Inhibition of Mammary Gland Differentiation in Vitro by 5-Bromo-2'-deoxyuridine*

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SUMMARY

Mammary epithelial cells in organ culture incorporate 5-bromo-2'-deoxyuridine (B UdR) into DNA during a single cycle of DNA replication. The specific milk proteins, casein and α-lactalbumin, are induced by prolactin in control mammary cells, but the induction of these proteins is markedly inhibited in cells which contain B UdR-DNA. This is a selective effect, since the induction of the galactosyltransferase of lactose synthetase is not inhibited, and the rate of total protein synthesis is only slightly decreased in such cells. Incubation of mammary cells with B UdR after completion of DNA synthesis did not inhibit the induction of casein or α-lactalbumin. Thus B UdR appears to selectively inhibit the expression of genes which are uniquely expressed in differentiated mammary secretory cells. Formation of transfer RNA, total rapidly labeled nuclear RNA, and ribosomal RNA was unaltered in cells containing B UdR-DNA. However, a marked reduction of prolactin-stimulated polysome formation was observed. Initiation of casein synthesis in these cells has been previously shown to be dependent upon the formation of prolactin-induced polysomes. B UdR inhibited milk protein induction by more than 90%. This result suggests that only mammary daughter cells containing the newly synthesized “sense” strand of DNA may express differentiated function.

The regulation of gene expression in mammary epithelial cells in vitro is dependent upon the sequential action of specific hormones. Stem cells may be induced to undergo a single cell division by insulin (1), and hydrocortisone is required to act upon the progeny cells before prolactin can synergize with insulin to induce the specialized proteins casein and lactose synthetase (2). Differentiation of stem cells into secretory cells which uniquely express the genetic information for differentiated function requires replication of the stem cell DNA and subsequent cell division (3, 4). Previous studies have demonstrated changes in chromosomal proteins which occur during this critical cell cycle (5, 6), and have provided evidence to indicate that new areas of the genome are transcribed in the differentiated cells (7). In the experiments to be described here, the structural characteristics required of the DNA template for cell differentiation have been probed. Mammary epithelial cells were permitted to incorporate 5-bromo-2'-deoxyuridine to form unifilar B UdR-DNA, and the effects on cell differentiation associated with this change in template structure have been studied in relation to the mechanisms for hormonal induction of specific milk proteins.

EXPERIMENTAL METHODS

Animals—Four-month-old midpregnant (10 to 12 days) mice of the C3H/HeJ strain were used in all experiments. Organ Culture Methods—Small explants of the thoracic, abdominal, and inguinal mammary glands were prepared and cultured on Medium 199 (Microbiological Associates, Bethesda, Maryland) as previously described (8). Hormones, prepared as fresh stock solutions, were added to a final concentration of 5 μg per ml. The culture medium was replenished at least every 48 hours.

Chemicals and Hormones—32P-Orthophosphate (carrier free) was obtained from Tracerlab, Waltham, Massachusetts. A mixture of uniformly labeled 14C-amino acids was obtained from New England Nuclear Corporation. Uridine-5-3H (specific activity, 22 Ci per mmole) and 5-bromo-2'-deoxyuridine-6-3H (specific activity, 12.7 Ci per mmole) were obtained from Schwarz BioResearch. Pancreatic ribonuclease (five times crystallized) and pancreatic deoxyribonuclease (electrophoretically pure) were obtained from Worthington. 5-Bromo-2'-deoxyuridine, 5-bromo-2'-deoxycytidine, 5-iodo-2'-deoxyuridine, and thymidine were purchased from Calbiochem. Sodium heparin was obtained from Fisher, and density gradient grade (ribonuclease free) sucrose was obtained from Mann. Bovine zinc insulin (23.6 units per mg) was a gift from the Eli Lilly Company, and ovine prolactin was provided by the Endocrinology Study Section, National Institutes of Health.

Casein Synthesis—Explants were exposed to medium containing 32P1 (25 μCi per ml) or 14C-amino acids (10 μCi per ml) for 4 hours. They were then quickly weighed and homogenized, and the homogenate was centrifuged at 100,000 × g for 60 min. 32P-casein or 14C-casein was isolated by coprecipitation with bovine casein carrier after addition of rennin and calcium ions to the 100,000 × g supernatant fluid, as previously described (9).

1 The abbreviation used is: B UdR, 5-bromo-2'-deoxyuridine.
Lactose Synthetase Assay—The activity of the lactose synthetase (UDP-galactose: d-glucose-1-galactosyltransferase; EC 2.4.1.22) system was measured by assays of its separate components. The galactosyltransferase was measured by the transfer of \(^{14}C\)-galactose from UDP-galactose to an N-acetylgalactosamine acceptor. a-Lactalbumin was measured by the rate of transfer of \(^{14}C\)-galactose from UDP-galactose to glucose in the presence of an excess of exogenous galactosyltransferase, as previously described (8).

Analysis of Polysomes—Polysomes were prepared from explant homogenates and were analyzed on 15 to 32\% sucrose isokinetic gradients as previously described (10, 11).

Ribosomal RNA Synthesis—Rapidly labeled nuclear RNA was labeled by exposing the explants to medium containing \(^{3}H\)-uridine, 4.0 \(\mu\)Ci per ml, for 20 min. Ribosomal RNA was labeled by exposing the explants to medium containing \(^{3}H\)-uridine at the same concentration for 4 hours. RNA was extracted by treatment of homogenates with 70\% phenol at 60\°, as previously described (12). The method of analysis of the RNA preparations on 5 to 27.9\% sucrose isokinetic gradients has been described previously (10).

Transfer RNA Assay—RNA was extracted from explant homogenates with 85\% phenol at 27\°, as described previously (13). Amino acid acceptor activity of the RNA preparations was measured by the extent of charging with a mixture of \(^{14}C\)-amino acids in the presence of an excess of amino acyl synthetases, magnesium ions, and ATP, as previously described (13).

Analysis of \(^{3}H\)-BUdR-DNA—Explants were exposed to medium containing insulin and \(^{3}H\)-BUdR (1.0 \(\mu\)Ci per ml) during the 24- to 48-hour or 72- to 96-hour periods of incubation. DNA was isolated from homogenates of the explants by a modification of the method of Marmur (14) as previously described (7). For analysis of the products of \(^{3}H\)-DNA hydrolysis, explants were homogenized in medium which was 0.25\% sucrose in the presence of an excess of amino acyl synthetases, magnesium ions, and ATP, as previously described (13).

Analysis of Incorporation of \(^{3}H\)-BUdR into DNA—Mammary explants were incubated on medium containing \(^{3}H\)-BUdR, 0.5 \(\mu\)Ci per ml, during the 0- to 72-hour period of incubation. The tissue was then fixed in Bouin's solution, sectioned at 5 \(\mu\), and autoradiographs of the sections made, as previously described (1).

RESULTS

Incorporation of \(^{3}H\)-5-bromo-2'-deoxyuridine into DNA—Mammary epithelial cells incubated with insulin are stimulated to initiate DNA synthesis and subsequently to divide. A maximal increase in the number of cells engaged in DNA synthesis is observed during the 24- to 24-hour period of incubation, but after 72 hours only 1 to 2\% of the epithelial cells incorporate \(^{3}H\)-thymidine into DNA (1). As shown in Table I, \(^{3}H\)-BUdR was incorporated into trichloroacetic acid-precipitable material at a high rate during a portion of the period of proliferation, and the rate of incorporation was correspondingly reduced during the 72- to 96-hour period when only 1 to 2\% of the epithelial cells were engaged in DNA synthesis. The \(^{3}H\)-BUdR labeled material was sensitive to treatment with DNase or hot trichloroacetic acid, but was not solubilized by RNase. During the 0- to 72-hour period of incubation, approximately 75\% of the epithelial cells became labeled with \(^{3}H\)-BUdR, as shown by autoradiography of tissue sections. This result is comparable to that previously reported for the number of cells which incorporate \(^{3}H\)-thymidine during this period (1, 15). Mitotic indices in the BUdR-treated tissues were the same as in control tissues, and were comparable to those previously reported (15, 16). As shown in Table II, more than 97\% of the 6-\(^{3}H\)-BUdR incorporated into DNA was recovered as

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Labeling period</th>
<th>cpm/100 mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>24 to 48 hours</td>
<td>125,000 11,600</td>
</tr>
<tr>
<td>DNase, 70 (\mu)g per ml, 37\°, 60 min.</td>
<td>30,000 3,300</td>
<td></td>
</tr>
<tr>
<td>10% trichloroacetic acid, 90\°, 10 min.</td>
<td>900 200</td>
<td></td>
</tr>
<tr>
<td>RNase, 100 (\mu)g per ml, 37\°, 60 min.</td>
<td>121,000 11,800</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Base</th>
<th>Radioactivity</th>
</tr>
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<tbody>
<tr>
<td>Guanine</td>
<td>0 0</td>
</tr>
<tr>
<td>Thymine</td>
<td>20 0</td>
</tr>
<tr>
<td>Cytosine</td>
<td>3 0</td>
</tr>
<tr>
<td>5-Bromouracil</td>
<td>14,313 97.3</td>
</tr>
<tr>
<td>Adenine</td>
<td>394 2.7</td>
</tr>
</tbody>
</table>

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TABLE III

Effect of 5-bromo-2'-deoxyuridine on hormonal induction of specific milk proteins

Mammary explants were incubated on media containing the indicated additions. During the final 4-hour period (106 to 112 hours) some explants in each system were exposed to medium containing \(^{32}\)P, 25 \(\mu\)Ci per ml, and \(^{32}\)P-casein was isolated and counted. Homogenates of other explants in each system were prepared at 112 hours and were assayed for lactose synthetase activities. I, insulin; H, hydrocortisone; P, prolactin. The concentration of BUdR was 10\(^{-4}\) M.

<table>
<thead>
<tr>
<th>Additions to medium</th>
<th>4P-Casein</th>
<th>Lactose synthetase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\mu)g/4 hrs/mg tissue</td>
<td>umoles/min/mg tissue</td>
</tr>
<tr>
<td>0 to 72 hours</td>
<td>72 to 112 hours</td>
<td></td>
</tr>
<tr>
<td>I + H</td>
<td>I + H</td>
<td>172</td>
</tr>
<tr>
<td>I + H</td>
<td>I + H + P</td>
<td>600</td>
</tr>
<tr>
<td>I + H + BUdR</td>
<td>I + H</td>
<td>176</td>
</tr>
<tr>
<td>I + H + BUdR</td>
<td>I + H + P</td>
<td>174</td>
</tr>
<tr>
<td>I + H</td>
<td>I + H + P + BUdR</td>
<td>600</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of the concentration of BUdR on prolactin-stimulated synthesis of casein in mouse mammary explants. Explants were incubated with insulin, hydrocortisone, and various concentrations of BUdR for 72 hours. The medium was then changed to include only insulin, hydrocortisone, and prolactin. During the 92- to 96-hour period the medium contained \(^{32}\)P (25 \(\mu\)Ci per ml), and the explants were then assayed for \(^{32}\)P-casein. Control explants incubated with insulin and hydrocortisone for 96 hours (without BUdR or prolactin) had a \(^{32}\)P-casein value of 265 cpm per mg of tissue per 4-hour period.

5-bromouracil after formic acid digestion of the DNA, while a small amount of radioactivity was found in adenine.

Effect of 5-Bromo-2'-deoxyuridine on Protein Synthesis—The differentiation of mammary epithelial cells involves the formation of secretory alveolar cells in which the specific milk proteins, casein and lactose synthetase, can be induced by insulin and prolactin. As shown in Table III, cells were formed during the 0- to 72-hour period of cell proliferation on medium containing insulin and hydrocortisone. The subsequent addition of prolactin to the medium resulted in a marked increase in the rate of \(^{32}\)P-casein synthesis and in the enzymatic activities of the two protein components of lactose synthetase, \(\alpha\)-lactalbumin and the galactosyltransferase, as previously described (8). Incorporation of BUdR into DNA by the proliferating cells during the 0- to 72-hour period did not significantly alter the “base line” values of these proteins in the differentiated cells of the explant cell population. However, the incorporation of BUdR markedly inhibited the induction of \(^{32}\)P-casein synthesis. The induction of \(\alpha\)-lactalbumin was also nearly completely inhibited, although the formation of the galactosyltransferase of lactose synthetase in response to prolactin stimulation was not detectably altered.

TABLE IV

Rate of incorporation of \(^{14}\)C-amino acids into protein by mammary explants incubated with 5-bromo-2'-deoxyuridine

Mammary explants of mice in midpregnancy were incubated on medium containing insulin and hydrocortisone, with or without BUdR (10\(^{-4}\) M) for 72 hours. During the 68 to 72-hour period the medium contained a mixture of \(^{14}\)C-amino acids (10 \(\mu\)Ci per ml). The explants were then weighed and homogenized in 4 ml of 0.15 M KCl-0.2% casein, and the homogenate was treated with an equal volume of 10% trichloroacetic acid. Labeled nucleic acids were hydrolyzed by heating at 90° for 15 min. The precipitates were washed three times in 5% trichloroacetic acid and then in ether-ethanol (1:3 v/v), dissolved in 5 M acetic acid, and counted in Bray's solution (17).

<table>
<thead>
<tr>
<th>System</th>
<th>(^{14})C-Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm/mg tissue</td>
</tr>
<tr>
<td>Control</td>
<td>4830</td>
</tr>
<tr>
<td>BUdR</td>
<td>4110</td>
</tr>
</tbody>
</table>

TABLE V

Effect of various halogenated deoxynucleosides on hormonal induction of casein

Mammary explants were incubated for 72 hours on medium containing insulin and hydrocortisone. Various analogues of uridine or cytidine were present in some systems. The explants were then transferred to medium containing insulin and hydrocortisone, with or without prolactin for the 72- to 96-hour period. \(^{32}\)P (25 \(\mu\)Ci per ml) was present in the medium during the 92- to 96-hour period, after which the tissues were assayed for \(^{32}\)P-casein. The percentage of inhibition of casein synthesis refers to the prolactin-dependent increase.

<table>
<thead>
<tr>
<th>Analogue present at 0 to 72 hours</th>
<th>Prolactin present at 72 to 96 hours</th>
<th>(^{32})P-Casein</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cpm/mg tissue</td>
<td>%</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>285</td>
<td>0</td>
</tr>
<tr>
<td>5-iodo-2'-deoxyuridine (10(^{-4}) M)</td>
<td></td>
<td>659</td>
<td>43</td>
</tr>
<tr>
<td>5-bromo-2'-deoxyuridine (10(^{-4}) M)</td>
<td></td>
<td>444</td>
<td>41</td>
</tr>
<tr>
<td>5-bromo-2'-deoxyuridine (10(^{-4}) M)</td>
<td></td>
<td>452</td>
<td>90</td>
</tr>
<tr>
<td>5-bromo-2'-deoxyuridine (10(^{-4}) M)</td>
<td></td>
<td>216</td>
<td>51</td>
</tr>
<tr>
<td>5-bromo-2'-deoxyuridine (10(^{-4}) M) + thymidine (10(^{-3}) M)</td>
<td></td>
<td>630</td>
<td>0</td>
</tr>
</tbody>
</table>
hibitory effect of BUdR was completely reversed by incubating
most active inhibitor tested, although significant inhibition was
by deoxycytidylate deaminase in other cell types (18). The in-
inhibited the induction of casein and cr-lacta'bumin by 90% or
inhibited by approximately 90%. A further increase to 10ea
bited in proportion to logarithmic increases in the concentration
of BUdR for half-maximal inhibition
was approximately 2
M.

Addition of BUdR to the medium during the postmitotic period
did not significantly interfere with the induction by prolactin and
insulin of the specific proteins.

Fig. 1 shows the effect of the concentration of BUdR on the
rate of casein synthesis in prolactin-stimulated cells. Cells were
allowed to proliferate in medium containing insulin, hydro-
cortisone, and various concentrations of BUdR for 72 hours.
Prolactin was then added to the medium and the rate of incor-
poration of 3P into casein during the 92- to 96-hour period of in-
cubation was determined. The rate of casein synthesis was in-
hibited in proportion to logarithmic increases in the concentra-
tion of BUdR over the range of 10-14 to 10-4 M. At a concentration
of BUdR of 10-4 M, prolactin-stimulated casein synthesis was
inhibited by approximately 90%. A further increase to 10-3
M resulted in a slight further increase in the degree of inhibition.
The concentration of BUdR for half-maximal inhibition was
approximately 2 × 10-4 M.

Table IV compares the rates of synthesis of total protein in
control mammary explants and in mammary explants exposed to
BUdR for 72 hours. At this point a nearly maximum number of
cells had incorporated BUdR into DNA under conditions which
inhibited the induction of casein and cr-lacta'bumin by 90% or
more. However, the rates of total protein synthesis in such cells
were decreased by only 15%.

The relative activities of several structural analogues of BUdR
for inhibition of the prolactin-induced synthesis of casein are
shown in Table V. At a concentration of 10-4 M, BUdR was the
most active inhibitor tested, although significant inhibition was
shown by 5-iodo-2'-deoxyuridine and 5-bromo-2'-deoxycytidine.
The latter compound has been shown to be converted to BUdR
by deoxycytidylate deaminase in other cell types (18). The in-
hibitory effect of BUdR was completely reversed by incubating
the cells with BUdR and an excess of thymidine.

Effect of 5 Bromo 2'-deoxurydine on RNA—Previous studies
have demonstrated that the prolactin-induced increases in casein
and lactose synthetase are inhibited by actinomycin D, and are
preceded by marked increases in the rates of synthesis of specific
classes of RNA (12, 19, 20). The earliest effect of prolactin on
RNA synthesis relates to the increased rates of formation of

![Fig. 2. Isokinetic sucrose density gradient profiles of "H-RNA extracted from mammary explants incubated on various media.](http://www.jbc.org/)

TABLE VI

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>µH-RNA cpm/20 min/mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 72 hours</td>
<td>214</td>
</tr>
<tr>
<td>72 to 60 hours</td>
<td>356</td>
</tr>
<tr>
<td>1 + H</td>
<td>210</td>
</tr>
<tr>
<td>1 + H + BUdR</td>
<td>348</td>
</tr>
</tbody>
</table>

TABLE VII

<table>
<thead>
<tr>
<th>Incubation system</th>
<th>Amino acid acceptor activity of RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 24 hours</td>
<td>cpm/45 µg RNA</td>
</tr>
<tr>
<td>24 to 12 hours</td>
<td>cpm/100 mg tissue</td>
</tr>
<tr>
<td>1 + H</td>
<td>2190</td>
</tr>
<tr>
<td>1 + H + BUdR</td>
<td>3340</td>
</tr>
<tr>
<td>1 + H + BUdR + P</td>
<td>6560</td>
</tr>
<tr>
<td>1 + H + BUdR + P</td>
<td>8060</td>
</tr>
<tr>
<td>1 + H + BUdR + P</td>
<td>9600</td>
</tr>
<tr>
<td>1 + H + BUdR + P</td>
<td>7800</td>
</tr>
</tbody>
</table>
DISCUSSION

These studies demonstrate that mammary epithelial cells incorporate BUdR into DNA during cell proliferation in vitro, and this observation provides an opportunity to test the effects of a structural alteration of the genome on the regulation of selective gene expression. The BUdR is incorporated into DNA as a structural analogue of thymidine (21), and its biological effects on the mammary epithelial cells could be completely reversed by providing an excess of thymidine in the medium during the period of DNA replication. BUdR had no detectable effects on the molecular processes measured in these experiments when added to cells in the postmitotic period, indicating that its effects derive largely from its insertion into the DNA.

The incorporation of BUdR into DNA had a strikingly selective effect on gene expression in the progeny cells formed by cell division, in that the induction of casein and α-lactalbumin by prolactin was nearly completely prevented. Synthesis of these proteins by mammary explants in the postmitotic period and prior to prolactin stimulation in vitro has been shown to represent the biosynthetic activity of the previously differentiated, non-dividing cells in the explant cell population (22), and the synthesis of casein is not detectable in the newly formed cells in vitro until after addition of prolactin to the medium (19). Synthesis of these milk proteins was selectively inhibited in the BUdR-DNA-containing cells, and not in the previously differentiated cells which did not incorporate BUdR into DNA in vitro. Inhibition of the induction of casein and α-lactalbumin was not a failure of the cells to respond to the hormones per se, since insulin-mediated cell division (as indicated by mitotic indices) was not inhibited, and increases in ribosomal RNA and transfer RNA occurred in the hydrocortisone and prolactin-treated cells. Furthermore, the induction by prolactin of the galactosyltransferase component of lactose synthetase was not inhibited. Although lactose synthesis and α-lactalbumin are unique features of the mammary gland, the galactosyltransferase enzyme is found in a wide variety of other cell types (23). The formation of other specific proteins which are not unique to the mammary gland was not studied; however, it was observed that total protein synthesis following the incorporation of BUdR was decreased by only 15%, in contrast to the 90% inhibition of casein and α-lactalbumin formation.

Thus the incorporation of BUdR into the mammary cell genome exerted a selectively inhibitory effect on the new expression of genes with protein products which are organ-specific, specialized secretory proteins.

The effect of BUdR on other differentiating cell types has been reported to share a number of similarities with those observed in the present studies. Proliferating myogenic cells grown for a single round of DNA synthesis in BUdR fail to fuse their membranes and fail to synthesize myosin and actin (24, 25). Amnion cells which replicate in the presence of BUdR fail to synthesize normal hyaluronic acid (26), and cultured BUdR-treated chondrocytes fail to synthesize chondroitin sulfate and do not have detectable levels of sulfokinase or UDPGNAc-4-sulfotransferase (27). All the evidence available from studies on these cells indicates that BUdR suppresses tissue-specific, specialized function as a result of its incorporation into DNA (25). In the present studies the inhibition by BUdR of the induction of casein and α-lactalbumin has been studied in terms of the formation of specific classes of RNA. Among the changes in RNA which occur prior to prolactin-mediated synthesis of casein and α-lactalbumin, only the numbers of polysomes in the BUdR-DNA-containing cells were found to be altered. Previous studies provided evidence to support the concept that the polysomes which form in response to prolactin stimulation contain the messenger RNAs for the synthesis of specific casein polypeptides (10). It is possible that the presence of BUdR in the genome inhibits the formation of these messenger RNAs. Genes for milk proteins may lack the high degree of redundancy which may exist for genes controlling unspecialized metabolic processes so that the former class of genes may be especially sensitive to pyrimidine analogue substitution. Alternatively, induction of milk proteins may depend upon differential gene amplification, from either a DNA or an RNA template, and the amplification process may be more selectively inhibited by analogue incorporation. Such amplification of genes would have to have occurred during the first 72-hour period of chromosomal replication, since the addition of BUdR after this period did not interfere with the subsequent action of

RNA to the same degree in BUdR-DNA-containing cells as in the control prolactin-stimulated system. Previous results (12) have shown that the pool size and specific activity of UTP in these tissues are not significantly increased by prolactin treatment, so that the increased labeling of nuclear RNA and ribosomal RNA may be taken as an indication of increased rates of formation of these RNAs.

After 6 hours of prolactin stimulation of mammary epithelial cells previously treated with insulin and hydrocortisone, an increase in polysomes is observed (10). This increase occurs concomitantly with the initiation of casein synthesis, and has been interpreted to represent the translation of new casein messenger RNAs on cytoplasmic polysomes (10). Fig. 3 shows a similar experiment in which prolactin caused a marked increase in polysome formation in the control explants, but this response was markedly reduced in explants previously treated with BUdR. This effect could not be explained by differences in polysome degradation, since RNase activities were similar in all systems, and there was no alteration of the general shape of the heavy polysomal profile. Some decrease in the total number of ribosomes in the BUdR-treated cells is also evident from these tracings.

Fig. 3. Isokinetic sucrose gradient sedimentation profiles of polysomes extracted from mouse mammary explants incubated on various media. A, insulin and hydrocortisone, 96 hours. A similar profile was obtained with explants incubated with insulin, hydrocortisone, and BUdR for 96 hours. B, insulin and hydrocortisone, 72 hours, then insulin, hydrocortisone, and prolactin, 72 to 96 hours. C, insulin, hydrocortisone, and BUdR (10⁻⁴ M), 72 hours, then insulin, hydrocortisone, and prolactin, 72 to 96 hours. In each case the polysomes were derived from 175 mg of tissue.
prolactin. The action of prolactin is not associated with detectable stimulation of DNA synthesis. Formation of the milk protein messengers may require activation at a pyrimidine-rich initiator site for transcription (28, 29), whereas genes for proteins serving unspecialized functions may have initiator sites with lower pyrimidine content or specificity. Genes which may selectively promote polysomal stability may also be altered, resulting in a loss of the ability of the cell to synthesize large numbers of secretory protein molecules. Prolactin-induced polysome formation was only inhibited by 50 to 75%, a finding consistent with the observation that induction of milk proteins was only 90% inhibited, and the induction of the galactosyltransferase of lactose synthetase by prolactin was uninhibited.

An interesting finding in these studies was the effect of the concentration of BUdR on the induction of casein. At high concentrations (10^{-4} to 10^{-3} M), the induction of casein was inhibited by 90% or more. This result would appear to imply that normally only one daughter cell formed in vitro by stem cell division under the present experimental conditions is differentiated. Since the DNA of mammalian cells appears to replicate in a semi-conservative manner, BUdR was presumably incorporated into only the newly synthesized polynucleotide strands during the single DNA synthetic cycle. Therefore only “hybrid” DNA molecules are present, each composed of one BUdR-free and one BUdR-labeled strand. In one daughter cell the “sense” strand would be labeled with BUdR, and the presence of BUdR could potentially affect gene transcription. In the other daughter cell only the “nonsense” strand would be labeled, permitting transcription to proceed normally. If the assumption can be made that intrastrand effects of BUdR predominate and interstrand effects do not alter the regulation of transcription, the nearly 100% inhibition by BUdR of milk protein induction indicates that only the daughter cell with a newly synthesized sense strand is differentiated. Otherwise, only a 50% or less degree of inhibition would have been anticipated if the cell containing the BUdR-free sense strand were capable of differentiated function.

We tentatively propose, therefore, that mammary stem cell division in vitro results in the formation of two daughter cells, only one of which can differentiate, the other presumably being a stem cell which can replenish the population of dividing cells. Under normal conditions, then, only the cell containing the newly synthesized DNA sense strand can express new genetic information. A number of possible mechanisms, including new recombinations of chromosomal proteins on the newly synthesized sense strand-containing chromosome, could possibly account for new gene expression by this model. More experiments are in progress to test this proposed model of gene regulation.

REFERENCES

Inhibition of Mammary Gland Differentiation in Vitro by 5-Bromo-2'-deoxyuridine
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