A SEARCH FOR DIFFERENTIAL POLYPEPTIDE SYNTHESIS THROUGHOUT THE CELL CYCLE OF HELA CELLS

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ABSTRACT

The polypeptides synthesized during the cell cycle of HeLa cells were analyzed by means of two-dimensional gel electrophoresis followed by fluorography under conditions in which the position of 700 polypeptides (acidic and basic) could be reproducibly assessed. Mitotic cells obtained by mechanical detachment and synchronized cells in other stages of the cell cycle were labeled with [^{35}S]methionine for 30-min pulses or for long terms starting at the beginning of each phase. Visual comparison of the polypeptide maps obtained in the different stages of the cell cycle showed that these were strikingly similar, and there was no indication that the synthesis of any of the detected polypeptides was confined to only one of the cell cycle phases. Quantitation of 99 abundant polypeptides (acidic and basic) in pulse-labeled and long-term labeled cells revealed that the relative amount (i.e., the rate of synthesis) of most polypeptides, including total actin, α-actinin, 6 abundant basic nonhistone proteins, and 13 major acidic proteins present in Triton cytoskeletons, remains constant throughout the cell cycle. Among the few variable polypeptides (markers), we have identified α- and β-tubulin (increase in M), the subunit of the 100-Å filament protein "fibroblast type" (decreases in M), and a 36,000 mol wt acidic cytoarchitectural protein that increases in S. A few other unidentified polypeptides have also been found to vary in M and in M and G_2, but no marker was found in G_1.

KEY WORDS cell cycle · high-resolution gel electrophoresis · isoelectric focusing · nonequilibrium pH gradient electrophoresis · polypeptide marker

The cell cycle of somatic cells is divided into four stages, namely, G_1, S, G_2 and mitosis (M) (17, 18). The S phase corresponds to the intermitotic stage that is engaged in chromosomal DNA synthesis. G_1 and G_2 correspond to the two gaps in the intermitotic stage, G_1 preceding the S phase and G_2 coming after it (10). Cell division takes place in M, a phase that is characterized by drastic changes in cell morphology and cell surface characteristics (3, 17, 18).

Studies of RNA synthesis during the cell cycle have indicated that most RNA synthesis takes place continuously during G_1, S, and G_2, but that it ceases in mitosis (25, 28). Similarly, it has been shown that protein synthesis takes place continuously throughout the mitosis cycle, though at a reduced rate (7, 11).

Central to understanding the processes that control cell proliferation is the elucidation of the mechanisms that regulate macromolecular synthesis and cell progression throughout the cell cycle. Toward this aim, we report here a detailed analysis of acidic and basic polypeptides synthesized during the life cycle of HeLa cells that was made using high-resolution two-dimensional gel electrophoresis (15, 16).

MATERIALS AND METHODS

Cells and Cell Synchrony

HeLa cells (GIBCO Bio-Cult Ltd., stock source, American Type Culture Collection) were grown routinely as monolayer cultures in Eagle's minimal essential medium (MEM) containing 10% fetal calf serum and antibiotics (100 U/ml penicillin, 50 μg/ml streptomycin). Mitotic cells were obtained by mechanical detachment essentially as described by Terasima and Tolmach (26). Two 250-ml flasks containing ~1–2 × 10^6 cells per flask were used. The homogeneity of the collected cells was assessed according to the criteria described by Terasima and Tolmach (26). The distribution of phases in mitotic populations was determined by phase-contrast microscopy. Counts of the distribution of phases in a mitotic population gave: prophase, 10%; metaphase, 66% (including early and late prometaphase); anaphase, 3%; telophase, 13%; and interphase, 8%.

Determination of the Duration of the Phases of the Cell Cycle

The average duration of the phases of the HeLa division cycle was determined in synchronous cultures as described by Terasima and Tolmach (26). The average duration of G_1 was 11.7 h; S was 8.8 h; and G_2 and M was 4 h. The division time was 24.5 h. 5% of the cell population in G_1 incorporated [^{3}H]thymidine after 7 h.

Labeling of Mitotic Cells with [^{35}S]-Methionine

Mitotic cells (1–3 × 10^4) harvested at 4°C and washed with cold Hanks' buffer were labeled at 37°C in 0.1 ml of Dulbecco's modified minimal essential medium (DMEM) without methionine that contained 1 g/liter of $NaHCO_3$, 10% dialyzed fetal calf serum, 100 μCi of [^{35}S]methionine (Amersham SJ204, England), and 1 mg/liter of cold methionine. At the end of the labeling period, the cells were washed in Hanks' buffer and resuspended in lysis buffer (15). On the average, the time between harvesting and the beginning of labeling varied from 10 to 15 min.

Labeling of Interphase Cells with [^{35}S]-Methionine

Mitotic cells detached as described above and resuspended in complete MEM were placed in 5-cm Petri dishes containing 9-mm^2 coverslips (Microcover glass, 3 × 3 mm, Bellco Glass, Inc., Vineland, N.J.). To label the synchronized interphase cells, the coverslips were washed three times in Hanks' solution and placed in 0.25-ml, round-bottomed microtiter plates (NUNC, Denmark) containing 0.1 ml of DMEM without methionine, 1 g/liter of $NaHCO_3$, 100 μCi of [^{35}S]methionine (Amersham, England), 1 mg/liter of cold methionine, and 10% dialyzed fetal calf serum (5). Cells were pulse labeled for 30 min starting at the beginning of each phase. For long-term labeling, cells were labeled for the following times: G_1, 5.5 h; S, 9 h; and G_2, 3 h. In all cases, cells were labeled from the beginning of each phase. The same pattern of polypeptides was obtained when the cells were labeled in the presence of 30 mg/liter of cold methionine. At the end of the labeling period, the slides were washed three times by dipping them into wells containing Ca^{2+}- and Mg^{2+}-free Hanks' solution and were placed immediately in 20 μl of lysis buffer (15).

Two-dimensional Gel Electrophoresis

The procedures essentially followed those described by O'Farrell and co-workers (15, 16). A 15% acrylamide gel was used in the second dimension. Approximately 2 × 10^5 TCA-precipitable cpm were applied per gel (average number of cells: M (30 min) = 18,000 cells; G_1 (30 min) = 8,000 cells; G_1 (5.5 h) = 800 cells; S (30 min) = 8,500 cells; S (9 h) = 425 cells; G_2 (30 min) = 5,700 cells; and G_2 (3 h) = 960 cells). The gels were processed for fluorography as described by Laskey and Mills (12). Polypeptides (isoelectric focusing [IEF] and nonequilibrium pH gradient electrophoresis [NEPHGE]) were numbered starting from the top of the first dimension and from the highest molecular weight. For quantitation, the gels were cut out, resuspended for 12 h in 10% Bio-solv solubilizer BBS-3 in toluene-based scintillator, and counted for 10 min. The identity of α-actinin, α-tubulin, β-tubulin, and total actin was determined by coelectrophoresis with purified markers and by analysis of polypeptides from Triton cytoskeletons treated with buffers of low and high ionic strength (22, 23, and Bravo, Small, and Celis, submitted for publication).

RESULTS

Synthesis of Acidic and Basic Polypeptides throughout the Cell Cycle. Pulse Labeling

Synchronized HeLa cells in different stages of the cell cycle were labeled with [^{35}S]methionine for 30-min pulses starting at the beginning of each phase or after collection, in the case of mitosis (see Materials and Methods). Analysis of the labeled polypeptides by two-dimensional gel electrophoresis (15, 16) followed by fluorography (12) revealed ~700 polypeptides (acidic and basic) whose position could reproducibly be assessed. The gel patterns were superimposable, and little radioactivity remained at the origin. Visual comparison of the polypeptide maps obtained in the different stages of the cycle indicated that they were strikingly similar, if not identical, and there was no indication that the synthesis of any of the visually detected polypeptides was confined to only one of the phases of the cell cycle inasmuch as all the polypeptides were represented in all four stages, though at a much reduced amount in a few cases (see below).

For calculation of the relative proportion of the polypeptide spot in the different stages of the cycle, 99 abundant polypeptides (66 acidic [IEF] and 33 basic [NEPHGE]) were chosen (Fig. 1 *A* and *B*). The individual polypeptides were excised from the gel, and their radioactivity was determined by scintillation counting. The relative amount of each polypeptide was calculated on the basis of the amount of radioactivity present in spots 12 (IEF) and 20 (NEPHGE) because the amount of these two polypeptides was remarkably

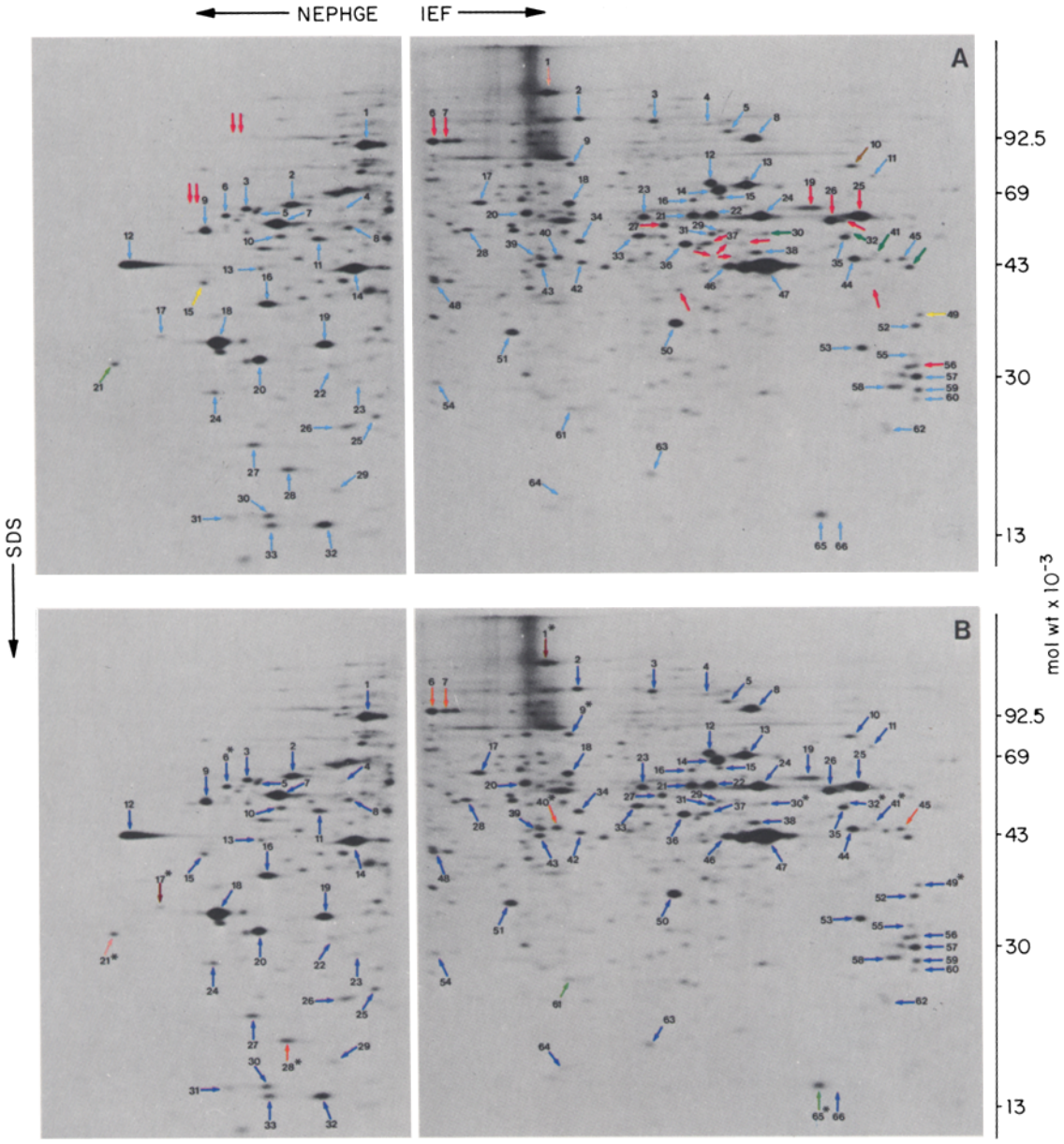

FIGURE 1 Two-dimensional gel electrophoresis maps of [^{35}S]methionine polypeptides from 100 asynchronous HeLa cells labeled for 24 h. In this pattern, one can see all the polypeptides synthesized throughout the cell cycle. (*A*) Summary of pulse labelings. Only numbered spots were quantitated (see Table I). Polypeptides whose relative proportion (Table I) varied by less than twofold during the cell cycle are indicated with a light blue arrow. The color indicates the phases in the cycle in which there is variation. Red = mitosis; yellow = S; light green (NEPHGE 21) = G_2; brown = M and S; green = M and G_2; apricot (IEF 1) = G_1 and S. Unnumbered arrows indicate obvious changes in intensities, but these spots were not quantitated. The gels have been exposed so as to keep the intensity of the spots within the linear range of the film response. Only 400 polypeptides of 700 detected in the fluorograms are seen in the photographs. Polypeptide spot 47 corresponds to α-, β-, and γ-actin. β- and γ-actin are the predominant variants in HeLa cells. (*B*) Comparison between short-term and long-term labeling (see Table I). Dark blue arrows indicate spots whose short-term/long-term labeling ratio in each of the interphases varies by less than twofold. Those spots whose ratio varies by more than twofold in any phase of the cycle are indicated with a colored arrow. Orange = variation in G_1; light green = variation in G_2; magenta (IEF 1) = variation in all phases; apricot (NEPHGE 21) = variation in G_1 and S; violet (NEPHGE 17) = variation in S and G_2. The asterisks indicate spots whose relative proportion varies by more than twofold when long-term labeled interphase cells are compared.

constant from cell cycle phase to cell cycle phase. As a first approximation, this value is a measure of the rate of synthesis of a given polypeptide. The relative amount of 49 of the 99 spots analyzed is

TABLE I

Relative Proportion of Several Polypeptides during the Cell Cycle

	Short-term labeling—relative proportion*				Ratio—short-term/long-term labeling‡		
Polypeptide	M	G_1	S	G_2	G_1	G	G_2
Acidic							
1	1.46	3.63	2.84	1.41	2.49	3.15	2.01
5	0.33	0.36	0.39	0.37	1.12	1.85	1.19
6	0.62	1.21	1.88	1.65	0.49	1.14	0.69
7	0.77	2.05	1.45	1.20	2.10	1.48	1.26
9	0.35	0.44	0.44	0.32	1.22	0.94	1.45
10	1.53	0.75	0.35	0.67	1.23	1.06	1.52
12	1.00	1.00	1.00	1.00	1.00	1.00	1.00
13	2.48	4.00	3.79	3.36	1.27	1.17	0.96
17	0.83	1.02	0.77	1.06	0.64	0.35	0.51
18	0.99	0.75	0.96	0.99	0.99	1.41	1.11
19	3.62	1.36	1.21	1.80	1.40	0.97	1.65
20	0.80	1.03	0.87	1.07	1.35	1.32	1.62
23	0.69	0.67	0.63	0.66	1.09	1.03	0.97
25	8.00	3.63	3.08	4.43	1.10	1.28	1.33
26	1.26	2.95	2.52	2.95	0.86	0.93	1.02
27	0.61	0.31	0.35	0.25	1.07	1.66	0.93
29	0.11	0.12	0.18	0.14	0.86	1.20	1.27
30	0.32	0.17	0.16	0.42	1.21	1.45	1.50
32	0.28	0.05	0.06	0.27	1.00	0.69	1.12
33	0.85	0.78	0.73	0.70	1.04	1.49	1.67
36	1.88	1.82	1.71	1.41	1.01	0.86	0.74
37	0.92	0.48	0.49	0.43	0.75	0.62	0.67
41	0.05	0.02	0.02	0.06	1.00	1.00	1.20
45	0.32	0.53	0.45	0.39	2.03	1.55	1.85
47	37.13	42.23	41.97	40.06	1.02	1.05	1.01
48	0.52	0.65	0.59	0.66	1.51	0.95	1.73
49	0.17	0.12	0.24	0.09	1.71	0.92	1.16
50	1.39	1.26	1.33	1.27	1.14	1.12	1.02
51	0.55	0.47	0.62	0.78	1.02	1.12	1.39
53	0.46	0.46	0.37	0.56	1.15	1.12	1.64
56	0.18	0.43	0.49	0.62	1.43	1.40	1.36
58	0.97	1.01	1.20	0.97	1.48	1.44	1.38
61	0.34	0.29	0.29	0.42	1.61	1.22	2.20
65	0.43	0.69	0.41	0.56	0.93	0.95	2.07
Basic							
1	1.44	2.03	2.12	1.64	0.84	1.00	1.13
5	0.13	0.12	0.11	0.11	0.63	0.78	0.85
6	0.24	0.16	0.20	0.20	0.94	0.87	0.60
7	3.02	2.91	2.40	2.87	1.01	0.80	0.80
9	0.99	1.19	0.98	0.92	1.27	1.44	0.92
12	4.52	3.52	3.68	2.86	1.21	1.53	0.96
14	3.18	3.54	2.94	3.14	1.18	0.85	0.89
15	0.30	0.38	0.16	0.29	1.40	0.80	1.38
17	0.26	0.18	0.17	0.17	1.20	2.12	2.12
18	5.56	6.49	5.75	4.82	1.34	1.13	0.93
20	1.00	1.00	1.00	1.00	1.00	1.00	1.00
21	0.91	0.66	0.78	0.33	2.06	5.20	1.57
26	0.30	0.22	0.20	0.18	1.69	1.54	0.52

TABLE I *Continued*

Polypeptide	Short-term labeling—relative proportion*				Ratio—short-term/long-term labeling‡		
	M	G_1	S	G_2	G_1	G	G_2
28	0.44	0.27	0.35	0.30	3.00	1.06	0.91
31	0.32	0.21	0.22	0.18	1.50	1.69	0.70

* Data based on 30-min pulses given at the beginning of each phase. The data are based on quantitations of two or more independent samples. The average proportion of each spot relative to spots 12 (IEF) and 20 (NEPHGE) is given for 48 of the 99 polypeptides analyzed. When 2×10^5 dpm were applied per gel, the average numbers of counts in spot 12 (IEF) were the following: M (30 min) = 391; G_1 (30 min) = 387; S (30 min) = 388; and G_2 (30 min) = 399. For spot 20 (NEPHGE), the following average numbers of counts were obtained: M (30 min) = 326; G_1 (30 min) = 336; S (30 min) = 344; and G_2 (30 min) = 381.

‡ The relative proportion of each spot in long-term labelings was calculated as above. Synchronous cells in each phase were labeled for the following times from the beginning of each phase: G_1, 5.5 h; S, 9 h; and G_2, 3 h. The average numbers of counts in spot 12 (IEF) (2×10^5 dpm applied to the gel) were the following: G_1 (5.5 h) = 398; S (9 h) = 425; and G_2 (3 h) = 402. Counts in spot 20 (NEPHGE) were the following: G_1 (5.5 h) = 354; S (9 h) = 372; and G_2 (3 h) = 337. From the data in this table, it is possible to determine the percent of any of these polypeptides relative to total protein label applied to the gel. The data have not been corrected for the efficiency of counting (50%).

given in Table I, and a summary of all 99 spots is given in Figure 1*A*. Given the reproducibility of the gels and the constancy of many polypeptides, we have arbitrarily defined as variable those whose relative intensities vary by twofold or more. 83 of the 99 polypeptides showed a less than twofold variation when their relative amounts in the four phases of the cycle are compared. These are indicated with a light blue arrow in Fig. 1*A*. Only 16 polypeptides varied by twofold or more (Table I), and these are indicated as follows (Fig. 1*A*): red arrows: (M), IEF spots 6 (decreases), 7 (decreases), 19 (α-tubulin [9], mol wt 57,000, increases), 25 (β-tubulin [9], mol wt 55,000, increases), 26 (100-Å filament subunit "fibroblast type" [4, 14], mol wt 55,000, decreases), 27 (increases), 37 (increases), 56 (decreases); yellow: (S), IEF spot 49 (increases), NEPHGE spot 15 (decreases); brown: (M and S), IEF spot 10 (high in M, low in S); green: (M and G_2), IEF spots 30, 32, and 41 (high in M and G_2), and apricot: (G_1 and S), IEF spot 1 (high in G_1 and S).

The spots indicated with unnumbered arrows in Fig. 1*A* correspond to polypeptides that showed obvious changes in intensities (M, red arrows; M and G_2, green arrows), but these were not quantitated because their counterparts in the same phase, or in other phases, even though visible after long exposures, did not lend themselves to quantitation. As an example, Fig. 2*A–C* shows the polypeptide patterns (IEF) of mitotic cells labeled for 22, 30, and 60 min, respectively. Only the portion of the gel where most changes occur is shown. Note that the quantity of some polypeptides varies considerably throughout mitosis (unnumbered and numbered arrows in Fig. 2, red arrows in Fig. 1*A*). Whether some of these polypeptides correspond to precursors, have a high turnover number, or are subject to modifications is not known.

Of the invariable polypeptides, we have identified total actin (IEF spot 47, mol wt 43,000, 16% of the total labeled proteins) (21, 27), α-actinin (IEF spot 8, mol wt 95,000, 0.8% of total label), and six abundant basic nonhistone proteins (NEPHGE spots 2, 3, 5, 9, 13, and 17) that are present mainly in karyoplasts isolated from HeLa cells prelabeled with [^{35}S]methionine and enucleated with cytochalasin B (Bravo, Mosses, Celis, and Celis, in preparation). Also, analysis of Triton cytoskeletons (23) has shown that IEF polypeptides 6, 7, 8, 12, 13, 24, 31, 33, 35, 36, 37, 52, and 65 correspond to major cytoarchitectural proteins (Bravo, Small, and Celis, manuscript submitted). Recent results obtained by Milcarek and Zahn from HeLa cells (13) have shown that actin synthesis remains constant during the cell cycle.

The percent relative to total protein label applied to the gel for all 49 polypeptides reported in Table I can be calculated using the data given in the legend of this table. Unfortunately, as a result of the poor labeling of the histone proteins with [^{35}S]methionine, we have been unable to study the synthesis of these proteins throughout the cycle. These proteins are synthesized in the S phase (8, 20), although there is evidence indicating that H_1 is also synthesized in G_1 but at a reduced rate (24).

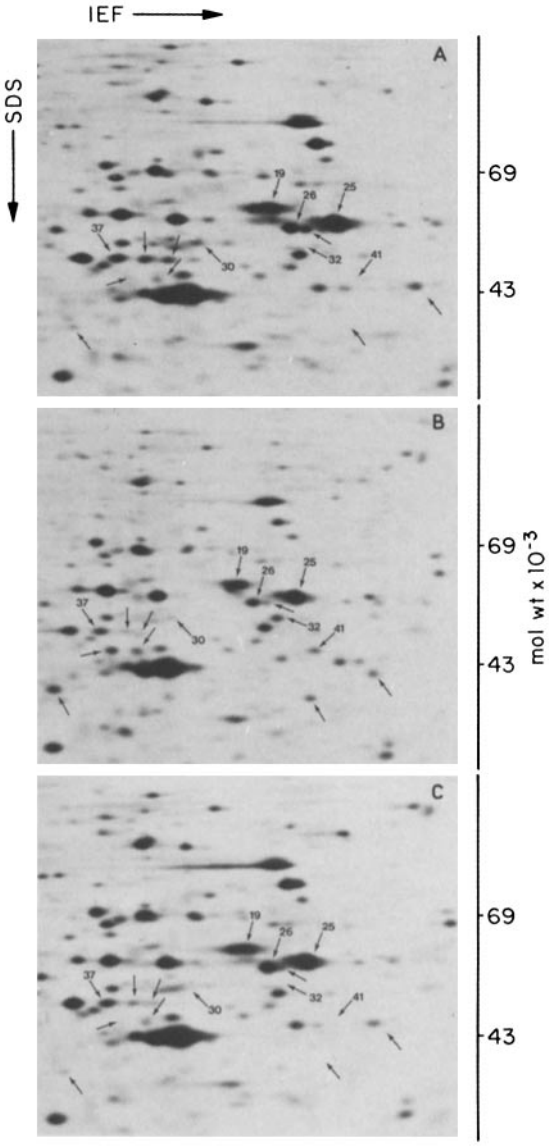

FIGURE 2 Two-dimensional gel electrophoresis analysis of polypeptides isolated from mitotic cells labeled for three different time periods. Only a fraction of the gel is shown in each case. Mitotic cells were labeled for (*A*) 22 min, (*B*) 30 min, and (*C*) 90 min. Only numbered spots have been quantitated.

Relative Intensities of Polypeptides in Long-term-labeled Interphase Cells Compared with Pulse Labelings

Synchronized HeLa Cells in G_1, S, and G_2 were labeled with [^{35}S]methionine for 5.5, 9, and 3 h, respectively, starting at the beginning of each phase. The polypeptides were analyzed by two-dimensional gel electrophoresis under the same conditions described for short-pulse labelings, and the relative proportion of each polypeptide was calculated relative to polypeptides 12 (IEF) and 20 (NEPHGE). The ratio of short-term vs. long-term labeling for 49 polypeptides is given in Table I, and a complete summary is given in Fig. 1 *B*. The ratio of 89 polypeptide spots varied by less than twofold when all three interphase stages of the cycle were compared, and these are indicated by dark blue arrows in Fig. 1 *B*. Those polypeptides whose ratio varies by twofold or more in any phase of the cycle are indicated as follows (Fig. 1 *B*): magenta arrow (the ratio varied in all three phases, IEF spot 1); orange (varied in G_1, IEF spots 6, 7, 40, 45, and NEPHGE spot 28); light green (varied in G_2, IEF spots 61 and 65); apricot (varied in G_1 and S, NEPHGE spot 21) and violet (varied in S and G_2, NEPHGE spot 17). At present, it is not known whether these changes reflect variations in the rate of polypeptide synthesis, in the rate of degradation, or both. It is also possible that some of these changes are caused by modifications.

Those polypeptides whose relative amount changes by twofold or more when only long-term-labeled G_1, S, and G_2 cells are compared are indicated by an asterisk in Fig. 1 *B*, i.e., IEF spot 1 (low in G_2); 9 (low in G_2); 30 (high in G_2); 32 (high in G_2); 40 (low in G_1); 41 (high in G_2); 49 (high in S); 65 (low in G_2); NEPHGE spot 6 (low in G_1); 17 (low in S and G_2); 21 (low in S and G_2); 28 (low in G_1). Of these polypeptides, IEF 30, 32, 41, and 49 vary in the same phase(s) as short-term-labeled cells, whereas polypeptides IEF 1 and 21 show differences as to the phase(s) in which there is variation. Polypeptides IEF 9 and NEPHGE 6 and 28 have been observed to vary in long-term-labeled cells only. Taken together, these results suggest that, in a few cases, the rate of polypeptide synthesis, degradation, or modification (phosphorylation, acetylation) may vary in a given phase or throughout the cell cycle.

DISCUSSION

Our reasons for undertaking these experiments were twofold. Firstly, we were interested in studying the effect of ionizing radiation on gene expression, and, secondly, we were interested in searching for polypeptide markers for tumorigenicity. In both cases, it is essential to know how much

variation there is in polypeptide synthesis throughout the process of cell division.

One of the striking features of our results is that all visually detected acidic and basic polypeptides are synthesized throughout the cell cycle and that, when differences appeared, they were clearly variations in relative intensity (rate of synthesis) rather than the appearance of new polypeptides. This observation is in agreement with similar studies by Milcarek and Zahn (13) but they contradict the findings of Al-Bader et al. (2) who, using Coomassie Blue staining, reported that at least 9 new polypeptides appear after the transition of HeLa cells from the S to the G_2 phase.

Even though our results are in general agreement with those of Milcarek and Zahn (13), they differ in the assignment of the polypeptides whose relative proportion changes during the cycle. In particular, these authors did not detect the changes we observed in mitosis. Of the polypeptides whose relative proportion varies significantly in mitosis, we have identified α- and β-tubulin (9) and the subunit of the 100-Å filament protein ("fibroblast type") (4, 14). In mitosis these proteins correspond to 1.4, 3.1, and 0.45%, respectively, of the total labeled protein as compared with 0.5, 1.3, and 1% found in interphase. An increase in the rate of synthesis of tubulin during mouse preimplantation development has been reported by Abreu and Brinster (1), and they have considered the possibility that this change could be the result of increased mitotic activity. The decreased synthesis of the 100-Å filament protein ("fibroblast type") is interesting inasmuch as we have observed a similar decrease in mouse 3T3B cells transformed with SV40 (manuscript submitted). Also, some cell types, such as mouse myeloma cells, have very little or none of this protein (unpublished observations).

The relative intensity of only a few polypeptides has been shown to vary consistently in interphase, and we will comment only on those polypeptides whose variation can be detected in short- as well as in long-term labelings (IEF spots 30, 32, 41, and 49). Polypeptide IEF 49 (mol wt 36,000) increases specifically in S and provides a polypeptide marker for this phase. This polypeptide is most interesting inasmuch as we have observed an increase in a similar polypeptide in spontaneously and virally transformed mouse cells (manuscript submitted). Currently, we are attempting to purify IEF spot 49 and are planning to microinject it into senescent skin fibroblasts in an effort to assess its function. It is relevant to note that Riddle et al. (19) have found a protein (mol wt 33,000) that increases in 3T3-arrested cells stimulated by serum.

A few polypeptides markers (IEF 30, 32, and 41) could be assigned to G_2 phase when only interphase cells were compared. The relative intensity of these polypeptides also increases in some stages of mitosis (Fig. 1*A*, green arrows; Fig. 2*A–C*), but this does not preclude their use as markers in synchronized interphase cells.

We are well aware of the fact that some of the changes we have observed could be the result of modifications rather than variations in the rate of synthesis, but, with the data at hand, it is not possible to decide between these possibilities.

Among the polypeptides whose rate of synthesis remains more or less constant throughout the cycle, we have identified total actin (comprising 16% of the total protein label), α-actinin (0.8% of the total protein label), 6 basic nonhistone proteins, and 13 major acidic cytoarchitectural proteins present in Triton cytoskeletons. Similar results with regard to the invariability of actin throughout the cell cycle have been reported by Milcarek and Zahn (13) in HeLa cells. Also, Elliot and McLaughlin (6) have reported invariability in the relative amount of polypeptides throughout the cell cycle of yeast cells.

Our results with regard to the percent of total actin (16% of total label) differ markedly from those reported by Milcarek and Zahn (2–4%) (13). Careful examination of the polypeptide maps and the molecular weight assignments published by these authors suggests to us that the spot they identified as actin may be different from ours.

Finally, it should be pointed out that we have analyzed only a fraction of the total polypeptides synthesized by HeLa cells and that there could be other important changes in minor components that escaped detection because of the present technical limitations. One way in which one can circumvent this problem is by studying the polypeptides of purified subcellular fractions. These experiments are now underway.

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This paper is dedicated to the memory of Prof. E. Zeuthen.

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