined by Cardelli et al. (1976).

Results

Isolation of Nuclear Envelope Bound RNA. Isolation of polysomes from purified nuclear envelope was not feasible because RNA degradation occurred rapidly upon nuclear lysis. Therefore, conditions were selected to liberate polysomes from nuclei without extensive leakage of intranuclear RNA while maintaining polysomal mRNA integrity. Leakage was monitored by use of a pulse of 100 μCi of [^{3}H]orotic acid per rat 5 min before sacrifice; this procedure would label primarily intranuclear RNA. The radioactivity liberated by a 2% Triton X-100 treatment in the presence of 0.5 mg of heparin/mL and high-speed supernatant was identical with that of the control, not treated with detergent. In addition to this evidence, high molecular weight nuclear RNAs such as heterogeneous RNA and ribosomal RNA precursors were not detected in our nuclear envelope bound polysomal RNA by sedimentation analysis of poly(A) mRNA (Figure 1A) and poly(A) minus RNA (F. J. Gonzalez and C. B. Kasper, unpublished experiments), indicating the absence of significant nuclear leakage.

Nuclear polysomal poly(A) mRNA was compared with mRNA isolated from rough endoplasmic reticulum bound polysomes. Sedimentation analysis of ^{3}H-labeled poly(A) mRNA on 15–30% sucrose gradients revealed that polysomal poly(A) mRNA bound to the nuclear envelope (Figure 1A) had the same average size as polysomal poly(A) mRNA bound to the rough endoplasmic reticulum (Figure 1B). Free polysomal poly(A) mRNA (Figure 1C) sedimentated at a slightly faster rate than both membrane-bound polysomal mRNA populations.

Table I: In Vitro Synthesis of Epoxide Hydratase, NADPH–Cytochrome *c* Oxidoreductase, and Serum Albumin by use of the Rabbit Reticulocyte System[a]

RNA isolated from	% of total translation products represented by: epoxide hydratase[b]	oxidoreductase[b]	serum albumin[c]
nuclear envelope	0.015 ± 0.002	0.017 ± 0.002	1.5
rough endoplasmic reticulum[d]	0.024 ± 0.003	0.023 ± 0.004	4.8
rapidly sedimenting endoplasmic reticulum	0.012 ± 0.003	0.015 ± 0.002	1.9

[a] RNA preparations isolated from each membrane-bound polysomal RNA fraction were subjected to translation in the reticulocyte system, and each protein was immunoprecipitated from 2 X 10^6 dpm of total translation products. The amount of radioactivity incorporated into each protein was computed as a percentage of the radioactivity incorporated into the total translation products. [b] These values represent the mean ± the standard deviations of three experiments. [c] These values represent the average of two experiments. [d] Taken from Gonzalez & Kasper (1980a,b).

Synthesis in Vitro of Epoxide Hydratase, NADPH–Cytochrome c Oxidoreductase, and Serum Albumin. Polysomal RNA, purified from nuclear envelope bound, rough endoplasmic reticulum bound, and rapidly sedimenting endoplasmic reticulum bound polysomes, was used to direct translation in a cell-free rabbit reticulocyte protein synthesizing system. Epoxide hydratase, oxidoreductase, and serum albumin were immunoprecipitated from polysome-released protein by monospecific antibodies. Each immunoprecipitate was solubilized with sodium dodecyl sulfate and electrophoresed on a sodium dodecyl sulfate–polyacrylamide gel. Autoradiographs of these gels revealed that each immunoprecipitate yielded a single radioactively labeled polypeptide that corresponded in molecular weight to the native enzyme, thus confirming the absence of prematurely terminated peptide chains (Gonzalez & Kasper, 1980a,b). This was found to be the case regardless of the RNA population used in the protein synthesis reaction. The respective antigens were excised and counted, and the percentage of polysome-released protein labeled with [^{35}S]-methionine represented by epoxide hydratase, oxidoreductase, and serum albumin synthesized from each fraction of membrane-bound polysomal RNA was determined (Table I). Rough endoplasmic reticulum bound polysomal RNA synthesizes approximately 2-fold more epoxide hydratase and oxidoreductase and 2–3-fold more serum albumin than do nuclear envelope bound and rapidly sedimenting endoplasmic reticulum bound polysomal RNAs. These differences may reflect the occurrence of other mRNAs in the latter two fractions that are absent from the rough endoplasmic reticulum bound polysomal RNA population. Since nuclei and rapidly sedimenting endoplasmic reticulum are not washed with high salt concentrations, they may contain low levels of loosely membrane bound polysomes (Gonzalez & Kasper, 1980a) that contribute mRNA not found on high salt washed rough endoplasmic reticulum.

Quantitation of Epoxide Hydratase, NADPH–Cytochrome c Oxidoreductase, and Serum Albumin mRNAs after Phenobarbital Administration. Rough endoplasmic reticulum bound and rapidly sedimenting endoplasmic reticulum bound polysomes were isolated from groups of three rats killed at

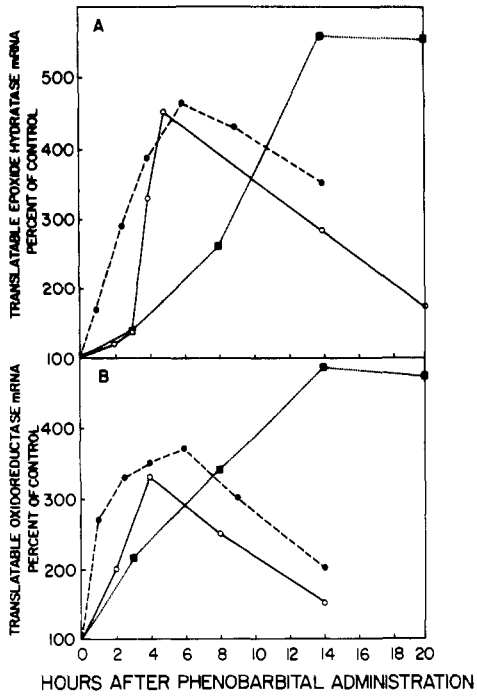


FIGURE 2: Quantitation of epoxide hydratase and NADPH–cytochrome *c* oxidoreductase mRNAs after phenobarbital administration. RNA was purified from nuclear envelope bound, rough endoplasmic reticulum bound, and rapidly sedimenting endoplasmic reticulum bound polysomes isolated at various times after administration of a single dose of phenobarbital, and in vitro translation was carried out as described under Materials and Methods. The percentage of epoxide hydratrase and oxidoreductase in the total translation products (2×10^6 dpm) was determined by immunoprecipitation with γ-globulin monospecific for each enzyme (Table I). The percentage of control (Table I) at each time point was determined for epoxide hydratase (A) and NADPH–cytochrome *c* oxidoreductase (B) translated from nuclear envelope bound (●), rough endoplasmic reticulum bound (○), and rapidly sedimenting endoplasmic reticulum bound (■) polysomal RNA. The data for rough endoplasmic reticulum bound polysomal oxidoreductase mRNA from Gonzalez & Kasper (1980a) are included for comparative purposes.

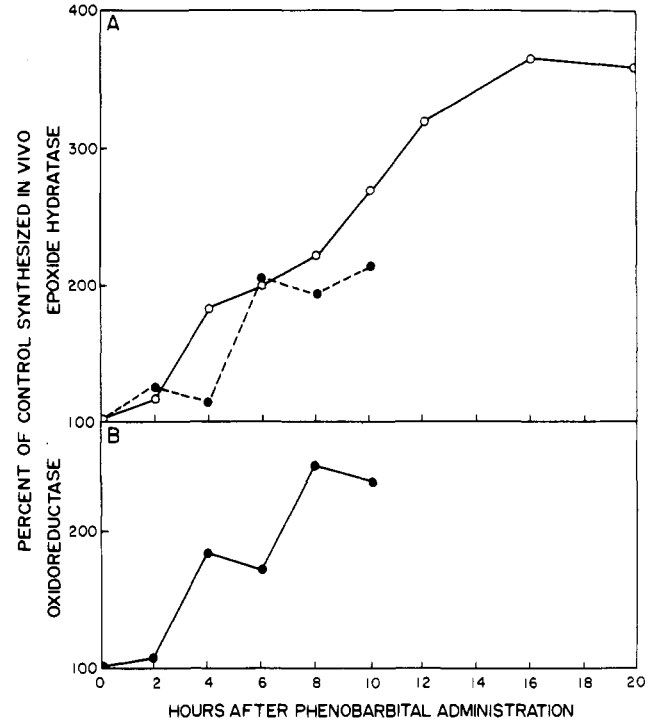


FIGURE 3: Synthesis in vivo of nuclear envelope and microsomal epoxide hydratase (A) and nuclear envelope NADPH–cytochrome *c* oxidoreductase (B). Groups of three rats (microsomes) and 15 rats (nuclear envelope) were administered phenobarbital at zero time and injected with 60 μCi of L-[^{3}H]leucine 40 min before killing. The specific radioactivity of epoxide hydratase in microsomes (○) and the nuclear envelope (●) was determined for each time point (A) as was that for the nuclear envelope oxidoreductase (●) (B) after precipitation and quantitation by immunochemical techniques. The percent of the control specific activity was computed for each time point. The specific radioactivities of control microsomal and control nuclear envelope epoxide hydratase were 4.5×10^3 and 2.5×10^3 dpm/mg, respectively. The specific radioactivity of control oxidoreductase in the nuclear envelope was 3.25×10^3 dpm/mg.

various times after the administration of a single dose of phenobarbital. Nuclear envelope bound polysomes were isolated from groups of 15 rats at each time point. RNA, extracted from different time points for each membrane-bound polysome preparation, was used to direct translation in the reticulocyte system. Epoxide hydratase and oxidoreductase were immunoprecipitated from polysome-released protein and quantitated as described under Materials and Methods, and the percentage of the total translation products represented by each enzyme was computed.

Translatable levels of nuclear envelope bound polysomal epoxide hydratase mRNA increased rapidly to 3-fold greater than control levels 2.5 h after administration of phenobarbital and reached a maximum level almost 5-fold above the control level at 6 h (Figure 2A). In the rough endoplasmic reticulum, translatable epoxide hydratase mRNA remained near the control levels for up to 3 h after administration of the drug, and then rapidly increased to approximately 5-fold above the control at 5 h. After maximal levels of epoxide hydratase mRNA were reached in the nuclear envelope and rough endoplasmic reticulum, they declined with an estimated half-life of 15 and 13 h, respectively. Coincident with this decline was an increase in the translatable level of epoxide hydratase mRNA in the rapidly sedimenting endoplasmic reticulum. A maximal level almost 6-fold above the control was reached at 14 h and was maintained for at least 20 h after the administration of phenobarbital. In order to determine if this flux of membrane-bound polysomes is a generalized phenomenon, translatable levels of NADPH–cytochrome *c* oxidoreductase mRNA were examined in the various RNA preparations. Translatable oxidoreductase mRNA increased in the same sequential manner as epoxide hydratase mRNA; however, the increases occurred earlier in all three membrane fractions (Figure 2B). In contrast, translatable levels of serum albumin mRNA remained at control levels after phenobarbital administration in all membrane-bound polysomal RNA fractions.

Synthesis in Vivo of Epoxide Hydratase Associated with the Nuclear Envelope and Endoplasmic Reticulum. The specific radioactivity of epoxide hydratase was determined from detergent-solubilized microsomes, and nuclear envelope, isolated from animals labeled with a 40-min pulse of [^{3}H]-leucine at various times after phenobarbital administration. The purpose of this experiment was to investigate whether the rate of synthesis in vivo of nuclear envelope and microsomal epoxide hydratase and oxidoreductase correlated with the increase in their respective mRNAs. Synthesis in vivo of microsomal epoxide hydratase during phenobarbital induction (Figure 3A) was found to parallel the increase in its translatable mRNA levels associated with rapidly sedimenting endoplasmic reticulum bound polysomes (Figure 2A). Translatable oxidoreductase mRNA levels associated with this fraction also closely correlated with the synthesis in vivo of the oxidoreductase associated with the microsomal fraction (Gonzalez & Kasper, 1980a). Synthesis in vivo of both enzymes in the nuclear envelope (Figure 3A,B) did not correlate with the initial rapid increase in their respective nuclear en-

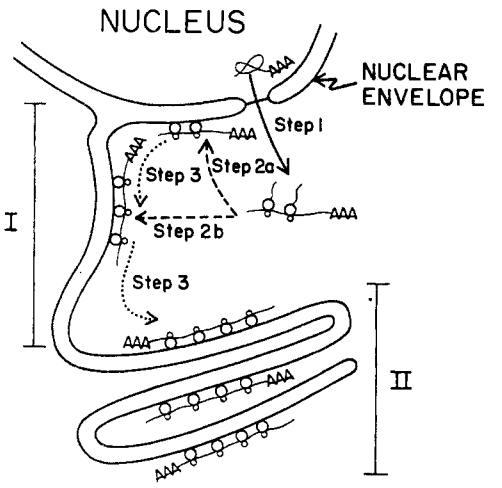
velope bound polysomal mRNAs (Figure 2A,B). This may be due to the time required for insertion of these enzymes into the nuclear envelope. Hence, translocation of polysomes from nuclear envelope to rough endoplasmic reticulum may be too rapid to allow epoxide hydratase and oxidoreductase to be fully synthesized and inserted. The increase in synthesis in vivo detected in the nuclear envelope at 6–10 h after administration of the drug, therefore, may be from concerted synthesis occurring in the rough endoplasmic reticulum and nuclear envelope during the translocation process.

Discussion

In studies directed toward the biogenesis of the nuclear envelope and its role in cellular metabolism, care must be taken to ensure that nuclei and nuclear envelope preparations are free of contaminating endoplasmic reticulum. Such contamination can be quite significant, since the cellular mass of the nuclear envelope is approximately 1% of that of the endoplasmic reticulum (Kasper, 1974b). The recently described microsomal 1-phosphatidylinositol 4-phosphate formation (Behar-Bannelier & Murray, 1980) and the camphor binding P-450 (Fahl et al., 1978), both of which are absent from highly purified nuclear envelope (Fahl et al., 1978; Lam & Kasper, 1978), allow an accurate determination of the degree of microsomal contamination of nuclear envelope preparations. Using these analyses in conjunction with electron microscopic examination, we estimated that nuclei contained a maximum of 1–2% microsomal contamination. Nuclear envelope bound polysomes, therefore, represent a distinct class of membrane-bound polysomes and do not result from contamination by rough endoplasmic reticulum bound polysomes.

In response to phenobarbital administration, proliferation of smooth endoplasmic reticulum occurs in concert with the increase in the levels of certain enzymes involved with oxidative drug metabolism (Conney et al., 1960; Remmer & Merker, 1963; Orrenius et al., 1965), including epoxide hydratase (Bresnick et al., 1977; Fahl et al., 1978) and NADPH–cytochrome *c* oxidoreductase (Orrenius et al., 1965; Jick & Shuster, 1966; Kasper, 1971). These enzymes, however, do not increase in the nuclear envelope (Kasper, 1971; Fahl et al., 1978) even though they represent the same molecular entities as their counterparts in the endoplasmic reticulum (Zimmerman & Kasper, 1978; Thomas et al., 1979). Our data demonstrate that both epoxide hydratase and oxidoreductase are synthesized by nuclear envelope bound polysomal mRNA, and the levels of these mRNAs increase soon after phenobarbital administration. These induced levels, however, decline with half-lives of 15 and 8 h, respectively. The inability of certain nuclear envelope enzymes to be induced by phenobarbital, therefore, does not result from the failure of their respective mRNAs to be induced, but instead may result from the transient association of phenobarbital-induced mRNA with nuclear envelope bound polysomes. Translatable levels of epoxide hydratase and oxidoreductase mRNAs associated with rough endoplasmic reticulum bound polysomes rise shortly after their rise in the nuclear envelope, and then decline with similar half-lives in both membranes. During the phase in which this decline occurs, a dramatic increase in the translatable levels of epoxide hydratase and oxidoreductase mRNAs appears in the rapidly sedimenting endoplasmic reticulum fraction. Induced levels of both mRNAs are then stabilized in this fraction up to 20 h after phenobarbital administration.

It has been reported previously that phenobarbital administration decreases ribonuclease activity and stabilizes polysomes (Louis-Ferdinand & Fuller, 1970; Seifert & Vacha, 1970; Venkatesan & Steele, 1975). Our data, however, illustrate that translatable serum albumin mRNA remains at the same level in all three membrane polysome fractions after phenobarbital administration. This would suggest that the induction and translocation of mRNA are specific for mRNAs coding for those enzymes induced by phenobarbital and are not the result of a nonspecific decrease in RNA degradation after phenobarbital administration.



Step 1 (⟶); Transport of mRNA out of nucleus
Step 2 (⇢); Binding of newly formed polysome to the nuclear envelope and adjacent rough endoplasmic reticulum (I)
Step 3 (⋯⇢); Translocation of polysomes along the nuclear envelope and rough endoplasmic reticulum (I) to rapidly sedimenting endoplasmic reticulum (II)

FIGURE 4: Diagrammatic scheme depicting the translocation of membrane-bound polysomes.

It is of interest that oxidoreductase mRNA is transported out of the nucleus, and its polysomes are shuttled to the rough endoplasmic reticulum more rapidly than those of epoxide hydratase mRNA (Figure 2). This suggests that the biochemical events that are stimulated by phenobarbital produce an initial burst of one mRNA (oxidoreductase), followed after a period of time by another (epoxide hydratase). This differential stimulation of two different mRNA species was not noted for the glucocorticoid induction response, in which both tyrosine aminotransferase and tryptophan oxygenase mRNAs were induced at similar rates (Roewekamp et al., 1976). Phenobarbital may therefore trigger nuclear processes via a mechanism different from glucocorticoids, even though the end result of both types of induction is cytoplasmic accumulation of certain mRNAs (Roewekamp et al., 1976; Gonzalez & Kasper, 1980a,b).

Our results support an intriguing intracellular translocation phenomenon, diagrammed in Figure 4. Upon stimulation of intracellular events by phenobarbital, levels of certain mRNAs, destined to become incorporated into membrane-bound polysomes, increase (step 1). These mRNAs are transported out of the nucleus and form polysomes, which begin to produce hydrophobic nascent polypeptide chains that bind the polysome to either the nuclear envelope (step 2a) or the rough endoplasmic reticulum adjacent to the nuclear envelope (step 2b). These polysomes are then translocated along the nuclear envelope and the rough endoplasmic reticulum to that region of the latter membrane network operationally defined as the rapidly sedimenting endoplasmic reticulum (step 3), where they are stabilized. This membrane translocation process suggests that specific regions of biochemical specialization exist

within the endoplasmic reticulum and that these regions are separable by physical methods such as centrifugation. Rapidly sedimenting endoplasmic reticulum has been shown to be arranged in stacked parallel arrays similar to its orientation seen in situ (Shore & Tata, 1977). Our data indicate that a portion of the rough endoplasmic reticulum contiguous with the cell nucleus may receive polysomes directly from the nuclear surface for transfer to the rapidly sedimenting endoplasmic reticulum. During nuclear isolation, that segment of the endoplasmic reticulum forming a continuum with the outer nuclear envelope is sheared from the nucleus and further fragmented into characteristic microsomal vesicles. In contrast, rapidly sedimenting endoplasmic reticulum maintains much of its native configuration, as clearly demonstrated by the electron micrographs of Shore & Tata (1977). This scheme introduces a new concept whereby mRNA contained in a functional polysomal unit can shuttle along the nuclear envelope and the endoplasmic reticulum until it reaches a segment of this latter membrane network, where it is stabilized and proceeds in coding for the synthesis of intrinsic membrane proteins.

Many intriguing aspects of the phenobarbital induction process and intracellular membrane biogenesis are beginning to emerge. Rapidly sedimenting endoplasmic reticulum serves as a reservoir of phenobarbital-induced polysomes, which synthesize enzymes that become associated with both preexisting and proliferating endoplasmic reticulum. During synthesis, these enzymes can be either inserted directly into the phospholipid bilayer of the endoplasmic reticulum or transferred into the cisternae. Indeed, evidence for the latter process has been reported for cytochrome P-450 (Craft et al., 1979). Movement of newly synthesized enzyme within the cisternae or lateral diffusion within the membrane would then result in a distribution of the induced enzymes throughout the endoplasmic reticulum of the cell. This type of enzyme movement could account for the 4-fold increase in the in vivo synthesis of epoxide hydratase and oxidoreductase that occurred in microsomes 16 h after phenobarbital administration [Figure 3 and Gonzalez & Kasper (1980a)]. Since rough endoplasmic reticulum isolated in the vesicular form (microsomes) did not contain elevated levels of polysomal mRNA coding for either enzyme at 16 h (Figure 2A,B; Gonzalez & Kasper, 1980a), these newly synthesized molecules are probably transferred from rapidly sedimenting endoplasmic reticulum, where the mRNAs are stabilized and found at elevated levels. Furthermore, synthesis in vivo of epoxide hydratase and oxidoreductase in the nuclear envelope also increased approximately 2-fold 6–8 h after phenobarbital administration (Figure 3); however, this increase more closely paralleled the rate of increase in their respective mRNAs associated with the rough endoplasmic reticulum and not the nuclear envelope. Hence, polysome translocation from the nuclear envelope to the rough endoplasmic reticulum may be too rapid to allow these enzymes to be fully synthesized and inserted into the outer envelope bilayer. Therefore, some of the increased in vivo synthesis detected in the envelope probably results from enzyme synthesis and insertion occurring on rough endoplasmic reticulum during polysome translocation and the subsequent transfer of these newly synthesized enzymes back to the nuclear envelope. Since the mass of the endoplasmic reticulum is much greater than that of the nuclear envelope, transfer of newly synthesized enzymes from the rapidly sedimenting endoplasmic reticulum pool of induced polysomal mRNA to the nuclear envelope probably does not occur to any significant extent. This would lead to a failure of phenobarbital-induced enzymes to accumulate in the envelope and to the explanation of their inability to be induced after chronic administration of the drug.

References

Agutter, P. S., Cockrill, J. B., Lavine, J. E., McCaldin, B., & Sim, R. B. (1979) *Biochem. J. 181*, 647–658.

Aviv, H., & Leder, P. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1408–1412.

Behar-Bannelier, M., & Murray, R. K. (1980) *Biochem. J. 187*, 147–156.

Blobel, G., & Potter, V. R. (1966a) *Proc. Natl. Acad. Sci. U.S.A. 55*, 1283–1288.

Blobel, G., & Potter, V. R. (1966b) *Science (Washington, D.C.) 154*, 1662–1665.

Bornens, M., & Courvalin, J.-C. (1978) *J. Cell Biol. 76*, 191–206.

Bresnick, E., Mukhtar, H., Stoming, T. A., Dansette, P. M., & Jerina, D. M. (1977) *Biochem. Pharmacol. 26*, 891–892.

Cardelli, J., Long, B., & Pitot, H. C. (1976) *J. Cell Biol. 70*, 47–58.

Conney, A. H., Davison, C., Gustel, R., & Burns, J. J. (1960) *J. Pharmacol. Exp. Ther. 130*, 1–8.

Craft, J. A., Cooper, M. B., Estall, M. R., Rees, D. E., & Rabin, B. R. (1979) *Eur. J. Biochem. 96*, 379–391.

Dwyer, N., & Blobel, G. (1976) *J. Cell Biol. 70*, 581–591.

Fahl, W. E., Jefcoate, C. R., & Kasper, C. B. (1978) *J. Biol. Chem. 253*, 3106–3113.

Franke, W. W., & Scheer, U. (1974) in *The Cell Nucleus* (Busch, H., Ed.) Vol. 1, pp 219–347, Academic Press, New York.

Fry, D. J., & Wishart, G. J. (1976) *Biochem. Soc. Trans. 4*, 265–266.

Gerace, L., & Blobel, G. (1980) *Cell (Cambridge, Mass.) 19*, 277–287.

Gonzalez, F. J., & Kasper, C. B. (1980a) *Biochemistry 19*, 1790–1796.

Gonzalez, F. J., & Kasper, C. B. (1980b) *Biochem. Biophys. Res. Commun. 93*, 1254–1258.

Harris, J. R. (1978) *Biochim. Biophys. Acta 515*, 55–104.

Jick, H., & Shuster, L. (1966) *J. Biol. Chem. 241*, 5366–5369.

Kashnig, D. M., & Kasper, C. B. (1969) *J. Biol. Chem. 244*, 3786–3792.

Kasper, C. B. (1971) *J. Biol. Chem. 246*, 577–581.

Kasper, C. B. (1974a) *Methods Enzymol. 31*, 279–293.

Kasper, C. B. (1974b) in *The Cell Nucleus* (Busch, H., Ed.) Vol. 1, pp 349–384, Academic Press, New York.

Kay, R. R., & Johnston, I. R. (1973) *Subcell. Biochem. 2*, 127–167.

Khandwala, A. S., & Kasper, C. B. (1973) *Biochem. Biophys. Res. Commun. 54*, 1241–1246.

Kruppa, J., & Sabatini, D. D. (1974) *J. Cell Biol. 77*, 414–427.

Lam, K. S., & Kasper, C. B. (1978) *Biochemistry 18*, 307–311.

Lam, K. S., & Kasper, C. B. (1979) *J. Biol. Chem. 254*, 11713–11720.

Louis-Ferdinand, R. T., & Fuller, G. C. (1970) *Biochem. Biophys. Res. Commun. 38*, 811–816.

Mancini, G., Carbonara, A. O., & Heremans, J. F. (1965) *Immunochemistry 2*, 235–254.

Masters, B. S. S., Baron, J., Taylor, W. E., Isaacson, E. L., & LoSpalluto, J. (1971) *J. Biol. Chem. 246*, 4143–4150.

Maul, G. R. (1977) *Int. Rev. Cytol. 6*, 75–186.

Mukhtar, H., Elmamlouk, T. H., & Bend, J. R. (1979) *Arch. Biochem. Biophys. 192*, 10–21.

Orrenius, S., Ericsson, J. L. E., & Ernster, L. (1965) *J. Cell Biol. 25*, 627-639.
Perry, R. P., LaTorre, J., Kelley, D. E., & Greenberg, J. R. (1972) *Biochim. Biophys. Acta 262*, 220-226.
Pezzuto, J. M., Yang, C. S., Yang, S. K., McCourt, D. W., & Gelboin, H. V. (1978) *Cancer Res. 38*, 1241-1245.
Ramsey, J. C., & Steele, W. J. (1976) *Biochemistry 15*, 1704-1712.
Remmer, H., & Merker, H. J. (1963) *Science (Washington, D.C.) 142*, 1657-1658.
Roewekamp, W. G., Hofer, E., & Sekeris, C. E. (1976) *Eur. J. Biochem. 70*, 259-268.
Sadowski, P. D., & Howden, J. A. (1968) *J. Cell Biol. 37*, 163-181.
Seifert, J., & Vacha, J. (1970) *Chem.-Biol. Interact. 2*, 297-307.
Shore, G. L., & Tata, J. R. (1977) *J. Cell Biol. 72*, 714-725.
Sikstrom, R., Lanoix, J., & Bergeron, J. J. M. (1976) *Biochim. Biophys. Acta 448*, 88-102.
Thomas, P. E., Korzeniowski, D., Bresnick, E., Bornstein, W. A., Kasper, C. B., Fahl, W. E., Jefcoate, C. R., & Levin, W. (1979) *Arch. Biochem. Biophys. 192*, 22-26.
Venkatesan, N., & Steele, W. J. (1975) *Chem.-Biol. Interact. 11*, 41-54.
Whittle, E. D., Bushnell, D. E., & Potter, V. R. (1968) *Biochim. Biophys. Acta 161*, 41-50.
Wunderlich, F., Berezney, R., & Kleinig, H. (1976) in *Biological Membranes* (Chapman, D., & Wallach, D., Eds.) Vol. 3, pp 282-333, Academic Press, New York.
Zbarsky, I. B. (1979) *Int. Rev. Cytol. 54*, 295-360.
Zimmerman, J. J., & Kasper, C. B. (1978) *Arch. Biochem. Biophys. 190*, 726-735.

Bovine $H1^0$ Histone Subfractions Contain an Invariant Sequence Which Matches Histone H5 Rather Than H1†

John R. Pehrson and R. David Cole*

ABSTRACT: Histone $H1^0$ differs substantially from H1 in a 37-residue region of the primary structure that is conserved between the usual H1 histones of fish and mammals. Instead, $H1^0$ is homologous to H5 in this region. Histones $H1^0$ and H5 might be classified together in a histone category distinct from the usual H1.

An inverse correlation between levels of $H1^0$ histone and rates of cell division has been established by comparing several mammalian tissues (Panyim & Chalkley, 1969a; Marsh & Fitzgerald, 1973). Recently, we reported that the amount of $H1^0$ in homogenous cell lines increased 3- or 4-fold when cell division was inhibited by high cell density or by serum deprivation (Pehrson & Cole, 1980). Since the synthesis of the major classes of histone is tightly coupled to DNA[1] synthesis (Prescott, 1966), the histone pool of nondividing cells would be expected to remain constant quantitatively and qualitatively. Unlike most histones, however, the accumulation of $H1^0$ in growth-inhibited cell cultures might be accomplished without accompanying DNA synthesis. This situation would be similar to that of histone H5 (Appels & Wells, 1972), which is apparently a variant of H1 unique to mature erythrocytes in birds. In broad terms, histones $H1^0$ and H5 might be considered as related functionally, since the former may suppress replication (Marsh & Fitzgerald, 1973) while the latter seems to suppress transcription (Brasch et al., 1974). This suggested to us that $H1^0$ and H5 might have some structural features in common. In fact, a partial determination of the primary structure, as reported below, has shown bovine $H1^0$ to be strikingly homologous to chicken H5 and rather different from the H1. While this work was in progress, Smith, Walker and Johns reported that a sequence of 17 residues in a particular subfraction of $H1^0$ was quite homologous with the corresponding region in H5 (Smith et al., 1980). Our work not only extends the earlier report by 37 residues but also removes the possibility that microheterogeneity in $H1^0$ includes molecular species very homologous to the usual H1 histones in addition to one homologous to H5.

Smith and Johns recently discovered that, like H1, $H1^0$ is microheterogeneous (Smith & Johns, 1980). Since H5 has been considered to be a variant of H1 (Morris, 1976), it could be postulated that the microheterogeneity in $H1^0$ included molecular species homologous to H1 as well as species homologous to H5. It is conceivable that the particular $H1^0$ subfraction whose sequence was studied by Smith et al. (1980) did not represent the entire population of $H1^0$ molecules. In our studies the sequence determination was made on unfractionated $H1^0$, and since no polymorphism was observed, it is clear that the close homology with H5 is a general characteristic of $H1^0$.

Experimental Procedures

Materials

PMSF, NBS, and Tris-base were obtained from Sigma Chemical Co. Ultra pure urea was from Schwarz/Mann. PCA, hydrochloric acid, trichloroacetic acid, and $NaHSO_3$ were from Malinkrodt, Inc. Acrylamide and bis(acrylamide) were from Bio-Rad Laboratories. Sequenator grade solvents were from Pierce Chemical Co. and Beckman Instruments, Inc.

Methods

Isolation of $H1^0$. Steer kidney cortex was homogenized at 4 °C in 50 mM Tris-base, 50 mM $NaHSO_3$, 5 mM $MgCl_2$, and 250 μm PMSF, pH 7.5, with a Waring blender. The homogenate was centrifuged at 1500*g* for 10 min, the su-

† From the Department of Biochemistry, University of California, Berkeley, California 94720. *Received September 2, 1980.* This work was supported by National Institutes of Health Grants GMS 20338 and NIEHS IT32 ES07075 and by the Agricultural Research Station.

[1] Abbreviations used: DNA, deoxyribonucleic acid; PMSF, phenylmethanesulfonyl fluoride; PCA, perchloric acid; NBS, *N*-bromosuccinimide; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol.

0006-2960/81/0420-2298$01.25/0 © 1981 American Chemical Society