GF do not exhibit interferon activity (*15*).

Our results indicate that the intraperitoneal administration of pristane to BALB/cAnPt mice results in the stimulation of cells capable of producing PCT-GF in vitro. This agrees with the observations of Metcalf (*4*) and suggests that PCT-GF levels in the oil granuloma may be substantially elevated, providing an environment in which early tumor cells can proliferate. The results presented here provide strong evidence that PCT-GF is distinct from IL-1, IL-2, IL-3, BSF-1, BCGF-II, macrophage-CSF, EGF, TGF-β, and γ- and β-interferons.

The identification of a factor that supports PCT growth in vitro presents the intriguing possibility that this factor is involved in the establishment and maintenance of PCT's in vivo and may thus be important in the tumorigenic process. Because there are other known and probably unknown factors present in the P388D1 supernatant, any evaluation of the possible role of PCT-GF in normal lymphocyte proliferation or differentiation will require purified preparations of this material. Experiments to elucidate the structure and function of this factor will thus be important to our understanding of normal and malignant cell growth.

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8. L. Neckers and R. Nordan, in preparation.
9. Purified factors were provided by the following investigators or laboratories: human IL-1, J. Schmidt [*J. Exp. Med.* **160**, 772 (1984)]; recombinant human IL-2, Biogen, Geneva, Switzerland; murine IL-3, J. Ihle [J. Ihle *et al.*, *J. Immunol.* **129**, 2431 (1982)]; murine BSF-1, J. Ohara and W. E. Paul [*Nature (London)* **315**, 333 (1985)]; murine EGF, R. Assoian [C. R. Savage and S. Cohen, *J. Biol. Chem.* **247**, 7609 (1972)]; human TGF-β, R. Assoian [R. K. Assoian *et al.*, *J. Biol. Chem.* **258**, 7155 (1983)]; murine γ-interferon, affinity purified, G. Spitalny [G. L. Spitalny and E. A. Havel, *J. Exp. Med.* **159**, 1560 (1984)]; murine β-interferon, P. Lengyel [B. M. Jayaram, H. Schmidt, O. Yoshie, P. Lengyel, *J. Interferon Res.* **3**, 177 (1983)]; human C3b, L. Fries [L. H. Hammer, G. H. Wirtz, L. Renfer, H. D. Gresham, B. F. Tack, *J. Biol. Chem.* **256**, 3995 (1981)]. Partially purified murine macrophage-CSF was a gift from M. K. Warren and S. Vogel [*J. Immunol.* **134**, 982 (1985)], partially purified murine BCGF-II was provided by S. Swain, personal communication [S. L. Swain and R. W. Dutton, *J. Exp. Med.* **156**, 1821 (1982)], and bovine thymosin F5 was obtained from A. Goldstein [M. M. Zatz and A. L. Goldstein, *Gerontology* **31**, 263 (1985)].
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The 35-Nucleotide Spliced Leader Sequence Is Common to All Trypanosome Messenger RNA's

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In Trypanosomatidae the messenger RNA's (mRNA's) that code for the variant surface glycoproteins (VSG's), tubulins, calmodulin, and at least a subset of other proteins contain a common 35-nucleotide leader sequence at their 5′ ends. Hybrid-arrested in vitro translation has been used to show that all mRNA's in both African and South American trypanosomes contain this 35-nucleotide sequence. Oligonucleotides complementary to this sequence blocked translation of all trypanosome mRNA's in a rabbit reticulocyte lysate system, but did not inhibit translation of mRNA's from other organisms lacking this sequence. An oligonucleotide complementary to the VSG mRNA downstream from the spliced leader sequence arrested only VSG synthesis. Thus, the 35-nucleotide leader sequence is a general feature of all trypanosome mRNA's. The high specificity of oligonucleotides complementary to the spliced leader for their target sequence suggests that analogues permeable to the cell membrane may be useful in the treatment of trypanosomal infections.

AMONG THE MANY UNUSUAL AND interesting features of trypanosomes is the fact that at least some of their messenger RNA's have a common 35-nucleotide leader sequence at their 5′ nontranslated ends (*1–10*). This sequence was first recognized in the complementary DNA's (cDNA's) for the VSG's, the surface coat proteins of African trypanosomes whose sequential expression enables the parasite to evade the host's immune system (*1*). This same sequence was subsequently found at the 5′ ends of the calmodulin and tubulin cDNA's of African trypanosomes (*5, 6*), and in some cDNA's of *Trypanosoma cruzi*, the South American trypanosome that does not undergo antigenic variation (*11*). The sequence has been termed the miniexon (*2*) or spliced leader sequence (*12*), and is encoded within a repeated DNA sequence in the genome. In the African trypanosome *T. brucei*, the best studied case, the spliced leader is transcribed from a 1.35-kb DNA sequence that is present in about 200 copies, most of which occur within tandem arrays (*13*). The initial transcript from these repeats is a 140-nucleotide RNA that contains the spliced leader (*14*) and a cap structure (*15*) at its 5′ end. Two models have been proposed for the transfer of the 35-nucleotide leader sequence to the ends of the mRNA's (*14*), a primer mechanism and a bimolecular splicing mechanism. Neither model has been proven nor is the biological function of the leader sequence known.

Tandem DNA repeats containing the coding region for a highly homologous sequence occur in other members of Trypanosomatidae, for example, *Leptomonas collosoma* (*16*), suggesting that this may be a general phenomenon for this family of protozoans. However, not all RNA's of these organisms have this sequence; it is not found in structural RNA's such as the large ribosomal RNA's and 5*S* ribosomal RNA, or in their

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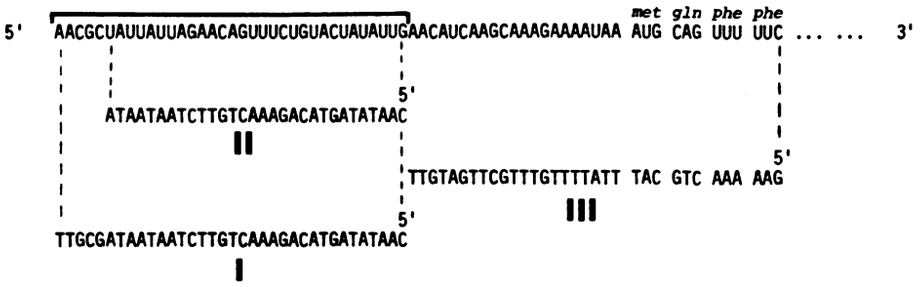

Fig. 1. The sequence at the 5′ end of the mRNA for the VSG expressed in *T. brucei* clone IaTat 1.2 (*19*). A line is drawn over the 35-nucleotide spliced leader sequence. Complementary oligonucleotides (I), (II), and (III) are shown below the mRNA sequence. The oligonucleotides were synthesized by the phosphoramidite method using a Beckman automated DNA synthesizer.

precursors (*17, 18*). Furthermore, to date only a limited number of the total cellular mRNA's have been shown to contain the spliced leader (*4–6*). For these reasons, we decided to determine whether the presence of the spliced leader is a general feature of all trypanosome mRNA's, or of just a discrete subset.

Figure 1 shows the spliced leader and the adjacent sequence at the 5′ end of the mRNA coding for the VSG expressed in *T. brucei* clone IaTat 1.2 (*19*). Two oligonucleotides complementary to the leader sequence were synthesized, one corresponding to the entire sequence (I), and a second in which there is a deletion of five nucleotides at the 3′ end (II). Both oligonucleotides completely blocked the translation in vitro of total RNA from *T. brucei* and *T. cruzi* in a rabbit reticulocyte lysate system. Figure 2A shows the translation products from *T. brucei* RNA in the presence of increasing amounts of (I). At a concentration of 4 μM of the oligonucleotide, translation was inhibited by 84% (Table 1). At concentrations of 8 μM and above, the inhibition of protein synthesis was essentially complete. This represents approximately a 1000-fold molar excess over the total poly(A)$^+$ message in the translation mixture. However, even at the highest concentration of the oligonucleotide studied (20 μM), there was no inhibition of translation of RNA from brome mosaic virus (lanes 6 and 7), or from rat liver. Likewise, in experiments in which brome mosaic virus RNA and trypanosome RNA were mixed, the oligonucleotide interfered only with trypanosome protein synthesis. Vast overexposure of the autoradiogram in Fig. 2A revealed the same pattern of bands in lanes 3 and 4 as first observed in lane 1, indicating that the small amount of remaining protein synthesis is not due to a discrete subset of trypanosome mRNA's that lack the leader sequence.

Similar results were obtained with oligonucleotide (II). At a concentration of 8 μM, this oligonucleotide completely inhibits translation of total RNA from both *T. brucei* and *T. cruzi* (Fig. 2B). Although the spliced leader sequences in *T. brucei* and *T. cruzi* differ in three positions (*20*), the oligonucleotide was equally effective in blocking translation of RNA from both organisms.

To demonstrate the specificity of the hybrid-arrested in vitro translation, an oligonucleotide complementary to the 35 nucleotides that follow the spliced leader in the IaTat 1.2 VSG mRNA was synthesized (Fig. 1). This oligonucleotide (III) prevents the synthesis of only the VSG (Fig. 3, lanes 2 to 4), a protein that immunoprecipitates with antiserum against the IaTat 1.2 VSG (lanes 6 and 7). Even at the highest concentration of the oligonucleotide (20 μM), there was no significant inhibition of the synthesis of other proteins (Table 1). Furthermore, an oligonucleotide with an unrelated sequence has no effect on the synthesis of the VSG (lane 5).

The data presented here demonstrate that oligonucleotides complementary to the spliced leader sequence selectively inhibit the translation of trypanosome mRNA's. All of the proteins of both *T. brucei* and *T. cruzi* appear to be equally affected. Overexposure of the autoradiograms in Fig. 2 did not reveal any minor proteins whose synthesis was unaffected by the presence of oligonucleotides (I) or (II). This result strongly suggests that all protein-coding RNA's of these protozoan parasites have the spliced leader sequence. Furthermore, the spliced leader sequence must be at the 5′ end of the

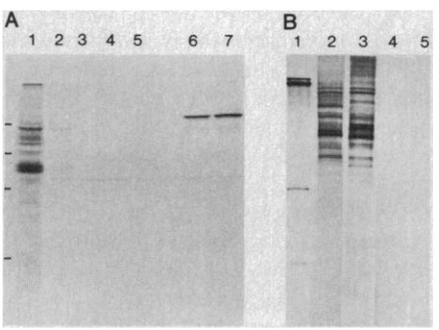

Fig. 2. The effect of oligonucleotides complementary to the spliced leader sequence on in vitro translation of trypanosome RNA. (A) (Lane 1) Total translation products of *T. brucei* RNA; (lanes 2 to 4) translation products synthesized in the presence of 4 μM, 8 μM, and 20 μM, respectively, of oligonucleotide (I); (lane 5) translation products in the absence of added RNA. The band at about 50 kD is an endogenous protein in the reticulocyte lysate; (lanes 6 and 7) translation products of brome mosaic virus RNA in the absence and in the presence of 20 μM oligonucleotide (I), respectively. Lines on the left indicate molecular weight markers of 92, 68, 43, and 30 kD. (B) Hybrid-arrested translation of *T. cruzi* RNA (lanes 2 and 4) and *T. brucei* RNA (lanes 3 and 5) with oligonucleotide (II). (Lanes 2 and 3) Total translation products; (lanes 4 and 5) translation products synthesized in the presence of 8 μM of the oligonucleotide; (lane 1) total translation products of brome mosaic virus RNA. Total RNA was isolated from *T. brucei* clone IaTat 1.2 (*19*) and *T. cruzi* Sylvio X-10/clone 4 (*27*) as described (*28*). Brome mosaic virus RNA was obtained from Promega Biotec. RNA (2.7 μg) was incubated either alone or in the presence of the oligonucleotide for 1 hour at room temperature in 11 μl of 10 mM tris-HCl (*p*H 7.5), 5 mM $MgCl_2$, 25 mM NaCl, 1 mM dithiothreitol. Translation was initiated by addition of 39 μl of a rabbit reticulocyte lysate system (Promega Biotec) that included 45 μCi of [^{35}S]methionine (Amersham). The indicated concentration of oligonucleotide is after addition of the reticulocyte lysate. After 1 hour at 30°C, 10-μl samples were removed, mixed with an equal volume of electrophoresis buffer, heated in a boiling water bath for 3 minutes, and loaded onto an acrylamide (4 to 12%) discontinuous slab gel (*29*). The gel was then fixed in fluorographic reagent (Amplify, Amersham), and exposed overnight at −80°C with an intensifying screen to preflashed Kodak XAR-5 film.

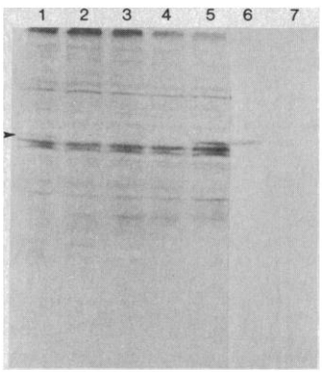

Fig. 3. Selective hybrid-arrested translation of *T. brucei* VSG mRNA. The conditions for the in vitro translations and the acrylamide gel electrophoresis are the same as in Fig. 2. (Lane 1) Total translations products of *T. brucei* IaTat 1.2 RNA; (lanes 2 to 4) translation products synthesized in the presence of 4 μM, 8 μM, and 20 μM, respectively, of oligonucleotide (III); (lane 5) translation products in the presence of 4 μM of an unrelated, nonspecific oligonucleotide; (lanes 6 and 7) immunoprecipitation of the translation products in lanes 1 and 4, respectively, with antiserum against the IaTat 1.2 VSG. The arrow on the left points to the VSG band.

Table 1. Hybrid-arrested translation of trypanosome RNA.

Oligonucleotide	Concentration (μM)	Translation products* (10^3 cpm)	Inhibition (%)
(I)	0	295	
	4	46	84
	8	6	98
	20	8	97
(III)	4	292	0
	8	313	−6
	20	259	12

*Samples (5 μl) of the translation mixtures in Figs. 2A and 3 were precipitated with 10% trichloroacetic acid, washed, and counted after being redissolved in 0.1*N* NaOH. The values reported are the average of two separate determinations.

message since it has been shown that oligonucleotides or cDNA fragments complementary to the coding regions do not effectively inhibit translation in rabbit reticulocyte lysates (*21–23*).

Oligonucleotide methylphosphonates, which have an uncharged internucleotide link, have been shown to enter cells and inhibit translation of their corresponding mRNA's (*24*). Capping the 5′- and 3′-hydroxyl groups with apolar substituents may also be sufficient to facilitate transport of the oligonucleotide into the cell and to inhibit degradation by exonucleases (*25, 26*). The high degree of specificity of the oligonucleotide for its target sequence suggests that this approach may be therapeutically useful. An oligonucleotide complementary to a portion of the spliced leader sequence should be lethal to the trypanosome, but would be without effect on host protein synthesis. Recent advances in DNA synthesis make it possible to test this approach.

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Regulation by Growth Hormone of Number of Chondrocytes Containing IGF-I in Rat Growth Plate

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Whether growth hormone stimulates longitudinal bone growth by a direct effect at the site of the growth plate or indirectly by increasing the concentration of circulating somatomedins (insulin-like growth factors) has been the subject of controversy. Immunohistochemical methods were used to explore the localization and distribution of insulin-like growth factor I (IGF-I) immunoreactivity in the epiphyseal growth plate of the proximal tibia of male rats. Cells in the proliferative zone of the growth plate of normal rats exhibited a bright immunofluorescence, whereas cells in the germinal and hypertrophic zones stained only weakly. In rats subjected to hypophysectomy, the number of fluorescent cells was markedly reduced. When the hypophysectomized rats were treated with growth hormone, either systemically or at the site of the growth plate, the number of IGF-I–immunoreactive cells in the proliferative zone was increased. The results show that IGF-I is produced in proliferative chondrocytes in the growth plate and that the number of IGF-I–containing cells is directly regulated by growth hormone. These findings suggest that IGF-I has a specific role in the clonal expansion of differentiated chondrocytes and exerts its function locally through autocrine or paracrine mechanisms.

ACCORDING TO THE SOMATOMEDIN hypothesis, growth hormone (GH) does not have a direct effect on cartilage, but instead stimulates chondrogenesis and subsequent growth indirectly through circulating peptide growth factors termed somatomedins (*1*). The insulin-like growth factor I (IGF-I), which is identical with somatomedin C (*2*), is growth hormone–dependent and generally considered to be the main mediator of the stimulatory effect of GH on somatic growth (*3–5*).

Investigators from our laboratory reported earlier that local injection of GH into the epiphyseal growth plate of the proximal tibia of hypophysectomized rats stimulates unilateral longitudinal bone growth (*6–8*). This observation has been confirmed by other investigators (*9*). We have also shown that chondrocytes isolated from rabbit ear and epiphyseal growth plate have specific binding sites for GH (*10*) and that GH stimulates DNA and proteoglycan synthesis in cultured chondrocytes from cartilage of rabbit ear and rat rib growth plate (*11, 12*). These results are inconsistent with an effect of GH mediated through circulating IGF-I.

Although IGF-I was traditionally considered to be produced in the liver, there is now evidence that IGF-I is synthesized in many different organs (*13–15*). We used immunohistochemical techniques to examine the possibility that IGF-I is produced in epiphyseal growth cartilage under the influence of GH. We studied the localization and distribution of IGF-I–like immunoreactivity in sagittal sections of the epiphyseal growth plate of the proximal tibia of normal and hypophysectomized male rats and observed

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