of **3**. Jones oxidation (*13*) and subsequent methanolysis gave **8** as in the case of **3**, thus **5** is the epimer of **3** at C-3.

The proton system for the moiety C-6 to C-15 in pavoninin-6 (**6**) was clarified by ^{1}H-NMR studies. As in **4**, the 5α-proton and 3β-hydroxyl configurations are based on NMR data of 10-methyl, 0.79 and 11.7 ppm, and 3-proton, 3.55 ppm (tt, 10, 4). Hydrogenation of **6** (over platinum in acetic acid) gave dihydro-**6**, which was identical with one of the two hydrogenation products of **5**. Furthermore, Jones oxidation and subsequent methanolysis of both dihydro-**6** and **4** gave the identical (25*R*)-15α,26-dihydroxy-5α-cholestan-3-one. The conversions described above chemically correlate all six pavoninins and thus establish their full structures (*16*).

Exposure of the dog shark *Mustelus griseus* to pavoninins suggested that they are repellents that act on the shark's olfactory sense, whereas the proteinaceous toxin is possibly an antifeedant that acts on its gustatory sense. Tests based on termination of tonic immobility (TI tests) of the lemon shark *Negaprion brevirostris*, have been developed for screening potential shark repellents (*17*). According to preliminary TI tests, the pavoninins have been shown to be relatively strong repellents by acting on buccal receptors and possibly on the olfactory rosette at 5 mg/ml, a concentration well below that of pavoninins in the secretion discharged by the sole. In contrast to pavoninins, the common steroidal saponins are only weakly active in these tests (*18*). Since the amount of pavoninins available from the sole fish is insufficient to carry out proper biological tests, pavoninin-4 and a simpler model are now being synthesized in gram quantities from diosgenin (*19*). Detailed biological tests remain to be determined (*20*).

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6. The ^{1}H-NMR spectra were recorded on Nicolet NT-360 at 360 MHz in CD_3 OD (**1**–**6**) or $CDCl_3$ (derivatives). The data represent chemical shifts from tetramethylsilane in parts per million (multiplicity, coupling constants in hertz; assignments). For details see K. Tachibana, M. Sakaitanai, K. Nakanishi, *Tetrahedron*, in press.
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10. Compare H. Liu and K. Nakanishi, *J. Am. Chem. Soc.* **103**, 5591 (1981).
11. Configurations at oxygenated methines, whether the oxygen is axially or equatorially attached, were derived from the ^{1}H-NMR coupling constants between the methine proton and its neighboring protons.
12. With (+)- or (−)-MPTA chloride in pyridine, 60°C. Induced shifts by 1.0*M* equivalent $Eu(fod)_3$ added to a solution of the diastereomeric mixture in $CDCl_3$: 0.68 ppm for the (+)-MPTA ester and 0.66 ppm for the (−)-MPTA ester; compare F. Yasuhara and S. Yamaguchi, *Tetrahedron Lett.* (1977), p. 4085.
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20. We thank T. Yoshino, Ryukyu University, for assistance in collecting the sole; the instrumental analysis team, this institute, for spectroscopic measurements: S. Uchida, director, Okinawa Expo Aquarium, for assistance in the shark assay, and S. H. Gruber for the TI tests.

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Growth Inhibitor from BSC-1 Cells Closely Related to Platelet Type β Transforming Growth Factor

Abstract. *Purified growth inhibitor from BSC-1 cells and type β transforming growth factor from human platelets are shown to have nearly identical biological activity and to compete for binding to the same cell membrane receptor. These findings suggest that the growth inhibitor and the type β transforming growth factor are similar molecules. The data also show that the same purified polypeptide can either stimulate or inhibit cell proliferation depending on the experimental conditions.*

The regulation of the growth of cells in culture is a highly complex process in which the cells interact with growth-inhibitory and growth-stimulatory polypeptides as well as with nutrients and ions. African green monkey kidney cells (BSC-1) produce a polypeptide growth inhibitor (GI) when arrested at saturation density (*1*). This GI has been purified from medium conditioned by these cells and shown to cause arrest of the same cells at low density in the G_1 phase of the cell cycle (*2*). Similar effects of the GI have also been observed with certain lung and mammary cell lines but not with Swiss mouse 3T3 cells or human skin fibroblasts (*2, 3*).

Transforming growth factors (TGF's) are operationally defined as polypeptides that stimulate anchorage-dependent cells to grow in soft agar and have been detected in neoplastic and non-neoplastic cells in culture and in tissues in vivo (*4*). Two types of TGF have been purified: TGFα, which binds to the epidermal growth factor (EGF) membrane receptor (*5*), shows significant sequence homology with both mouse and human EGF (*6*), and has biological activities similar to those of EGF when purified free of TGFβ (*7*); and TGFβ, a different molecule that is a potent growth stimulator of mouse embryo-derived AKR-2B cells in soft agar with or without added EGF (*8*) and of rat kidney-derived (NRK) cells when added in the presence of EGF (*4*). However, TGFβ has been reported to have little or no mitogenic activity for NRK (*9, 10*) or AKR-2B (*11*) cells in monolayer culture. Recently, TGFβ has been shown to have specific membrane receptors on responsive cells (*12*).

Both the GI (*2*) and TGFβ (*9*) were shown by sodium dodecyl sulfate–polyacrylamide gel electrophoresis to have apparent molecular weights in the 24,000 to 26,000 range and, when reduced with β-mercaptoethanol, exhibit a single band at approximately 12,500. The apparently similar molecular weights and subunit composition of the GI and TGFβ, the observation that TGFβ occurs widely (*4*), and initial data indicating that TGFβ is actually inhibitory for EGF- and insulin-stimulated mitogenesis in serum-free monolayer cultures of AKR-2B cells, suggest that the GI and TGFβ may be the same molecule. We now show that GI purified from medium conditioned by BSC-1 cells and TGFβ purified from human platelets have almost identical biological activities in stimulation of the

growth of AKR-2B cells in soft agar and inhibition of DNA synthesis in AKR-2B, BSC-1, and CCL-64 (mink lung) cells. The GI and TGFβ also bind to the same cell membrane receptor. This suggests that the GI and TGFβ are similar molecules.

Type β TGF purified from outdated human platelets by a modification (*12*) of the method described (*9*) stimulated growth of mouse AKR-2B (clone 84A) cells in soft agar, with a half-maximum response at 1 ng/ml (Fig. 1). An assay for stimulation of DNA synthesis in AKR-2B cells arrested in the G_1 phase has been developed with the use of defined serum-free conditions (*11*). Under these circumstances, EGF and insulin stimulate most of the cells to enter the S phase, with a peak of [^{3}H]thymidine incorporation at 22 hours. The addition of TGFβ inhibited the stimulation of DNA synthesis caused by EGF and insulin, with a dose response similar to that obtained for stimulating these cells to grow in soft agar (Fig. 1).

Similar experiments were carried out with the GI purified from medium conditioned by BSC-1 cells (*2*). The results were similar to those obtained with TGFβ (Fig. 2). The GI stimulated growth of AKR-2B (clone 84A) cells in soft agar, with half-maximum stimulation at 0.8 ng/ml and maximum response at 2 ng/ml. The GI also inhibited EGF and insulin stimulation of DNA synthesis in serum-free monolayer cultures with a similar dose response (Fig. 2). A decrease in both soft agar stimulation and monolayer inhibition effects was observed at concentrations of 4 ng/ml or higher. The reason for this decreased response at higher concentrations is not known.

The effects of TGFβ and the GI on growth of BSC-1 and CCL-64 cells were also determined. Inhibition was observed with both compounds on both cell lines (Fig. 3). Half-maximum inhibition of BSC-1 cells was obtained with approximately 0.7 ng of GI and 0.15 ng of TGFβ per milliliter. The CCL-64 cell line known to be highly responsive to the GI (*3*) was also much more responsive to the inhibitory effects of TGFβ (Fig. 3). The relative specific activity of the GI and TGFβ on BSC-1 and CCL-64 cells appears to differ somewhat. The reason for this difference is not known. Because the GI and TGFβ preparations are from different species, and because GI is a secreted protein whereas TGFβ is extracted from platelets, there may be minor differences in structure.

These data show that the GI and TGFβ have similar biological activities. In experiments to determine whether these growth modulators bind to the same cell membrane receptor, GI was tested for its ability to compete with ^{125}I-labeled TGFβ binding by means of the radioreceptor assay described (*12*). The GI competed effectively in this assay, with 95 percent inhibition of ^{125}I-labeled TGFβ at a dose of 20 ng/ml (Fig. 4). Half-maximum inhibition was obtained at approximately 2 ng/ml with GI in comparison to approximately 1 ng/ml with unlabeled TGFβ (Fig. 4). These are similar dose responses, especially considering the different sources of the proteins as discussed above and the possible error in determining the protein content of the GI and TGFβ preparations. These data indicate that the GI and TGFβ interact with the same cell membrane receptor.

It is interesting that the same molecule has both growth-stimulatory and growth-inhibitory activities. The reason for this different response is unknown. One possible explanation is that the shape of a

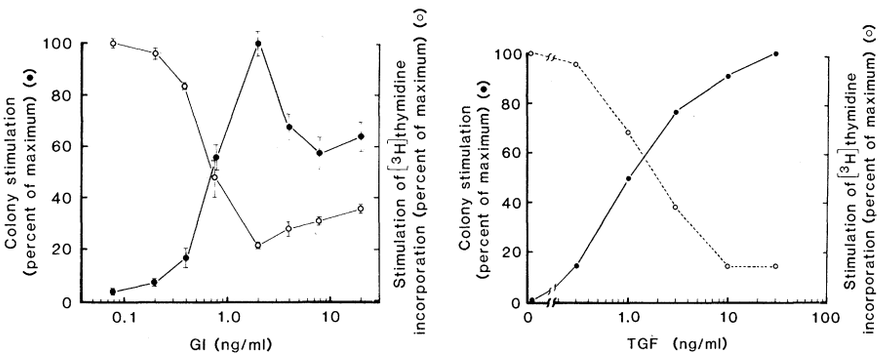

Fig. 1 (left). Comparison of stimulation of colony formation in soft agar and inhibition of EGF- and insulin-stimulated [^{3}H]thymidine incorporation in AKR-2B cells by TGFβ. Type β TGF from outdated human platelets was purified as described (*9*) with the addition of a final reversed-phase C-18 high-performance liquid chromatography step (*12*). The assay for the stimulation of colony formation of AKR-2B (clone 84A) cells in soft agar (●) by various concentrations of TGFβ was performed as described (*8*) with the use of a Quantimet 800 image analyzer (Cambridge Instruments) to measure the colony formation after 7 days of growth. Maximum colony formation was 2400 colonies (>50 μm in diameter) per 7500 cells seeded. The AKR-2B cells in serum-free monolayer cultures were maximally stimulated with 10 ng of EGF and 500 ng of insulin per milliliter, and [^{3}H]thymidine incorporation was measured between 22 and 23 hours later as described (*11*). At time zero, when the EGF and insulin were added, various concentrations of TGFβ were also added and the amount of [^{3}H]thymidine incorporated measured (○). The incorporation of [^{3}H]thymidine into AKR-2B cells stimulated by EGF and insulin was 300 times greater than that in untreated control cultures. Fig. 2 (right). Comparison of stimulation of colony formation in soft agar and inhibition of EGF- and insulin-stimulated [^{3}H]thymidine incorporation of AKR-2B cells by growth inhibitor (GI) produced by BSC-1 cells. The GI was purified from conditioned medium as described (*2*). The assay for the stimulation of colony formation of AKR-2B (clone 84A) cells in soft agar (●) by GI was performed as described in the legend of Fig. 1. Maximum colony formation was 2100 colonies per 7500 cells seeded. The inhibition of EGF- and insulin-stimulated [^{3}H]thymidine incorporation by GI (○) was performed as described in the legend of Fig. 1.

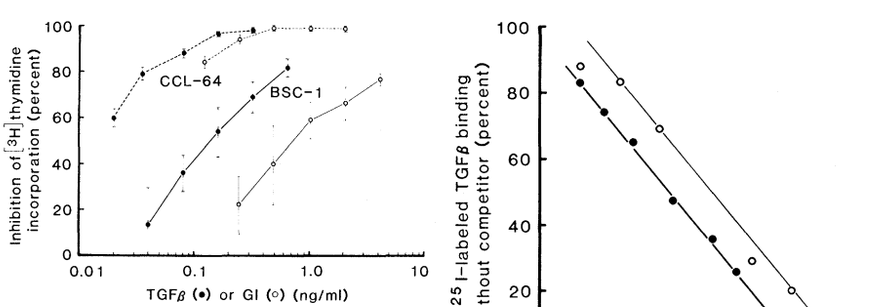

Fig. 3 (left). Comparison of inhibition of [^{3}H]thymidine incorporation by GI (○) and TGFβ (●) in BSC-1 cells (——) and CCL-64 cells (- - - -). The incorporation of [^{3}H]thymidine between 20 and 25 hours after addition of GI or TGFβ was determined as described (*2*). Fig. 4 (right). Comparison of the inhibition of ^{125}I-labeled TGFβ binding to AKR-2B (clone 84A) cells by unlabeled TGFβ (●) from human platelets and GI (○) from the conditioned medium of BSC-1 cells. Various concentrations of TGFβ and GI were added with a tracer amount of ^{125}I-labeled TGFβ and incubated on AKR-2B cells for 2 hours at room temperature (22°C). The amount of bound ^{125}I-labeled TGFβ was determined as described (*12*).

cell in culture or its attachment (or lack thereof) to a solid substrate influences the response of the cell to growth factors and serum (*13*). Rounded, unattached cells in soft agar appear to respond to different growth factors than those attached to plastic or glass (*5*). The possibility cannot be excluded that the postreceptor pathways activated by GI or TGFβ lead to a stimulatory response when the cells are rounded and unattached and to an inhibitory response when the cells are attached and flattened. Another possible explanation for the differences in response is cell-type differences. The growth of certain cells is significantly inhibited by the GI whereas the growth of others is inhibited minimally or not at all (*2*, *3*). Type β TGF stimulates certain cells to grow in soft agar but not others (*14*). BSC-1 cells are epithelial whereas the AKR-2B cells are mesenchymal, and for a single growth-modulating polypeptide to exhibit growth stimulation of mesenchymal cells while inhibiting epithelial cells could have some physiological significance.

Although the GI and TGFβ bind to the same cell surface receptor, it is not known whether the cell surface receptors for GI or TGFβ that lead to growth stimulation are the same receptors that lead to growth inhibition. Different receptors may be involved in the different actions. Alternatively, if the same receptors are involved, a primary action of GI or TGFβ, such as an effect on Na^+ fluxes (*15*), may be growth inhibitory in one setting and growth stimulatory in another.

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Transcription and Promoter Usage of the *myc* Gene in Normal Somatic and Spermatogenic Cells

Abstract. *In somatic cells the level of* myc *transcription is not restricted to particular cell types but correlates closely with the rate of cell division. Such transcription involves the use of two active* myc *promoters and produces two messenger RNA species that are differentially represented among the transcripts of different tissues. In apparent contrast to somatic cells, mitotically and meiotically dividing germ cells have very few* myc *transcripts and appear to proliferate, at least for a few divisions, in the absence of* myc *transcription. These results raise interesting questions regarding the role of the* myc *gene product in terminally differentiating cells, particularly of the germ line series.*

The *myc* gene is transcriptionally active in cells of the hematopoietic lineage, yet its expression is low in liver and fibroblasts (*1*). The apparently limited number of cell types expressing *myc* transcripts at high levels has led to the suggestion that the *myc* gene may be preferentially expressed in lymphocytes (*2*) or else may play a role in cellular differentiation (*3*). The *myc* gene has been shown to be transcriptionally activated in both fibroblasts and lymphocytes by various competence-inducing and mitogenic agents, suggesting that the gene product may function during the transition through the G_1 phase of the cell cycle (*4*); this observation, in contrast to the hypothesis relating *myc* function to differentiation, suggests a more fundamental role for the gene product. In consideration of the concept of *myc* being a participant in cell division, we have examined its transcriptional activity in normal tissues by means of a sensitive S1 nuclease protection assay.

The probe used in this assay extends from near the 3′ end of the first exon of the *myc* gene to beyond the two promoter-like sequences that have been identified at the 5′ end of the first exon (*5*) (Fig. 1). This probe allows assessment of whether both promoters are used as well as determination of the total amount of *myc* transcripts. The results (Fig. 1) indicate that *myc* transcripts can be detected in all the tissues and organs examined, with high levels occurring in the ovaries, uterus, preputial gland, and thymus. The other tissues had fewer transcripts, and in some tissues, such as skeletal muscle, transcripts were barely detectable. Although it appeared as though both promoters were active, there were tissue-specific differences in the relative abundance of the transcripts. In tissues such as spleen and thymus the ratio of longer to shorter transcripts was approximately 1:4, whereas in the preputial gland the ratio was closer to 1:20. It has been suggested that the two different transcripts may behave differently in their processing, stability, or efficiency of translation (*6*, *7*).

There appeared to be a general correlation between the number of *myc* transcripts and the overall rate of cellular proliferation within each tissue. For example, in the thymus and ovaries the relatively high degree of cell division coincided with substantial amounts of *myc* RNA. In contrast, skeletal muscle, which has few dividing cells, had barely detectable *myc* transcripts. Because it is extremely difficult to obtain accurate estimates for the total rate of cell division within a whole tissue or organ, we were not able to rule out the possibility that factors other than cellular proliferation contributed to the degree of *myc* expression. One apparent exception to this correlation is adult testes, which have substantial numbers of actively proliferating cells and low amounts of *myc* messenger RNA (mRNA). This apparent discrepancy has been examined further by measurements of changes in the amount of *myc* RNA during sexual maturation of the mouse testis (*8*). After a brief collagenase treatment, the developing testes were separated into two fractions: the