more complex explanation of our results is that, although reflex activity promotes crusher development, another signal to the CNS inhibits its induction in the contralateral claw. Consequently, if inhibition took priority over induction, then exercise of both claws would result in paired cutter claws even though reflex activity induces crusher formation. In either case, our results demonstrate that control of claw asymmetry resides in the CNS. Although asymmetry of the vertebrate brain with respect to vocalization in song birds (*11*) and language processing in humans (*12*) may be experimentally altered by ablation and deprivation, we do not know the degree to which use and disuse determines the original asymmetry. Our experiments with lobster claws show how use promotes asymmetry. Moreover, the neuronal changes involved in determining such asymmetry may be more easily understood in lobsters because of the relative simplicity of their nervous system as compared to that of vertebrates. Insights can be gained here that will be of help in understanding neural asymmetry in vertebrates—for example, cerebral lateralization in humans (*13*).

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Interferon Inhibits the Establishment of Competence in G_0/S–Phase Transition

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Addition of mouse interferon-α/β (IFN) to confluent, quiescent BALB/c 3T3 (clone A31) mouse fibroblasts resulted in a block or delay in serum-induced activation of the cell cycle. It was necessary to add IFN within 6 hours after serum stimulation to inhibit nuclear labeling with [^{3}H]thymidine. This is consistent with the time required for platelet-derived growth factor (PDGF) to induce cells to become competent to respond to additional growth factors present in platelet-poor plasma. Simultaneous addition of IFN with PDGF inhibited the PDGF-induced synthesis of a 29-kilodalton and a 35-kilodalton protein that normally occurs within 1 hour after PDGF addition. IFN also suppressed the general increase in protein synthesis that occurs by the fifth hour after PDGF addition. These results show that IFN antagonizes the action of PDGF, thereby interfering with the activation of G_0 cells for G_1 traverse and S-phase entry.

INTERFERONS (IFN's) CAN INHIBIT cell proliferation by blocking cells in the G_0/G_1 phase of the cell cycle (*1–5*). Serum factors control the traverse of cells from G_0/G_1 to S phase. Platelet-derived growth factor (PDGF) initiates proliferation by rendering quiescent fibroblasts "competent" to respond to the progression activity contained in platelet-poor plasma-derived serum (PPP) (*6–8*). Plasma factors, which include epidermal growth factor (EGF) and somatomedin C (SmC), govern cellular progression through early G_1 phase to a mid-G_1 point (*8*). Only SmC is required for progression from mid-G_1 into S phase (*8*). We have investigated the antagonism between interferons and growth factors and report that mouse IFN-α/β inhibits the traverse of serum-stimulated quiescent mouse BALB/c 3T3 cells from G_0 to S phase by acting on processes that take place within the first 6 hours. IFN both selectively inhibits the early synthesis of at least two PDGF-induced proteins and suppresses the PDGF-induced increase in the overall level of protein synthesis.

We first defined the conditions under which mouse IFN-α/β (2.6×10^7 U/mg) inhibited the proliferation of a subline (*7*) of BALB/c 3T3 cells (clone A31). An exponential relationship was observed between IFN concentration and reduction in growth rate of logarithmically growing cells as determined over a 3-day interval (Fig. 1). A 20% reduction in growth rate was evident at 100 U/ml and a 46 and 51% reduction occurred at 5000 and 10,000 U/ml, respectively. The BALB/c 3T3 subline used in the present studies possessed an IFN sensitivity intermediate between the lower sensitivity of BALB/c 3T3 cells used by Sokawa *et al.* (*1*) and the higher sensitivity of cells used by Balkwill and Taylor-Papadimitriou (*2*).

The ability of IFN to delay or block serum-stimulated entry into S phase was evaluated by determining the percent of nuclei that incorporated [^{3}H]thymidine at various intervals after addition of serum to density-arrested BALB/c 3T3 cells (Fig. 2a). After an initial lag of 10 hours, the percent of labeled nuclei increased with time over the ensuing 14 hours in cultures that received serum without IFN. Treatment with IFN-α/β at either 1500 or 5000 U/ml delayed entry into S phase in a concentration-dependent manner. In addition, the rate of increase in DNA-synthesizing cells was clearly reduced in the presence of 5000 U/ml. These results are consistent with previous observations (*1, 2*).

The establishment of competence by PDGF is both concentration- and time-dependent. With an estimated PDGF concentration of the order of 1 ng or 1 U/ml in the medium containing 10% calf serum, the great majority of the cells that achieve competence do so within 6 hours (*7*). The inhibition of S-phase entry as a function of the time of IFN addition was measured in order to determine the time in the cell cycle at which the IFN-mediated block occurred. 3T3 cells were stimulated by the addition of fresh medium supplemented with 10% calf serum, and IFN (final concentration 5000 U/ml) was added simultaneously or at various intervals after serum addition. Cultures were allowed to incorporate [^{3}H]thymidine and the percent of cells that entered S phase

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was determined at 24 hours. Under these conditions, a maximal inhibition of S-phase entry (60%) was observed upon simultaneous addition of serum and IFN-α/β. The ability of IFN to inhibit entry into S phase decreased progressively as the time interval between serum stimulation and IFN addition was increased (Fig. 2b). There was essentially no inhibition of S-phase entry when IFN addition was delayed for 6 hours after serum stimulation (Fig. 2b). Pretreatment with IFN for 24 hours followed by concurrent treatment with fresh IFN did not appreciably enhance the inhibition of S-phase entry above that obtained when IFN and serum were added simultaneously. These results indicate that the major block by IFN on the G_0/S–phase transition takes effect very rapidly and involves events that occur during the initial phase (corresponding to transit out of G_0 and through early G_1).

An elevation in the overall rate of intracellular protein synthesis becomes demonstrable by 3 hours after stimulation, and the rate increases progressively through the second half of the prolonged G_1 phase of PDGF-activated quiescent cells (Table 1). This increase in protein synthesis reflects the transition of cells to a cycling state (*9*) and does not occur in cells exposed to PPP, which lacks PDGF. Simultaneous addition of IFN with PDGF largely suppressed this increase in protein synthesis (Table 1). IFN treatment alone did not affect the basal level of protein synthesis in quiescent cells. The inhibition by IFN of PDGF-induced enhancement of protein synthesis was still evident at 9 hours after PDGF addition.

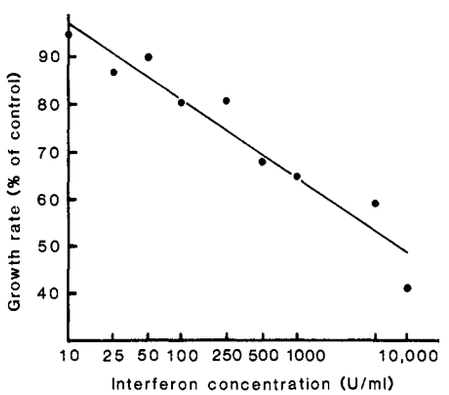

Fig. 1. Relationship between IFN-α/β concentration and inhibition of growth rate of BALB/c 3T3 cells. BALB/c 3T3 (clone A31) mouse cells were seeded at a density of 1.5×10^3 to 2.0×10^3 cells per square centimeter into 25-cm^2 flasks containing 5 ml of Eagle's reinforced minimum essential medium (*25*) + 10% calf serum (growth medium) and incubated at 37°. Twenty-four hours later, cultures were refed with growth medium containing mouse IFN-α/β (2.6×10^7 U/mg) at varying concentrations and cell counts were determined immediately and at 24-hour intervals (*26*). The growth rate of IFN-treated cultures was calculated and expressed as a percent of the growth rate of control cultures that had been refed with growth medium alone. The data plotted are based on three experiments. The coefficients of variation ranged from 1.9 to 6.7%, with a mean of 4.8%. The mean doubling time in control cultures was 20.6 ± 3.5 hours (mean ± SD) on the basis of seven experiments.

Competence is transferable by cytoplasts (*10*) and its acquisition may correlate with the induction by PDGF of proteins of 29 (pI) and 35 (pII) kilodaltons (kD) (*11, 12*). The enhanced synthesis of pI was detectable within 1 hour, was maximal by 3 hours, and declined by 5 hours after PDGF addition (Fig. 3a) (*12*). With PDGF added at 15 U/ml, 2–3 hours of exposure are sufficient to render the majority of the density-arrested cells responsive to PPP (*7*). In cells to which IFN-α/β (10,000 U/ml) had been added simultaneously with PDGF, the enhancement of pI synthesis by PDGF was markedly inhibited as determined at 1 and 3 hours after addition of PDGF (Fig. 3a), and it remained suppressed at 5 hours. The synthesis of pII increased progressively over the initial 5 hours after PDGF addition (*11, 13*), and IFN suppressed this PDGF-induced increase (Fig. 3b).

Tominaga and Lengyel (*14*) have reported that IFN-pretreatment of BALB/c 3T3 cells for 48 hours neither altered the pattern of cell-associated PDGF-induced proteins, nor inhibited the ability of PDGF to increase the levels of PDGF-inducible messenger RNA's (mRNA's) JE, KC, and *myc*. However, coaddition of IFN with PDGF, in the absence of IFN pretreatment, inhibits the PDGF-induced expression of c-*fos*, c-*myc*, and ornithine decarboxylase genes (*15*). It is possible that IFN-induced proteins, which could be responsible for the inhibition of induction of PDGF-induced mRNA's, may decline to basal levels in 48 hours (*15*). This would be consistent with the observation that a 48-hour pretreatment with IFN does not result in the suppression of certain PDGF-induced mRNA's (*14*) while the simultaneous addition of IFN and PDGF does (*15*).

Pretreatment of cells with IFN for 48 hours, followed by exposure to PDGF in the presence of freshly added IFN did, however, inhibit the accumulation of PDGF-induced, secreted proteins (*14*). IFN has been shown to inhibit the secretion of plasminogen activator (*16*). Recently, Scher *et al.* (*13*) demonstrated that pII represents a group of secreted, phosphorylated glycoproteins that are identical to the 35-kD proteins; these proteins are called major excretion proteins (MEP's) and are constitutively produced and secreted into the medium by Kirsten sarcoma virus–transformed NIH 3T3 cells (*17*). Our finding of an IFN-mediated reduction in the intracellular levels of pII indicates that reduced accumulation of at least some of the PDGF-induced, secreted proteins in the medium surrounding IFN-treated cells might be due to reduced synthesis, rather than failure of secretion of the proteins.

The initial events in the serum-stimulated traversal of cells from G_0 through early G_1 are governed by PDGF. Previous studies indicated that the IFN-mediated delay in S-phase entry of serum-stimulated BALB/c 3T3 cells was due to IFN action during the middle to late G_1 phase (*1, 18*). Our demonstration that the simultaneous addition of

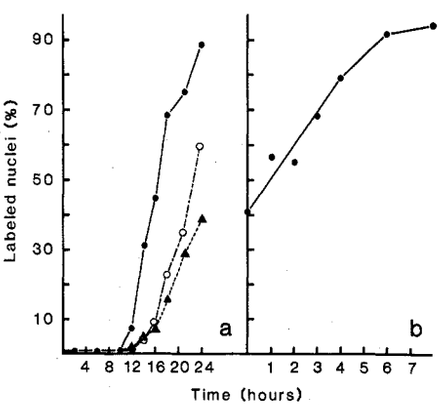

Fig. 2. S-phase entry of control and IFN-treated BALB/c 3T3 cells. (a) Time course after simultaneous addition of serum and IFN to quiescent cells. 3T3 fibroblasts were seeded in each well of a 96-well cluster dish at a density of 1.0×10^4 to 1.5×10^4 cells per square centimeter and allowed to reach confluence in 3 days. Medium was removed, cultures were refed with fresh Eagle's reinforced minimum essential medium supplemented with 1% platelet-poor plasma-derived serum (PPP) and incubated an additional 3 days to achieve quiescence. Monolayers were then stimulated to synthesize DNA by the addition of 10% calf serum (●). IFN was added simultaneously at a concentration of either 1500 U/ml (○) or 5000 U/ml (▲). The fresh medium also contained [methyl-^{3}H]thymidine (1 μCi/ml; 6 Ci/mmol). At various times after serum addition, monolayers were fixed and subjected to autoradiography as previously described (*27*), and the percent of labeled nuclei was determined. Data plotted are from a representative experiment. (b) Percent of S-phase cells as a function of delayed addition of IFN. Quiescent 3T3 cells were stimulated by the addition of 10% calf serum in medium containing [methyl-^{3}H]thymidine (1 μCi/ml). IFN-α/β was added simultaneously with, or at various times after, serum stimulation. The incorporation of [^{3}H]thymidine was allowed to proceed for 24 hours after serum stimulation. Monolayers were fixed and subjected to autoradiography (*27*). The number of labeled nuclei in the IFN-treated cultures was expressed as a percent of the number of labeled nuclei in cultures stimulated with serum in the absence of IFN. The data plotted are based on four experiments. The coefficients of variation ranged from 10 to 18%, with a mean of 13%.

Table 1. The effects of IFN-α/β on PDGF-enhanced protein synthesis. 3T3 cells were planted in 35-mm culture dishes at a density of 1.2×10^4 cells/cm^2 and allowed to reach quiescence as described in Fig. 2. Cultures were transferred to 1 ml of methionine-free medium + 0.1% PPP to which PPP (5%), PDGF (15 U/ml), IFN-α/β (5000 U/ml), or PDGF + IFN-α/β had been added. At various times after addition, [^{35}S]methionine (≧400 Ci/mmol; final concentration 50 μCi/ml; New England Nuclear) was added and cultures were incubated for an additional hour. Monolayers were then lysed in RSB buffer + 1% NP-40 and the number of trichloroacetic acid (TCA)–precipitable counts was determined as described (*23*). The means of acid-precipitable counts of duplicate samples from two experiments were normalized with respect to zero-hour levels of cultures that received no additions. The coefficient of variation between experiments averaged 7.0% and did not exceed 15%. Between experiments, the coefficient of variation was not significantly larger than within duplicate samples of one experiment. PDGF (>25% pure) from outdated human platelets was used for all experiments. The PDGF preparations used were free of β-transforming growth factor (*24*).

Time (hr)	Incorporation of [^{35}S]methionine into proteins (count/min)			
	PPP	PDGF	PDGF + IFN	IFN
0	61,900	—	—	—
1	61,400	51,500	60,100	61,400
3	54,000	65,300	60,500	60,200
5	53,000	102,800	67,600	57,100
9	59,700	147,600	69,400	58,300

IFN with PDGF results in the inhibition of the synthesis of PDGF-induced proteins that appear within the first hour after PDGF addition, establishes a time interval of 1 hour within which IFN exerts at least some of its effects. Simultaneous addition of IFN and PDGF to BALB/c 3T3 cells also inhibits the PDGF-induced disaggregation of actin fibers within 1 hour (*19*). Our kinetic evidence suggests that IFN blocks early serum-stimulated events required for the transition of cells out of the G_0 phase and through the early G_1 phase, rather than events occurring within the second half of G_1 and the transition into S phase. This conclusion is further strengthened by the observation that the extent of inhibition of entry into S phase was similar in cells either exposed to PDGF and IFN for 5 hours in 0.1 to 5.0% PPP and then incubated in 5% PPP alone for 24 hours, or exposed to PDGF and IFN continually for 24 hours in the presence of 5% PPP. Moreover, IFN-treatment of cells that have been arrested in a competent state by nutrient starvation in the presence of PDGF (*20*), prevents neither the appearance of a 55-kD protein that occurs 3 hours after the addition of 5% PPP in complete medium, nor entry into S phase.

Our observation that IFN-mediated growth inhibition affects the mechanism for the acquisition of competence suggests that some of the reported antagonistic effects on progression factors (*5*) could be indirect and secondary effects of IFN. This is consistent with a previous demonstration (*21*) of a requirement in human melanoma cells for a "G_0" component in the cell cycle in order for these cells to display sensitivity to the growth-inhibitory effects of IFN. However, we have not excluded a direct action of IFN on some early EGF-induced processes during the first half of the prolonged G_1 of activated quiescent cells.

The mechanism by which IFN inhibits the acquisition of competence remains to be defined. It should also be noted that, conversely, PDGF is able to inhibit IFN action by an as yet unknown mechanism (*22*). Substances known to induce competence have been shown to enhance synthesis of a set of proteins, which includes pI and pII (*11, 13*). Our demonstration that IFN inhibits the induction of both pI and pII by PDGF is consistent with the possibility that the expression of these proteins may be required for the establishment of competence.

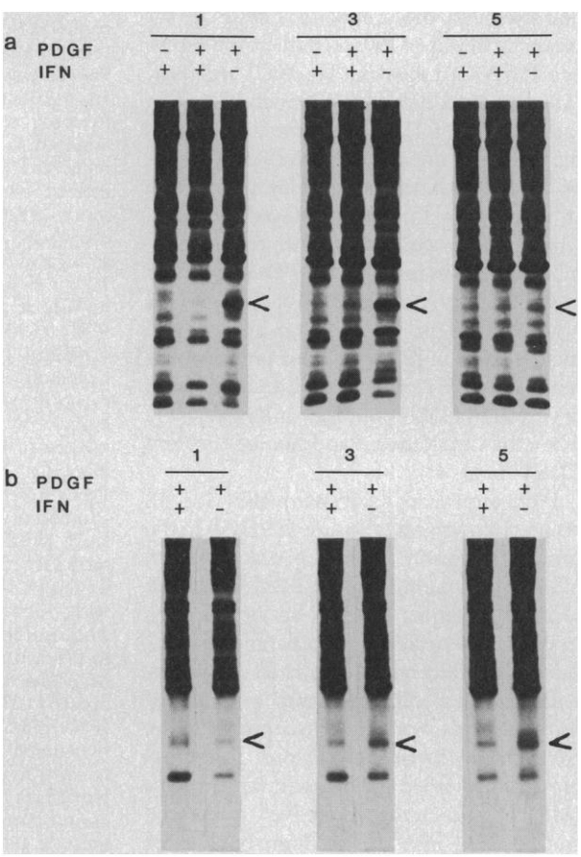

Fig. 3. Inhibition of the synthesis of PDGF-induced proteins by IFN. Quiescent monolayers of 3T3 cells were fed with methionine-free medium + 0.1% PPP. PDGF (15 U/ml) was added with or without IFN-α/β (10,000 U/ml). At 1, 3, and 5 hours after addition of PDGF, cells were labeled with [^{35}S]methionine for 1 hour as described (Table 1). Monolayers were then lysed and extracts were clarified by centrifugation at 13,000*g* for 5 minutes to remove cytoskeletons and cell debris (*11*). The supernatants were separated on (a) 12% or (b) 12.5% sodium dodecyl sulfate–polyacrylamide gels; an equal number of TCA-precipitable counts were placed in each lane. The (<) indicates the position of pI in (a) and the position of pII in (b).

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Temperature Regulation of Bacterial Activity During the Spring Bloom in Newfoundland Coastal Waters

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While the spring phytoplankton bloom in Newfoundland coastal waters is in progress during April and May, at water temperatures between −1° and +2°C, bacterial growth and respiratory rates remain low. Microbial community respiration is not measurable at −0.2°C. Particulate materials that would be utilized by microorganisms in 2 to 3 days at 20° to 25°C require 11 days at 4°C and 18 days at −0.2°C. Thus, photosynthesis is active but microbial utilization of the products is suppressed. High secondary production in cold water may result from the low rate of microbial decomposition, enabling herbivores to utilize much of the primary production.

HIGH SECONDARY PRODUCTIVITY in cold waters has been an enigma, because the rates of primary production do not seem to be large enough to support it (*1*). Cold-water food chains have been thought to be short and energetically efficient, but microbial pathways of energy flow are now known to occur in cold waters (*2*), in addition to the metazoan grazer-predator relationships (*3*). Dissolved organic materials and nonliving particulate materials are converted to living biomass by bacteria that are subsequently grazed by protozoa and other small eukaryotes. Since energy losses are 50 to 90% at each step in a food chain, a significant fraction of primary production is lost through energy conversions in this microbial loop. If, however, bacteria in cold water are less active metabolically than in warm water, the microbial loop will consume less energy and more primary biomass will be available to metazoans. Heterotrophic marine psychrophiles are inhibited by very low temperature (*4, 5*). For one bacterium isolated from water at 62°S, the increase in rate of the chemical reaction for each 10°C increase in temperature (Q_{10}), was measured; the extrapolated Q_{10} of respiration between +1° and 4°C was 142, whereas between 7° and 10°C it was 1.7. The growth rate declined from a maximum of 0.19 generation hour^{-1} at 4°C to 0.08 hour^{-1} at +1°C (*5*).

Phytoplankton photosynthesis also is suppressed by low temperature, but the decline in photosynthetic rate occurs at lower temperatures than does the decline in bacterial growth and metabolism (Fig. 1). Thus, between +1° and −1°C, photosynthesis is substantial but rates of bacterial growth and metabolism are low. This is potentially important where water temperature is in that critical range during the spring bloom period, when much of the annual primary production occurs (*6*). To examine these differential responses during the spring bloom, we incubated samples of natural seawater and appendicularian houses from Logy Bay, Newfoundland, at temperatures near 0°C and +4.2°C.

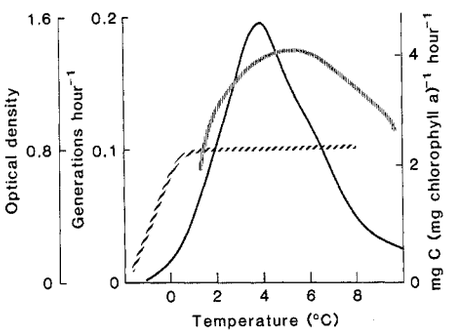

Fig. 1. Differential growth of phytoplankton and bacteria at very low temperatures. Growth of psychrophilic bacteria in culture at various temperatures (*4*), expressed as optical density of the culture (continuous line) and (*5*) expressed as generations hour^{-1} (line of short vertical marks), compared with potential production of phytoplankton, measured in the field (*11*), expressed as milligrams C (milligrams of chlorophyll *a*)$^{-1}$ hour^{-1} (dashed line).

Appendicularian houses are common, naturally occurring, macroparticulate matter on which bacteria grow. They support rates of photosynthesis and respiration much higher per unit volume than that in the surrounding water (*7*). Samples of water and living appendicularians, *Oikopleura vanhoeffeni*, were collected during April 1984 and 1985 by divers. Abandoned appendicularian houses were placed in glass vials with 23 ml of seawater from the collection site, and these together with control vials of seawater were held at controlled temperatures with natural illumination from laboratory windows (*8*). During April 1985, the change in oxygen concentration in control vials at −0.2°C was not significant (analysis of variance, F test, $P \geqq 0.50$) over 21 days of observation (Fig. 2). However, the presence of appendicularian houses resulted in a significant increase in oxygen concentration, $+2.64 \pm 2.02$ μM day^{-1} ($P \leqq 0.01$). At 4.2°C, oxygen concentration decreased at the same linear rate in both the vials with houses, -4.52 ± 2.76 μM day^{-1}, and the control vials, -4.50 ± 1.33 μM day^{-1} (analysis of covariance, t test, $P > 0.20$).

This experiment demonstrates that at ambient temperature of −0.2°C photosynthesis was greater than respiration, with some fraction of the primary producers attached to large particles, while at +4.2°C heterotrophic respiration exceeds photosynthesis.

At −0.2°C, the numbers of free-living bacteria were initially 1.2×10^4 ml^{-1}, increasing after a week to 10^6 ml^{-1} and remaining in the range of 2×10^6 to 7×10^6 ml^{-1} until week 3, when numbers of free bacteria were 1×10^5 to 5×10^5 ml^{-1}. Some bacteria were attached to particles, but on the order of 99% were free-living. Few flagellates were seen. At 4.2°C, however, bacteria attached to particles, in colonies too dense to be counted, formed the dominant bacterial biomass. Free-living bacteria were in the range 1×10^5 to 5×10^5 ml^{-1}

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