7. J. H. Werren, U. Nur, D. Eickbush, *Nature (London)* **322**, 75 (1987).
8. DNA from *psr* males was partially digested with the restriction enzyme Sau 3A; 15- to 25-kb fragments isolated from a 5 to 20% sucrose gradient were ligated to the arms of Charon 35 [W. A. M. Loenen and F. R. Blattner, *Gene* **26**, 171 (1983)]. The recombinant DNA was packaged in vitro, plated, and amplified on strain KH802.
9. The Charon 35 library was plated at 1000 plaques per 150-mm petri plate. Two plaque lifts to nitrocellulose paper [W. D. Benton and R. W. Davis, *Science* **196**, 180 (1977)] were conducted of each plate. One set of filters was hybridized to 0.1 μg of *psr* male DNA labeled in vitro by nick-translation, while the second set of filters was hybridized to 0.1 μg of nick-translated control DNA. Hybridization was conducted at 65°C in 0.6*M* saline solution as described (Fig. 1). Final washes were at 65°C in 0.015*M* saline, 0.1% SDS.
10. D. L. Brutlag, *Annu. Rev. Genet.* **14**, 121 (1980); R. Flavell, *Annu. Rev. Plant Physiol.* **31**, 569 (1980); M. F. Singer, *Int. Rev. Cytol.* **76**, 67 (1982).
11. Because the size of the *N. vitripennis* genome is unknown, the absolute number of repeats per genome cannot be determined. However, a conservative estimate based on the size of the genome in *Drosophila melanogaster* (2×10^8 bp), would place their number at nearly 20,000 copies per haploid genome.
12. U. Nur, J. H. Werren, D. G. Eickbush, W. D. Burke, T. H. Eickbush, unpublished data.
13. Testes from *psr* and control male pupae were dissected out and kept for 1/2 hour in a solution containing 0.8% KCl and 0.2% colchicine. The testes were then rinsed in 70% ethanol, fixed in a 4:1 mixture of 95% ethanol, glacial acetic acid for 5 minutes, and stained and squashed in Lacto-aceto orcein.
14. A. Amos and G. Dover, *Chromosoma* **81**, 673 (1981); A. Lima-de-Faria *et al.*, *Hereditas* **99**, 203 (1983).
15. G. B. Saul II, *Z. Vererbungsl.* **92**, 28 (1961); S. L. Ryan and G. B. Saul II, *Mol. Gen. Genet.* **103**, 24 (1968).
16. S. L. Ryan, G. B. Saul II, G. W. Conner, *J. Hered.* **76**, 21 (1985).
17. For the incipient *psr* chromosome to be selected to cause paternal genome loss, however, the species must have a female-biased sex ratio. The necessary conditions are $Bx > 1 - x$ where x = proportion of eggs fertilized, and B = proportion of sperm from *psr* males carrying the *psr* chromosome. See J. H. Werren, *J. Theor. Biol.* **124**, 317 (1987); S. W. Skinner, *Heredity* **59**, 47 (1987).
18. R. N. Jones and H. Rees, *B Chromosomes* (Academic Press, New York, 1982).
19. G. Ostergren, *Bot. Not. Suppl.* **1945**, 157 (1945).
20. Because *psr* causes the production of all-male families, it is also expected to have a negative effect on populations carrying it, either by lowering the growth rate of these populations, or by causing them to become extinct. Laboratory experiments confirm this (J. H. Werren, unpublished data).
21. C. Yanisch-Perron *et al.*, *Gene* **33**, 103 (1985).
22. F. Sanger, S. Nicklen, A. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977).
23. Supported by NSF BSR-8796222, NIH GM31867, and NSF BSR-8306286.

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Scanning Tunneling Microscopy of recA-DNA Complexes Coated with a Conducting Film

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A link between scanning tunneling microscopy (STM) and conventional transmission electron microscopy has been established for biological material by applying STM on freeze-dried recA-DNA complexes coated with a conducting film. The topography of the complexes observed by means of STM revealed a right-handed single helix composed of about six recA monomers per helical turn.

SCANNING TUNNELING MICROSCOPY (STM) images structural and electronic properties of metal and semiconductor surface on an atomic scale (*1*). Imaging can be performed under atmospheric conditions, in insulating liquids, and even in electrolytes (*2–4*). The possibility of obtaining three-dimensional high-resolution images under various conditions makes STM an interesting technique for biological applications, with the ultimate hope of working in a biological environment that should preserve the biological sample in its native conformation. Exploratory STM efforts on DNA in vacuum (*5*) and on biological or organic matter under atmospheric conditions have been reported (*6–8*).

This report is on STM experiments on DNA and on complexes of DNA and recA, a protein involved in recombination, that were freeze-dried and coated with a conducting film. We have chosen recA-DNA complexes because their structure is a long, filamentous, well-characterized helix (*9, 10*) that is easy to distinguish on the support (Fig. 1). The periodicity is a signature to check reliability and reproducibility of imaging in different regions of the specimen. We used recA-DNA complexes formed in the presence of ATP[S] (the nonhydrolyzable analog of adenosine triphosphate). Image averaging and reconstruction (*9, 11*) from micrographs of negatively stained complexes and information from shadowed specimens show that this type of recA-DNA complex has a structure of a deeply grooved, right-handed helical filament of 10-nm diameter with about six recA monomers per helical turn. Very frequently, the complexes are not complete and have part of the DNA uncovered (Fig. 2).

Formed recA-DNA complexes (*9*) were purified from unbound recA protein on a Sepharose 2B column and subsequently adsorbed on platinum-carbon films (Pt-C) that were about 2 nm thick. The Pt-C film was evaporated on freshly cleaved mica with an electron gun. The tunneling current on this support was quite stable and not as noisy as on pure carbon films. The Pt-C films showed a granular structure with a typical grain size of 1.5 to 2 nm laterally and about 0.6 nm vertically. The adsorbing properties of Pt-C films for biological material were similar to those of carbon films that are routinely used for transmission electron microscopy (TEM) samples.

Freeze-drying followed by heavy-metal shadowing is an established TEM approach for routine structural studies of dehydrated biological specimens. Slow freeze-drying eliminates strong structural alterations due to effects of dehydration (*12*). To minimize film alterations after shadowing (such as oxidation or metal clustering caused by air contact), the metal films were stabilized with a relatively thick (5 nm) carbon-backing layer (*13*). Such a carbon coat would blur fine surface details when investigated with the tunneling microscope.

We found that at the right composition platinum-iridium-carbon films (Pt-Ir-C) remained three dimensionally stable after transferring to atmospheric conditions and that such films allowed, in contrast to pure carbon or Ir-C films, stable tunneling. In addition, Pt-Ir-C films showed a much smaller granularity than the standardly used Pt-C films, both in TEM and STM. Pt-Ir-C films were obtained by electron beam–induced evaporation of Pt-Ir cylinders (diameter 1.5 mm, 25% Ir) inserted in a carbon rod (diameter 2 mm). During evaporation the fraction of platinum decreased while the fraction of iridium and carbon increased. For stable tunneling a substantial content of metal was necessary, whereas for the three-

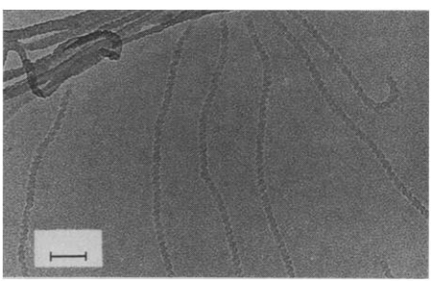

Fig. 1. TEM micrograph of freeze-dried recA-DNA complexes coated with a Pt-Ir-C film. The bar in the inset denotes a distance of 50 nm.

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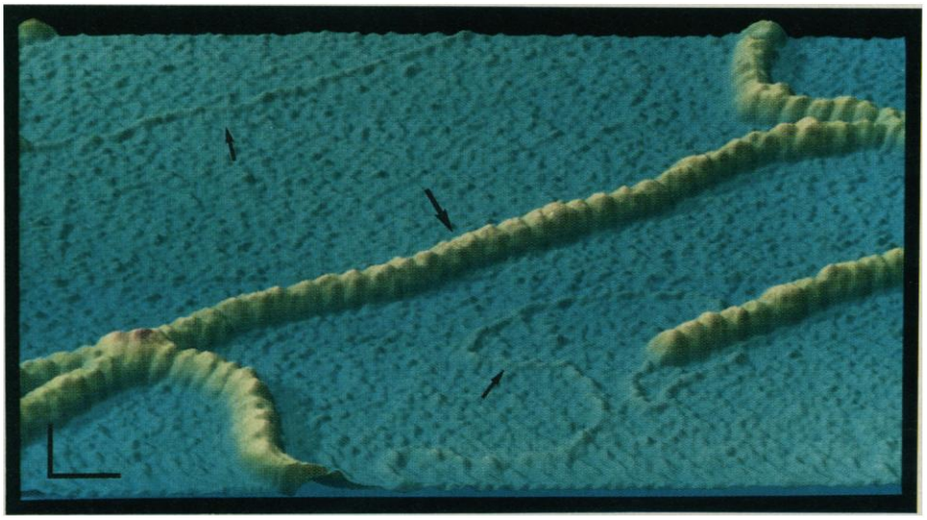

Fig. 2. Processed STM image with simulated shading of freeze-dried recA-DNA complexes coated with a Pt-Ir-C film. The area corresponds to 236 nm by 192 nm. Large arrows point to recA-DNA complex; small arrows point to free DNA. The bars denote a distance of 20 nm in each direction.

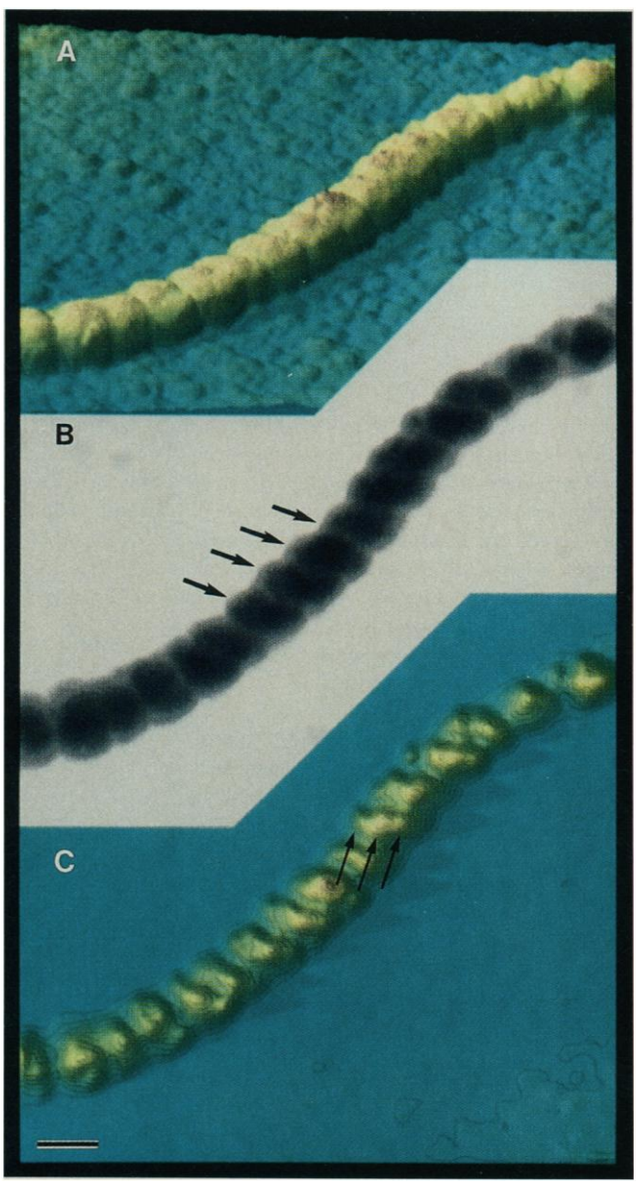

Fig. 3. Processed STM images of a segment of freeze-dried recA-DNA complex coated with Pt-Ir-C film; (**A**) Three-dimensional representation with simulated shading. (**B**) Top view in gray-tone representation. The striations, caused by the right-handed single helix, are pointed out by thick arrows. (**C**) A top view with simulated shading of the segment with the filament structures enhanced and the background corrugation suppressed. The tripartite structure of one striation is indicated by thin arrows. The bar denotes a distance of 10 nm.

dimensional stability, a substantial content of carbon was necessary (*14*).

Tunneling experiments were performed under atmospheric conditions with a pocket-size–type STM (*15*). A tunneling voltage of about 0.8 V, tip negative, proved to be suitable. We used cut or mechanically sharpened Ir-Pt tips or Au- tips that were chemically etched in concentrated HCl from wires 0.5 mm in diameter. Tips that imaged the objects in a properly symmetric way were carefully selected by trial and error. The images obtained in the constant current mode, that is, with the tip tracing contours of constant tunnel current, are referred to as STM images or topographs. Such images reflect the true topography only when the surface is electronically homogeneous as in the case of a conducting film.

Figure 1 presents recA-DNA complexes as they appeared in the TEM when they were prepared in the same way as for STM, except for the support, which was in this case a carbon film. The striation of the complexes was caused by the 10-nm pitch of their helical structure. The Pt-Ir-C coating film appeared to be nearly grainless.

In Fig. 2, a processed (*16*) STM image of freeze-dried recA-DNA complexes, adsorbed on Pt-C film and shadowed with Pt-Ir-C, is shown. The thick filamentous complexes with pronounced striation are clearly visible. This structural information is similar to that obtained by TEM (Fig. 1). Note that uncomplexed DNA segments extending from complexed regions are also clearly visible as free thin filaments.

Figure 3 shows three differently processed presentations of a segment of a freeze-dried recA-DNA complex coated with a Pt-Ir-C film. In Fig. 3A, in which tilt and simulated shading are introduced, the directly recorded three-dimensional structure of recA-DNA complexes can be seen. Figure 3B is a top view in a gray-tone scale, where the height is increased from bright to dark, and clearly shows a right-handed single helix. Figure 3C is a top view with shading of the recA-DNA segment, where protrusions of the filament are enhanced against the background corrugation. Many striations in top view show a tripartite structure that indicates that one helical turn of the complex contains about six such parts, which corresponds to the established number of recA monomers per turn (*9*). The width of the complexes amounts to 12 nm (similarly to TEM data, Fig. 1), and the height to 7 nm indicates flattening of the helical filaments caused by adsorption.

Studies by STM of biological samples coated with a conducting film promises to be a reliable technique with a resolution exceeding that of TEM. We prepared many

independent samples of recA-DNA complexes and always obtained images of similar quality. Our results prove that relief data at macromolecular level can be achieved directly without averaging. In case of recA-DNA complexes, the STM showed directly the number of recA monomers per helical repeat of the complex, whereas successful determination of this number with TEM required averaging (*9*). In TEM, the third dimension must be calculated from averaged different projections (*17*). When averaging is difficult, STM seems to be a straightforward, reliable way to reveal a three-dimensional structure, particularly for small relief corrugations that are difficult to reconstruct from projections.

REFERENCES AND NOTES

1. G. Binnig and H. Rohrer, *IBM J. Res. Develop.* **30**, 355 (1986).
2. G. Miranda *et al.*, *Appl. Phys. Lett.* **47**, 367 (1985).
3. S. Park and C. F. Quate, *ibid.* **48**, 112 (1986).
4. R. Sonnenfeld and P. K. Hansma, *Science* **232**, 211 (1986).
5. G. Binnig and H. Rohrer, in *Trends in Physics 1984*, J. Janta and J. Pantoflicek, Eds. (European Physical Society, Prague, 1984), pp. 38–46.
6. A. M. Baro *et al.*, *Nature (London)* **315**, 253 (1985).
7. A. Stemmer *et al.*, *Surf. Sci.* **181**, 394 (1987).
8. G. Travaglini, H. Rohrer, M. Amrein, H. Gross, *ibid.*, p. 380.
9. E. H. Egelman and A. Stasiak, *J. Mol. Biol.* **191**, 677 (1986).
10. A. Stasiak, E. DiCapua, Th. Koller, *ibid.* **151**, 557 (1981).
11. A. Stasiak and E. H. Egelman, in *DNA Replication and Recombination*, vol. 47 of the *UCLA Symposia on Molecular and Cellular Biology*, R. McMacken and T. J. Kelley, Eds. (Liss, New York, 1987), p. 619.
12. H. Gross in *Cryotechniques in Biological Electron Microcopy*, R. A. Steinbrecht and K. Zierold, Eds. (Springer-Verlag, Berlin, 1987), p. 205.
13. H. Gross, T. Müller, I. Wildhaber, H. Winkler, *Ultramicroscopy* **16**, 287 (1985).
14. We used preevaporated rods to ~60% of the Pt-Ir cylinder. The samples were freeze-dried for 3 hours and rotary shadowed (elevation angle 60°) at 193 K with ~1 nm of Pt-Ir-C by using a standard high-vacuum freeze-etching apparatus (Balzers BAF 400T).
15. Ch. Gerber, G. Binnig, H. Fuchs, O. Marti, H. Rohrer, *Rev. Sci. Instrum.* **57**, 221 (1986).
16. E. Stoll and O. Marti, *Surf. Sci.* **181**, 222 (1987).
17. D. J. DeRosier and A. Klug, *Nature (London)* **217**, 130 (1968).

18. We thank our colleagues H. Rohrer, A. Baratoff, P. Ballone, Th. Koller, J. Sogo, and N. Garcia for valuable discussions. We also thank F. Rohner and R. Florin for technical assistance and B. Müller for providing us with recA protein.

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Interferon-γ: The Major Mediator of Resistance Against *Toxoplasma gondii*

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Mice were injected with a monoclonal antibody to interferon-γ to examine the importance of endogenous production of this lymphokine in resistance against infection with the sporozoan parasite *Toxoplasma gondii*. Mice with intraperitoneal infections of *T. gondii* that received no antibody survived and developed chronic *T. gondii* infection, whereas the infected mice that received the monoclonal antibody died of toxoplasmosis. The activation of macrophages, which kill *T. gondii* in vivo, was inhibited by administration of the monoclonal antibody, but the production of antibodies to *T. gondii* was not suppressed. The fact that an antibody to interferon-γ can eliminate resistance to acute *Toxoplasma* infection in mice suggests that this lymphokine is an important mediator of host resistance to this parasite.

DATA FROM STUDIES PERFORMED in vitro and in vivo suggest that the major mechanism of resistance against *Toxoplasma gondii* is cell-mediated (*1*). Adoptive transfer of lymphocytes confers strong resistance against challenge infection with *T. gondii* in laboratory animals (*2*), whereas passive transfer of antibodies does not (*3*). Recently, the high prevalence of toxoplasmic encephalitis reported in patients with acquired immunodeficiency syndrome (AIDS) and the fact that almost all such cases occur in individuals who have serologic evidence of prior infection with *T. gondii* have underscored the importance of T cell–mediated immunity against this organism. The major immunological defect in patients with AIDS is the decreased numbers and function of CD4 lymphocytes (*4*).

On the basis of studies in vitro, we (*5*) and others (*6, 7*) have postulated that the activated macrophage is the effector arm of cell-mediated immunity against *T. gondii*. Interferon-γ (IFN-γ) has the capacity to activate mouse macrophages in vitro (*7, 8*) and in vivo (*9*), and human macrophages in vitro inhibit or kill this parasite (*8, 10*). When IFN-γ was administered in vivo, significant protection against *T. gondii* was observed in a mouse model of toxoplasmosis (*9*). These observations suggest that IFN-γ is of primary importance in host resistance against *T. gondii*. In all of these studies, exogenous IFN-γ was used as the macrophage-activating factor. Thus, the relative importance of IFN-γ to resistance against this parasite has not been evaluated during the natural host response to infection with *T. gondii*. Our experiments reveal that IFN-γ is essential for resistance to acute *T. gondii* infection in vivo.

All control mice that received saline or normal hamster immunoglobulin G (IgG) survived intraperitoneal or oral infection with an avirulent strain of *T. gondii* (strain ME49). In contrast, seven out of seven mice injected with a monoclonal antibody to IFN-γ (H22.1) died after intraperitoneal inoculation with the parasite, and four of five died after peroral infection (Table 1). This H22.1 antibody (100 or 200 μg) caused no apparent illness or mortality in noninfected BALB/c mice. The effect of H22.1 on host resistance to infection with *T. gondii* was dose-dependent (Fig. 1). Whereas all mice that received normal hamster IgG survived the infection, a dosage of the monoclonal antibody as small as 7.4 μg resulted in death due to toxoplasmosis in 83% (5/6) of the mice. All mice that received 2.5 μg of the antibody survived, but all mice that received 67 μg of the monoclonal antibody died of the infection. Buchmeier and Schreiber (*11*) reported that this same monoclonal antibody, H22.1, significantly reduced resistance against *Listeria*

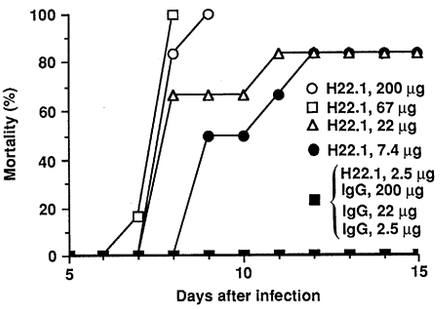

Fig. 1. Dose effect of monoclonal antibody to IFN-γ on resistance to infection with *T. gondii*. Mice were injected intraperitoneally with various doses of H22.1 or normal hamster IgG diluted in saline. There were six mice in each group. One day later, mice were infected intraperitoneally with 18 cysts of *T. gondii* strain ME49 prepared as described (Table 1). No deaths occurred in the group treated with 2.5 μg of H22.1 alone or in any of the three groups treated with IgG alone. The latter results are depicted as solid squares on the abscissa.

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