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12. The bottles were placed on a rotator (2 rev min^{-1}) in low light (3 to 20 μeinstein m^{-2} sec^{-1}; 12/12 light/dark) and 19°C. Samples were analyzed by cryofocusing on silylated glass beads in a liquid nitrogen bath, followed by chromatographic separation on a Chromosil 330 (Supelco) column with a flame photometric detector (Varian 3700) [see J. W. H. Dacey, S. G. Wakeham, B. L. Howes, *Geophys. Res. Lett.* **11**, 991 (1984)]. We prepared DMS standards by diluting a stock solution in filtered, gas-stripped seawater in identical 1-liter glass bottles. Headspace samples were withdrawn and analyzed in the same fashion.
13. The filters were placed in silylated serum tubes and stoppered with Teflon-faced stoppers; 2*N* KOH was added to the tubes, and the DMS headspace concentration was measured after 24 hours. DMSP is the only naturally occurring compound known to decompose to DMS in cold base (*8*).
14. Our unpublished data suggest that DMSP can be broken down to DMS by enzymes released during cell lysis. C. Wagner and E. R. Stadtman [*Arch. Biochem. Biophys.* **98**, 331 (1962)] and C. Wagner *et al.* [*J. Biol. Chem.* **241**, 1923 (1966)] described microbial degradation of DMSP to DMS by species of *Clostridium* and *Pseudomonas*.
15. In our experiment, daily DMS production in *G. nelsoni* equaled 0.3% of intracellular DMSP per day. We estimate from the data of Vairavamurthy *et al.* (*7*) that DMS production in *H. carterae* equaled about 1.4% of intracellular DMSP. DMS release is certainly dependent on physiological state, although Vairavamurthy *et al.* (*7*) suggest it is not a function of growth rate. All calculations are for DMSP-containing phytoplankton species only. Those phytoplankton without DMSP are not believed to produce DMS and are also unlikely to release DMS on ingestion.
16. For example, N. A. Welschmeyer and C. J. Lorenzen [*Limnol. Oceanogr.* **30**, 1 (1985)] concluded that the phytoplankton growth rate in the Pacific gyre is 0.2 per day and that all this production is consumed by zooplankton. They concluded that similar steady-state conditions usually exist in other environments, although growth rates are generally higher in coastal areas. This balance between phytoplankton growth and zooplankton grazing does not always exist, since phytoplankton blooms do occur.
17. We thank N. H. Marcus for making zooplankton and phytoplankton cultures available to us for study and for guiding us in their handling. We thank L. Hare and P. Alatalo for assistance in the conduct of the research. Supported by NASA grant NAGW-606 and NSF grant OCE-8416203. Contribution 6165 of the Woods Hole Oceanographic Institution.

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Static and Initiator Protein–Enhanced Bending of DNA at a Replication Origin

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DNA bending has been suggested to play a role in the regulation of gene expression, initiation of DNA replication, DNA packaging, and the recognition of specific DNA sequences by proteins. It has recently been demonstrated that DNA bending can be sequence-directed. Bent DNA has also been observed as a consequence of sequence-specific binding of proteins to DNA. In this report DNA of plasmid pT181 is shown to contain a bend at the replication origin. Furthermore, this bend is enhanced by the binding of the pT181 replication initiator protein, RepC, to the origin.

THE RESULTS FROM RECENT STUDIES have demonstrated the existence of sequence-directed static bends in DNA (*1–5*), as well as bending in response to binding of specific proteins (*1, 6, 7*). A role of DNA bends in such processes as gene expression, initiation of DNA replication, and DNA packaging is yet to be established. However, static bends in DNA may facilitate recognition and binding by specific proteins to these regions, and protein-induced or -enhanced bending of DNA may be critical for providing local structural alterations that are required for DNA transcription, replication, or packaging. DNA containing static bends has been isolated from the kinetoplasts of trypanosomes (K-DNA) (*1, 2*) and the origins of the replication of phage λ (*3*) and simian virus 40 (*4*). Furthermore, it has been suggested that bends in DNA may constitute recognition sites for the replication proteins in the origins of replication of simian virus 40 and λ (*3, 4*). In the R6K plasmid, the binding of the replication initiator protein induces a bend in the DNA at the replication origin (*6*). In addition, DNA bending is enhanced when the λ initiator protein, O, is bound to the origin (*7*). We now demonstrate that DNA from the plasmid pT181 origin of replication contains a bend, and that this bending is enhanced by the binding of the pT181 replication initiator protein, RepC.

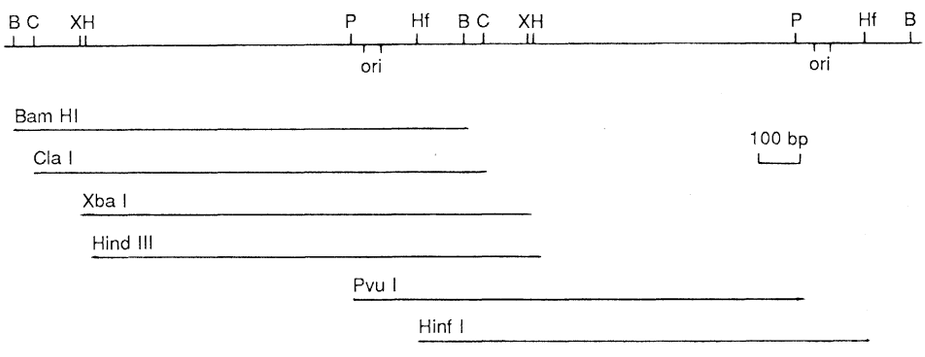

Fig. 1. DNA fragment used for bending analysis. A tandem duplication of the *repC* gene was constructed by partial digestion of plasmid pSK179 (a pUC7 derivative containing the *repC* gene cloned at the Hinc II site) with Bam HI, and inserting the Bam HI fragment from pSK179 that contained the pT181 DNA (*8*). Transformants were isolated, analyzed for orientation of the Bam HI fragment, and plasmid DNA purified by CsCl-ethidium bromide centrifugation. The DNA was digested with restriction enzymes that cut only once within the *repC* gene to generate a series of fragments, each of which was 1075 bp in length. The fragments were purified from acrylamide gels, precipitated with alcohol, and used for migrational analysis. The upper line shows the duplicated DNA with the restriction sites designated as follows: B, Bam HI; C, Cla I; H, Hind III; Hf, Hinf I; P, Pvu I; and X, Xba I. The pT181 origin of replication is designated *ori*. The fragments generated are shown below the restriction map and are designated by the enzyme used to produce them.

We have previously purified the replication initiator protein of pT181, RepC (*8*), and have shown that it binds to a 32–base pair (bp) sequence within the genetically defined origin of replication (*9*). The origin of replication containing the RepC binding site is located within the structural gene for RepC protein (*10, 11*). The protein nicks a single strand of the DNA within the origin and replication probably proceeds by the rolling circle mechanism (*12*). To test for the possibility of a bend in the pT181 origin, we constructed a plasmid that contains a tandem duplication of the *repC* gene (Fig. 1). Permuted restriction fragments, each consisting of 1075 bp, were generated to have the origin of replication located at different sites relative to the end of the fragment (*1*). The location of the origin region varied between 5 and 35% from one end of the fragment. The isolated DNA fragments were incubated in the presence and absence of RepC protein and analyzed by polyacrylamide gel electrophoresis. The series of permuted fragments shows migrational differences in the absence of RepC

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(Fig. 2, lanes 1 to 6) and the difference in migration is enhanced in the presence of RepC (lanes 7 to 12). In both cases the fragments that migrated most slowly were those that contained the pT181 origin nearest to the center of the fragment (lanes 4, 5, 10, and 11).

The results from Fig. 2 are depicted graphically in Fig. 3. Wu and Crothers (*1*) showed that, when the relative migration of a series of permuted fragments containing a bend was plotted against the position of the restriction sites, the resulting curve was sigmoidal. Furthermore, the lowest point on the curve corresponded to the bend site (*1*). This is consistent with our results (Fig. 3A). The data indicate that the center of the bend lies at 195 ± 15 bp (mean ± 1 SD) from the right end of the fragment (Fig. 1). When DNA is complexed with RepC protein (Fig. 3B), migrational differences are enhanced and the bend center appears to lie at nucleotide 215 ± 15 bp. These regions correspond to pT181 map coordinates 92 to 62 for the static bend and from 72 to 42 for the protein-enhanced bend (Fig. 4). The difference in results may be due to the asymmetric binding of RepC to the static bend in the DNA. It is likely that the center of the induced bend corresponds to the RepC binding site (*9*) that extends from nucleotide 37 to 68 on the pT181 map (positions 204–235 from the right end of the fragment in Fig. 1). These results are consistent with RepC binding to and enhancing a bent configuration within the origin. When the permuted DNA fragments are heat denatured and analyzed by electrophoresis, they display identical mobilities, indicating that the migrational anomaly is due to an altered helix configuration. We therefore conclude that the anomalous migration of the different fragments seen in Fig. 2 is the result of a bend in the DNA.

The most extensive examination of bent DNA has been conducted with the K-DNA from the trypanosomes (*1, 2*). The bend center was shown to lie within a segment of DNA containing four repeats of the sequence $CA_{5-6}T$ at 10-bp intervals (*1, 2*). This sequence was shown to be responsible for the bent configuration because replacement of one of the central A residues or changing the spacing between repeats eliminates the bend (*13*). A similar sequence motif is also found in the phage λ origin of replication, which has been shown to contain a static bend (*3*). In contrast to the above studies, crystal structural and physical analysis of the dodecamer CGCGAATTCGCG has shown that this DNA also contains a bend (*14–17*). The bend appears to involve the alternating purines and pyrimidines, resulting in steric interference that leads to an altered helical structure (*16, 17*). The DNA sequence of the pT181 origin of replication does not contain a sequence analogous to the K-DNA (*10, 11*). The origin does, however, contain a stretch of 21 nucleotides with the sequence 5′(Pu–Py)$_5$Pu(Pu–Py)$_5$3′ between nucleotides 37 and 57 (Fig. 4). This region partially overlaps the RepC-enhanced bend site.

Although the average base composition of pT181 DNA is about 30% G+C, the origin region contains about 50% G–C pairs (*10, 11*). This is in contrast with the observation that most origins of replication in the Gram negative bacteria are A+T–rich (*18*). Bent DNA has been associated primarily with A+T–rich DNA sequences (*1–3*). The situation appears to be reversed for pT181, where both the origin of replication and a bent DNA configuration are present within a G+C–rich segment, making it unusual in both respects. Although we favor a DNA bend as an interpretation of our results, another possible explanation would be the formation of a cruciform structure within the origin region. Such a structure can be inferred from the sequence of the origin (Fig. 4). A fragment containing a cruciform structure displays an altered gel mobility analogous to a bend (*19*). However, this structure is unlikely to form in linear DNA and has only been seen in supercoiled DNA (*19*).

In addition to static bends, protein-in-

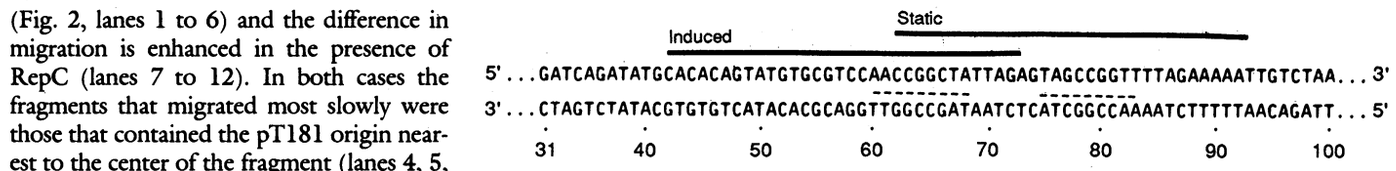

Fig. 4. DNA sequence of the pT181 origin region. Numbers correspond to the published sequence of pT181 (*10*). Solid lines indicate the probable bend site or sites. Broken lines indicate sequences that may be involved in the formation of a hairpin structure.

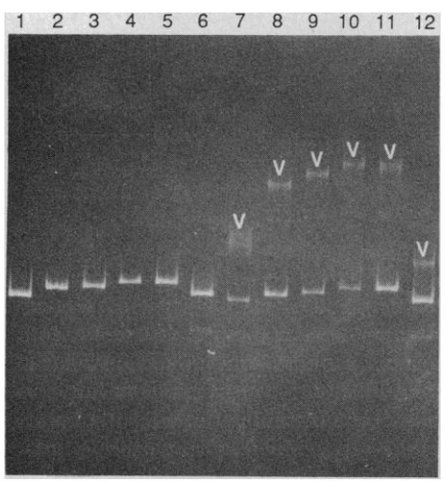

Fig. 2. Analysis of permuted fragments by polyacrylamide gel electrophoresis. The fragments, generated as described in the legend to Fig. 1, were separated by electrophoresis in a 5% polyacrylamide gel (acrylamide to bisacrylamide ratio of 70:1). The gels were run for 4 hours at 10 V/cm in a buffer containing 45 m*M* Tris-borate, *p*H 8.0, and 10 m*M* EDTA, stained with ethidium bromide, and photographed under ultraviolet illumination. Lanes 1 to 6 contain the fragments generated by Hinf I, Bam HI, Cla I, Xba I, Hind III, and Pvu I, respectively. Lanes 7 to 12 contain the same DNA's to which RepC protein was added at a protein to DNA molar ratio of approximately 1:1, and loaded on the gel after incubation at 20°C for 5 minutes. Arrows indicate the positions of the protein-DNA complexes.

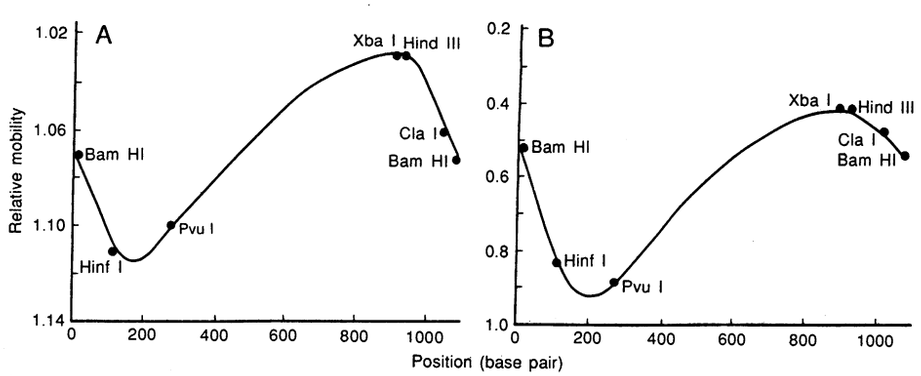

Fig. 3. Relative migration of the permuted fragments. The migrations of the fragments in Fig. 2 were compared with the migration of a 1206-bp marker fragment used as a standard. The results are plotted against the position of the restriction cleavage site (as measured from the right end of the fragment in Fig. 1). (A) DNA without RepC protein. (B) DNA incubated with RepC protein. In both cases extrapolation of the lowest point of the plot identifies the bend site (*1*).

duced and protein-enhanced bending of DNA has been observed for the origins of replication of plasmid R6K (*6*) and phage λ (*7*), as well as for the cyclic adenosine monophosphate gene-activating protein interaction with its binding site on the *lac* DNA, which shows limited homology to the RepC-enhanced bend site (*1*). These induced bends may have functional significance, such as bringing the protein into the proper spatial relation with its recognition sequence (*1, 4*), or opening the helix to allow replication (*6*). The static bends may act as recognition sites for protein-DNA interactions.

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A New Approach to the Adoptive Immunotherapy of Cancer with Tumor-Infiltrating Lymphocytes

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The adoptive transfer of tumor-infiltrating lymphocytes (TIL) expanded in interleukin-2 (IL-2) to mice bearing micrometastases from various types of tumors showed that TIL are 50 to 100 times more effective in their therapeutic potency than are lymphokine-activated killer (LAK) cells. Therefore the use of TIL was explored for the treatment of mice with large pulmonary and hepatic metastatic tumors that do not respond to LAK cell therapy. Although treatment of animals with TIL alone or cyclophosphamide alone had little impact, these two modalities together mediated the elimination of large metastatic cancer deposits in the liver and lung. The combination of TIL and cyclophosphamide was further potentiated by the simultaneous administration of IL-2. With the combination of cyclophosphamide, TIL, and IL-2, 100% of mice (n = 12) bearing the MC-38 colon adenocarcinoma were cured of advanced hepatic metastases, and up to 50% of mice were cured of advanced pulmonary metastases. Techniques have been developed to isolate TIL from human tumors. These experiments provide a rationale for the use of TIL in the treatment of humans with advanced cancer.

THE TREATMENT OF HUMANS WITH advanced metastatic cancer represents a major therapeutic challenge. One new approach to treating metastatic cancer is adoptive immunotherapy, a treatment in which immune cells with antitumor reactivity are transferred to the tumor-bearing host (*1*). The major obstacle to the use of adoptive immunotherapy has been the inability to generate, from cancer patients, immune cells with antitumor reactivity in numbers sufficient for cancer treatment. Virtually all prior experimental applications of this approach have utilized lymphocytes from highly immunized syngeneic animals, a cell source not available in the human (*2*).

In 1980, we described the lymphokine-activated killer (LAK) cell phenomenon, a method for generating cells capable of lysing fresh tumor by the incubation of lymphocytes in interleukin-2 (IL-2) (*3*). We reported that the adoptive transfer of LAK cells in conjunction with IL-2 was capable of mediating the regression of a variety of advanced metastatic cancers in humans (*4*). Of 55 patients treated with this approach, objective regression of cancer has been achieved in 21 patients, with complete regression of malignancy in five individuals (*4, 5*). Extensive testing of LAK cell therapy in vivo was conducted in various murine tumor models before this treatment was attempted in humans (*6*). These studies showed that tumor regression was optimally induced when both LAK cells and IL-2 were systemically administered and that LAK cells proliferated in vivo under the influence of IL-2 and maintained their cytolytic activity. Although highly effective in mediating tumor regression in some individuals, this treatment approach is cumbersome and can be toxic. Very large numbers of cells are required to mediate cancer regression (about 10^8 cells in a mouse and 3×10^{10} to 3×10^{11} cells in a human). In addition, the high doses of IL-2 required (approximately 100,000 units per kilogram every 8 hours) mediate toxic side effects, the most common of which is a capillary permeability leak syndrome that results in major fluid retention. We have thus sought means of generating cells with more antitumor reactivity and reducing the requirement for the administration of high doses of IL-2.

We have now identified a cell population that can be obtained from tumor-bearing patients and that appears to be 50 to 100 times more potent than LAK cells when used for adoptive immunotherapy. These cells, which constitute a subpopulation of lymphocytes that infiltrate into growing cancers, can be expanded in IL-2 to numbers sufficient to mediate the regression of large metastatic tumors. The adoptively transferred cells are effective in the absence of administered IL-2, although low doses of IL-2 can enhance their therapeutic efficacy. Successful therapy with these tumor-infiltrating lymphocytes (TIL) is dependent upon immunosuppression of the host at the time of treatment with either a high dose of cyclophosphamide or total body irradiation. TIL are highly effective in mediating the regression of advanced pulmonary and hepatic metastases in several mouse model tumors and are effective in curing mice with tumor burdens unaffected by the LAK/IL-2 adoptive immunotherapy system. We have recently developed techniques for isolating these TIL from human tumors (*7*). The experimental results presented in this report provide the rationale for testing this treatment approach in humans with advanced cancer.

A transplantable murine sarcoma and a murine colon adenocarcinoma in syngeneic C57BL/6 mice were used in these studies. The MCA-105 sarcoma was induced in our

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