Communication

Induction of Specific Protein Tyrosine Phosphatase Transcripts during Differentiation of Mouse Erythroleukemia Cells*

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We reported previously that most of the phosphotyrosine-containing cellular proteins were quickly dephosphorylated at the very early stage of erythroid differentiation of mouse erythroleukemia (MEL) cells. These and other experimental results implicated a specific protein tyrosine phosphatase(s) (PTPase(s)) involved in the commitment of the erythroid differentiation. We have investigated the pattern of transcripts of PTPases during MEL cell differentiation and found that while the transcripts of most PTPases were unchanged or undetected in the cells, transcripts for two PTPases (PTP2 and RIP) exhibited distinct patterns of induction at a very early stage of differentiation. Some of the mutant cells defective in differentiation did not show the induction of these PTPase transcripts. We discuss the possible role played by the PTPases in the commitment of MEL cell differentiation.

Although cell differentiation has been the subject of extensive studies in a number of experimental systems in vivo and in vitro, the nature of the molecular cascade, particularly that of the early reactions leading to cellular commitment to differentiation, remains unknown. Mouse erythroleukemia (MEL) cells differentiate into erythroid cells in vitro in response to a number of compounds with a wide variety of molecular structure and, presumably, different biological functions, which include dimethyl sulfoxide (Me2SO), hexamethylene-bis-acetamide (HMBA), sodium butyrate, trichostatic acid, and herbimycin A, as well as other compounds (1–7). This suggests that the molecular cascade is diversified at the initial stage but converges to a common and critical step for cellular commitment to differentiation. Previously, we reported that a series of inhibitors of protein tyrosine kinases are very effective inducers of MEL cell differentiation (6, 16–18). More recently, we showed that phosphotyrosine moieties of most of the cellular phosphotyrosine-containing proteins were dephosphorylated at the very early stage of MEL cell differentiation and that Na3VO4, a specific inhibitor of protein tyrosine phosphatases (PTPases) (9), prevented the dephosphorylation and erythroid differentiation (8). Mutant MEL cells resistant (defective) to differentiation were also resistant to dephosphorylation (8). The in vitro interaction between two differentiation inducing factors also indicated the involvement of a dephosphorylation step in MEL cell differentiation (10). Based upon these results, we are inclined to conclude that phosphotyrosine dephosphorylation of specific cellular proteins is a critical reaction that results in the commitment of MEL cell differentiation.

To identify a specific PTPase(s) that is involved in the dephosphorylation step, we examined the level of transcripts of 16 PTPases during MEL cell differentiation. Here we report that while the transcripts of most of the PTPases were unchanged or undetected in the cells, several specific PTPase transcripts exhibited distinct patterns of induction. Some of the mutant MEL cells resistant to differentiation did not show such alterations. The possible role of the PTPases in MEL cell differentiation is discussed.

EXPERIMENTAL PROCEDURES

Materials—HMBA was a gift from Dr. T. Yamane (Bell Laboratories). Mouse monoclonal anti-phosphotyrosine antibody (PY20, lot 32188) and sheep anti-mouse IgG horseradish peroxidase-conjugated antibody were purchased from ICN and Amersham Corp., respectively. Minimal essential medium was purchased from Nisui Seiyaku. Fetal calf serum was obtained from United Biotechnologies.

Cells and Cell Culture—MEL (Friend) cells (745A) were supplied by Dr. M. Terada (National Cancer Center Institute, Tokyo, Japan). Differentiation-resistant MEL cells (Dif-1, -2, -3, and -4; formerly DM-0 and RIP) exhibited distinct patterns of induction at a very early stage of differentiation. Some of the mutant cells defective in differentiation did not show the induction of these PTPase transcripts. We discuss the possible role played by the PTPases in the commitment of MEL cell differentiation.

RESULTS AND DISCUSSION

As we reported recently, dephosphorylation of phosphotyrosine-containing proteins was observed as early as 12 h after addition of inducers, and most of the phosphotyrosine-containing proteins were dephosphorylated by 24 h of incubation (8). Mutant MEL cells defective in differentiation did not exhibit such extensive dephosphorylation (Fig. 1). The presence of

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† The abbreviations used are: MEL, mouse erythroleukemia; PTPase, protein tyrosine phosphatase; HMBA, hexamethylene-bis-acetamide.

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The RNA were then subjected to Northern blot analysis. For Na$_3$VO$_4$, a specific inhibitor of PTPases, not only inhibited the control (uninduced) cells, but their level was virtually unchanged during MEL cell differentiation, particularly at the early stage and immunoblotted using anti-phosphotyrosine antibody as described under "Experimental Procedures."

PTPase cDNAs. The cDNA clones used as probes are listed in these, transcripts for PTPp2 (previously BET; see Ref. 27) as exemplified by LRP and SHP in Fig. 2 were present in the commitment to differentiation should occur by 20-30 h (12). Cells were incubated in the presence of Me$_2$SO, a typical erythroid-inducing agent, and poly(A)$^+$ RNA were prepared at different time intervals up to 48 h. Under the condition employed, the differentiation was completed in 100-120 h but the cellular commitment to differentiation should occur by 20-30 h (12). The RNA were then subjected to Northern blot analysis. For the PTPase probes, we used 16 different mammalian PTPase cDNA probes, which included many recently cloned PTPase transcripts detected up to 48 h after addition of the inducer is shown in Fig. 2. Transcripts for several PTPases were apparent as two molecular weight forms, decreased immediately after addition of Me$_2$SO but the level recovered to even higher level between 18 and 24 h. Transcripts for PTPp2 (previously BET; see Ref. 27), as well as for CD45 and RIP (previously MTC; see Ref. 27), exhibited a drastic increase at the very early stage of differentiation (group 3). As shown in Fig. 2, transcripts for these PTPases were at very low level before addition of Me$_2$SO, but sharply increased between 6 and 12 h (PTPβ2 transcripts exhibited a slight decline at 6 h of incubation). Transcripts for PTPε, which were apparent as two molecular weight forms, decreased im-

| Time(hours) | Wild type(745A) | Dif-2 |
|-------------|-----------------|-------|
| 0           | 24              | 48    | 72   | 96  |
| 0           | 24              | 48    | 72   | 96  |
| 0           | 24              | 48    | 72   | 96  |

**Fig. 1.** Alteration of cellular phosphotyrosine-containing proteins during erythroid differentiation. MEL (745A) cells and a mutant MEL (Dif-2) cell were incubated in the presence of Me$_2$SO (280 μM). At the times (h) indicated, samples were withdrawn and cell-free extracts were prepared. Proteins (50 μg) were separated on SDS-polyacrylamide gels, transferred to polyvinylidene difluoride membrane, and immunoblotted using anti-phosphotyrosine antibody as described under "Experimental Procedures."

N$_2$V$_4$, a specific inhibitor of PTPases, not only inhibited the dephosphorylation but also the differentiation itself (8). As a natural extension of these results, we sought to identify a PTPase(s) that is responsible for the dephosphorylation and possibly for triggering the differentiation. Initially, we examined whether the transcripts of any specific PTPases are induced during MEL cell differentiation, particularly at the early stage (0-12 h) of differentiation when the pattern of cellular phosphotyrosine-containing proteins started to be altered. MEL cells were incubated in the presence of Me$_2$SO, a typical erythroid-inducing agent, and poly(A)$^+$ RNA were prepared at different time intervals up to 48 h. Under the condition employed, the differentiation was completed in 100-120 h but the cellular commitment to differentiation should occur by 20-30 h (12). The RNA were then subjected to Northern blot analysis. For the PTPase probes, we used 16 different mammalian PTPase cDNA clones, which included many recently cloned PTPase cDNAs. The cDNA clones used as probes are listed in Table I.

Table I: **List of PTPase clones**

| PTPase clone | Ref. |
|--------------|-----|
| LRP          | Matthews et al. (19) |
| SHP          | Matthews et al. (20) |
| CDC25C       | Sadhu et al. (21)    |
| STEP         | Lombruso et al. (22) |
| PTP-S        | Swarup et al. (23)   |
| MPTP         | Mosinger et al. (24) |
| SH-PTP2      | Kakizuka et al. (25) |
| PTPβ2 (BET)  | Thomas et al. (27)   |
| RIP (MTC)    | Thomas et al. (27)   |
| CD45         | Thomas et al. (28)   |
| PTPε         | Thomas et al. (27)   |
| PTPδ         | Thomas et al. (27)   |
| PTPκ (MBR)   | Thomas et al. (27)   |
| PEP          | Matthews et al. (20) |
| PTPμ         | Gebbink et al. (29)  |

**Fig. 2.** Alteration of PTPase transcripts at the early stage (0-48 h) of MEL cell differentiation. Exponentially grown (MEL 745A) cells were exposed to Me$_2$SO (280 μM) when the cell density reached 2-3 x 10$^5$ cells/ml. Cells (5 x 10$^5$) were collected at 0, 6, 12, 18, 24, and 48 h of incubation with Me$_2$SO, and poly(A)$^+$ RNA was prepared and subjected to Northern blot analysis using various $^{32}$P-labeled PTPase cDNA probes.
induction apparently corresponded to, or preceded, the start of the dephosphorylation of phosphotyrosine-containing proteins.

In order to obtain further evidence for the possible involvement of PTPβ2 and/or RIP in MEL cell differentiation, we examined the level of transcripts of PTPβ2 and RIP in differentiation-resistant (defective) mutant MEL cells. Poly(A)+ RNA were prepared from four independently isolated mutant MEL cells (Dif-1, -2, -3, and -4) after addition of Me2SO4 and subjected to Northern blot analysis. These mutant MEL cells are resistant (defective) to erythroid differentiation, as well as dephosphorylation of phosphotyrosine-containing proteins induced by Me2SO4, HMBA, and other typical inducing agents (8). After addition of Me2SO4, the transcripts of these PTPases increased in two of the mutant cells, Dif-3 and Dif-4, the pattern being not significantly different from those of the control (745A) cells (data not shown). In Dif-1 and Dif-2, the level of PTPβ2 transcripts exhibited a similar pattern to that of the wild type (745A) cells (data not shown); however, no RIP transcripts were detected in Dif-1 even after 48 h of incubation with Me2SO4, and significantly lower levels of transcripts were detected in Dif-2 at least up to 12 h of incubation, although the level may be increased at the later stage of differentiation (Fig. 5). Patterns similar to those shown in Fig. 5 were also observed with poly(A)+ RNA from HMBA-treated Dif-1 and -2 cells (data not shown). It seems that the induction of transcription of RIP was affected by the introduction of the mutation(s), which results in the deficiency of erythroid differentiation.

We examined whether the level of any of the PTPase transcripts increased or was altered during MEL cell differentiation. Among 16 PTPases examined, PTPβ2 and RIP PTPases exhibited drastic increase of their transcripts at the very early stage of differentiation. Among a number of biochemical, morphological, and physiological changes specific to MEL cell differentiation, dephosphorylation of phosphotyrosine-containing proteins and the increase of specific PTPase transcripts reported here are the earliest events so far known, preceding by at least 10 h the cellular commitment to differentiation that occurs between 20 and 30 h after incubation with inducing agents. One of the cytoplasmic differentiation inducing factor (Dif-II) implicated by our previous cell and cytoplast fusion also reached the maximum approximately 6–8 h after addition of
inducers (14, 15). The very early increase of the transcripts of these PTPases and coincidence of the timing with the start of the dephosphorylation of phosphotyrosine containing proteins, as well as the induction of the putative differentiation inducing factor, may support the view that the induction of specific reactions leading to MEL cell differentiation by dephosphorylating specific cellular proteins. In this connection, it is interesting that mutant MEL cells (Dif-1) defective in differentiation apparently lacked the transcripts for RIP and had no signs of their induction after Me2SO or HMBA treatment. This suggests that the mutation responsible for the defectiveness in Dif-1 is located either at or before the dephosphorylation step by RIP in the differentiation cascade, although the possibility that the inhibition of the differentiation cascade somehow suppresses the transcription of RIP gene cannot be excluded. From this rather circumstantial evidence alone, it is too early to conclude that RIP and/or PTPβ2 are responsible for dephosphorylation of phosphotyrosine-containing cellular proteins, which leads to cellular commitment (6, 8, 16–18). We are currently in the process of determining whether expression of PTPβ2 and RIP can induce MEL cell differentiation.

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REFERENCES
1. Friend, C., Scher, W., Holland, J. G., and Sato, T. (1971) Proc. Natl. Acad. Sci. U.S.A. 68, 378–382
2. Reuben, R. C., Wife, R. L., Breslow, R., Rifkind, R. A., and Marks, P. A. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 862–866
3. Leder, A., and Leder, P. (1975) Cell 5, 319–322
4. Takahashi, E., Yamada, M., Saito, M., Kobayama, M., and Ogawa, K. (1975) Gann 66, 577–589
5. Yoshida, M., Nomura, S., and Beppu, T. (1987) Cancer Res. 47, 3688–3691
6. Kondo, K., Watanabe, T., Sasaki, H., Uehara, Y., and Oishi, M. (1988) J. Cell Biol. 109, 285–303
7. Oishi, M., and Watanabe, T. (1980) Mechanism of Differentiation, Vol. 2; pp. 129–141, CRC Press, Boca Raton, FL.
8. Watanabe, T., Kume, T., and Oishi, M. (1992) J. Biol. Chem. 267, 17116–17120
9. Swarup, G., Cohen, S., and Garbers, D. L. (1982) Biochem. Biophys. Res. Commun. 107, 1104–1109
10. Watanabe, T., and Oishi, M. (1992) Cell Growth Diff. 3, 865–871
11. Sambrook, J., Priscu, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
12. Nudel, U., Salomon, J., Fibach, E., Terada, M., Rifkind, R. A., Marks, P. A., and Bank, A. (1977) Cell 12, 463–469
13. Sasaki, H., Watanabe, T., Nomura, S., and Oishi, M. (1987) Jpn. J. Cancer Res. 78, 776–779
14. Kaneko, T., Nomura, S., and Oishi, M. (1984) Cancer Res. 44, 1756–1760
15. Watanabe, T., and Oishi, M. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 6481–6485
16. Watanabe, T., Shiraishi, T., Sasaki, H., and Oishi, M. (1989) Exp. Cell Res. 185, 325–342
17. Watanabe, T., Kondo, K., and Oishi, M. (1991) Cancer Res. 51, 764–768
18. Watanabe, T., Kume, T., Tunezumizumi, K., Kondo, K., Shiraishi, T., and Oishi, M. (1992) Exp. Cell Res. 199, 269–274
19. Matthews, R. J., Cahir, E. D., and Thomas, M. L. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 4444–4445
20. Matthews, R. J., Browne, D. B., Flores, E., and Thomas, M. L. (1992) Mol. Cell. Biol. 12, 2396–2405
21. sodhu, K., Reed, S. I., Richardson, H., and Russell, P. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5139–5143
22. Lombroso, P. J., Murdoch, G., and Lerner, M. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 7242–7246
23. Swarup, G., Kawatkar, S., Radho, V., and Rewa, V. (1991) FEBS Lett. 280, 65–69
24. Mosinger, B., Jr., Tillmann, U., Westphal, H., and Tremblay, M. L. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 499–503
25. Kakizuka, A., Sebaatian, B., Borgmeyer, U., Borgmeyer, I. H., Balado, J., Hunter, T., Hoekstra, M. F., and Evans, R. M. (1992) Genes & Dev. 6, 578–590
26. Freeman, R. M., Jr., Plutzky, J., and Neel, B. G. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 11339–11243
27. Thomas, M. L., Bowne, D. B., Cahir McFarland, E., Flores, E., Matthews, R. J., Pingel, J. T., Roy, G., Shaw, A., and Shenes, H. (1983) Proc. Immunol. 6, 213–219
28. Thomas, M. L., Reynolds, P. J., Chain, A., Ben-Neriah, Y., and Trowbridge, I. S. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 5360–5363
29. Gebbink, M. F. B. G., van Etten, I., Hatakeov, G., Sujkerbuijk, R., Beijersbergen, R. L., Geurts van Kessel, A., and Moobenaar, W. H. (1991) FEBS Lett. 290, 123–130