Persistence of the Mitogenic Response to Platelet-derived Growth Factor (Competence) Does Not Reflect a Long-term Interaction between the Growth Factor and the Target Cell

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ABSTRACT Quiescent BALB/c-3T3 cells exposed briefly to platelet-derived growth factor (PDGF) become "competent" to replicate their DNA even if PDGF is removed from cell culture medium prior to the onset of DNA synthesis. We have suggested that persistence of the PDGF-induced competent state reflects a rapidly induced and relatively stable biochemical change within the target cells. Others suggest that the phenomenon reflects a long-term association between PDGF and its target cells or perhaps between PDGF and the cell culture dish. This controversy has been addressed (a) by examining the effect of anti-PDGF antibodies on PDGF-induced competence and (b) by studying the chemical fate of ^{125}I-labeled PDGF.

Anti-PDGF antibodies inactive both soluble and surface-bound PDGF. However, if quiescent 3T3 cells are exposed to PDGF for as little as 30 min, subsequent addition of these antibodies to the culture medium does not prevent the mitogenic response. Under conditions where the PDGF-induced competent state decays stochastically with a $t_{1/2}$ of 18–20 h, cell-associated ^{125}I-PDGF decays with a $t_{1/2}$ of ~50 min. These data do not support the concept that persistence of the PDGF-induced competent state reflects a long-term association between PDGF and the target cells or between PDGF and the culture dish.

Platelet-derived growth factor (PDGF) is a connective tissue mitogen that has been purified to homogeneity from clinically outdated human platelets (1–4). The salient feature of PDGF action is induction of a long-term cellular memory that we have termed "competence (5). Quiescent BALB/c-3T3 cells exposed even briefly to PDGF become competent to replicate their DNA and divide. The PDGF-treated cell cultures do not "progress" efficiently through the G_0/G_1 phase of the cell cycle into S phase unless they are exposed continuously to a second set of growth factors contained in platelet-poor plasma (6); the "progression factors" in plasma appear similar to, and can be replaced by, epidermal growth factor (EGF) and insulinlike growth factors (somatomedins) (7, 8). In the absence of platelet-poor plasma, PDGF-treated cells remain competent to replicate their DNA but few do so. Readdition of plasma to PDGF-treated cells at later times allows expression of the mitogenic response.

The ability of PDGF to trigger a growth response long after it has been removed from the culture medium sets it apart from agents such as EGF, insulin, and the somatomedins which are required continuously in the culture medium to sustain a growth response (6–10). Somatic cell fusion analysis (11) together with other data (12) led us to suggest that persistence of the mitogenic response to PDGF in its physical absence from the culture medium reflected acquisition of a relatively stable cytoplasmic "second signal." Other workers have challenged this view of PDGF action, opining that competence may simply reflect a long-term association between PDGF and its target cells (13, 14). Since purified preparations of PDGF adhere tenaciously to laboratory plasticware (15), it has also seemed possible that competence reflects the sustained action of PDGF adsorbed adventitiously to the cell culture dish.

In studies described here, we used anti-PDGF antibodies (16) to study the effects of transient exposure to PDGF under conditions which were not contingent upon the physical removal of PDGF from the culture dishes. We used ^{125}I-PDGF

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to measure duration of the interaction between PDGF and BALB/c-3T3 cells under conditions where competence is induced. The data do not support the view that persistence of the PDGF-induced competent state reflects a long-term association between PDGF and the culture dish. By contrast, a large body of data is consistent with the view that competence reflects the intracellular accumulation of relatively stable PDGF-regulated gene products.

MATERIALS AND METHODS

Cell Culture: Stock cultures of BALB/c-3T3 cells (clone A-31) were maintained as previously described (5). All experiments were conducted on density-arrested monolayers of 3T3 cells which were prepared by seeding stock cell cultures in a density of $\sim 10^4$ cells/cm^2 and culturing them for 5 d in Dulbecco-Vogt modified Eagle's medium supplemented with 10% calf serum.

PDGF: Human PDGF was purified to electrophoretic homogeneity by a modification of our previous protocol (1) that employs high performance liquid chromatography (HPLC) at two stages. Reverse phase chromatography on an HPLC C-18 column (Micromeritics Instruments Co., Norcross, GA) was used in lieu of the isoelectric focusing step described earlier. The PDGF was applied to the C-18 column in 0.1% trifluoroacetic acid, 20% acetonitrite, 6% isopropyl alcohol, and eluted with an isopropanol gradient to 36% final concentration. Molecular sieving chromatography on an HPLC sizing column (Waters Associates, Milford, MA) was employed in lieu of the SDS gel electrophoresis step described earlier (1). The solvent for the sizing chromatography step was 1 M ammonium acetate, 10% isopropyl alcohol. The revised procedure results in PDGF preparations that appear as a single band of 33,000 daltons by SDS gel electrophoresis and are mitogenic at protein concentrations of 0.5–1 ng/ml. One experiment was conducted with partially purified PDGF carried through the Biogel P150 step of our purification protocol (1). By multiple criteria, the mitogenic response of BALB/c-3T3 cells to pure and to partially purified PDGF is identical (1, 11, 12).

Radio-iodination: Human PDGF was labeled with ^{125}I by a modification of the Hunter-Greenwood procedure (17) as described by Singh et al. (18). Specific activity of the radio-labeled PDGF ranged from 0.2 to 0.3 mol I/mol PDGF in various preparations. The radio-labeling procedure did not alter the mitogenic activity of PDGF as assayed on BALB/c-3T3 cells under standard conditions (1).

Anti-PDGF: Rabbit anti-PDGF (16) was a generous gift from Dr. C. H. Heldin, Dr. A. Wasteson, and Dr. B. Westermark of Uppsala University. The antibody preparation was a Staph A-purified Ig fraction which at a protein concentration of 50 μg/ml would efficiently inactivate 10–12 ng/ml of pure PDGF. Control experiments demonstrated that (*a*) the anti-PDGF preparation itself had no mitogenic activity in our standard assay system (1), (*b*) that the anti-PDGF was not toxic to 3T3 cells, and (*c*) that nonimmune rabbit immunoglobin had no anti-PDGF action.

RESULTS

Early Addition of Anti-PDGF Antibodies Is Required to Prevent the Induction of Competence

We used anti-PDGF antibodies to study the induction of competence under conditions that are not contingent upon physical removal of PDGF from the culture dishes. In the experiment summarized by Fig. 1, pure PDGF, at a final concentration of either 12 or 4 ng/ml, was added to quiescent density-arrested monolayers of 3T3 cells. At intervals thereafter, anti-PDGF was added directly to the tissue culture medium. As negative control, the PDGF was preincubated with antibody and the PDGF:anti-PDGF mixture was then added to the tissue culture cells. As a positive control, cells were incubated with PDGF alone. All of the cultures were incubated in the presence of [^{3}H]thymidine and 5% platelet-poor plasma for 24 h, fixed, and processed for autoradiography. The data (Fig. 1) show (*a*) that the antibody preparation will neutralize ~12 ng/ml of PDGF within a 1-h period of time at 37°C, and (*b*) that if the cells are exposed to PDGF for even a few hours, the antibody cannot effectively block the subsequent mitogenic response.

In previous studies, it was shown that the length of time required for induction of competence was a function of PDGF concentration. At lower concentrations of PDGF, several hours' exposure was required while at higher doses a 30-min "pulse" was effective. In these studies with antibodies, the PDGF at 12 ng/ml makes the cells "competent" for DNA synthesis more quickly than at 4 ng/ml. The antibody data are thus very similar to those described previously in which PDGF was removed by a medium change (5).

PDGF-induced Competent State Persists for Many Hours after Washout of PDGF

To accurately measure stability of the PDGF-induced competent state, we "pulsed" cells with PDGF and then transferred them to media containing a suboptimal (0.5%) concentration of plasma. At periodic intervals, the medium was supplemented with additional plasma to an optimal final concentration of 5%. The data (Fig. 2) show that few of the PDGF-treated cells enter S phase before the addition of optimal (5%) plasma. After the addition of 5% plasma to PDGF-treated cells, a lag time of 8 to 11 h precedes the onset of DNA synthesis. 24 h following the addition of plasma to PDGF-treated cells, the number of cells entering S phase has attained a maximum value. The percentage of competent cells is defined as this maximum value minus the "background" of cells that were not pulsed with PDGF and enter S phase in response to optimal (5%) plasma alone. With prolonged incubation in low plasma medium, some cells within the monolayer detach. A percentage of the remaining cells are stimulated by subsequent addition of optimal (5%) plasma in a phenomenon resembling the classic "wound healing response" (19). The "background" thus tends to increase with time, but with careful handling it can be held to <10%. We have found that for prolonged experiments 0.5% plasma facilitates cell attachment while not allowing optimum expression of the growth response to PDGF. For short-term experiments, 0.25% plasma as used previously is acceptable (5).

Data from the experiment outlined in Fig. 2 are replotted as the percentage of competent cells vs. incubation time in the

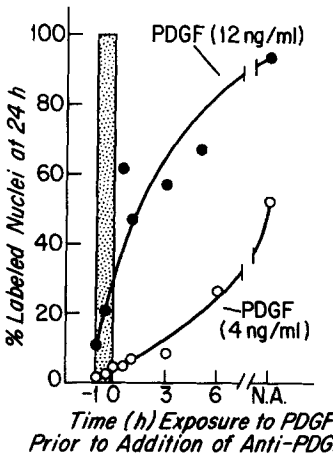

FIGURE 1. Early addition of anti-PDGF antibodies is required to prevent the induction of competence. Confluent monolayers of BALB/c-3T3 cells were prepared in microtiter tissue culture wells as previously described (1). Either 2.4 ng (●) or 0.8 ng (○) of pure PDGF was added together with 200 μl of Dulbecco's modified Eagle's medium (DME) supplemented with 5% platelet-poor plasma and [^{3}H]thymidine (5 μCi/ml). At the indicated time intervals, anti-PDGF was added to a final concentration of 50 μg/ml. As negative control, the PDGF solution was preincubated for 1 h or for ½ h at 37°C with antibody (shaded area) and the PDGF:anti-PDGF mixture was then added to the tissue culture cells. As a positive control, the cells were incubated with PDGF alone (*N.A.*). All of the cultures were then incubated for 24 h, fixed, and processed for autoradiography.

absence of platelet-poor plasma (Fig. 3). As can be seen from Fig. 3, the PDGF-induced competent state in BALB/c-3T3 cells decays with apparent first order kinetics and a $t_{1/2}$ of 18–20 h. From experiment to experiment the decay time of PDGF-induced competence shows some variability but always falls within the range of 10 to 20 h.

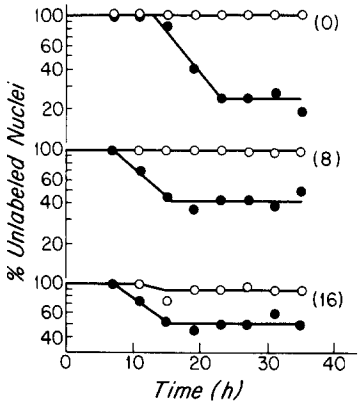

FIGURE 2 Decay of the PDGF-induced competent state. Quiescent density-arrested monolayers of BALB/c-3T3 cells were exposed for 3 h at 37°C to 50 U/ml of PDGF which had been purified through the Biogel-P150 stage (1). This concentration of Biogel-P150 PDGF corresponds to a protein concentration of ~250 ng/ml. Following 3-h exposure to PDGF, the culture medium was aspirated and the monolayers were washed twice with PBS. The PDGF-treated cell monolayers (●) and also control cell cultures treated with solvent only (○) were subsequently incubated with DME containing suboptimal (0.5%) platelet-poor plasma plus [^{3}H]thymidine (5 μCi/ml). At 0, 4, 8, 12, 16, and 20 h, platelet-poor plasma was added to PDGF-treated monolayers to a final optimum concentration of 5%. At timed intervals following the addition of platelet-poor plasma, cultures were fixed and processed for autoradiography. The data are plotted as percentage of unlabeled nuclei vs. time following the addition of plasma to 5%. For simplicity's sake, only the data from the 0 delay, 8-h delay, and 16-h delay measurements are shown. Data from all six time points are consolidated and replotted as shown in Fig. 3.

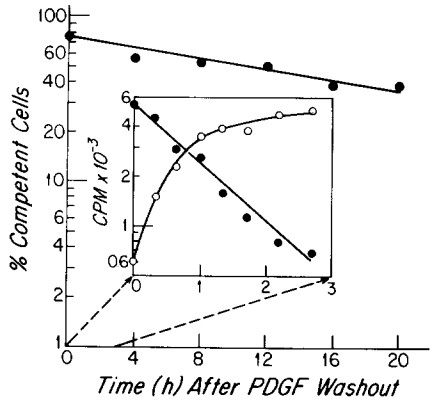

FIGURE 3 Stability of the PDGF-induced competent state exceeds the stability of cell associated PDGF. Decay of the PDGF-induced competent state (●) was determined from data of the experiment described in Fig. 2. Percentage of competent cells is defined as the fraction of PDGF-treated cells entering S phase following the addition of plasma to 5% (plateau value) minus the fraction of cells which entered S phase without being exposed to PDGF. Stability of cell-associated PDGF (*inset*: note expanded time scale) was measured in the following way: Quiescent density-arrested monolayers of BALB/c-3T3 cells were exposed to 2 ng of ^{125}I-PDGF in 200 μl of DME–0.25% plasma (equivalent to 50 U/ml used in Fig. 2). After 3 h, the culture medium was aspirated and the monolayers were washed twice with PBS. The cells were subsequently incubated in DME–0.25% plasma. At the indicated time intervals cultures were harvested. The TCA-insoluble radioactivity associated with the cell monolayer (●) and TCA-soluble radioactivity in the cell culture media (○) were determined.

PDGF-induced Competence Persists in the Presence of Anti-PDGF Antibodies

The salient feature of the PDGF-induced competent state is its relative stability. The experiment summarized in Table I shows that decay of the PDGF-induced competent state is unaffected by the presence of anti-PDGF antibodies. The fraction of cells which are rendered "competent" by a 3-h exposure to PDGF is somewhat reduced when anti-PDGF antibodies are added after the PDGF washout. However, subsequent decay of the competent state in the antibody-treated cultures occurs at a comparable if not slower rate. From the data of Table I, the $t_{1/2}$ of the competent state is 15 h for control cells vs. 21 h for antibody-treated cultures although the differences calculated from these two point measurements are probably not significant. The observation that fewer cells are rendered competent when a 3-h "pulse" of PDGF is followed by addition of anti-PDGF antibodies probably reflects the fact that some PDGF remains associated with the cells for an hour or two following wash-out from the tissue culture medium (see below).

Cell-associated PDGF Is Degraded with a $t_{1/2}$ of <1 h

If 3T3 cells are incubated with PDGF for more than a few hours, anti-PDGF antibodies cannot prevent induction of the competent state; moreover, the PDGF-induced competent state persists for many hours in the presence of anti-PDGF antibodies. These data could reflect the accumulation and long-term retention of PDGF within a cellular compartment which is inaccessible to antibodies. To determine the relationship between stability of cell-associated PDGF and stability of the PDGF-induced competent state, we exposed quiescent 3T3 cells to pure ^{125}I-PDGF at 37°C under conditions paralleling those employed for the experiments summarized in Figs. 2 and 3. At the end of 3-h incubation with ^{125}I-PDGF at 37°C, unbound radioactivity was removed by aspiration. The treated monolayers were washed four times with PBS and the cultures were subsequently incubated in DME–0.25% platelet-poor plasma. At periodic intervals, the cell monolayers were har-

TABLE I

Persistence of the PDGF-induced Competent State in the Presence of anti-PDGF Antibodies

	% Competent cells	
	Plasma added at zero time	Plasma added after 8 h
No AB	87	61
+AB	40	32

Quiescent microtiter cultures of BALB/c-3T3 were exposed to 4 ng of pure PDGF in DME–0.5% platelet-poor plasma (40 μl) for 3 h at 37°C. PDGF-containing medium was then removed and the monolayers were washed twice with phosphate-buffered saline. Half of the cultures were transferred to 200 μl of Dulbecco's modified Eagle's medium supplemented with [^{3}H]thymidine and an optimal (5%) concentration of platelet-poor plasma with or without anti-PDGF antibodies (10 μl). The other half of the cultures were transferred to medium containing a suboptimal (0.5%) concentration of platelet-poor plasma with or without anti-PDGF antibodies. After 8-h incubation in suboptimal plasma, the latter cultures received a supplement of platelet-poor plasma to a final concentration of 5%. At 24 h after exposure to 5% plasma medium, all cultures were fixed with methanol and processed for autoradiography. The data are shown as percent competent cells (as defined in Fig. 2) after correction for the percentage of cells which became labeled without exposure to PDGF (12%). The data points represent the mean of experiments done in duplicate or triplicate.

vested as well as the overlying cell culture medium. We monitored trichloroacetic acid (TCA)-insoluble radioactivity associated with the cell monolayer and TCA-soluble radioactivity associated with the culture medium. The data indicate that cell-associated PDGF is lost in exponential fashion with a $t_{1/2}$ of ~50 min and that this loss is quantitatively accounted for by an increase of TCA-soluble radioactivity in the cell culture medium (Fig. 4, *inset*). By comparing the two decay curves shown in Fig. 4, it is clear that stability of PDGF-induced competence does not coincide with stability of cell-associated PDGF.

Persistence of Competence Is Not Mediated by PDGF Adherent to the Surface or Side of the Culture Dish

Purified PDGF adheres tenaciously to laboratory plasticware; moreover, surface-bound PDGF is mitogenically active (15). For these reasons, we wished to determine whether persistence of the PDGF-induced competent state was mediated by material adventitiously bound to the surface or sides of the culture dish. The experiments summarized in Fig. 4 and Table I argue against this possibility. The data show (*a*) that the amount of PDGF that binds to the surface of culture dishes containing 3T3 cell monolayers is quantitatively insufficient to account for competence and (*b*) that anti-PDGF antibodies (which block neither the induction nor the persistence of competence) inhibit surface-bound PDGF.

To determine the amount of PDGF which adheres to the surface of plastic culture dishes, we incubated ^{125}I-PDGF at 37°C in empty cell-culture dishes and also in dishes that contained confluent monolayers of 3T3 cells. We measured the ^{125}I-PDGF that adhered to the bottoms and sides of the dishes as a function of the ^{125}I-PDGF concentration (Fig. 4). The empty culture dishes exhibit a high capacity for PDGF binding. The kinetics of binding are linear with PDGF concentration up to the highest levels tested (300 ng/ml) and the bound radioactivity resists extraction with Triton X-100. By contrast, only limited quantities of ^{125}I-PDGF bind to culture wells which contain 3T3 cell monolayers. The binding becomes curvilinear at higher concentrations reflecting the fact that most of the PDGF is associated with saturable receptors in the cell monolayer (Triton X-100 soluble radioactivity) rather than the plastic culture dish.

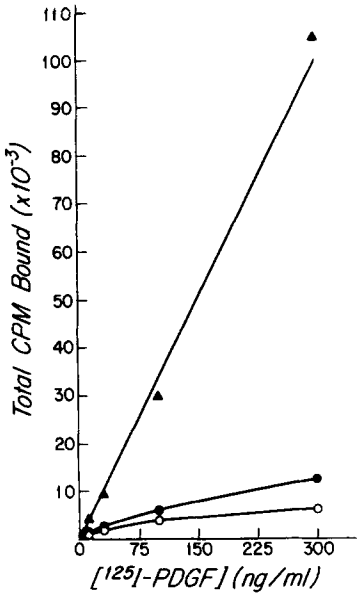

FIGURE 4 PDGF adherent to culture dishes which contain 3T3 cell monolayers is quantitatively insufficient to account for competence. ^{125}I-PDGF was added as indicated to either unused empty microtiter culture wells or to identical wells containing BALB/c-3T3 cells which had been cultured to the confluent monolayer stage as previously described (5). The culture wells were incubated at 37°C for 1 h. Unbound PDGF was aspirated and the wells were washed five times with PBS. All wells were then extracted initially with 200 μl of 1% Triton X-100–10% glycerol; this extraction removes cell-associated PDGF but not PDGF which has adhered adventitiously to plastic (2, 15). The wells were then extracted twice with 200 μl of 1 M NaOH. The total bound ^{125}I radioactivity (Triton X-100 + NaOH extractions) is indicated for the empty culture wells (▲) and for wells which had contained 3T3 cell monolayers (●). The cell-associated PDGF (radioactivity in first Triton extraction) is indicated for wells which contained 3T3 cells (○). Less than 10% of the radioactivity in unused culture dishes was Triton X-100–extractable at any given data point, so these data were not plotted separately.

The small quantity of radioactivity which binds to plastic in the presence of 3T3 monolayers is 75% associated with the side of the culture dish and 25% associated with the bottoms (this was determined by punching out the bottoms of the microtiter wells and counting radioactivity separately—data not shown). When microtiter cultures of 3T3 cells are exposed to 10 ng/ml of PDGF—a concentration sufficient to induce competence—the amount of material adherent to the sides of the dish would form a PDGF solution of only 0.045 ng/ml if it were all resolubilized in the medium (200 μl media/microtiter well). This concentration of PDGF is inadequate to induce a growth response (1). The PDGF adherent to the bottom of the culture dishes could conceivably be in direct contact with 3T3 cells and could present as many as 3.3×10^3 molecules of PDGF per cell. This quantity of PDGF would occupy no >2% of the available receptors on BALB/c-3T3 cells (18). Under the standard conditions employed for our PDGF bioassay (1) and our PDGF binding assay (18), at least 33% of the total receptors must be occupied for 100% mitogenic activity and 10% must be occupied for 50% mitogenic activity. Thus the quantity of PDGF adherent to the bottom of cell culture dishes is probably inadequate to account for competence.

In separate experiments, PDGF was allowed to bind to empty microtiter tissue culture wells as previously described (15). Anti-PDGF antibodies were then added to the treated culture wells; positive controls received no antibody and negative controls were not pretreated with PDGF. After a 1-h preincubation at 37°C, quiescent 3T3 cells were plated at near-confluent density into the treated culture wells. The cultures were incubated overnight in DME–5% plasma and [^{3}H]thymidine, fixed, and processed for autoradiography. The data (Table II) show that anti-PDGF antibodies are capable of inactivating surface-bound PDGF.

DISCUSSION

Transient exposure to PDGF suffices to trigger the mitogenic response of BALB/c-3T3 cells in culture (5). Other workers have shown that transient exposure to PDGF induces growth of monkey arterial smooth muscle cells (20), human diploid fibroblasts (21, 22), and Swiss 3T3 cells (13). Thus, the phenomenon of the PDGF-induced "competent" state is broadly based although its biochemical basis has yet to be defined.

By inference from experiments conducted with TPA or with thrombin, Dicker and Rozengurt (13) and Van Obberghen-Schilling et al. (14) suggested that persistence of PDGF action following removal from the cell culture medium reflects a long-term association between PDGF and the target cell. The direct experiments reported here do not support such a conclusion. Anti-PDGF antibodies block the mitogenic action of surface bound (Table II) as well as soluble PDGF. Yet, when 3T3 cells are exposed even briefly to PDGF, subsequent addition of

TABLE II

Anti-PDGF Antibodies Inactivate Surface-bound PDGF

Pretreatment	# Labeled cells/total (% Label) Exp. #1	Exp. #2
None	305/2,256 (13.5%)	9/948 (0.9%)
Plates precoated with PDGF	1,052/3,151 (33.3%)	150/1,410 (10.6%)
Plates precoated with PDGF followed by addition of anti-PDGF	287/2,598 (11.0%)	28/980 (2.8%)

Empty microtitre tissue culture wells were pretreated with pure PDGF (4 ng in 40 μl) or a solvent control for 4 h at 37°C as previously described (15). The PDGF solution was then removed and the empty wells were washed several times with PBS. 200 microtiters of DME-5% plasma plus [^{3}H]thymidine (5 μCi/ml) was pipetted into each well. One of the PDGF-treated wells also received anti-PDGF to a final concentration of 50 μg/ml. All wells were preincubated for 1 h at 37°C. Quiescent density-arrested 3T3 cells were then harvested by trypsin digestion and pipetted into the wells (1.5×10^4 cells/well). The culture wells had been precoated with collagen to facilitate rapid cell attachment as previously described (15). All cultures were incubated for 4 h at 37°C, fixed, and processed for autoradiography.

anti-PDGF antibodies prevents neither the induction (Fig. 3) nor the persistence (Table I) of competence. The data obtained with PDGF and anti-PDGF antibodies contrast markedly with those of similar studies conducted with EGF and anti-EGF antibodies. The EGF studies lead to the conclusion that the mitogenic response to EGF requires persistent occupation of high affinity EGF receptors (10).

There is no evidence that 3T3 cell cultures ingest or retain PDGF in a compartment that is inaccessible to antibodies. The interaction between PDGF and its target cells is quite similar to that described for growth factors such as EGF which are required continually for mitogenic action. Cell-associated EGF is degraded by 3T3 cells with a $t_{1/2}$ of between 1–2 h (9). Cell-associated PDGF is degraded with a half-life of <1 h in BALB/c-3T3 cells (Fig. 3). Degradation of PDGF also occurs rapidly ($t_{1/2}$) 60–90 min) in human diploid fibroblasts (23) and Swiss 3T3 cells (24, 25).

Quantitative analysis with ^{125}I-PDGF (Fig. 4) and direct analysis with anti-PDGF antibodies (Table II) indicate that persistance of the competent state is not mediated by PDGF adherent to the surface of the culture dish. Moreover, we noted in previous studies (11) that competence persists even when PDGF-treated cells are harvested by trypsin digestion and replated into fresh culture dishes (11). It should be noted in passing that the phenomena of adventitious binding is not unique to PDGF. Insulin, which needs to be present continuously to promote growth, binds with high affinity to artificial surfaces such as common talc (26).

Our studies with ^{125}I-PDGF do not strictly exclude the possibility that PDGF-induced competence is mediated by a long-term association between the target cells and a degradation product of PDGF or the PDGF-receptor complex; however, there is no compelling evidence of such a long-term association. By contrast, a growing body of evidence suggests that persistence of the mitogenic response to PDGF following its removal from cell culture medium reflects the intracellular accumulation of stable, PDGF-regulated gene products. Thus, (*a*) cytoplasts derived from PDGF-treated cells initiate replicative DNA synthesis in quiescent 3T3 cells when fused to them with Sendai virus, (*b*) acquisition of the active components in such cell fusion studies is blocked when RNA synthesis is inhibited during the period of PDGF treatment (11), (*c*) PDGF treatment induces a prompt (within 40 min) accumulation of several cytoplasmic proteins (12), (*d*) PDGF regulates these proteins at a pre-translational level (27), and (*e*) variants of the 3T3 cell line which express these cytoplasmic proteins constitutively can proliferate in the absence of PDGF (12, 27).

PDGF is not the only regulatory agent which functions in a discontinuous manner on mammalian cells. Agents such as pituitary fibroblast growth factor (28), calcium phosphate (7), and the fibroblast-derived growth factor (13) mimic the action of PDGF rendering cultured fibroblasts competent to respond to a second set of growth factors and divide (7). A brief exposure to plant lectins renders mouse lymphocytes competent to replicate when secondary growth factors contained in serum are subsequently provided (29). In the erythropoietic pathway, transient exposure to soluble T cell factors primes undifferentiated erythroid precursor cells to respond to erythropoietin with proliferation and formation of "bursts" of erythrocytes (30–32). It would appear that PDGF is but one example of a class of regulatory signals which are needed only transiently to induce a mitogenic response. Thus insights into the molecular nature of "competence" in BALB/c-3T3 cells may take on broader significance in the area of cell growth regulation.

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