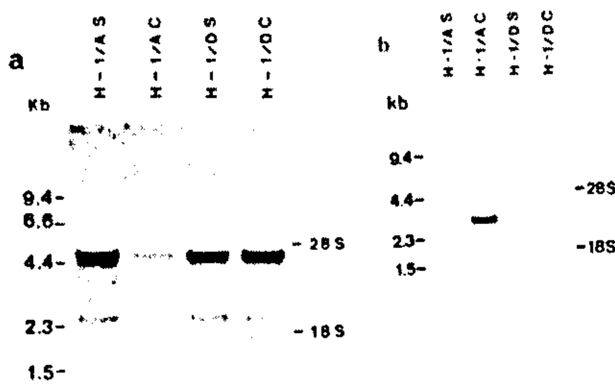

FIG. 1. Expression analysis of CSF-1 and GPD RNA during adipocyte differentiation of H-1/A cells. (a and b) Autoradiograms of RNA blot analysis from semiconfluent H-1/A cells (H-1/AS), H-1/A cells at 7 days postconfluence (H-1/AC), semiconfluent H-1/D cells (H-1/DS), and H-1/D cells at 7 days postconfluence (H-1/DC). Ten micrograms of the total RNA was electrophoresed in each lane. The blots were hybridized with a probe of the CSF-1 cDNA (a) or GPD cDNA (b), as described in Materials and Methods. The positions of the size markers and the 28S and 18S rRNA markers are indicated. The filters were exposed for 72 h at −80°C, using an intensifying screen.

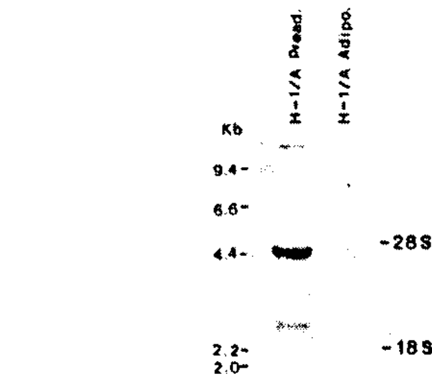

FIG. 2. Expression of CSF-1 RNA in preadipocytes and adipocytes of H-1/A. RNAs were extracted from H-1/A preadipocytes (Pread.) or isolated adipocytes (Adipo.). Ten micrograms of the total RNA was electrophoresed in each lane. The blot was hybridized with the CSF-1 probe as described in Materials and Methods. The positions of the size markers and the 28S and 18S rRNA markers are indicated. The filter was exposed for 72 h at −80°C, using an intensifying screen.

Even after 12-h treatments of H-1/A with various concentrations of phytohemagglutinin, concanavalin A, hydrocortisone, dbcAMP plus caffeine, l-epinephrine, recombinant IL-1α or IL-1β, or lipopolysaccharide, no detectable levels of hybridization were found with H-1/A RNA by G-CSF, GM-CSF, and IL-3 cDNA probes (data not shown).

H-1/A cells developed many fat droplets in their cytoplasm after reaching confluence, while few fat droplets were detected long after H-1/D cells reached confluence. Equal amounts of the total RNA from the semiconfluent and confluent cultures of H-1/A and H-1/D were subjected to blot hybridizations with the CSF-1 probe. In the confluent H-1/A culture in an adipocyte state, the CSF-1 mRNA levels decreased to approximately one-tenth of those of the semiconfluent H-1/A culture in a preadipocyte state (Fig. 1a). On the other hand, no reduction of the RNA levels was observed in H-1/D after confluence was reached (Fig. 1a). In contrast, the levels of GPD mRNA clearly increased in the confluent H-1/A cells compared with those in the semiconfluent culture (Fig. 1b). However, no detectable bands of GPD mRNA were observed in either semiconfluent or confluent H-1/D cells.

To obtain more accurate assessments of the change in the CSF-1 mRNA levels during differentiation, we purified adipocytes from the confluent H-1/A culture by using the procedure of Spiegelman et al. (44). The percentage of cells in the adipocyte preparation that were not adipocytes was found to be less than 1%. No detectable bands of CSF-1 mRNA were observed in the adipocyte preparation (Fig. 2). CSF-1 mRNA decreased as a result of differentiation of H-1/A cells into adipocytes rather than as a result of general RNA degradation, as similar amounts of rRNA were recovered from the preadipocytes and adipocytes.

We performed a study to follow the time course of CSF-1, actin, and GPD expression during differentiation (Fig. 3). The expression of CSF-1 and actin genes began to decrease just after the culture reached confluence, followed by a delayed increase of GPD expression.

H-1/A cells showed a fibroblastlike morphology, and no adipocytes were observed until they reached confluence. After reaching confluence, H-1/A cells differentiated efficiently into lipid-accumulating cells (Fig. 4a and 5). Cachectin/TNF is widely known to inhibit adipocyte differentiation (43, 47). By the treatment of the H-1/A culture at confluence with 200 or 1,000 U of cachectin/TNF per ml, the cells maintained their fibroblastoid morphology and did not accumulate any fat droplets for at least 10 days after reaching confluence (Fig. 4b and 5). Therefore, the lipid accumulation of the marrow preadipocytes, H-1/A, was prevented by

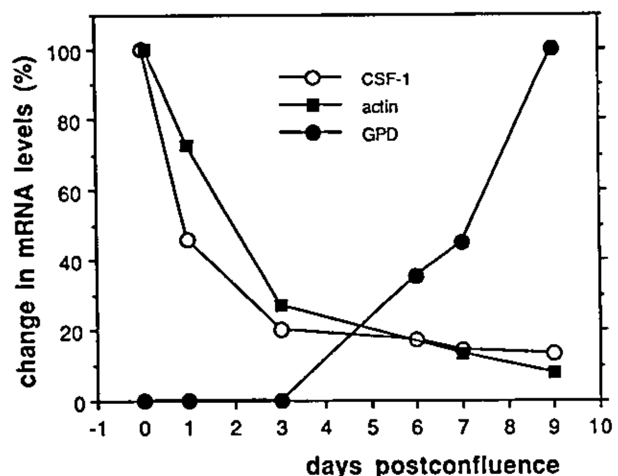

FIG. 3. Time course of CSF-1, GPD, and actin expression in differentiating H-1/A cells. CSF-1, actin, and GPD mRNA levels were determined densitometrically from autoradiograms of RNA blot analysis. At the indicated day postconfluence, RNA was isolated. Five micrograms of total RNA was electrophoresed in each lane. The blots were hybridized with each probe. The amounts of CSF-1 and actin mRNA at confluence (day 0) and GPD mRNA at day 9 postconfluence were regarded as equal to 100%.

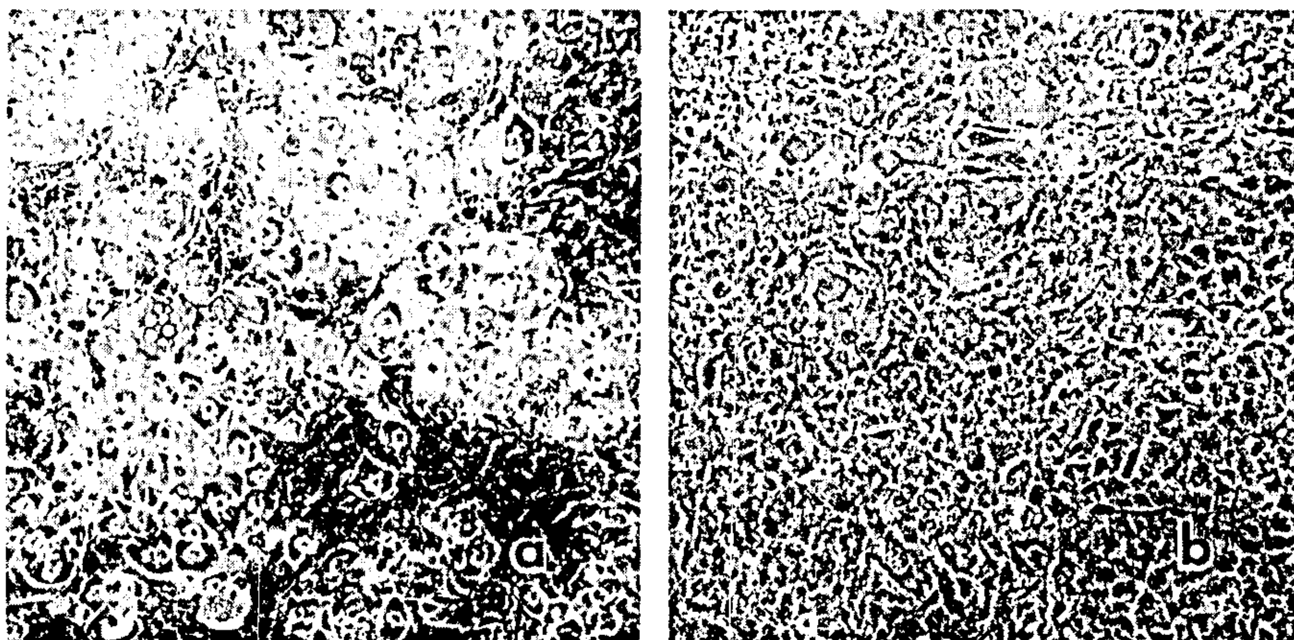

FIG. 4. Differentiation of untreated H-1/A cells and cachectin/TNF-treated H-1/A cells. (a) H-1/A adipocytes at 6 days after confluence. Obvious fat droplets in the cytoplasm were observed, which are an indication of differentiation. (b) H-1/A cells at 6 days after treatment with 200 U of cachectin/TNF per ml. Cachectin/TNF was added to the growth medium when cells reached confluence. The culture medium containing cachectin/TNF was changed every 3 days.

cachectin/TNF, as also found for TA 1 adipocytes (47). At confluence, the CSF-1 expression was significantly enhanced at 12 and 24 h after treatment with 200 U of cachectin/TNF per ml (Fig. 6a). However, GPD was not expressed in either the control cultures or the cachectin/TNF-treated cultures. For comparison, we analyzed the expression of the actin gene on the same blot. The level of

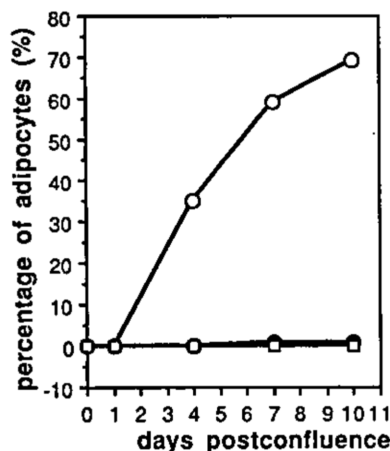

FIG. 5. Time course of cell differentiation in H-1/A cells, as measured by lipid-accumulating cells after the treatment with cachectin/TNF. H-1/A cells reached confluence at day 0. Cells were treated with 200 (●) or 1,000 (□) U of cachectin/TNF per ml at day 0. Cachectin/TNF was not added to the growth medium of a control culture (○). To estimate adipocyte differentiation, four random photomicrographs were taken for each dish, and about 2,000 cells were then counted in each dish. The mean percentage of adipocytes was calculated from four dishes.

actin mRNA did not change with cachectin/TNF treatment. To see whether cachectin/TNF increases CSF-1 expression transiently, we exposed the H-1/A cells to cachectin/TNF just at confluence. The culture medium containing 200 U of cachectin/TNF per ml was changed every day in order to avoid the effect by the degradation of cachectin/TNF in the medium. The CSF-1 transcripts increased and after reaching a peak on the second day began to decrease (Fig. 6b). On the ninth day after the treatment, the level of the CSF-1 was lower than that in the untreated H-1/A cells. We also saw the effects of cachectin/TNF on completely differentiated adipocytes. A 12-h exposure to 200 U of cachectin/TNF per ml was toxic to the differentiated H-1/A adipocytes.

To determine whether cachectin/TNF induced the production of active CSF-1, the colony assay was performed by using conditioned medium from the cachectin/TNF-treated H-1/A cells (Table 1). Cachectin/TNF increased CSF-1 activity in H-1/A culture medium. Neither cachectin/TNF nor growth medium alone stimulated the clonal proliferation of macrophages. The results suggest that cachectin/TNF induced CSF-1 at both mRNA and protein levels.

To determine whether these changes in the amounts of CSF-1 RNA were due to transcriptional or posttranscriptional events, we performed nuclear run-off transcription assays. When cells differentiated into adipocytes, the transcription rates of CSF-1-specific RNA did not change significantly, while those of actin RNA decreased by 60 and 70% in two independent experiments, respectively (Fig. 7a). From these results, we conclude that the change of RNA stabilization or any other posttranscriptional events contributed to the decrease of CSF-1-specific cytoplasmic RNA during adipocyte differentiation. In contrast, the induction of CSF-1 by cachectin/TNF exposure in the H-1/A cells at confluence occurred at the transcriptional level (Fig. 7b), although cachectin/TNF did not affect the transcription rate

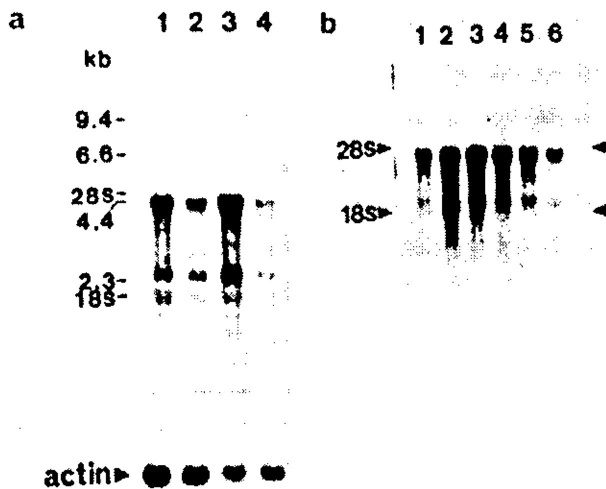

FIG. 6. Expression of CSF-1 (a and b) and actin (a) genes in H-1/A cells with exposure to cachectin/TNF. (a) H-1/A cells were treated with 200 U of cachectin/TNF per ml when cells reached confluence. RNAs were extracted from H-1/A cells at 12 (lane 1) and 24 (lane 3) h after treatment. Lanes 2 and 4 were the control cultures (without cachectin/TNF) for lanes 1 and 3, respectively. Ten micrograms of total RNA was electrophoresed in each lane. The blot was hybridized with the CSF-1 probe (upper gel) first. Then, the blot was dehybridized with a solution of boiled 0.01% SDS and 0.01× SSC 5 times and rehybridized with the actin probe (lower gel). (b) H-1/A cells at confluence were treated with 200 U of cachectin/TNF per ml. The culture medium containing cachectin/TNF was changed every day. RNAs were extracted from untreated H-1/A cells at confluence (lane 1) and H-1/A cells at 1 (lane 2), 2 (lane 3), 3 (lane 4), 6 (lane 5), and 9 (lane 6) days after the cachectin/TNF treatment. Ten micrograms of total RNA was electrophoresed in each lane. The blot was hybridized with the CSF-1 probe. The conditions of hybridization were described in Materials and Methods (a and b). The positions of the size markers and the 28S and 18S rRNA markers are indicated to the left of the gels. The filters were exposed for 48 (upper gel in panel a), 8 (lower gel in panel a), or 24 (panel b) h at −80°C, using an intensifying screen.

of the actin gene. The increased amounts of CSF-1-specific mRNA in response to cachectin/TNF exposure may also have been caused by a prolonged half-life of CSF-1 RNA. We isolated RNAs from the H-1/A cultures at confluence at different times after the addition of dactinomycin and determined the amounts of CSF-1-specific RNA. The quantitative evaluation of the decay of CSF-1 RNA by densitometry revealed that the half-life of CSF-1 RNA was about 2 h in both the cachectin/TNF-treated and untreated cultures (Fig. 8). The actin RNA used as an internal control was stable for more than 8 h. On the basis of these findings, we conclude that the changes in the transcription rates significantly contribute to the increased level of CSF-1 RNA in response to cachectin/TNF treatment.

DISCUSSION

Marrow stromal cells, H-1/A, accumulate fat droplets in their cytoplasm after confluence was reached. This morphological change represents adipocyte differentiation rather than uptake of fat from the culture medium, because expression of the GPD gene was induced after confluence was reached. GPD, which provides the glycerophosphate backbone for acylation, is one of the key enzymes for synthesis of triglyceride, a marker for adipocyte differentiation (44). Neutral triglyceride is synthesized from acetates in H-1/A cells (16).

In H-1/A cells, the CSF-1 gene is expressed, but the G-CSF, GM-CSF, and IL-3 genes are not. The expression of CSF-1 gene is reduced after the differentiation of H-1/A cells into adipocytes. Our RNA blot analysis clearly demonstrates that the decrease of colony-stimulating activity levels after the adipocyte differentiation of H-1/A (30) can be explained by changes in the CSF-1 mRNA levels, not by those in its secretion levels. The isolation of adipocytes helped to clarify these results. The down-regulation of CSF-1 gene seems to be linked with the processes of differentiation per se, rather than with the culture condition, since another differentiation-incompetent subline, H-1/D, did not show any similar alterations in the differentiation or CSF-1 regulation. In the human promyelocytic cell line HL-60, down-regulated expression of myeloblastin is reported to cause arrest of growth and differentiation (2). In the differentiation of the murine erythroleukemia cell, c-*myc* mRNA levels decrease dramatically and the decline of c-*myc* expression is required for the commitment to the differentiation (6, 23). In the present study, CSF-1 mRNA dramatically decreased before GPD expression increased. The induction of CSF-1 by treatment with cachectin/TNF was accompanied with the inhibition of GPD expression. This suggests that the down-regulated expression in CSF-1 mRNA may be a prerequisite for the commitment of the H-1/A cells to enter the differentiation program. Alternatively, the down-regulation of the

TABLE 1. CSF-1 production in H-1/A cells after exposure to cachectin/TNF for 3 days[a]

Growth factor	Concn (U/ml) of cachectin/TNF	No. of days exposed	No. of cells plated/ml (10^4)	No. of plates	No. of colonies
CM H-1/A[b]	0	3	3	4	57 ± 8
	200	3	3	4	195 ± 25
	1,000	3	3	4	282 ± 8
	0	3	1	4	34 ± 5
	200	3	1	3	71 ± 6
	1,000	3	1	3	169 ± 27
Cachectin/TNF[c]	0		3	4	0 ± 0
	200		3	4	0 ± 0
	1,000		3	4	0 ± 0
	0		1	4	0 ± 0
	200		1	4	0 ± 0
	1,000		1	4	0 ± 0

[a] H-1/A cells were cultured with either 200 or 1,000 U of cachectin/TNF per ml for 3 days in Fischer medium supplemented with 10% horse serum. The conditioned medium were collected and tested (10% vol/vol) for their ability to stimulate the clonal proliferation of normal murine monocytic stem cells (CSF-1 activity). Fresh bone marrow cells were obtained from 8- to 12-week-old female C57/B1 mice by flushing their tibias and femurs. 3 × 10^4 or 1 × 10^4 fresh marrow cells were cultured in 1 ml of Iscove medium containing 0.5% agar and 10% conditioned medium in plastic dishes. After incubation for 6 days at 37°C in an atmosphere of 7.5% CO_2 in humidified air, the specimens were fixed with absolute methanol, air-dried, and stained. Colonies with more than 100 cells were scored with the aid of a Nikon optical microscope.

[b] CM H-1/A, Conditioned medium of H-1/A cells.

[c] As a control, the culture medium containing cachectin/TNF was incubated in the absence of cells. The culture medium containing cachectin/TNF was then used for the colony assay in the same manner as for CM H-1/A.

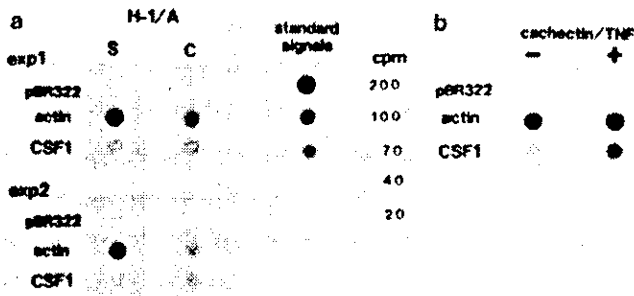

FIG. 7. Nuclear run-off transcription of H-1/A cells at semiconfluence and postconfluence (a) and cachectin/TNF-treated and untreated cells (b). (a) Semiconfluent H-1/A (H-1/AS) and H-1/A cells at 10 days postconfluence (H-1/AC) were harvested from four dishes, and nuclei were isolated. Almost 70% of the cells differentiated into adipocytes at 10 days postconfluence. The in vitro ^{32}P-labeled nuclear RNA was hybridized to the CSF-1 cDNA, and actin cDNA was immobilized to the nylon filter. The transcription rate of CSF-1 gene was estimated by measuring the amount of labeled RNA transcribed in nuclei isolated from the H-1/A cells. The amount of CSF-1-specific transcription was quantitated by hybridization to an excess of the CSF-1 cDNA bound to a Nylon filter. To demonstrate the specificity of observed signals, pBR322 DNA was used for nonspecific binding of the labeled nuclear RNA. pBR322 DNA gave weak signals. For the standard signals, ^{32}P-labeled DNAs of known radioactivity were dotted on the filter, as shown to the right of the gel. Spots on the autoradiograms were scanned with a densitometer. The areas of peaks for the standard signals on a recording sheet provide the standard curve. The intensity of sample signals was converted to counts per minute by using this standard curve. The value for the irrelevant DNA dot (pBR322 DNA) was subtracted from the values for the CSF-1 and actin signals. The ratios presented in the text were obtained by dividing the subtracted values for CSF-1 and actin of H-1/A cells at 10 days postconfluence by those of semiconfluent cells, respectively. The sample and signal-control filters were both exposed for 48 h at −80°C, using an intensifying screen. (b) H-1/A cells at confluence were treated with 800 U of cachectin/TNF per ml. After incubation for 15 h, cells were harvested for analysis. The ratio of the CSF-1 signal (cachectin/TNF-treated cells/untreated cells) was found to be 4.8, while that of the actin signal was found to be 1.0. The ratios were obtained as described above. The filter was exposed for 48 h at −80°C, using an intensifying screen.

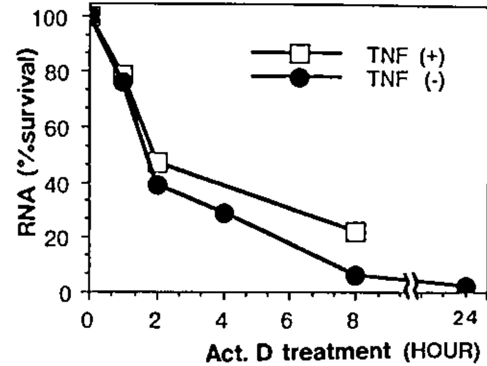

FIG. 8. Decay of CSF-1-specific mRNA in cachectin/TNF-treated (□) and untreated (●) H-1/A cells at confluence. H-1/A cells at confluence were treated with 200 U of cachectin/TNF per ml for 12 h followed by dactinomycin (Act. D) (5 μg/ml). The total RNA extracted at the times indicated after the addition of dactinomycin was analyzed by Northern blots, using a CSF-1 probe. Five micrograms of the total RNA was electrophoresed in each lane. The amount of the total RNAs was checked by 28S and 18S rRNAs stained with methylene blue. The decay rates of the CSF-1-specific RNA were determined from the band intensities from the autoradiograms of two or three independent RNA blots by densitometric scanning. The means for each set of data were plotted at the times indicated. The amount of RNA at the time of dactinomycin addition was regarded as equal to 100%. The cachectin/TNF-treated cells were killed with 24-h exposure to dactinomycin.

CSF-1 may result from the onset of differentiation. To address this question, we need to conduct further experiments to determine whether the constitutive expression of exogenous CSF-1 gene inhibits differentiation.

During the differentiation of H-1/A cells into adipocytes, the reduction of CSF-1 transcripts is due to a posttranscriptional event, such as the degradation of CSF-1 mRNA, rather than to a decreased rate of transcription. Posttranscriptional events are known to be one of the mechanisms which regulate cytoplasmic mRNA levels (7). In the terminal differentiation of erythroblasts, erythrocyte-specific mRNAs such as those of the globin gene appear to be conserved, while the other mRNAs are specifically destroyed (1). When the developmental cycle of the slime mold is interrupted, developmentally regulated mRNAs are specifically destroyed (5).

On the other hand, both CSF-1 protein and mRNA synthesis were induced by cachectin/TNF treatment in semiconfluent H-1/A cells. The induction of growth factors such as G-CSF and GM-CSF is reported in cachectin/TNF-treated lung fibroblasts and endothelial cells, although the transcription rates have not been studied (3, 22, 29). Recently, CSF-1 transcription has been investigated in human monocytes (17, 32, 42). In H-1/A cells at confluence, CSF-1-specific mRNA was induced at a transcriptional level by cachectin/TNF treatment. The half-life of the CSF-1 mRNA in the cachectin/TNF-treated H-1/A cells at confluence was almost the same as that in the untreated cells.

In the monocytes, the induction of CSF-1 by cachectin/TNF is transient and the level of CSF-1 transcripts returned to that of the control cells by 24 h (42). In H-1/A cells, the induction was observed for at least 3 days after treatment and the level of CSF-1 transcripts then returned to the level of the untreated cells. The possibility still remains that cachectin/TNF inhibited GPD expression only transiently. When cells were exposed to 200 and 1,000 U of cachectin/TNF per ml continuously, no adipocyte differentiation was observed for at least 10 days after confluence was reached and no GPD expression was detected for at least 9 days after confluence. Therefore, cachectin/TNF inhibits the expression of lipogenic enzymes, rather than delaying their expression.

The 4.5- and 2.5-kb transcripts of CSF-1 were expressed in H-1/A, while L cells express high levels of the 4.5-kb species as well as several other species of about 3.8, 2.3, and 1.4 kb (34). Multiple CSF-1 transcripts have been reported in a human pancreatic carcinoma cell line, MIA PaCa-2 (21), which arise from alternative splicing of a large primary transcript (24). In H-1/A, the 3′ sequence of the 4.5-kb transcript was not detected in the 2.5-kb species. The two major mRNA species may result from alternative splicing in H-1/A cells as well. The gene coding CSF-1 did not demonstrate any rearranged structure by Southern analysis of the H-1/A DNA with several restriction enzymes (data not shown).

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Gap-junctional Communication of Bone Marrow Stromal Cells Is Resistant to Irradiation in vitro

Akihiro Umezawa, Kenichi Harigaya,[1] Hitoshi Abe, and Yonosuke Watanabe

Department of Pathology, Keio University School of Medicine, Shinjuku-ku, Tokyo; and [1]Department of 1st Division of Pathology, Chiba University School of Medicine, Inohana, Chiba, Japan

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Abstract. Bone marrow is one of the most radiosensitive organs. Irradiation causes a marked decrease in the total number of hematopoietic cells in the bone marrow. The reticular meshwork structure of marrow stromal cells, however, is relatively resistant to irradiation. Unimpaired stromal cell structure has been thought to be a prerequisite for the repopulation of hematopoietic cells during recovery from the effects of irradiation. The reticular framework is maintained by cell adhesion apparatuses such as gap junctions. The in vitro radiobiologic survival values of a cloned stromal cell line, H-1/A, were studied ($\tilde{n} = 1.8$, $D_0 = 138$ cGy). Radiation doses of up to 4000 cGy had no detectable effects on the production of colony-stimulating factor 1. H-1/A cells communicate with each other via gap junctions as determined by the sensitive dye-transfer method. Gap-junctional communication between H-1/A cells was resistant to different levels of irradiation (500 to 10,000 cGy), but it was lost during adipocyte differentiation of H-1/A cells. Marrow stromal cells, which are important in the recovery of hematopoiesis, seemed capable of coordination with each other through gap junctions even when exposed to radiation.

Key words: Intercellular junction — Stromal cell — Radiation

Gap junctions are the unit membrane conduits that allow ions and molecules with molecular weights <1500 daltons to pass directly from the inside of one cell to that of another [1]. Cells connected by gap junctions share many of their small molecules, such as inorganic ions, metabolites, and messengers. Thus, gap junctions are clearly important in coordinating the activity of these cells and expressing a cell's individuality. Gap junctions between cells are present in many tissues and in a wide range of species. cDNAs for a human and rat liver gap-junction protein have been cloned, and it appears that there are tissue-specific gap junctions [2, 3]. Gap junctions are found in most tissues specialized for secretion. Gap junctions have also been detected between marrow stromal cells in vivo by means of electron microscopy [4]. In vitro, gap junctions are reported in bone marrow culture and cell lines; they are detectable by means of electron microscopy [5–8] and the dye-transfer method [9].

The dye-transfer method is known to be available as a means of detecting gap-junctional communications [10–12]. Although dyes such as fluorescein penetrate nonjunctional membranes to some extent, the transfer of Lucifer Yellow CH, a polar tracer used in these studies, occurs via gap-junctional communications. Because in some cells the only direct contact between cells observed by electron microscopy is at gap junctions, it is concluded that dye transfer is mediated by gap junctions [10, 12, 13].

Bone marrow is known to be one of the most radiosensitive tissues [14]. Hematopoietic cells are easily impaired by irradiation, as are the cells lining the intestines, perhaps because cell replication is frequent. To reconstitute hematopoiesis, early progenitor cells with mitotic capacity seed the bone marrow after rescue transfusion of irradiated mice [15, 16]. Although hematopoietic cells are lost as a result of irradiation, marrow stroma is unimpaired [17]. The undamaged stroma is believed to contribute to hematopoiesis via colony-stimulating factors (CSFs) [18] and cell-to-cell interactions, including interaction via gap junctions during recovery. Because the radioresistance of the reticular structure of the stroma is determined by the cells' own viability and the undamaged cell-to-cell adhesion apparatus, we used the dye-transfer method to determine whether irradiation affects gap-junctional communication between marrow stromal cells in vitro. Cell morphology dramatically changed after irradiation. However, communication remained intact despite varying levels of irradiation.

Naparstek et al. [19] reported that trypsinized, replaced, viable, adherent, but nondividing, x-irradiated D2XRII cells can be maintained after 10,000 cGy of irradiation. X-irradiation of plateau-phase D2XRII cells to 10,000 cGy reduces colony-stimulating factor 1 (CSF-1) production by 50% [19]. Because 10,000 cGy irradiation is above the lethal level for stromal cells, the lethal effect of x-rays on proliferative capacity is very different from their biological effect on the physiological functions of adherence and growth factor production. Broxmeyer et al. showed that CSF production by human marrow cells is affected by 600 or 1000 cGy irradiation in vitro as detected by bioassay [20]. In the present study, the effect of irradiation on CSF production and expression of H-1/A cells is reported.

Materials and methods

Stromal cell line. H-1/A cells (passage 47) were routinely cultured in Fischer media (Irvine Scientific, Santa Ana, California) and 10%

Address offprint requests to: Dr. Akihiro Umezawa, Department of Pathology, Keio University School of Medicine, 35 Shinanomachi Shinjuku-ku, Tokyo 160, Japan.

horse serum (Flow Laboratories) with antibiotics as previously reported [21]. The cells were incubated at 33°C and 5% CO_2.

Communication assay. Intercellular transfer of fluorescent Lucifer Yellow CH [12] (Sigma, St. Louis, Missouri) was measured after direct microinjection of the dye into a cell was performed using a phase-contrast microscope; observation of the dye transfer to neighboring cells was made using a fluorescence microscope. Microinjection was carried out using a micromanipulator (Narishige Company, Tokyo, Japan). A 10% solution of Lucifer Yellow CH in 0.3 M lithium chloride solution was transferred to a glass capillary needle that was prepared from a capillary tube using an automatic magnetic puller (Narishige Company). H-1/A cells were impaled with capillary needles close to the nucleus, and dye was injected by manual pressure. The transfer of dye into surrounding cells was monitored using a phase-contrast–fluorescence microscope after injection. The percentage of first-order neighbors in contact with the injected cell and the number of all fluorescent cells were scored after each injection. H-1/A preadipocytes were injected with Lucifer Yellow CH. If adipocytes were found to be first-order neighbors of the injected cells, we did not count the adipocytes when we totaled the number of first-order junctions of the injected cells, because Lucifer Yellow CH is not transferred to H-1/A adipocytes via gap junctions [9, 22]. The H-1/A cells were exposed to varying levels of irradiation (200, 500, 1000, and 2000 cGy) using an ML-6MA Linac (Mitsubishi Electric Corporation, Tokyo, Japan) at a rate of 200 cGy/min. In another series of experiments, the H-1/A cells were exposed to 5000 and 10,000 cGy.

Bone marrow cell colony-forming units (CFUc) assay. The medium was harvested from irradiated or untreated H-1/A cells grown for 3 days in Fischer's medium supplemented with 10% fetal bovine serum and antibiotics. It was filtered through a 0.22-μm Millipore filter and stored at −20°C until use. CFUc were allowed to proliferate in semisolid soft agar cultures as described [23]. Fresh murine bone marrow cells were obtained from 8- to 12-week-old female C57/BL mice by flushing their tibias and femurs. Fresh marrow cells were inoculated in 1 ml of complete Iscove's medium containing 0.3% agar (Difco Laboratories, Detroit, Michigan) and 10% appropriate conditioned media in plastic petri dishes. After incubation for 8 days at 37°C in an atmosphere of 7.5% CO_2 in humidified air, the specimens were air-dried and stained by means of a double-staining technique using naphthol-ASD-chloroacetate and α-naphthyl butyrate as substrates [24].

RNA blot analysis. Irradiated H-1/A cells (1000 or 2000 cGy) were harvested for RNA blot analysis. RNA was prepared from cultured cells by homogenizing them in guanidinium isothiocyanate followed by centrifugation over a cesium chloride cushion [25]. The RNA was then electrophoresed in 1.0% agarose gel, transferred to a nitrocellulose filter (Du Pont Company NEN Products), and hybridized with cDNA inserts labeled with [^{32}P]CMP by the random-primer method [26] at 65°C for 14–16 h in a buffer containing 5× saline sodium phosphate ethylenediaminetetraacetic acid (SSPE), 5× Denhardt's solution, 0.02% poly A, and 1% sodium dodecyl sulfate (SDS). The CSF-1 probe was a 3.9-kb Eco R1 fragment of pGEM2MCSF-10 from J.S. Price, Cetus Corporation [27]. The blots were washed with 2× saline sodium citrate (SSC) containing 1% SDS at room temperature and 65°C. Final washings were done with 0.1× SSC containing 0.1% SDS at 65°C. The blots were exposed to x-ray film at −80°C using an intensifying screen.

Results

H-1/A cells exhibited a fibroblast-like morphology, and no adipocytes developed until they reached confluence. After confluence, H-1/A cells differentiated into adipocytes [21]. When an individual cell was microinjected into H-1/A cell culture just after confluence, dye was transferred to the adjacent cells in the same manner as previously described [9]. In order to examine the effects of irradiation on intercellular communication capacity, we exposed H-1/A cells to different doses of radiation (200, 500, 1000, and 2000 cGy). On days 1 and 7 following irradiation, gap-junctional communication was found to be unchanged by exposure to 0–2000 cGy (Table 1A and Fig. 1). In another series of experiments, the H-1/A cells were irradiated at high doses (5000 and 10,000 cGy). On days 1 and 8 following irradiation, there was no significant change in the incidence of permeable junctions among the cells (Table 1B and Fig. 2). To eliminate the possibility that an increase in cell permeability (especially endocytosis or exocytosis) in response to irradiation interferes with the results of the dye-transfer method, we examined endocytosis and phagocytosis of H-1/A cells irradiated with 1000 or 2000 cGy. H-1/A cells were incubated for 5 or 60 min with excess amounts of free fluorescent dye in the medium or 10^7 heat-inactivated yeast cells per milliliter of medium in order to observe endocytosis and phagocytosis [28] at room temperature. No phagocytosis of yeast cells was observed in either the nonirradiated or the irradiated H-1/A cells. Although endocytosis of free dye was observed after 1 h of exposure to excess amounts of free dye in the nonirradiated and irradiated H-1/A cells, no endocytosis was observed within 5

Table 1. Effects of irradiation on the intercellular communication capacity of H-1/A cells

Radiation dose (cGy)	Percentage of first-order neighbors in contact with the injected cell	
	Day 1	Day 7
	A	
0	49.6 (133, 22, 8.8)	27.6 (163, 28, 3.6)
200	39.3 (122, 20, 4.3)	34.9 (172, 27, 4.1)
500	35.1 (111, 20, 3.1)	37.2 (145, 23, 4.1)
1000	51.0 (135, 21, 6.0)	38.3 (162, 32, 3.5)
2000	38.1 (118, 20, 4.3)	38.3 (111, 20, 3.5)
	B	
	Day 1	Day 8
0	59.2 (125, 20, 15.2)	40.6 (212, 33, 6.8)
5000	59.0 (100, 17, 10.4)	40.6 (106, 21, 3.9)
10,000	36.0 (125, 21, 5.0)	31.9 (141, 24, 4.4)

Cells were grown in Fischer medium plus 10% horse serum before irradiation. H-1/A cultures were exposed to the indicated levels of radiation. Intercellular transfer of fluorescent Lucifer Yellow CH was measured after direct microinjection of the dye into a cell was performed using a phase-contrast microscope; observation of the dye transfer to neighboring cells was made using a fluorescence microscope. An individual cell was microinjected with Lucifer Yellow CH. The transfer of dye into surrounding cells was monitored using a phase-contrast–fluorescence microscope after injection. The percentage of first-order neighbors in contact with the injected cell and the numbers of all fluorescent cells were scored after each injection. If adipocytes were found to be first-order neighbors of the injected cells, we did not count the adipocytes when we totaled the number of first-order junctions of the injected cells. Percentages shown in the table were obtained by dividing the number of dye-transferred first-order neighboring cells by the total number of first-order neighboring cells.
(**A**) Semiconfluent cultures were exposed to 200, 500, 1000, or 2000 cGy. On day 1 or 7 after irradiation, the cells were microinjected with Lucifer Yellow CH.
(**B**) Semiconfluent cultures were exposed to 5000 or 10,000 cGy. On day 1 or 8 after irradiation, the cells were microinjected with Lucifer Yellow CH. The numbers in parentheses represent the total number of first-order neighboring cells, the number of injections, and the number of dye-transferred cells per injection.

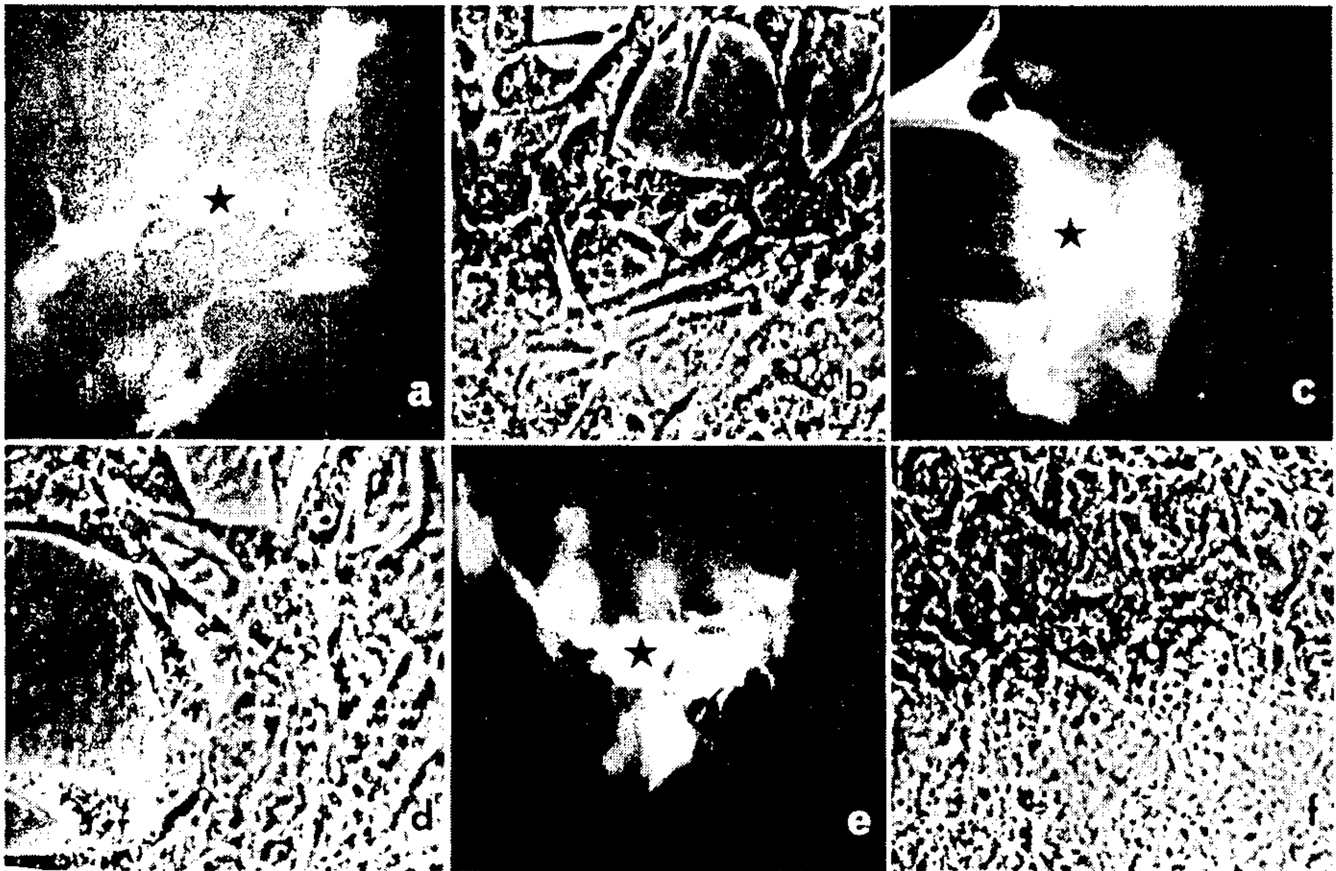

Fig. 1. Effects of irradiation (500–2000 cGy) on cell-to-cell interaction in H-1/A cells. Cells were injected with Lucifer Yellow CH 7 days after the irradiation. (**a**) Fluorescent photomicrograph of H-1/A cells that were not irradiated; (**b**) phase-contrast micrograph of (**a**); (**c**) fluorescent photomicrograph of H-1/A cells irradiated with 1000 cGy; (**d**) phase-contrast micrograph of (**c**); (**e**) fluorescent photomicrograph of H-1/A cells irradiated with 2000 cGy; and (**f**) phase-contrast micrograph of (**e**). The cells were photographed within 10 min after injection. Cells injected with fluorescent dye are indicated by *asterisks*. The dye was transferred into first-order neighbor cells within 10 s (**a**, **c**, and **e**). The percentage of first-order neighbor cells that contained fluorescent dye was dose independent. The vacuoles in the cells' cytoplasm (**d**), which was Oil Red O-negative, were not fat droplets. Therefore, these cells containing vacuoles were not adipocytes. These cells could be easily distinguished from true differentiated cells containing fat droplets using a phase-contrast microscope.

min in either cell type when we assayed dye-transfer. Dye-transfer into first-order neighbor cells occurred within only 10 s. Dye uptake by the cells from the extracellular space could, therefore, be ignored.

The H-1/A cells exhibited drastic changes in cell morphology as a result of irradiation. During the growth phase, H-1/A cells were fibrocytic and had no fat droplets in their cytoplasm. At >1000 cGy, the cells became flattened and their cytoplasm extended. Giant cells also appeared. Irradiation (0–2000 cGy) had no significant effect on adipocyte differentiation.

In order to determine whether our dye-transfer method (communication assay) is able to detect changes in gap-junctional communication, we used the present method to investigate the gap junctions of H-1/A cells during adipocyte differentiation in comparison with 3T3-L1 cells [22]. Whereas H-1/A preadipocytes communicate with neighboring cells, H-1/A adipocytes do not (Fig. 3). The fluorescent dye was able to extend to 68.0% and 5.6% of first-order adjacent cells on average when a single preadipocyte or adipocyte, respectively, was microinjected (Table 2). The present communication assay clearly demonstrates a difference in communication capability. The decrease in gap-junctional communication in H-1/A cells is linked to the processes of differentiation per se, rather than to the culture conditions, because another differentiation-incompetent subline, H-1/D, did not decrease dye transfer 7 days after confluence under the same conditions using the same method as in the case of H-1/A cells. To determine whether H-1/A adipocytes have less surface available to take up free fluorescent dye, we also examined the phagocytosis and endocytosis of H-1/A preadipocytes and adipocytes. H-1/A cells were incubated for 5 or 60 min with excess amounts of free fluorescent dye or 10^7 heat-inactivated yeast cells per milliliter of medium at room temperature [28]. No phagocytosis of yeast cells was observed in H-1/A preadipocytes or adipocytes. Although uptake of free dye was observed after 1-h exposure to excess amounts of free dye in the H-1/A adipocytes and preadipocytes, no dye uptake was observed within 5 min in either of the cells when we assayed dye transfer.

X-ray survival curves of H-1/A cells were plotted using 200-cGy/min x-rays with doses ranging from 50 to 1000 cGy

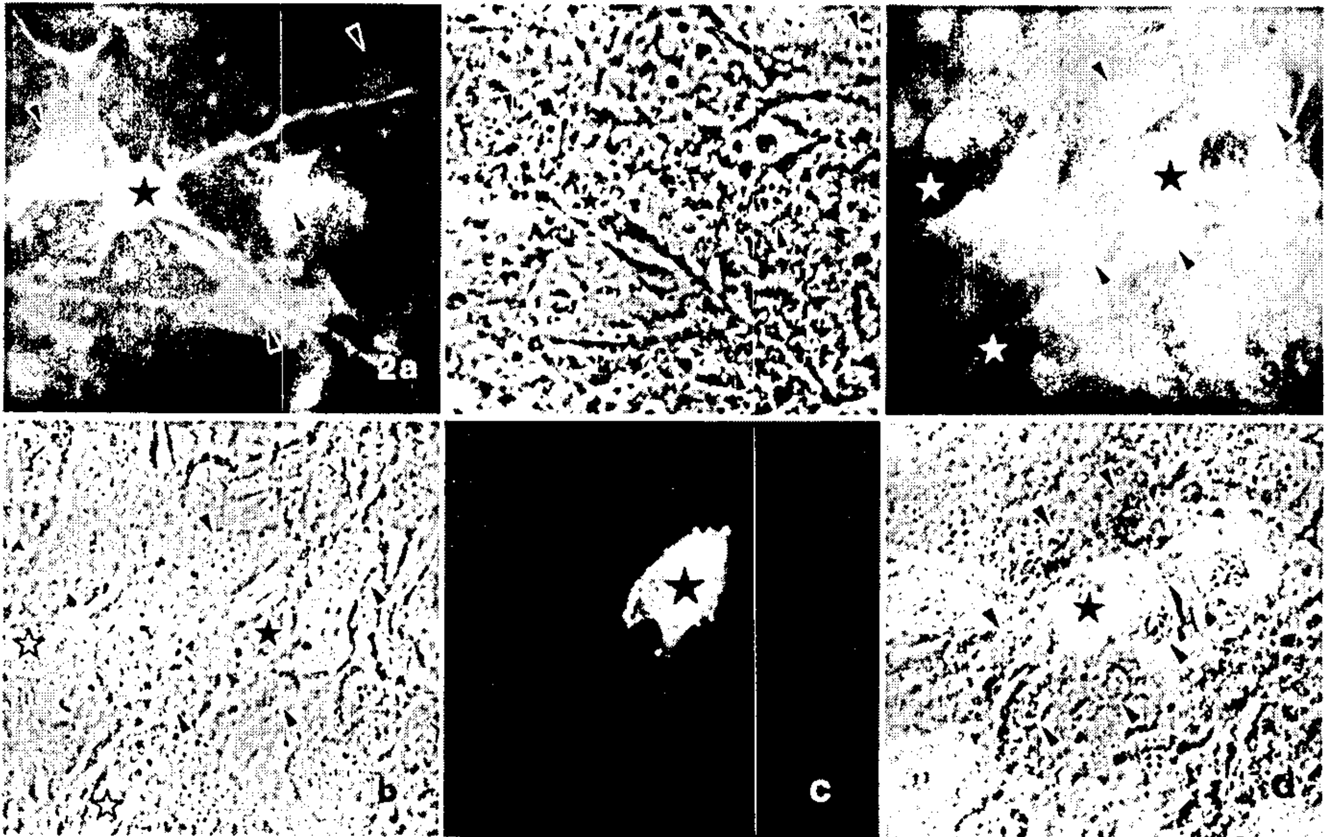

Fig. 2. Effects of irradiation (10,000 cGy) on cell-to-cell interaction in H-1/A cells. The cells were injected with Lucifer Yellow CH 8 days after 10,000-cGy irradiation. The dye was transferred from the injected cells (*asterisks*) to neighboring cells (*arrowheads*), although the cells were damaged by the irradiation. Injected cells irradiated with 10,000 cGy had elongated cytoplasmic processes. Dye spread to cytoplasmic processes within 1 min and demarcated cell shapes clearly. Photographs were taken within 10 min after injection.

Fig. 3. Dye transfer in a differentiated H-1/A adipocyte and an H-1/A preadipocyte. (**a, b**): An H-1/A preadipocyte (*black asterisk*) was injected with Lucifer Yellow CH. The fluorescent dye was transferred to adjacent cells extensively. The dye has spread to four first-order neighboring cells (*arrowheads*, 4/4). However, the dye was unable to extend to adipocytes (*white asterisks*), which have small fat droplets in their cytoplasm. (**c, d**): A differentiated H-1/A adipocyte (*black asterisk*) was injected with Lucifer Yellow CH. No dye transfer to any of the six first-order neighboring cells (*arrowheads*) was observed (0/6). (**a, c**) Fluorescent photographs; (**b, d**) phase-contrast micrographs of (**a**) and (**c**), respectively.

(Fig. 4); they were then compared with those of D2XRII cells [19]. Cells were trypsinized immediately after irradiation and replated at levels of 0.25, 0.5, 1.0, and 2.0 × 10^4 cells per dish. Adherent fibrocytic colonies of ≥50 cells were scored on day 7. Using regression analysis, D_0 and $\bar{n}$ were calculated for the H-1/A cultures. The D_0 was found to be 138, and the $\bar{n}$ was found to be 1.8. The number of dye-transferred cells decreased as a result of irradiation. The decrease may have been caused by a decrease in cell density, because cell growth was inhibited by increasing the level of irradiation.

Because irradiation induced certain marrow stromal cells to produce CSFs [29], we also performed colony assay and blot hybridization to determine the effect of irradiation on the production and expression of CSF-1 in H-1/A cells. H-1/A cell cultures were exposed to doses of 1000, 2000, and 4000 cGy. Doses of up to 4000 cGy had no detectable effect on the CSF production (Fig. 5). Nor were any significant changes in CSF-1 mRNA observed in H-1/A RNA after irradiation with 1000 and 2000 cGy (Fig. 6).

Discussion

The conclusion of this report is that gap-junctional communications between marrow stromal cells are resistant to irradiation in vitro. We investigated the effect of irradiation on gap-junctional communication in vitro using the dye-transfer method. This method is suitable for quantifying such communication, compared with electron microscopic study and immunohistochemistry. Although gap junctions are detected in the bone marrow in vivo and in vitro by electron microscopy [4–6], it is difficult to estimate the frequency of gap junctions because the incidence of gap junctions is reported to be very low in cultured cells [6]. In this series of experiments, we scored the incidence of permeable first-order junctions as an index of communication [22] from individual trials rather than the total number of fluorescent cells [9], because a decrease in cell number does not affect the incidence of dye transfer. At 0–2000 cGy of irradiation, the incidence of dye transfer was unaltered. In order to rule out

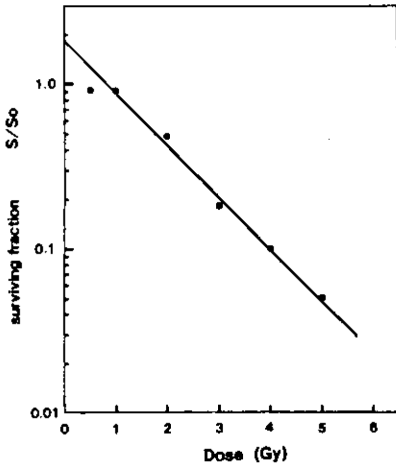

Fig. 4. x-ray survival curve of cloned stromal cell line H-1/A. Cultures of H-1/A cells were irradiated at 200 cGy/min, and cells were trypsinized, replated at 1 × 10^4/dish, and scored for fibroblast colonies of ≥50 cells per colony on day 7. S/S_0 = number of colonies scored at that dose/number in nonirradiated control plates. The plating efficiency was 1.6%, D_0 was 138 cGy, and $\bar{n}$ was 1.8. After 1000 cGy of irradiation, no ≥50 cell-adherent colonies were detected after replating the irradiated cells.

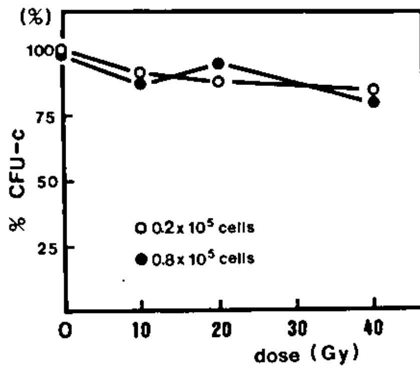

Fig. 5. The effect of radiation of confluent H-1/A cell cultures on induction of femoral bone marrow CFUc in agar. Confluent H-1/A cultures were exposed to radiation at the *dose* indicated. The CFUc assay was performed using medium harvested from irradiated and nonirradiated cells. Colonies were scored on day 8. Results of four independent experiments were pooled and presented as the mean percent of maximum number of colonies in terms of nonirradiated H-1/A cultures. The number of colonies in the control groups was 34.3 ± 1.7 for 0.2 × 10^5 cells per plate and 82.8 ± 2.1 for 0.8 × 10^5 cells per plate. The SD was ≤10% of the mean for each *point*.

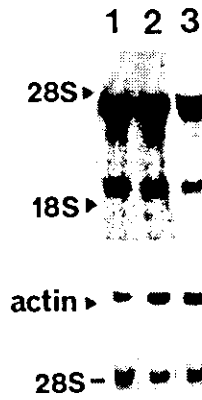

Fig. 6. RNA blot analysis of CSF-1 in H-1/A cells after irradiation. Shown is an autoradiogram of RNA blot analysis from nonirradiated H-1/A cells (*lane 1*) and H-1/A cells irradiated with 1000 cGy (*lane 2*) and 2000 cGy (*lane 3*). Five micrograms of total RNAs were electrophoresed on each lane. The blot was hybridized with CSF-1 (*top*) and actin (*middle*) probes. The CSF-1 probe was a 3.9-kb Eco R1 fragment of pGEM2MCSF-1 [27]. The actin probe was a 6.8-kb Eco R1 fragment of pSP62-PL from N. Davidson [32]. In the case of the RNA blots, the 28S and 18S rRNAs are indicated as *markers*. Almost exactly the same amounts of the 28S ribosomal RNA of H-1/A cells were detected in the control and the irradiated cultures (*bottom*). The autoradiogram was exposed for 72 h at −80°C using an intensifying screen.

Table 2. Intercellular communication capacity of H-1/A preadipocytes and adipocytes

Cells	Number injected	Communication rate[a] (percent)	Total number of dye-transferred cells per injection
H-1/A semiconfluent	71	68.0 (± 4.7)	17.6
H-1/A confluent	69	5.6[b] (± 2.3)	0.4
H-1/D semiconfluent	181	63.3 (± 6.7)	—
H-1/D confluent	183	70.0 (± 5.7)	—

Cells were grown to confluence in Fischer medium plus 10% horse serum. Fluorescent dye was injected into H-1/A and H-1/D cells. H-1/A cells developed many fat droplets in their cytoplasm after confluence, whereas few fat droplets were detected long after the confluence of H-1/D cells. In the H-1/A cultures, 65.9% of the cells on the 7th day after confluence (H-1/A confluent) differentiated into adipocytes, whereas only 1.3% of the cells of another subline, H-1/D, did (H-1/D confluent).

[a] Mean (± SE). Communication rate represents the percentage of first-order neighbor cells containing fluorescent dye, as evidenced by detectable permeable junctions within the first 5 min after injection.

[b] Statistically significant ($p < 0.01$) compatible with data for H-1/A semiconfluent cells. —, not counted.

the possibility that the lack of change was due to low doses of irradiation, H-1/A cells were irradiated at high doses, and the frequency of dye transfer remained unchanged. As far as gap-junctional communication is concerned, no radiation effects were detected in spite of changes in cell growth and morphology. Our results suggest that marrow stromal cells are coordinated with each other through gap junctions even after exposure to radiation.

In observations of nonirradiated bone marrow in situ, stromal cells are found to have gap junctions with adjacent cells [4]. Stromal cells were believed to communicate with each other via gap junctions. Even after in vivo irradiation, which failed to induce recovery of hematopoiesis in the short term, the marrow stroma remained unchanged morphologically [15]. In other words, reticular structure and sinus venules were intact in spite of the high radiation dose. These observations seemed to depend not only on the radioresistance of marrow stromal cells [30], but also on the radioresistance of cell adhesion apparatuses such as gap junctions. As shown in this study, gap-junctional communication between the cells of a stromal cell line was resistant to irradiation. This in vitro observation seems to be compatible with the

finding that the reticular meshwork is maintained during irradiation in vivo, although in vitro results cannot be applied directly to in vivo phenomena. Also, marrow adipocytes, which have a basement membrane around the cells, do not have gap junctions with neighboring cells in vivo [4]. These ultrastructural findings are consistent with results indicating that stromal cells lose their gap-junctional communications during adipocyte differentiation.

The H-1/A cells (D_0 = 138 cGy) used in this study were more sensitive to radiation than D2XRII stromal cells (D_0 = 198 cGy) [19]. Although the proliferative capacity of H-1/A cells was inhibited by 1000 cGy of irradiation, the CSF-1 production of H-1/A cells was unchanged by doses up to 4000 cGy, and the amount of CSF-1 mRNA was also unaffected by irradiation. The difference in radiation effects on proliferative capacity and expression and production of CSF-1 in H-1/A cells are compatible with the results in D2XRII cells [19].

Marrow stromal cells are heterogeneous functionally and morphologically. Some stromal cells, including H-1/A cells, express CSF-1, whereas other cells express granulocyte CSF and granulocyte–macrophage CSF [31]. Ultrastructurally, stromal cells are divided into endothelial cells, adventitial reticular cells, adipocytes, and macrophages [4]. It has also been reported that each component of stromal cells varies in its radiosensitivity [30]. The rate of growth inhibition of each stromal cell in response to radiation is different. Hence, the radioresistance of gap-junctional communication between H-1/A cells may not be applicable to all stromal cell types in bone marrow.

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Bone Marrow Stromal Cells Lose their Gap-Junctional Communication *In Vitro* during the Differentiation to Adipocytes

A. UMEZAWA†, K. HARIGAYA and Y. WATANABE

Department of Pathology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo

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Gap junctions which couple the cells ionically and metabolically are important in coordinating the cells. We studied the gap-junctional communication of adherent cells in long-term bone marrow (LTBM) culture and a clonal preadipocyte cell line, as probed with microinjected fluorescent tracer, Lucifer Yellow CH. In LTBM culture, fibrocytic stromal cells are dye-coupled and adipocytes are not. In the preadipocyte cell line, preadipocytes are dye-coupled and they lose dye transfer during the adipose conversion. We conclude that the differentiation of stromal cells to adipocytes causes the interruption of gap-junctional communication in bone marrow.

KEY WORDS: Continuous marrow culture, intercellular junction, adipose tissue, stromal cell.

INTRODUCTION

The long-term bone marrow (LTBM) murine culture has been developed by Dexter[1] and has facilitated the study of the proliferation and differentiation of hematopoietic stem cells. In this system, there are two basic components: adherent and non-adherent cells. Recently, a clonal

† Correspondence

preadipocyte cell line from the adherent cell layer of a LTBM culture has been established.[2]

With an electron microscope, the direct connection of gap junctions are observed between marrow stromal cells *in vivo*[3] and *in vitro*.[4] This study was undertaken in culture *in vitro* with the dye transfer method[5] to see whether the gap-junctional communication is present in stromal cells of LTBM culture and a murine marrow stromal cell line. The results clearly indicate that adherent cells in LTBM culture and the stromal cell line have gap-junctional communication between their neighboring cells and their conversion to adipocytes results in the loss of gap-junctional communication.

MATERIALS AND METHODS

Continuous marrow culture and stromal cells

LTBM cultures were set up as previously described.[1] Bone marrow was obtained from the femurs and tibiae of C3H/He mice by flushing the marrow cavity with cold media. Bone marrow cells (10^7) were placed in 7 ml of fischer media (Irvine Scientific, Santa Anna, California, USA) and 20% horse serum (Flow laboratory) with antibiotics. The cells were incubated at 37°C and 5% CO_2. Cultures were fed every seven days by removal of 4.5 ml growth medium and the addition of an equal volume of fresh medium. At the third weekly feeding when the adipocytes appeared, the cultures were prepared for the dye injection. The H-1/A cells were routinely cultured as previously described.[2]

Communication assay

Intercellular transfer of fluorescent Lucifer Yellow CH (Sigma, St Louis) was measured after direct microinjection of the dye into a cell under a phase-contrast microscope and observation of its transfer to neighboring cells under a fluorescence microscope. Microinjection was carried out using an Olympus injectoscope. A 10% solution of Lucifer yellow CH in 0.3 M lithium chloride solution was transferred to a glass capillary needle, which was prepared from a capillary tube using an Automatic Magnetic Puller (Narishige Co., Tokyo, Japan). Adherent cells of LTBM cultures and H-1/A cells were impaled with capillaries close to the nucleus, and dye was injected by hand pressure. The number of fluorescent cells after each injection was counted.

RESULTS

Fluorescent Lucifer Yellow CH was rapidly transferred to the neighboring fibrocytic cells, but not to adipocytes, when one particular fibrocytic stromal cell was microinjected in LTBM culture (Figure 1a, b). When the dye was injected into a fibrocytic cell, the number of cells to which the dye was transferred was 4.5 on average (Table I). When the dye was injected into an adipocyte containing multiple small fat droplets, dye transfer to neighboring adipocytes and fibrocytic cells seldom occurred (Figure 1c, d).

In low frequencies, dye coupling between fibrocytic stromal cells and hematopoietic cells was also seen. However, no dye coupling between adipocytes and hematopoietic cells was observed.

In order to examine whether the absence of gap-junctional communication in adipocytes correlates with the process of the differentiation of stromal preadipocytes, we used cultures of murine marrow stromal cell line, H-1/A. Cells convert to adipocytes after confluence and the manner of adipose conversion was similar to that discussed

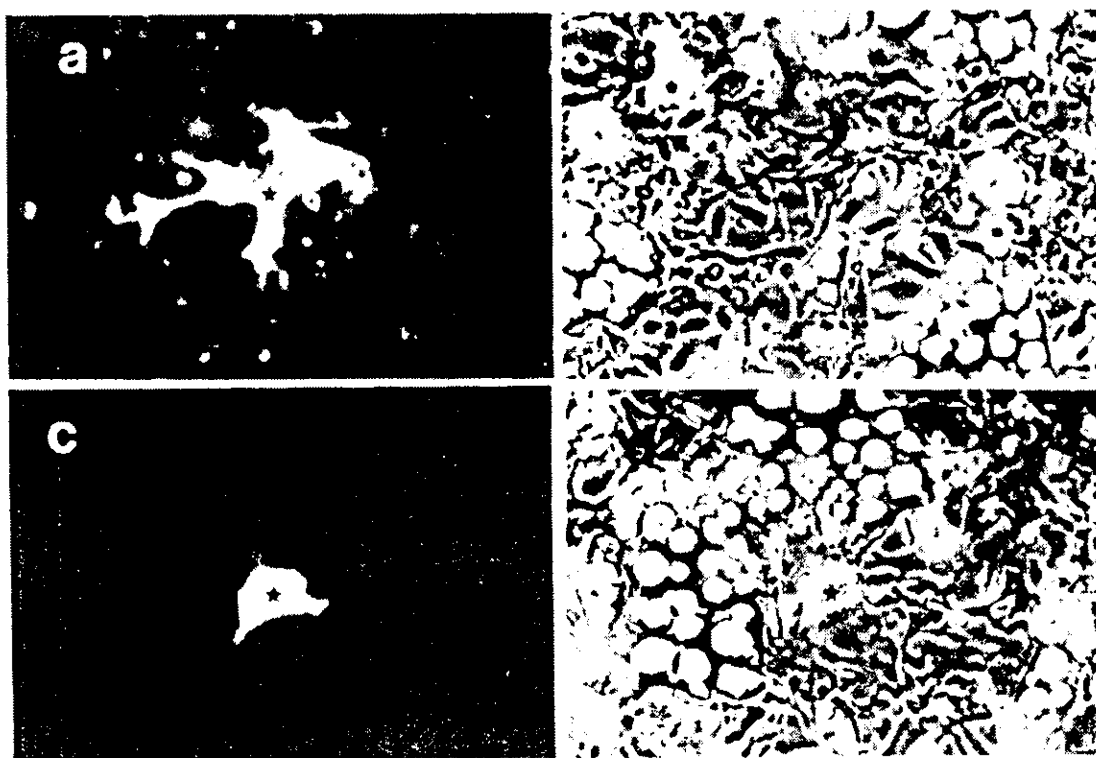

FIGURE 1 Dye transfer in fibrocytic cells and adipocytes in LTBM culture. (a) Fluorescent photomicrograph of fibrocytic cells; (b) phase contrast micrograph of (a); (c) adipocyte; (d) Phase contrast micrograph of (c). The cell which was injected, has multiple small droplets. The cells were injected with Lucifer Yellow CH and photographed within 30 min. ★, cells injected with Lucifer Yellow CH.

TABLE I
Intercellular communication capacity of adherent cells in LTBM cultures

Adherent cells	Percentage of uncoupled cells[a]	No. of dye-coupled cells/injection
Fibrocytic cells	10.3	4.5 ± 4.2[b] (29)[c]
Adipocytes	87.5	0.5 ± 1.3 (24)[†]

[a] The designation "uncoupled" refers to cells failing to transfer dye to contacting neighbors.
[b] Mean ± s.d.
[c] Numbers in parentheses, number of injections.
[†] Statistically significant ($P < 0.01$) to data of fibrocytic cells.

previously.[6] The dye was able to extend to 25.3 fibrocytic cells, on average (25.3 ± 11.8), when one particular fibrocytic cell was microinjected. On the contrary, almost no dye transfer was observed when an adipocyte was injected (Figure 2, Table II). No dye transfer from fibrocytic cells to adipocytes was seen at all.

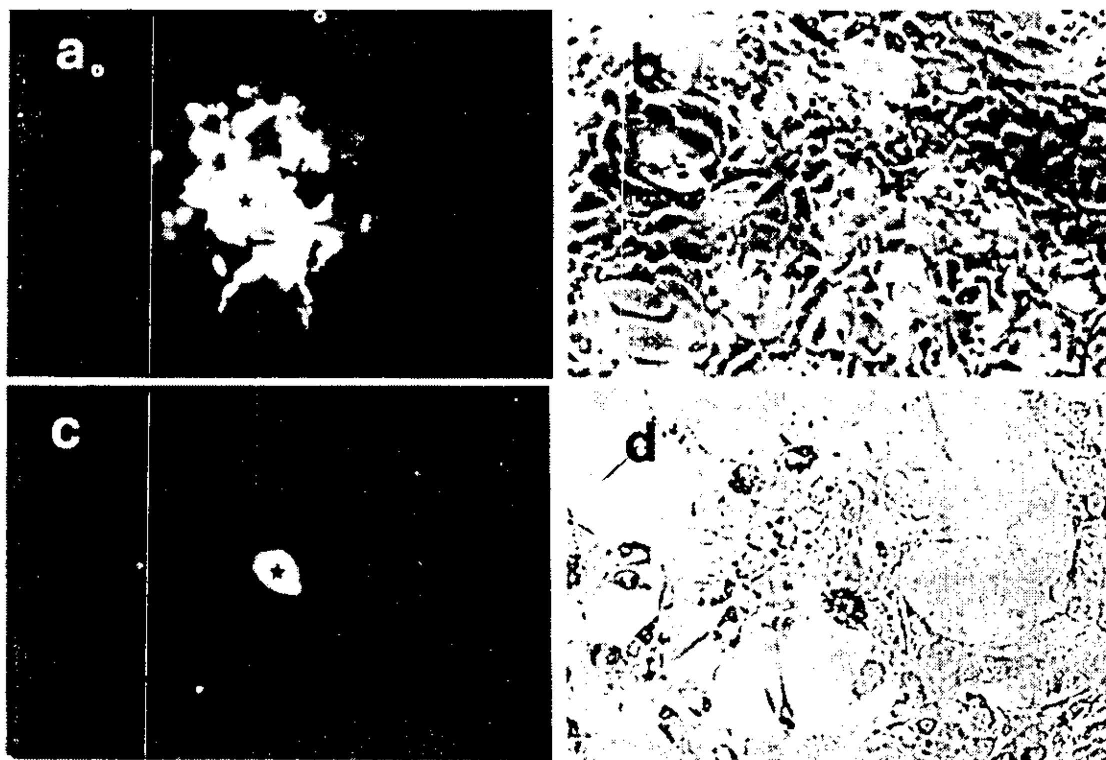

FIGURE 2 Dye transfer before and after adipose conversion in H-1/A cells. (a) Fluorescence photomicrograph of fibrocytic cells; dye was not transferred to adipocytes. (b) Phase contrast micrograph of (a); (c) adipocytes; no coupling is observed. (d) Phase contrast micrograph of (c). ★, cells injected with Lucifer Yellow CH.

TABLE II
Effect of adipocyte conversion on H-1/A clonal cells in their intercellular communication capacity

	Cell density (10^4 cells/cm^2)	Percentage of uncoupled cells[a]	No. of dye-coupled cells/injection
Fibrocytic cell, semiconfluent	2.4	11.8	3.6 ± 3.7[b] (17)[c]
Fibrocytic cell, confluent	18	0.0	25.3 ± 11.8 (13)
Adipocyte	18	91.7	0.3 ± 0.9[†] (12)

[a] The designation "uncoupled" refers to cells failing to transfer dye to contacting neighbors.
[b] Mean ± s.d.
[c] Numbers in parentheses, number of injections.
[†] Statistically significant ($P < 0.01$) to data of fibrocytic cells, confluent.

DISCUSSION

Gap junctions are composed of clusters of protein channels that allow ions and molecules of molecular weight less than 1500 to pass directly from the inside of one cell to that of another. Cells connected by gap junctions share many of their small molecules and gap junctions are clearly important in coordinating the activities of cells. Since Lucifer Yellow CH, which has a molecular weight of 457, cannot diffuse through membranes, its transfer into neighboring cells is believed to occur by gap-junctional communication.[5]

In LTBM culture, fibrocytic adherent cells are dye coupled and adipocytes are not. This indicates that fibrocytic cells have direct communication between the neighboring cells, but adipocytes communicate with neither fibrocytic cells nor adipocytes. Direct transfer of low-molecular-weight substances may occur between fibrocytic cells. By the study of the marrow preadipocyte cell line, it is demonstrated here that marrow preadipocytes have gap-junctional communication and the differentiation to adipocyte causes the interruption of communication, since conversion to adipocytes in the H-1/A cell line represents a process of differentiation.[7]

It has been shown that adipocytes in bone marrow differ from those in other sites.[7] Marrow adipocytes have a higher proportion of neutral fats and have different fatty acid profiles from adipocytes in other sites.

3T3-L1 cells derived from Swiss mouse embryo cells lose cell-to-cell communication during the adipocyte differentiation.[8] Bone marrow preadipocytes, as well as 3T3-L1 cells, behave in the same manner in terms of losing gap-junctional communication.

In a secretory cell system, correlation between secretion and gap junction has been studied. Gap junction development is correlated with the insulin content of B cells in the Islets of Langerhans.[9] We previously observed that H-1/A cells contribute to granulopoiesis during the fibrocytic stage through the production of colony-stimulating activity (CSA), while the CSA released by H-1/A cells decreases when they are converted to adipocytes.[6] Our results suggest that loss of gap-junctional communication is correlated with the decrease in production of CSA, although it is uncertain whether loss of communication is a cause or consequence.

In LTBM culture, gap-junctional communication between fibrocytic stromal cells and hematopoietic cells was found, but no communication between adipocytes and hematopoietic cells was observed. Since the cell–cell membrane channels of gap junctions are the likely conduits of growth-controlling signals,[10] it is conceivable that fibrocytic stromal cells make a contribution to hematopoiesis *via* direct cellular communication and their conversion to adipocytes results in the reduction of the influence on hematopoiesis.

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講　座

細胞の全能性と部分全能性

慶應義塾大学医学部病理学教室
梅澤明弘・秦　順一

Key Word： differentiation, cell conversion, cell therapy, marrow stromal cells, embryonal carcinoma cells

成人ヒトには，多くの分化した細胞が存在し，個体の維持のためにそれぞれが機能しています．大部分の細胞は分化しきっているため，その細胞の系列に分化することはあっても別の分化形質を示すことはありません．もちろん，血液細胞や消化管といった分裂の多い組織では未分化な幹細胞が存在し，いろいろな細胞になります．また，がん細胞はひとつの分化形質だけでなく，ふたつの分化形質を示したり，いくつかの分化形質を示すことがあります．でも，原則として，血液の細胞は神経の細胞になることはありませんし，心臓の細胞が肝臓の細胞になることはないのです．

その中でわたしが一番知りたいことは，どのようなメカニズムで細胞はいろいろな細胞になる能力を維持しているのであろうかということです．いろいろな細胞というのは，心臓，脳(神経)，肝臓，腎臓，肺，胃，骨，筋肉などの成人組織では，すべての細胞は受精卵に由来します．受精卵からすべての細胞ができるわけで，最初の受精卵はすべての細胞になることができるという全能性を有しているわけです．すべての細胞は免疫系の細胞を除けばすべての遺伝子のセットを有しており，さまざまな細胞になる潜在能力を持っています．

全能性を有している細胞は受精卵だけでなく，胎児性がん細胞や胎児性幹細胞があります[1,2]．胎児性がん細胞は精巣ないし卵巣の胚細胞由来のがんであり，いろいろな細胞に分化することで知られています．がんでありながら，ひとつの細胞が神経，膵臓，気管，軟骨，胃，皮膚などの組織ができあがります．自分が経験した患者さんでは，精巣の胚細胞由来の癌が転移先でさまざまな組織に分化して治癒しました．胚細胞由来の細胞ですので，癌細胞でありながら受精卵としての特性，すなわち多分化能を有しています．多分化能といっても，「血液幹細胞が好中球，赤血球，巨核球になる多分化能」や「骨髄間質細胞が骨，心，脂肪になる多分化能」よりも，高次の多分化能を胎児性がん細胞は有しています．胚盤胞にこれらの細胞を注入すれば，体を構成するすべての細胞になれますから，多分化能と言うよりも全能性があると言ったほうが適切でしょう[3]．

1. 細胞の多分化能―いくつかの異なる細胞に分化するメカニズム―

ヒト胎児性癌細胞を培養し，分化誘導を行い，ひとつのコロニーをみてみると，必ず何種類かの細胞が含まれます(第1図)．分化形質のマーカーを検討すると，デスミンを発現する筋肉細胞，ケラチンを発現する上皮細胞，ヒト絨毛ゴナドトロピンを産生する胎盤の栄養膜細胞など，上皮にも間葉系にも分化し，胎児にも胚外外胚葉にもなります[1]．これらの事実は，マウスの胎児性がん細胞を胚盤胞に注入したところ，体中のさまざまな細胞に分化した事実と合致します．

では，一種類の細胞が何種類の細胞になる機構はどのようなことが考えられるでしょうか．幹細胞が異なる2種類の細胞に分裂する不均等分裂の際の分子機構について簡単にふれます．ひとつの機構として考えられるのは，Notchなどの分子で知られている機構です[4,5]．それは一つの細胞から隣接する細胞に対して別の細胞になるようなシグナル伝達が生じます(第2図A)．別の細胞になっていくその細胞は元の細胞に対して異なる細胞が平面上で均等に並んでいくことになります．2つめはゲノムの修飾されかたが分裂した時に異なるような機構です（第2図B)．ゲノムの修飾とはクロマチンやメチル化状態を指し，分裂した細胞でその状態が異なれば遺伝子の発現も異なり別の細胞になるという考えです．もうひとつは分裂する際に蛋白質なりmRNAなりが不均等に分配さ

Differential Potential of Embryonic Stem Cells and Mesenchymal Stem Cells
Akihiro Umezawa, MD and Jun-ichi Hata, MD
Department of Pathology, School of Medicine, Keio University