Journal of Supramolecular Structure 13:489–499 (1980)
Control of Cellular Division and Development: Part A: 141–151

Regulation of the Balb/c-3T3 Cell Cycle-Effects of Growth Factors

C.D. Stiles, W.J. Pledger, R.W. Tucker, R.G. Martin, and C.D. Scher

Harvard Medical School, Boston, Massachusetts 02115 (C.D.S., C.D.S.), Johns Hopkins University Medical School, Baltimore, Maryland 21218 (R.W.T.), University of North Carolina, Chapel Hill, North Carolina 27514 (W.J.P.), and National Institutes of Health, Bethesda, Maryland 20205 (R.G.M.)

The platelet-derived growth factor (PDGF), which is found in serum but not in plasma, has been purified to homogeneity; it stimulates replication at a concentration of 10^{-10}M. Brief treatment with PDGF causes density-inhibited Balb/c-3T3 cells to become competent to synthesize DNA; pituitary fibroblast growth factor (FGF) or precipitates of calcium phosphate also induce competence. Continuous treatment with plasma allows competent, but not incompetent, cells to synthesize DNA. A critical component of plasma is somatomedin, a group of hormones with insulin-like activity; multiplication-stimulating activity (MSA) or insulin replace plasma somatomedin in promoting DNA synthesis.

We have studied the molecular correlates of competence and the role of SV40 gene A products in regulating DNA synthesis. Treatment of quiescent cells with pure PDGF or FGF causes the preferential synthesis of five cytoplasmic proteins (approximate molecular weight 29,000, 35,000, 45,000, 60,000, and 72,000 detected by SDS-PAGE under reducing conditions). Two of these competence-associated proteins (29,000 and 35,000 daltons) are found within 40 min of PDGF addition; they are not induced by plasma, insulin, or epidermal growth factor (EGF). PDGF, FGF, or calcium phosphate induce an ultrastructure change within the centriole of 3T3 cells; this ultrastructural modification of the centriole is detectable by immunofluorescence within 2 h of PDGF treatment. Plasma, EGF, or MSA do not modify the centriole. SV40 induces replicative DNA synthesis in growth-arrested 3T3 cells but does not cause this alteration in centriole structure.

Gene A variants of SV40, including a mutant with temperature-sensitive (ts) T-antigen (ts A209), a deletion in t-antigen (dl 884), and several ts A209 strains containing t-antigen deletions were used to induce DNA synthesis in Balb/c-3T3 cells. Like wild type SV40, all strains induced DNA synthesis equally well under permissive or nonpermissive conditions. Addition of PDGF or plasma had little effect on SV40-induced DNA synthesis. Thus, the viral function that induces replicative DNA synthesis in Balb/c-3T3 cells is not t and is not temperature sensitive. This SV40 gene function overrides the cellular requirement for hormonal growth factors. It does not induce transient centriole deciliation, a hormonally regulated event.

Key words: PDGF, somatomedin, SV40, cell cycle

Received March 21, 1980; accepted July 25, 1980.

0091-7419/80/1304-0489$02.30

Circulating blood platelets contain a polypeptide growth factor that stimulates proliferation of cells derived from embryonic mesoderm [1–4]. This platelet-derived frowth factor (PDGF) is sequestered within the α-granules of circulating platelets [5, 6] and is only released into serum when blood clots [2–4]. For this reason, early passage embryo fibroblasts [1], arterial smooth muscle cells [2], normal glial cells [7], and mouse 3T3 cells [8] proliferate much more efficiently in vitro when the cell culture medium is supplemented with clotted blood serum than when the medium is supplemented with platelet-poor plasma. PDGF has recently been purified to homogeneity and characterized as a heat stable, basic (pI 9.8) polypeptide with a native molecular weight of approximately 35,000 daltons by the criterion of SDS-gel electrophoresis under non-reducing conditions [9, 10].

Although PDGF is required for the growth of normal connnective tissue cells, it is not sufficient to promote growth. In 1977, our laboratories discovered that a second set of growth factors, contained in platelet-poor plasma, was required for optimal expression of the mitogenic action of PDGF on Balb/c-3T3 cells [11]. Detailed analysis of the mitogenic response revealed that transient exposure to PDGF rendered quiescent, density-arrested Balb/c-3T3 cells competent to replicate their DNA; however, PDGF-treated, competent 3T3 cells did not progress through the G_0/G_1 phase of the cell cycle into the S phase until they were exposed to a second set of growth factors contained in plasma [12, 13]. Our discovery that the early mitogenic response of density-arrested 3T3 cells to serum growth factors comprises two temporally distinct phases, competence and progression, and has provided a useful conceptual framework in which to analyze the mitogenic action of a wide variety of agents including polypeptide growth factors, tumor promoters, and tumor viruses. The goal of this article is to review and integrate some of these recent data on the relationship between growth factors, tumor promoters, and tumor viruses in regulation of the cell cycle.

THE MITOGENIC RESPONSE OF DENSITY ARRESTED BALB/c-3T3 CELLS TO PDGF AND PLASMA (SERUM)

When grown to the confluent monolayer stage in medium supplemented with 10% calf serum, Balb/c-3T3 cell cultures become growth arrested with a G_1 DNA content. When fresh serum containing medium is added to such cell cultures, replicative DNA synthesis in the cultures is reinitiated. The first cells enter the S phase following a minimum lag of 12 h; thereafter, cells enter the S phase asynchronously in a pseudo-first-order fashion [14]. We have shown that the mitogenic response to serum can be duplicated by the simultaneous addition of PDGF and plasma. Density-dependent growth arrest of Balb/c-3T3 cells reflects starvation for the PDGF component of serum; the addition of PDGF alone to cell-depleted medium will induce mitosis, whereas the addition of plasma to depleted medium will not [11]. The mitogenic response can be plotted on linear graph paper as the percent of cells in S vs time (Fig. 1A) or on semilogarithmic paper as the percent of cells remaining in G_1 vs time, after the fashion of Smith and Martin [14] (Fig. 1B). The latter method has some advantages and will be used in the remainder of this discussion.

PHASE 1 OF THE MITOGENIC RESPONSE: COMPETENCE

Transient exposure to the PDGF component of serum renders quiescent Balb/c-3T3 cells competent to replicate their DNA. However, in the absence of other growth factors

contained in the platelet poor plasma component of serum, competent 3T3 cells do not progress through a G_0/G_1 towards S phase. Rather, they remain at growth-arrested at least 12 h before progressing. The cellular response to PDGF is not inhibited by a short treatment with cycloheximide or inhibitors of pinocytosis. As shown in Table 1, several other agents can mimic the action of PDGF by inducing competence in 3T3 cells [13]; these include fibroblast growth factor (FGF) [15], $Ca_3(PO_4)_2$, and wounding [13]. Step 1 of the mitogenic response is summarized schematically in Figure 2.

PHASE 2 OF THE MITOGENIC RESPONSE: PROGRESSION

The addition of platelet-poor plasma to competent Balb/c-3T3 cells initiates progression towards S phase. Under optimal conditions, the first cells enter S phase 12 h after the addition of plasma; thereafter, cells enter S in an apparent first-order fashion. The first-order rate of entry into S is controlled by the concentration of plasma; however, the 12 h lag time is independent of the plasma concentration (Fig. 3). The 12 h lag time is also unaffected by the time of plasma addition. Thus, pretreating the 3T3 cells with plasma

TABLE I. Identification of Progression Factors and Competence Factors*

Factor or treatment	Progression activity		Competence activity	
Somatomedin A (partially pure)	Potent:	1 unit = 20 ng	Weak:	some activity at 330 ng
Somatomedin C	Potent:	1 unit = 2 ng	None:	tested to 200 ng
ILA (partially pure)	Potent:	1 unit = 100 ng	Not tested	
MSA	Potent:	1 unit = 2 ng	None:	tested to 200 ng
BRLC medium	Potent:	1 unit = 6 μl	Weak:	some activity at 50 μl
Insulin	Weak:	1 unit = 160 ng	None:	tested to 120 ng
Hydrocortisone	Weak:	1 unit = 724 ng (10^{-5}M)	None:	tested to 724 ng (10^{-5}M)
Growth hormone (50% pure)	None:	was tested to 200 ng	None:	tested to 200 ng
T-3	None:	tested to 132 ng (10^{-6}M)	Not tested	
NGF	None:	tested to 200 ng	None:	tested to 200 ng
EGF	Partial:	see text	None:	tested to 200 ng
FGF	None:	tested to 200ng	Potent:	2 ng is active
PDGF (partially pure)	None:	tested to 50 μg	Potent:	5 ng is active
$Ca_3(PO_4)_2$	None:	tested to 18 mM	Potent:	3 mM is active
Wounding	None		Potent	
Normal human serum	Potent:	1 unit = 2 μl	Potent:	5% serum is active
Normal human platelet poor plasma	Potent:	1 unit = 2 μl	None:	tested to 100%
SV40	Potent:	1 pfu/cell is active	Potent:	1 pfu/cell is active

*Growth factors were tested for progression factor activity according to the protocol outlined by Stiles et al [13]; a unit of progression factor is defined as the quantity of material that must be added to 3% hypophysectomized-rat plasma to give progression activity comparable to that of 3% normal rat plasma. For competence testing, quiescent density-arrested microtiter cultures of Balb/c-3T3 were exposed for 3 h to various quantitites of growth factor; control cultures were exposed for 3 h to 5% normal human plasma. The culture medium was then aspirated. Cell monolayers were washed once with saline containing 28 mM β-mercaptoethanol and once with saline only. The treated cultures were then incubated for 36 h with DME containing 5% normal human plasma and ^{3}H-dThd (5 μCi/ml). After 36 h, the cells were fixed and processed for autoradiography. Under these conditons, less than 5% of cells from control cultures were stimulated to synthesize DNA; those factors with competence activity stimulated 60%–95% of the cells to become labeled. Final concentrations of all growth factors can be derived by multiplying the quantities indicated × $5ml^{-1}$.

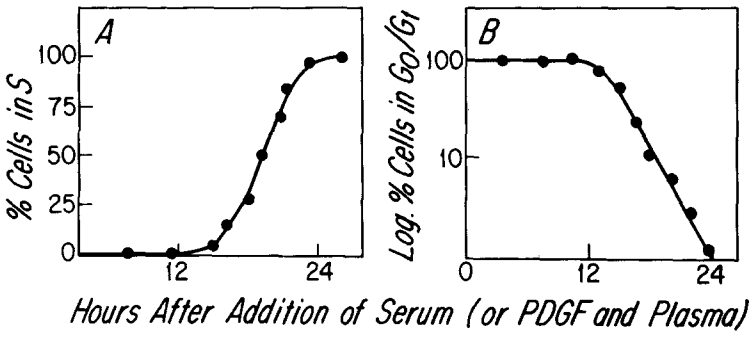

Fig. 1. Schematic illustration of the rate at which density-arrested cultures of Balb/c-3T3 cells enter S phase following the addition of fresh culture medium containing serum (or PDGF and plasma). A) The data are plotted as percent cells in S vs time. B) Same data replotted on a semilogarithmic scale after the fashion of Smith and Martin [14].

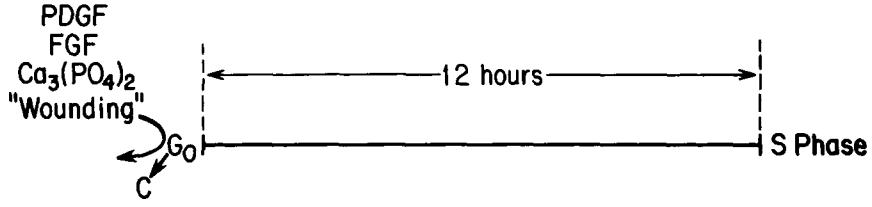

Fig. 2. Schematic illustration of the effects of "competence factors" (PDGF, FGF, $Ca_3(PO_4)_2$, and "wounding") on density-arrested Balb/c-3T3 cells. Transient exposure to any of these agents renders density-arrested Balb/c-3T3 cells in the G_0 phase of the cell cycle competent (C) to replicate their DNA. However, competent cells do not enter S phase until at least 12 h following the addition of platelet-poor plasma to the culture medium. Unlike competence factors that are required only transiently, plasma is required continually for entry into S phase to occur.

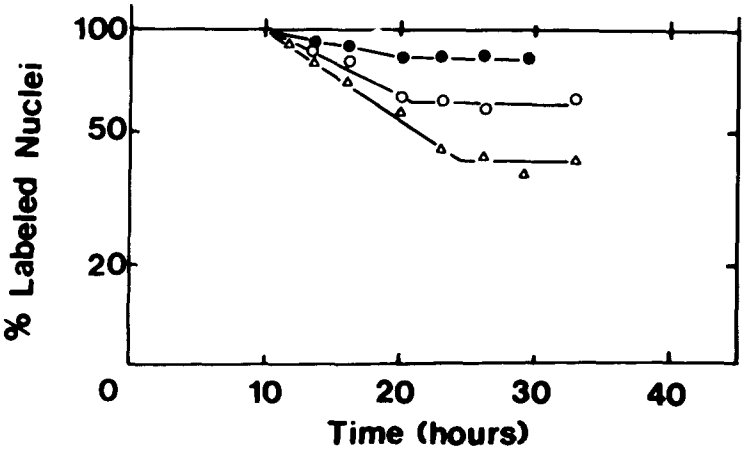

Fig. 3. Effect of concentration of platelet-poor plasma on the rate of cell entry into S phase. Density-arrested Balb/c-3T3 cells were treated with PDGF at 37°C in culture medium supplemented with ^{3}H-thymidine and platelet-poor plasma (●, 0.25%; ○, 2.5%; △, 5%). Cultures were fixed at the times indicated and processed for autoradiography. Adapted from Pledger et al [11].

does not shorten the lag time to S phase. Delaying the addition of plasma to PDGF-treated competent cells delays the onset of DNA synthesis by a corresponding amount of time, so that the minium lag after plasma addition always remains a constant 12 h (Fig. 4). In the presence of a constant concentration of plasma, eg, 5%, the rate that cells enter the S phase and the total number of cells that synthesize DNA is governed by the concentration of PDGF [Pledger et al; in preparation]. In the presence of cycloheximide, progression is totally blocked; cells remain competent, but growth is arrested 12 h prior to S [16].

SOMATOMEDINS ARE REQUIRED FOR PROGRESSION

PDGF-treated, competent Balb/c-3T3 cells were placed in medium containing plasma from surgically hypophysectomized animal donors. Relative to plasma from control animals,

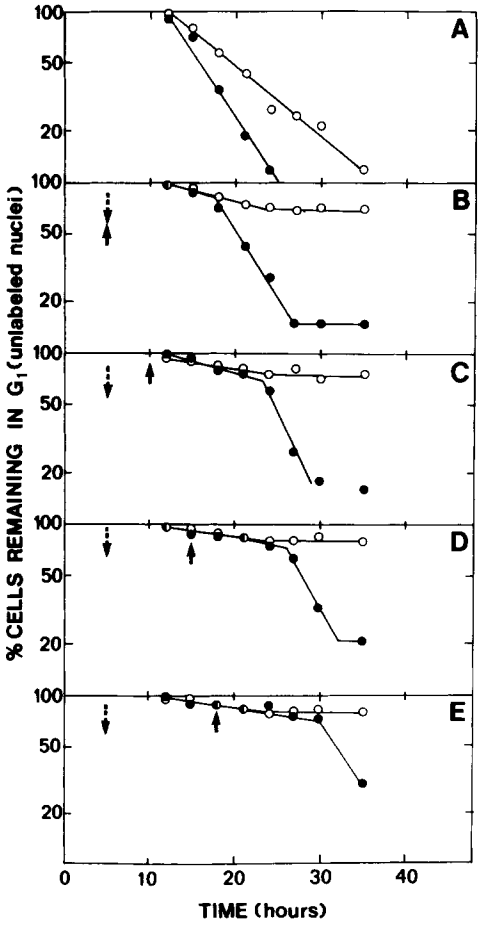

Fig. 4. The stability of the PDGF-induced competent state. A) Cultures were treated with PDGF at 37°C in medium containing 5% platelet-poor plasma and ^{3}H-thymidine. At the indicated times, cultures were fixed and processed for autoradiography. B–E) Cultures were treated with PDGF for 5 h (↓) at 37°C, washed, and returned to medium containing radioactive thymidine but lacking plasma. At the times indicated by (↑), the medium was suplemented with platelet-poor plasma (●, 5%; ○, 0.25%). The cultures were fixed and processed for autoradiography at time intervals. Adapted from Pledger et al [11].

the hypophysectomized animal plasma was deficient in progression activity; only a small fraction of the PDGF-treated cells entered S phase in the medium containing hypophysectomized animal plasma (Fig. 5a). The addition of ng per ml quantities of pure somatomedin C to the hypophysectomized animal plasma totally restored the "progression activity" (Fig. 5b). In a temporal analysis of the cellular response to somatomedin C, density-arrested Balb/c-3T3 cells were exposed briefly to PDGF and then transferred to medium containing plasma from hypophysectomized animals (Fig. 5c) [17]. As noted previously (Fig. 5a), only a small fraction of PDGF-treated cells entered S phase when incubated subsequently in the hypophysectomized plasma medium; however, when pure somatomedin C was added to the PDGF-treated culture that had been incubated in hypophysectomized plasma for 21 h, the cells resumed entry into S phase. The lag time between the addition of somatomedin C and the cellular response was not 12 h; rather, a small but signficiant in-

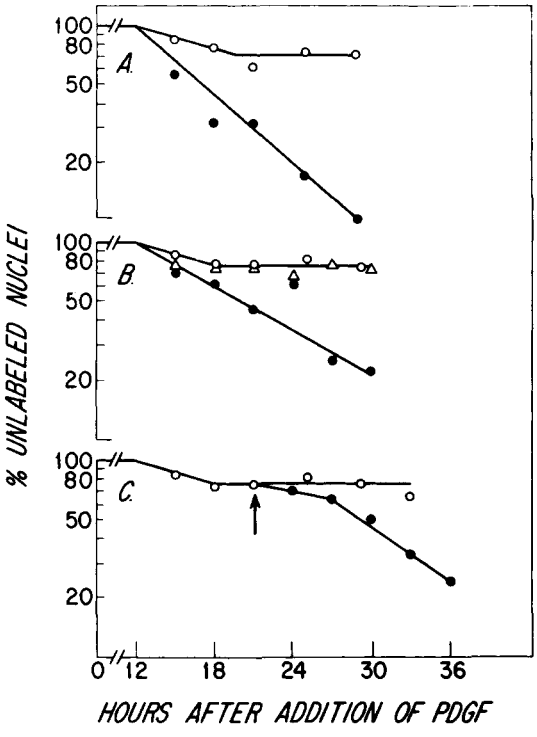

Fig. 5. The addition of pure somatomedin C to plasma from hypophysectomized animals restores "progression" activity. Quiescent density-arrested cultures of Balb/c-3T3 cells were exposed to PDGF for 3 h. The PDGF was removed, and the cells were washed and returned to medium containing ^{3}H-thymidine. A) The medium was supplemented with 3% plasma from either normal (●) or hypophysectomized (○) rats. B) Culture medium was supplemented with 3% plasma from hypophysectomized animals (○), 3% plasma from hypophysectomized animals plus 3 ng/ml pure somatomedin C (●), or 3 ng/ml pure somatomedin C only (△). C) Culture medium was supplemented with 3% plasma from hypophysectomized rats. At the time indicated (↑), somatomedin C (30 ng/ml) was added to some cultures (●), while others (○) received an equivalent volume of saline. At periodic intervals, the cells were fixed and processed for autoradiography. Adapted from Stiles et al [17].

crease in the rate of cellular entry into S phase was noted immediately after the addition of somatomedin C. Then, at 6 h, a major increase in the rate of entry into S phase was noted (Fig. 5c). We interpreted this result in the following way: The "early progress" of PDGF-treated competent cells towards S phase is mediated by an agent contained in platelet-poor plasma. The agent which allows early progress to occur is not under pituitary control since it is unaffected by surgical hypophysectomy. "Late progress" of PDGF-treated competent cells is regulated by somatomedins that, in turn, are regulated by pituitary growth hormone. In medium containing plasma from hypophysectomized animals, most PDGF-treated Balb/c-3T3 cells become growth arrested at a point "V" located 6 h prior to the G_1/S phase boundary; a few of the cells traverse "V" to become growth-arrested at a second point "W" located immediately prior to the G_1/S boundary [12]. The addition of somatomedin to PDGF-treated cells that have been cultured with plasma from a hypophysectomized donor thus causes a biphasic increase in the rate of cellular entry into S: A small fraction of the cell population responds immediately, and the majority responds within 6 h. This interpretation is summarized schematically in Figure 6.

We noted that pure somatomedin C and pure multiplication stimulating activity (MSA) were equipotent in promoting late progress. Impure preparations of somatomedin A and (at high concentration) insulin were also active. Hydrocortisone was also active but only at pharmacologic concentrations. These hormones with progression activity had no competence activity. Conversely, the agents with competence activity (see above) had no progression activity. These results are summarized in Table I.

It should be noted that somatomedins alone will not support the efficient progression of PDGF-treated cells from quiescence into S phase; as indicated in Fig. 5B, another set of components contained in the plasma from hypophysectomized rats is also required. Preliminary studies from our laboratories indicate that the plasma from these rats can be replaced by epidermal growth factor (EGF).

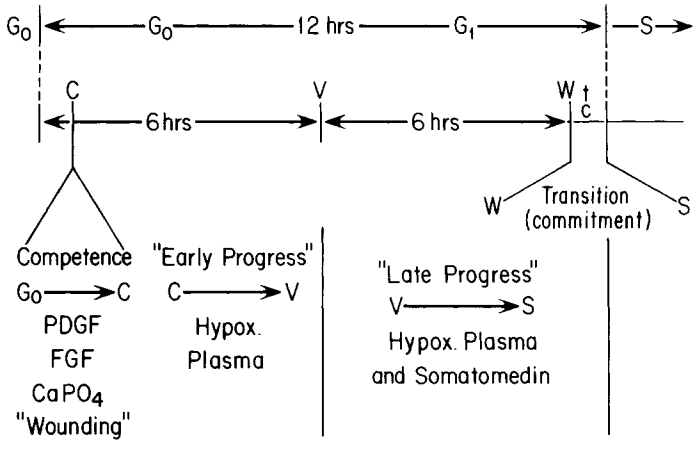

Fig. 6. Hormonal control of early events in the mitogenic response of Balb/c-3T3 cells to serum growth factors. Adapted from Stiles et al [17].

SOMATOMEDIN REGULATORY EVENT IN G_0/G_1 IS RELATED TO NUTRIENTS

In a metabolic analysis of competence and progression, we exposed 3T3 cells to PDGF and then incubated the cells in medium that contained an optimal concentration of plasma but was deficient in essential amino acids [18]. Under these conditions, very few cells entered the S phase. When the missing amino acids were restored to the culture medium, the rate of entry into S increased in a biphasic fashion idential to that noted in our somatomedin experiment. A few of the cell responded immediately to amino acid addition, and after a 6 h lag, a major increase in the rate of cellular entry into S phase was noted. This observation suggests that previous studies on growth-arrest mediated by nutrient starvation were relevant to the phenomena of late progress. In our experiments, amino acid deficiency did not affect the induction of competence by PDGF.

PREPARATION FOR A SECOND ROUND OF CELL DIVISION: PDGF CAN STIMULATE CELLS IN S PHASE

The addition of fresh serum (or of PDGF and plasma) to density-arrested populations of Balb/c-3T3 cells causes them to leave the G_0 state of the cell cycle and replicate their DNA following a minimum lage time of 12 h. Medium that contains serum (or PDGF and plasma) will also sustain the exponential growth of subconfluent cultures of Balb/c-3T3 cells; in exponentially growing 3T3 cell populations, however, the mean lag time between mitosis and the onset of DNA synthesis is 5–6 h. Evidently, serum contains a factor that can prevent cells from becoming growth-arrested when conditions are suitable for sustained exponential growth. We have recently shown that this factor is, in fact, PDGF [19]. In experiments with synchronized cell cultures, we demonstrated that when cells were treated briefly with PDGF during the S, G_2, or very early postmitotic fraction of the cell cycle, a subsequent round of DNA synthesis was initiated following a minimum lag of only a few hours rather than 12 h. Thus, PDGF is a hormone with a dual function. PDGF can stimulate quiescent 3T3 cells to enter the proliferative phase of the cell cycle; PDGF can also stimulate 3T3 cells in the S, G_2, or early postmitotic phase to prevent them from becoming quiescent.

BIOCHEMICAL AND ULTRASTRUCTURAL CORRELATES OF COMPETENCE AND PROGRESSION

Treatment of quiescent Balb/c-3T3 cells with pure PDGF induced the preferential synthesis of five cytoplasmic proteins (approximate molecular weight 29,000, 35,000, 45,000, 60,000, and 72,000 daltons detected by SDS-PAGE under reducing conditions) [Pledger et al, in preparation]. Two of these PDGF-induced proteins (29,000 and 35,000 daltons are found within 40 min of PDGF addition; they are not induced by plasma, insulin, or EGF. The PDGF-induced proteins appear to be associated in some way with the cellular state of competence. Fibroblast growth factor from bovine pituitary, which mimics the action of PDGF in the induction of competence, triggers preferential synthesis of the same group of cytoplasmic proteins; by contrast, plasma, insulin, or epidermal growth factor do not affect these cytoplasmic proteins and do not induce competence.

An interesting ultrastructural correlate of competence also exists. As proliferating cells replicate their DNA and proceed through mitosis, the centriole must also duplicate so that chromosomes can be distributed to the daughter cells. Tucker et al [20] demonstrated that density-arrested Balb/c-3T3 cells contain one centriole pair which forms a primary

cilium; upon serum stimulation, such cells undergo an early (1–2 h) transient deciliation followed by another deciliation associated with centriole duplication and DNA synthesis. The initial centriole deciliation, observed very rapidly after serum stimulation, is controlled by the PDGF component of serum [21]. In dose-response studies, only doses of PDGF that produced centriole deciliation were capable of inducing competence for DNA synthesis. Plasma alone or somatomedins produced neither centriole deciliation nor competence; however, these agents were required for the optimum progression of competent cells into DNA synthesis and were required for the second centriole deciliation, which was associated with DNA synthesis.

ROLE OF SV40 IN CONTROL OF COMPETENCE AND PROGRESSION

Our cell cycle analysis demonstrated that the mitogenic response of Balb/c-3T3 cells can be resolved into two stages, competence and progression, under the control of separate growth factors. These studies provided a useful new context in which to study the role of tumor viruses and chemical tumor promoters in control of cell growth. SV40 was analyzed in this context. In contrast to growth factors, which provided either compentence or progression activity (but never both), SV40 infection provided both competence and progression activity [13]. As shown in Figure 7, induction of host cell DNA synthesis in SV40-infected Balb/c-3T3 cells was strictly a function of virus multiplicty of infection (MOI); neither PDGF nor plasma enhanced the mitogenic response to SV40.

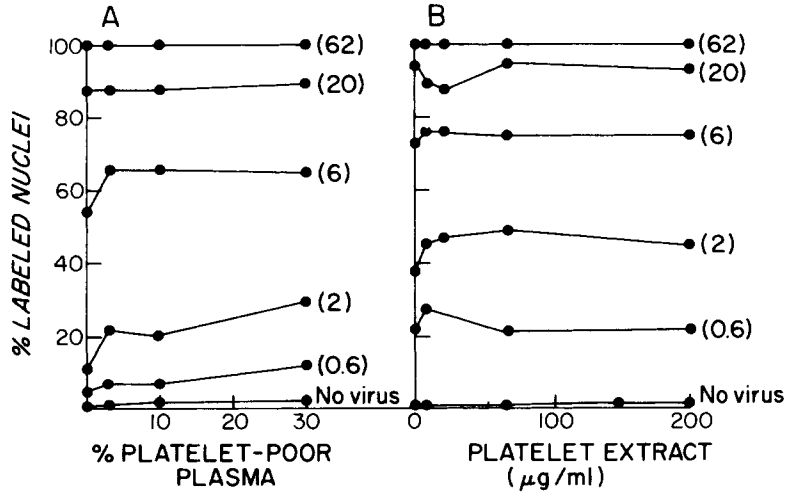

Fig. 7. SV40 induced both competence and progression. A) Gradient-purified SV40 was added to density-arrested Balb/c-3T3 cells at the MOI indicated in parenthesis. After 3 h, the medium was removed, and the cells were washed. Fresh medium containing ^{3}H-thymidine and normal human plasma as indicated (●) was added to the cultures. B) Density-arrested cultures of Balb/c-3T3 cells were treated for 3 h with PDGF as indicated (●). After 3 h, the medium was removed and the cells were washed. Fresh medium containing ^{3}H-thymidine and 0.25% normal human plasma was added together with SV40 at the MOI in parenthesis. After 36 h, all cultures were fixed and processed for autoradiography. Adapted from Stiles et al [13].

The mitogenic response to SV40 differed from the mitogenic response to growth factors in another very signficant way. A transient deciliation of the centriole is associated with the competence stage of the mitogenic response; this transient centriole deciliation was never observed when host cell DNA synthesis was induced by SV40 infection, although the time course of the mitogenic response to serum factors and SV40 was otherwise identical [21].

Our experiments with SV40 suggested that this small DNA tumor virus contained sufficient genetic information to regulate both the competence and progression phases of the mitogenic response. This observation was of special interest since the gene A region of SV40 codes for two transformation specific proteins: The small "t" and large "T" antigens (see [22] for review).

We have begun to use gene A variants of SV40 to explore the separate roles of the "t" and "T"-antigens in control of the mitogenic response. Quiescent Balb/c-3T3 cells were infected with SV40 strains containing a temperature sensitive (ts) T-antigen (ts A209), a deletion in t-antigen (dl 884), and several ts A209 strains containing t-antigen deletions. Surprisingly, all strains of SV40 induced DNA synthesis equally well under permissive or nonpermissive conditions. Treatment with ultraviolet light inactivated the virus preparations ability to induce DNA synthesis. The synthesis of T-antigen by these cells has not been studied. Addition of PDGF or plasma had little effect on DNA synthesis induced by any of the viral strains. Thus, the SV40 viral function(s) that induces replicative DNA synthesis in quiescent Balb/c-3T3 cells does not appear to be little t-antigen and appears to be unaffected by ts lesions in the large T-antigen.

ROLE OF TUMOR PROMOTER IN CONTROL OF HOST CELL DNA SYNTHESIS

The active ingredient of croton oil, 12-0-tetradecanoyl-phorbal-13-acetate (TPA) has been shown to stimulate the proliferation of Balb/c-3T3 cells in culture. TPA is also a widely used model compound in studies on tumor promotion; for this reason, we analyzed the mitogenic action of TPA in the context of competence and progression [23]. Using Balb/c-3T3 cells, we found that TPA functions in a fashion that differs from that of growth factors and also differs from SV40. Growth factors modulate either the competence or the progression events but never both. SV40 provides both competence and progression activity. TPA functions as a competence factor since it enhances the growth stimulatory activity of progression factors. TPA also functions as a progression factor since it enhances the growth stimulatory activity of competence factors. TPA alone, however, does not stimulate cell replication thus distinguishing this compound from SV40.

CONCLUSIONS

Regulation of Cell Cycle Events by Serum Growth Factors

Clotted platelet-rich serum stimulates the growth of normal connective tissue cells. Serum contains PDGF, a polypeptide factor not found in unclotted blood but carried in the α-granules of platelets. Thus, in vivo, PDGF is in a cryptic state and is not available to stimulate the replication of cells.

PDGF initiates the replication of density-arrested Balb/c-3T3 cells by making the cells competent to respond to other growth factors contained in platelet-poor plasma. Several other agents mimic the action of PDGF in inducing competence; these include FGF from bovine pituitary and microprecipitates of calcium-phosphate.

Platelet-poor plasma contains several components required for the growth of PDGF-stimulated Balb/c-3T3 cells; these components include, but are not restricted to, somatomedins; EGF may also play a role. The somatomedins are a family of insulin-like polypeptide hormones whose concentration in blood is controlled to a large extent by a pituitary growth hormone (see [24] for review).

Temporal analysis of the mitogenic response of Balb/c-3T3 cells to serum growth factors indicates that PDGF and somatomedins regulate separate and sequential events in the cell cycle. These data provide a useful context in which to study the molecular action of serum growth factors. Moreover, by examining the cellular growth response to tumor viruses and chemical tumor promoters in the context of competence and progression, it may be possible to establish functional analogies between these agents and specific polypeptide growth factors found in serum.

REFERENCES

1. Balk, SD: Natl Acad Sci USA 68:271, 1971.
2. Ross R, Glomset B, Kariya B, Harker L: Proc Natl Acad Sci USA 71:1207, 1974.
3. Kohler N, Lipton A: Exp Cell Res 87:297, 1974.
4. Scher CD, Shepard RC, Antoniades HN, Stiles CD: Biochim Biophys Acta 560:217, 1979
5. Kaplan DR, Chao FC, Stiles CD, Antoniades HN, Scher CD: Blood 53:1043, 1979.
6. Kaplan KK, Broekman MJ, Chernoff A, Witte L, Linder B: (Abstract). Clin Res 26:349, 1978.
7. Heldin CH, Wasteson A, Westermarke B: Exp Cell Res 109:429, 1977.
8. Scher CD, Pledger WJ, Martin P, Antoniades HN: J Cell Physiol 97:371, 1978.
9. Antoniades HN, Scher CD, Stiles CD: Proc Natl Acad Sci USA 76:1809, 1979.
10. Heldin C, Westmark B, Watson A: Proc Natl Acad Sci USA 76:3722, 1979.
11. Pledger WJ, Stiles CD, Antoniades H, Scher CD: Proc Natl Acad Sci USA 74:4481, 1977.
12. Pledger WJ, Stiles CD, Antoniades H, Scher CD: Proc Natl Acad Sci USA 75:2839, 1978.
13. Stiles CD, Capone GT, Scher CD, Antoniades HN, Van Wyk JJ, Pledger WJ: Proc Natl Acad Sci USA 76:1279, 1978.
14. Smith JA, Martin L: Proc Natl Acad Sci USA 70:1263, 1973.
15. Gospodarowicz D: J Biol Chem 250:2515, 1975.
16. Frantz CH, Stiles CD, Pledger WJ, Scher CD: J Cell Physiol 105:439, 1980.
17. Stiles CD, Pledger WJ, Van Wyk JJ, Antoniades HN, Scher CD: In Sato GH and Ross R (eds): "Hormones and Cell Culture." Cold Spring Harbor Conferences on Cell Proliferation 6:425, 1979.
18. Stiles CD, Isberg R, Pledger WJ, Antoniades HN, Scher CD: J Cell Physiol 99:395, 1979.
19. Scher CD, Stone M, Stiles CD: Nature 281:390, 1979.
20. Tucker RW, Pardee AB, Fujiwara K: Cell 17:527, 1979.
21. Tucker RW, Scher CD, Stiles CD: Cell 18:1065, 1979.
22. Bouck N, Beales N, Shenk T, Berg P, DiMayorca G: Proc Natl Acad Sci USA 75:2743, 19.
23. Frantz CN, Stiles CD, Scher CD: J Cell Physiol 100:413, 1979.
24. Van Wyk JJ, Underwood LE: In Litwach G (ed):"Biochemical Actions of Hormones." 5:101, 1978.