cording to Eq. 1, which gives the path length of a layer of 10^5 K plasma with thickness $\Delta\ell$ that produces the observed line intensity. The path-length distribution ranges from 0.1 to 10 km. These lengths are much smaller than the observed 2400-km extent of the structures along the slit.

The spicules and fibrils at transition zone temperatures are composed of structures that are well below the instrumental resolution. It is worthwhile to construct simple geometrical models that incorporate subresolution structures so that we may interpret the observed intensities and dimensions. Two models seem most readily applicable. In one, the transition zone consists of a thin layer that is wrapped around the chromospheric spicule. In the other, the transition zone is composed of a number of subresolution filamentary structures that are distributed throughout the spicular volume. The thickness of the transition zone in the first ("onionskin") model would be roughly half the value of the path length $\Delta\ell$ derived above. However, the 10^5 K transition zone emission is not observed in a thin skin wrapped around 10^4 chromospheric spicules but rather in higher altitude extensions of the spicules (*7*). The filamentary model constructs the spicular transition zone from a loose assembly of fine filaments. If we assume each filament has the same density and cross-sectional area, then we find that the radius of each individual filament ranges between 3 and 30 km for spicules consisting of ten filaments. The derived radii scale inversely with the square root of the number of filaments assumed to fill the spicular volume. A single filament cannot explain the observed width of spicules, so it is possible to set 70 km (0.1 arc sec), the width derived assuming there is a single filament, as an upper limit to the size of these subresolution filaments. It is not possible to determine a lower limit to their sizes.

The small area fill factor of the transition zone has immediate consequences for derived fluxes of material and energy through the transition zone. For example, it is estimated that coronal energy losses in the quiet sun are dominated by the conductive losses through the transition zone (*8*). This conclusion is based on the assumption of a plane-parallel solar atmosphere. When the areal fill factor of spicular structures (10^{-5} to 10^{-2}, calculated for the filamentary models but approximately the same for the onionskin model) is taken into account, the derived conductive losses will be reduced by this same factor. Furthermore, the observed temperature gradients along magnetic field lines in the quiet sun are on the order of two magnitudes lower than those derived under the plane-parallel assumption. Thus, the conductive flux from the corona through observed transition zone structures is negligible compared to coronal radiative losses.

The microscopic transition zone structures must be intimately related to the local heating mechanism, which might operate on microscopic scales but would also explain fundamental macroscopic characteristics of the transition zone. The heating is local because the shallow temperature gradients in the transition zone do not support a significant conduction of energy from the corona. Recently, it has been shown that coronal loops can be heated by the resonant absorption of Alfvén waves and that this dissipation occurs on spatial scales of 0.3 to 250 km, transverse to the magnetic field direction (*9, 10*).

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A Cell-Cycle Constraint on the Regulation of Gene Expression by Platelet-Derived Growth Factor

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In density-arrested monolayer cultures of Balb/c 3T3 cells, platelet-derived growth factor (PDGF) stimulates expression of the c-*myc* and c-*fos* proto-oncogenes, as well as the functionally uncharacterized genes, JE, KC, and JB. These genes are not coordinately regulated. Under ordinary conditions, c-*fos*, JE, KC, and JB respond to PDGF only when the cells are in a state of G_0 growth arrest at the time of PDGF addition. The c-*myc* gene is regulated in opposition to the other genes, responding best to PDGF in cycling cultures.

PLATELET-DERIVED GROWTH FACTOR (PDGF) exerts its mitogenic effect on mouse fibroblasts, in part, by inducing the expression of otherwise silent genes (*1–3*). The proto-oncogenes c-*myc* and c-*fos* are among these genes, and appear to function as intracellular mediators of the growth response to PDGF (*4, 5*). PDGF also induces the expression of several other genes, designated JE, KC, and JB, which are functionally uncharacterized (*1*). As a first step toward understanding the function of these genes, we have undertaken experiments to analyze the controls governing their PDGF-induced expression. If genes like JE, KC, and JB have functions analogous to those of c-*myc* and c-*fos*, we would expect that their expression might be regulated in similar ways.

Contrary to expectations, noncoordinate control of PDGF-inducible gene expression was displayed during the course of a cell growth experiment (Fig. 1). Balb/c 3T3 cells were plated at very low density and then grown to confluence in serum-supplemented medium (*6*). Steady-state levels of c-*myc* messenger RNA (mRNA) were highest when cells were sparse and decreased steadily as the cells approached confluence and growth arrest. In contrast, JE mRNA was undetectable in sparse cultures and rose to high levels only as the cultures approached the confluent monolayer state. The c-*myc* gene still showed some expression at high cell density because of continuous exposure to serum.

Serum contains several factors that regulate 3T3 cell growth. To determine whether this cell-density effect on gene expression reflected a differential response of the c-*myc* and JE genes to PDGF, we plated Balb/c 3T3 cells sparsely (about 1/40 of confluent density) and incubated them in PDGF-free medium supplemented with 5% platelet-poor plasma (PPP) (*7*). The steady-state level of c-*myc* mRNA in these sparse, PDGF-starved cell cultures was low (Fig. 2A). Treatment with PDGF led to a sizable

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induction of c-*myc*, but did not induce JE expression. When cells were grown to confluence and similarly deprived of PDGF for 24 hours, treatment with PDGF led to expression of JE as well as c-*myc*, although the level of c-*myc* expression was lower than it was in the sparse cells. Averaged over ten closely agreeing experiments, PDGF induced 14 times as much JE mRNA and approximately one-half (0.55) as much c-*myc* mRNA in confluent cells as in sparse cells. However, because of the high basal level of c-*myc* expression in sparse cells, the magnitude of c-*myc* induction (the amount of c-*myc* in PDGF-treated relative to that in untreated cells) was the same in sparse and confluent cells. The regulation of c-*fos*, JB, and KC genes is similar to that of JE, that is, they were induced by PDGF preferentially in confluent cultures [Fig. 2A and (*8*)].

To establish the level at which JE gene expression is blocked in sparse cell cultures, we performed run-on transcriptional analysis on nuclei from sparse or confluent cells, before and after exposure to PDGF (*9*). PDGF treatment of confluent cells stimulated transcription of both JE and c-*myc* (Fig. 2B). In sparse cell cultures, the JE gene was not transcribed and PDGF did not appreciably stimulate transcription. The c-*myc* gene appeared to be constitutively transcribed in sparse cell cultures. PDGF did not enhance the constitutive transcription of c-*myc* although PDGF clearly enhanced the level of c-*myc* mRNA detected by Northern blotting (Fig. 2A). We conclude, first, that the response of JE to PDGF is channeled through a "second message," which can only be generated or is only functional at high cell density; second, that the PDGF-mediated induction of c-*myc* in confluent cell cultures reflects enhanced transcription of the gene; and third, that the PDGF-mediated induction of c-*myc* in sparse cells is achieved largely by posttranscriptional means.

Although PDGF cannot induce JE expression in sparse cells, other agents can do so. We have previously shown that the synthetic double-stranded RNA poly(rI):poly(rC) can induce JE in confluent cells (*10*). Figure 2C shows that the JE gene responds to poly(rI):poly(rC) equally well in sparse and confluent cultures, as does c-*myc*.

The ability of PDGF to induce JE in confluent cells does not depend on cell-to-cell contact. Such contact was prevented by placing 3T3 cells in methylcellulose suspension culture (*11*). PDGF can induce both c-*myc* and JE under these circumstances (Fig. 2D). Parenthetically, this suggests that the anchorage requirement for 3T3 cell growth is not the result of disruption of the PDGF receptor-signal transduction system in suspended 3T3 cells.

What accounts for the differential response of the JE gene to PDGF in sparse and confluent cell cultures? The experiment shown in Fig. 3 suggests that the JE gene responds to PDGF only when cells are in the G_0/G_1 phase of the cell cycle. Flow microfluorometry (FMF) revealed that treating 3T3 cells with trypsin and diluting them 1:40 into sparse cell culture is sufficient to trigger one to two rounds of cell division, even if cells are kept in PDGF-free medium for 24 hours (the conditions we used in Fig. 2, A to C). A large fraction of 3T3 cells treated in this way displays a DNA content typical of the S or G_2 phase (Fig. 3). It is not until sparse cells have been deprived of PDGF for 48 to 72 hours that the FMF profile shifts to a G_0/G_1 DNA content. As the percentage of cells in G_0/G_1 increases, the ability of PDGF to induce JE expression increases proportionately (Fig. 3). (In the experiment shown in Fig. 3, cell number was adjusted so that the number of cells per dish after 72 hours in PPP was identical to the number of cells per dish after 24 hours in PPP in the experiments of Figs. 1 to 3.)

In summary, the JE gene, together with c-*fos*, KC, and JB, is regulated in opposition to c-*myc*. The latter gene responds to PDGF preferentially in sparse cell culture as suggested by others (*12*). The former genes respond to PDGF almost exclusively in

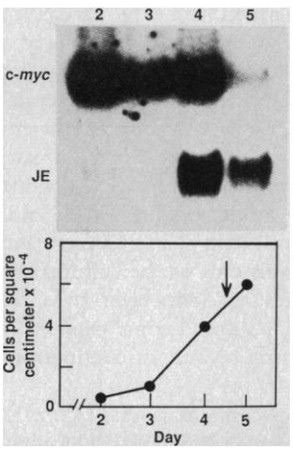

Fig. 1. Effect of cell density on steady-state levels of c-*myc* and JE mRNA. (**Top**) Cells (*6*) were plated at 250,000 cells per 150-cm² dish in DMEM with 10% BCS, and medium was replenished with fresh serum on days 2 and 4 after plating. RNA was collected daily (8 hours after refeeding on days 2 and 4) and analyzed by Northern blot (*3, 19*). The same nitrocellulose filter was used to probe for both c-*myc* and JE. (**Bottom**) Cell number per dish was determined for each day. The point at which confluence was reached is indicated by an arrow.

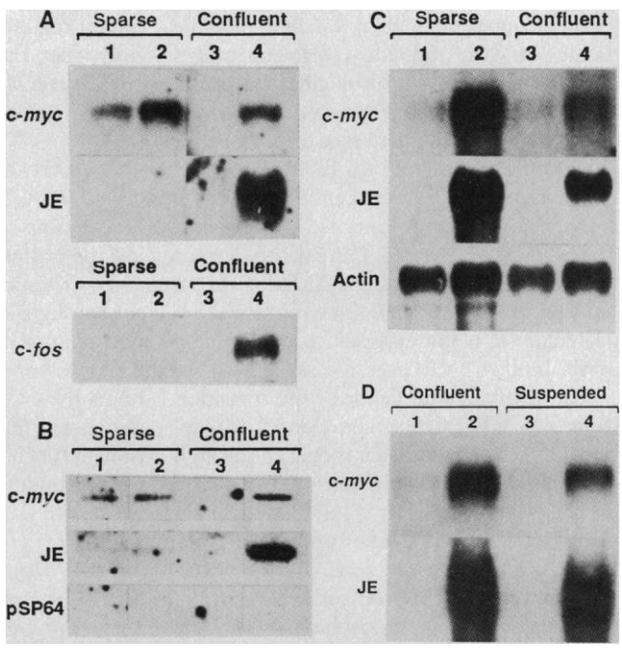

Fig. 2. Effect of cell density on PDGF-inducible gene expression. For sparse cultures, cells were plated at a density of 250,000 cells per 150-cm² plate in DMEM with 10% BCS for 12 hours. Medium was then changed to 5% PPP for 24 hours. For confluent cultures, cells were grown to confluence and density arrest in DMEM with 10% BCS, then medium was changed to 5% PPP for 24 hours. (**A**) (Top) One-half of the sparse and confluent plates were treated with 300 U/ml PDGF for 2 hours (*7*) (lanes 2 and 4) followed by RNA extraction. There was no addition to lanes 1 and 3. Levels of c-*myc* and JE mRNA were determined by Northern blot analysis (*9*). The same blot was stripped by boiling and reprobed for each mRNA. (Bottom) Cells were treated as above except that the PDGF treatment (lanes 2 and 4) was for 30 minutes. (**B**) Cells were treated as in (A), and nuclei were collected for run-on transcriptional analysis (*3, 19*). Lanes 1 and 3, no addition; lanes 2 and 4, PDGF treatment for 2 hours. We confirmed that equal amounts of radioactivity were added to each sample by hybridization to 100 ng of Balb/c liver DNA (not shown). (**C**) Cells were treated as in (A), except that they were treated for 2 hours (lanes 2 and 4) with 50 μg/ml poly(rI):poly(rC) instead of PDGF. Lanes 1 and 3, no addition. (**D**) Cells were suspended in 1.5% methylcellulose supplemented with DMEM and 5% PPP (*11*). After 72 hours, cells were harvested by centrifugation and resuspended in 1.5% methylcellulose with DMEM and 5% PPP alone (lanes 1 and 3) or with PDGF (300 U/ml) for 2 hours (lanes 2 and 4). Cells were then collected and RNA extracted for Northern analysis. A Northern blot with RNA from confluent, attached cells is shown for comparison.

dense cell cultures. The differential responsiveness is not due to cell-to-cell contact. Rather, PDGF can only induce JE, c-*fos*, KC, and JB if cells are in the G_0/G_1 phase of the cell cycle. There is no such constraint on c-*myc* expression (*13*). Another explanation for this noncoordinate gene induction by PDGF would be that 3T3 cells secrete a factor that functions in an autocrine way to facilitate the response of JE-like genes to PDGF. Dense cell cultures would quickly accumulate a sufficient concentration of this factor, whereas sparse cultures would require several days to do so. This model could reconcile some minor differences between the conclusions reached here and data presented by Bravo *et al.* (*14*).

The constraint on responsiveness of genes such as JE to PDGF may shed some light on the functions of these genes. The expression of c-*fos*, JE, KC, and JB is restricted to cells in G_0, a physiological condition approximating the "differentiated" state of fibroblasts in vivo. Recent studies on c-*fos* suggest that expression of this nuclear protein is important in the first round of cell division, in which cells exit G_0, but not during subsequent rounds of division which involve transit from mitosis directly into G_1 (*4*). As for the other genes, recent sequence and expression data suggest that JE may be a secretory glycoprotein (*15, 16*). Furthermore, unlike c-*myc* and c-*fos*, the expression of JE is lineage-restricted. Fibroblasts, endothelial cells, monocytes, and macrophages express JE after the appropriate stimulus (*17*), but activated T lymphocytes do not (*18*). Thus some of the PDGF-inducible genes appear to encode the secretory products of differentiated cells. Such proteins may have little to do with mediating the growth response of fibroblasts to PDGF, and more to do with influencing the behavior of other cells in the vicinity of these fibroblasts.

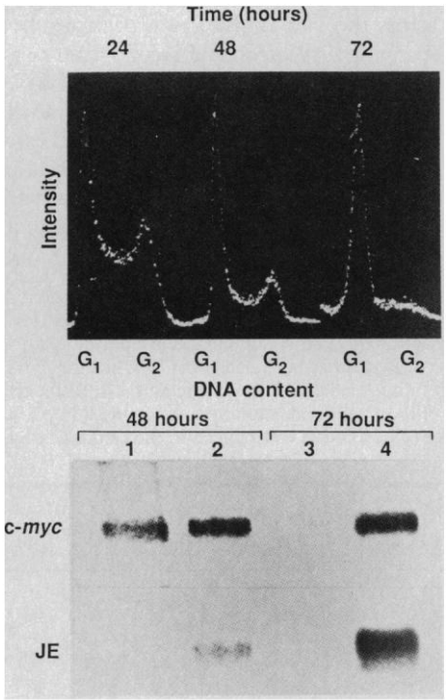

Fig. 3. Effect of cell cycle on PDGF-inducible gene expression. Cells were plated at a density of 150,000 cells per 150-cm^2 dish in DMEM with 10% BCS for 12 hours. Medium was then changed to 2.5% PPP, and cells were collected at 24, 48, and 72 hours for counting and fluorescence microfluorometric (FMF) analysis. RNA was collected at 48 and 72 hours for Northern blot analysis. (Top) FMF analysis; (bottom) Northern blot analysis; the same blot was stripped and used for both probes. Lanes 1 and 3, no addition; lanes 2 and 4, PDGF treated. Cell number at 72 hours was 410,000 cells per 150-cm^2 dish, the same as the cell number at 24 hours in 5% PPP in the sparse cultures of Figs. 1 and 2.

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9. After the appropriate treatment, cells (four 150-cm^2 plates of confluent cells or sixty 150-cm^2 plates of sparse cells per treatment) were scraped into cold phosphate-buffered saline. Nuclei were isolated by NP-40 lysis and centrifugation and stored at −70°C in a final volume of 200 μl in glycerol buffer (*3*). To radiolabel newly transcribed RNA, we thawed the nuclei in the presence of an equal volume of transcription buffer containing ribonucleotide triphosphates [including 5 μ*M* unlabeled uridine 5′-triphosphate (UTP)] and 200 μCi of [α-^{32}P]UTP (600 Ci/mmol, New England Nuclear). Run-on transcription was allowed to proceed at 27°C for 30 minutes. Nuclei were pelleted at 200*g* and resuspended in 400 μl of 10 m*M* tris-HCl (*p*H 8.0), 1 m*M* EDTA, 100 m*M* NaCl, 20 m*M* $MgCl_2$ along with 50 μg of transfer RNA and 3 μl of RNasin (Promega). DNA was digested with 15 μl of RQ deoxyribonuclease (Promega). This was followed by a proteinase K digest (BRL) and extraction with phenol, phenol + chloroform, and chloroform. The RNA was ethanol-precipitated twice. Equal amounts of radioactive RNA were hybridized to DNA immobilized on nitrocellulose filters in 10 m*M* 2-{[tris(hydroxymethyl)methyl]amino}ethanesulfonic acid (TES) (*p*H 7.4), 0.2% SDS, 10 m*M* EDTA, and 300 m*M* NaCl at 65°C for 36 hours. Addition of equal amounts of radioactive RNA was confirmed by hybridization to 100 ng of Balb/c liver DNA. Filters were washed in 2× standard saline citrate (SSC) at 65°C for 2 hours, then digested with ribonuclease A (10 μg/ml) (Sigma) in 2× SSC at 37°C for 30 minutes. Target DNA sequences (5 μg each) were denatured in 0.1*M* NaOH before blotting and included p54, c-*myc* complementary DNA (K. Marcu), cJE-1, JE complementary DNA (*15*) in pGEM-1 (Promega), pSP64 (Promega), and 100 ng of Balb/c liver DNA.
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19. For RNA analysis, cells were scraped directly into a solution of 4*M* guanidine isothiocyanate, 25 m*M* sodium citrate (*p*H 7.0), and 100 m*M* 2-mercaptoethanol. Total RNA was isolated by centrifugation through a cushion of 5.7*M* CsCl followed by ethanol precipitation [J. Chirgwin, A. Aeyble, R. McDonald, W. Rutter, *Biochemistry* **18**, 5294 (1979)]. Twenty micrograms of purified RNA was separated by electrophoresis through a 1.5% agarose and 2.2*M* formaldehyde gel and transferred to nitrocellulose filters in 20× SSC. Baked filters were hybridized at 42°C in solutions described in (*3*). Final washes for all blots were 0.1× SSC at 65°C for 1 hour. Probes were nick-translated to a specific activity of greater than 10^8 cpm per microgram and used at 3×10^6 cpm per milliliter. Probes were as follows: c-*myc*, 600-bp Pst I fragment of P54 [L. W. Stanton, R. Watt, K. B. Marcu, *Nature (London)* **303**, 401 (1983)] mouse c-*myc* cDNA containing the third exon, a gift of K. Marcu; JE, a 650-bp Eco RI fragment of pcJE-1 (*15*).

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