It is difficult to detect virus in mature circulating monocytes (*16*), and virus has not been recovered from mature granulocytes. In vivo, therefore, it is likely that only a very small proportion or a subpopulation of $CD34^+$ bone marrow progenitor cells are infected with HIV-1 and the remainder differentiate normally into mature blood elements. This could explain why cytopenias of granulocyte and monocyte lineage in HIV-1 infection are relatively uncommon (*2, 3*). The extent to which infection of bone marrow progenitor cells can explain the observed hematologic abnormalities in HIV-1 infection is uncertain. The studies of Donahue *et al.* (*6*) suggest that immune-mediated response to infected progenitor cells may have a significant role in the pathogenesis of these abnormalities. Our results further indicate that in the absence of growth factors the monocytoid cells that are derived from these progenitors may persist without detectable cell division, since mitotic figures were never observed and cell numbers did not increase during the culture period of 40 to 60 days. This minimizes the likelihood of infection of a subset of $CD34^+$ cells and subsequent outgrowth of a minor infected population. Nevertheless, even if only a small proportion of precursor cells could accumulate the quantity of viral particles in vivo as was observed in our in vitro system, this could provide an important reservoir of HIV-1 and could contribute to the dissemination of virus. Furthermore, there is precedent for bone marrow serving as a reservoir for retroviral infection. In the feline leukemia virus system a latent form of the virus resides predominantly in myelomonocytic precursors of the bone marrow (*17*).

The fact that these progenitor cells could be infected with HIV-1 in the initial absence of detectable surface or intracellular CD4 protein does not rule out its involvement (*18*). Clearly, undetectable CD4 could be present and serve as the HIV-1 receptor in these cells. Alternatively, phagocytosis might serve as a mode of viral entry. The finding that CD4 develops during differentiation of these cells might also represent the mechanism of viral spread in the cultures and explain the need to culture cells for an extended time before detection of virus. More important, the gradual differentiation of these cells into cells of the monocytoid lineage is consistent with monocytes serving as viral reservoirs (*19*) and with their potential for disseminating infection to other organs.

REFERENCES AND NOTES

1. *Morb. Mortal. Wkly. Rep.* **35**, 334 (1986).
2. J. L. Spivak, B. S. Bendar, T. C. Quinn, *Am. J. Med.* 77, 224 (1984).
3. D. R. Schneider, L. J. Picker, *Am. J. Clin. Pathol.* **84**, 144 (1985).
4. F. Delacretaz, L. Perez, P. M. Schmidt, J. P. Chave, J. Costa, *Virch. Arch. A* **411**, 543 (1984).
5. M. Busch, J. Beckstead, D. Gantz, G. Vyas, *Blood* **68** (suppl. 1), 122a (1986).
6. R. E. Donahue, M. M. Johnson, L. I. Zon, S. C. Clark, J. E. Groopman, *Nature* **326**, 200 (1987).
7. D. Klatzman *et al.*, *Science* **225**, 59 (1984); M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *ibid.* **224**, 497 (1984); A. G. Dalgleish *et al.*, *Nature* **312**, 763 (1984); D. Klatzman *et al.*, *ibid.* **312**, 767 (1984); J. S. McDougal *et al.*, *J. Immunol.* **135**, 3151 (1985); J. S. McDougal *et al.*, *Science* **231**, 382 (1986).
8. D. D. Ho, T. R. Rota, M. S. Hirsch, *J. Clin. Invest.* **778**, 1712 (1986); J. K. A. Nicholson, G. D. Gross, C. S. Collaway, J. S. McDougal, *J. Immunol.* **137**, 323 (1986); S. Gartner *et al.*, *Science* **233**, 215 (1986); S. Roy and M. A. Wainberg, *J. Leuk. Biol.* **43**, 91 (1988).
9. C. I. Civin *et al.*, *J. Immunol.* **133**, 157 (1984).
10. R. G. Andrews, J. W. Singer, I. D. Bernstein, *Blood* **67**, 842 (1986); L. Lue *et al.*, *J. Immunol.* **139**, 1823 (1987).
11. S. W. Kessler, D. Vembu, A. T. Black, *Blood* **70** (suppl. 1), 321a (1987).
12. S. W. Kessler, *Fed. Proc.* **46**, 1363 (1987).
13. Adherent and nonadherent, and infected and uninfected cells were prepared for TEM. Nonadherent cells were centrifuged (600*g*), fixed overnight in 2.5% glutaraldehyde in 0.1*M* cacodylate buffer, *p*H 7.4, postfixed in 1% OsO_4, block-stained in saturated uranyl acetate (UA) in 50% ethanol, dehydrated in graded ethanol and propylene oxide, and embedded in Spurr's plastic. Attached cells were processed in situ through 100% ethanol, removed from the well after adding propylene oxide, and embedded in Spurr's (method of C. Oliver, NIH, personal communication). Thin sections were stained with uranyl acetate and lead citrate and examined on a Zeiss EM 10A.
14. T. M. Folks *et al.*, *J. Immunol.* **140**, 1117 (1988); S. M. Hammer, J. W. Gillis, J. E. Groopman, R. M. Rose, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8734 (1986); P. Clapham *et al.*, *Virology* **158**, 44 (1987); C. J. Petit, F. G. Terpstra, F. Miedema, *J. Clin. Invest.* **79**, 1883 (1987); J. A. Levy *et al.*, *Virology* **147**, 441 (1985).
15. T. M. Folks *et al.*, *Science* **238**, 800 (1987).
16. S. Z. Salahuddin *et al.*, *Blood* **68**, 281 (1986); A. Ranki *et al.*, *Lancet* **ii**, 589 (1987).
17. R. G. Olsen, *Cancer Metast. Rev.* **6**, 243 (1987); J. I. Mullins, C. S. Chen, E. A. Hoover, *Nature* **319**, 333 (1986).
18. J. Sodroski, W. C. Goh, C. Rosen, K. Campbell, W. A. Hazeltine, *Nature* **322**, 470 (1986); J. D. Lifson *et al.*, *Nature* **323**, 725 (1986); J. A. Hoxie *et al.*, *Science* **234**, 1123 (1986).
19. H. E. Gendelman *et al.*, *J. Exp. Med.* **167**, 1428 (1988); J. M. Orenstein, M. S. Meltzer, T. Phipps, H. E. Gendelman, *J. Virol* **62**, 2578 (1988).
20. For above details on the cells and the purification procedures, see S. W. Kessler, in preparation.
21. R. Wiley *et al.*, *J. Virol.* **62**, 139 (1988).

22. We thank the Bone Marrow Procurement Team of the NMRI Tissue Bank and A. T. Black and D. M. Smoot for technical assistance. Supported by Naval Medical Research & Development Command Work Unit No. 63706N.M0095.003.1007. We also thank S. Honig and R. Roberts for technical assistance and B. Potts for the macrophage tropic HIV-1 strain.

9 September 1988; accepted 15 September 1988

Transcriptional Activation of a Cutinase Gene in Isolated Fungal Nuclei by Plant Cutin Monomers

GOPI K. PODILA, MARTIN B. DICKMAN, P. E. KOLATTUKUDY *

The molecular mechanism by which fungal spores that land on plants sense the contact and consequently trigger cutinase gene expression to gain entry into the plant was studied in isolated nuclei. Nuclear runoff experiments showed that the induction involved transcriptional activation. Monomers, unique to cutin, and a soluble protein factor from the fungal extract selectively activated cutinase gene transcription, probably by promoting initiation in nuclei isolated from uninduced *Fusarium solani pisi*. This in vitro transcriptional activation produced normal-sized cutinase messenger RNA. Alterations in the monomer structure diminished the transcriptional activation.

FUNGAL PATHOGENS PENETRATE THE cuticular barrier of plants by using cutinase, an extracellular enzyme produced by the germinating spore (*1*). The fungal spore senses the contact with the plant surface when the small amount of cutinase carried by the spore releases 10,16-dihydroxyhexadecanoic acid and 9,10,18-trihydroxyoctadecanoic acid from the insoluble structural polyester of plant cuticle. These monomers, which occur in nature only as components of cutin (*2*), were found to be potent inducers of cutinase in the spores of *Fusarium solani pisi (Nectria haematococca)* (*3*). We now show that these monomers from the plant cuticle trigger transcription of a cutinase gene in the fungal pathogen. We also show that the dihydroxy fatty acid component of plant cuticle, together with a soluble protein factor from the fungus, induces selective transcription of the cutinase gene in isolated nuclei. We used this in vitro transcriptional activation as an experimental system to elucidate how a plant cuticle component is used by the pathogen as a molecular signal to sense contact with the plant and how this signal is used to transcribe the gene whose product is necessary for fungal entry into the host.

Ohio State Biotechnology Center and Department of Biochemistry, Ohio State University, Columbus, OH 43210.

*To whom correspondence should be addressed at Biotechnology Center, Ohio State University, Rightmire Hall, Columbus, OH 43210.

Nuclear runoff experiments showed that the cutin monomer induced cutinase transcription in *F. solani pisi*. When nuclei, isolated from the mycelia after different periods of induction with 10,16-dihydroxyhexadecanoic acid, were incubated with ^{32}P-labeled uridine triphosphate ([^{32}P]UTP), the amount of label incorporated into cutinase transcripts increased with increasing periods of induction and reached a maximum at about 28 hours (*4*). This incorporation represented transcriptional activity, as shown by inhibition of labeling by the RNA-polymerase inhibitor α-amanitin. The cutinase transcript generated in these experiments was identical in size to cutinase mRNA. The appearance of cutinase in the culture medium showed a lag as expected (*4*).

We then attempted to induce transcription of the cutinase gene in isolated nuclei. When nuclei isolated from uninduced mycelia were incubated with [^{32}P]UTP, little label was incorporated into cutinase transcripts isolated by hybridization with cutinase cDNA. Addition of different concentrations of 10,16-dihydroxyhexadecanoic acid or 9,10,18-trihydroxyoctadecanoic acid together with [^{32}P]UTP did not result in labeling of any cutinase transcript (Fig. 1A). However, when the cutin monomer was added with an aliquot of the supernatant remaining after sedimenting the nuclei, a large increase in the incorporation of label into the cutinase transcript was observed. The supernatant factor alone had no effect on labeling of the cutinase transcript. When the supernatant was boiled or treated with immobilized protease, its cutinase transcription-stimulating activity was lost. When the supernatant was subjected to gel filtration on Sephadex G-25, the transcription-stimulating factor was excluded. These results suggest that the factor involved in the activation of cutinase gene transcription is proteinaceous.

To test whether the stimulation of cutinase gene transcription in the isolated nuclei by the cutin monomer and the protein factor is specific to the cutinase gene, we examined transcription of an actin gene. The total incorporation of label into RNA by the isolated nuclei was not affected by the inducing agents. Labeling of an actin gene transcript isolated by hybridization with actin cDNA (*5*) was unchanged by the addition of cutin monomer and the protein factor under conditions that caused large stimulation of cutinase transcript formation (Fig. 1B). Thus, the cutin monomer selectively induces cutinase gene transcription in the isolated nuclei from *F. solani pisi*.

Novobiocin, an antibiotic that inhibits the initiation of transcription (*6, 7*), prevented activation of cutinase gene transcription by the cutin monomer and the protein factor in the isolated nuclei (Fig. 1C). On the other hand, novobiocin had no effect on incorporation of label into cutinase transcripts by nuclei isolated from induced cultures in the typical nuclear runoff experiments (*4*). These results suggest that the cutin monomer and the protein factor or factors affect the preinitiation complex formation and thus promote initiation of cutinase gene transcription.

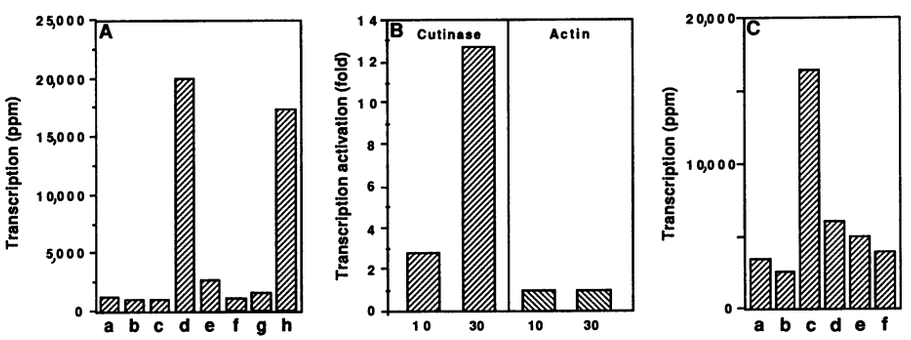

Fig. 1. (**A**) Activation of cutinase gene transcription in isolated nuclei. Each reaction mixture (200 μl) containing nuclei (5 μg of DNA) isolated (*10*) from glucose-grown *F. solani pisi* culture (*11*) was incubated with [^{32}P]UTP and the other components needed for transcription (*12*) in the presence of 10,16-dihydroxyhexadecanoic acid or other components indicated below. Transcription rate (in parts per million), measured by hybridization to cutinase cDNA (*13*), was calculated with respect to ^{3}H-labeled cutinase cRNA (*12*). (Lane a) Control with no cutin monomer or cell extract; (lane b) 10,16-dihydroxyhexadecanoic acid (40 μg) from cutin; (lane c) supernatant from cell extract containing 20 μg of protein; (lane d) cutin monomer as in lane b plus cell extract supernatant as in lane c; (lane e) cutin monomer plus boiled cell extract supernatant; (lane f) cutin monomer plus cell extract supernatant treated for 1 hour with immobilized *Staphylococcus aureus* V_8 protease (Sigma); (lane g) cutin monomer plus cell extract supernatant included in Sephadex G-25; and (lane h) cutin monomer plus cell extract supernatant excluded by Sephadex G-25. (**B**) Differential effects of cutin monomer and the cell extract supernatant on transcription of cutinase and actin genes. Nuclei were incubated with or without cutin monomer plus cell extract supernatant for 10 min or 30 min, and transcripts of cutinase and actin (*5*) were measured. (**C**) Effect of novobiocin on activation of cutinase gene transcription in isolated nuclei. (Lane a) Control with no monomer or protein factor; (lane b) same as lane a with 0.1 m*M* novobiocin; (lane c) monomer plus protein factor; and (lanes d, e, and f) same as lane c with 0.1 m*M*, 1 m*M*, or 10 m*M* novobiocin, respectively.

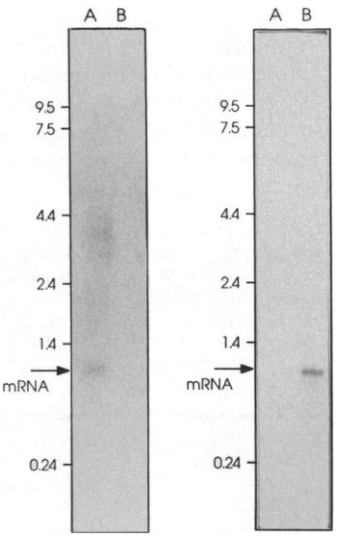

Fig. 2. Electrophoresis of the cutinase transcript generated by the isolated nuclei. (**Left**) Lane A, with, and lane B, without cutin monomer plus protein factor. Cutinase transcripts isolated by hybrid selection were separated by electrophoresis on a denaturing gel (*14*). (**Right**) RNA blot of transcripts generated by the nuclei in the absence (lane A) or presence (lane B) of monomers and protein factor. Unlabeled cutinase RNA generated by isolated nuclei was separated by electrophoresis, and ^{32}P-labeled cutinase cDNA (*13*) was used as the probe. Sizes of cutinase mRNA (arrow) and RNA markers (in kilobases) are indicated.

If the transcription of cutinase gene, stimulated by cutin monomer and the protein factor in the isolated nuclear preparation, is correctly initiated and terminated, the resulting processed transcript should be of the same size as the normal mRNA generated by the fungus. Electrophoresis of the labeled transcripts isolated from nuclei incubated with [^{32}P]UTP, the protein factor, and 10,16-dihydroxyhexadecanoic acid followed by autoradiography revealed that the labeled transcript was identical in size to the cutinase mRNA (Fig. 2, left). RNA blot analysis of unlabeled transcripts generated by the isolated nuclei showed a single band that hybridized with ^{32}P-labeled cutinase cDNA, and the size was identical to that of cutinase mRNA (Fig. 2, right).

The cutinase transcription-stimulating activity showed a dose-dependent response for both the cutin monomer and the protein factor. As the amount of dihydroxyhexadecanoate was increased, cutinase transcription increased with a maximum at about 200 μg/ml; larger amounts of the monomer showed less than maximal stimulation (Fig. 3A). Increasing the amount of protein factor

increased the stimulation of cutinase transcription to a maximum at 100 μg/ml, with an inhibitory effect at higher concentration (Fig. 3B). The time course of cutinase transcription stimulation showed a lag of about 30 min, and near maximal stimulation was observed in about 1 hour (Fig. 3C). To test whether the requirement for this period represents the time required for the transformation of the dihydroxy fatty acid into a transcription-stimulating factor, we incubated the acid with the protein factor for 1 hour before addition to the transcription reaction mixture. This preincubation did not affect the time course of transcriptional activation; maximal stimulation still required about 1 hour. Similarly, preincubation of nuclei with either the monomer or the protein factor alone did not reduce the time required for maximal stimulation (data identical to those in Fig. 3C). Preincubation with both the monomer and the protein factor (or factors) before addition of the [^{32}P]UTP eliminated the lag period (Fig. 3D). Thus, both the monomer and the protein factor are required to trigger biochemical reactions for selective activation of cutinase gene transcription.

Table 1. Activation of cutinase gene transcription in isolated nuclei of *F. solani pisi* by cutin monomers and their analogs in the presence of protein factor. In each case 40 μg of the hydroxy acid and 20 μg of protein factor were used in 200 μl of reaction mixture as described in Fig. 1. Control transcription rate (1075 ppm) was subtracted from all the treatments.

Structure	Transcriptional activation (ppm)
$CH_2(CH_2)_5CH(CH_2)_8COOH$ OH OH 10,16-Dihydroxyhexadecanoic acid	7350
$CH_2(CH_2)_7CH–CH(CH_2)_7COOH$ OH OH OH 9,10,18-Trihydroxyoctadecanoic acid	4500
$CH_2(CH_2)_5CH–CH(CH_2)_7COOH$ OH OH OH 9,10,16-Trihydroxyhexadecanoic acid	2580
$CH_3(CH_2)_5CHCH_2CH=CH(CH_2)_7COOH$ OH 12-Hydroxyoctadec-9-enoic acid	1840
$CH_3(CH_2)_{13}CHCOOH$ OH α-Hydroxyhexadecanoic acid	1760
$CH_2(CH_2)_{14}COOH$ OH ω-Hydroxyhexadecanoic acid	1730

The cutinase transcription-activating protein factor remained in the supernatant upon centrifugation at 105,000*g* for 90 min. Upon gel filtration of the high-speed supernatant on a Sepharose 6B column, the transcription-activating protein was retarded and eluted in fractions that indicated a molecular size of about 100 kD. The transcription-activating factor emerged at the void volume when the fractionation was done under low ionic strength in 10 m*M* tris-Cl buffer, suggesting aggregation.

If the cutinase transcription-stimulating effect observed in the isolated nuclear preparation represents cutinase induction by the monomer in germinating spores (*3*), the in vitro system should show specific requirement for the cutin monomer structure. When the midchain hydroxyl group of 10,16-dihydroxyhexadecanoic acid was removed, the transcription-stimulating effect was greatly decreased; the ω-hydroxy acid was much less effective (Table 1). α-Hydroxy fatty acids also could not substitute for the cutin monomer. The ω-hydroxyl group was found to be essential for the transcription-activating effect, as ricinoleic acid (12-hydroxyoctadec-9-enoic acid), which has a midchain hydroxyl group but not an ω-hydroxyl group, could not substitute for the cutin monomer. 9,10,18-Trihydroxyoctadecanoic acid, another cutin monomer (*2*), also stimulated cutinase transcription. 9,10,16-Trihydroxyhexadecanoic acid, a molecule similar to 9,10,18-trihydroxyoctadecanoic acid, showed some stimulation of cutinase gene transcription. The stringent structural requirement for the activation of cutinase gene transcription is consistent with the conclusion that the observed transcriptional activation represents the mechanism by which the germinating fungal spore (*3*) senses contact with the plant surface with the resultant activation of genes necessary for penetration into the plant.

Our results show that cutin monomers trigger transcription of cutinase, the enzyme involved in fungal penetration into plants. We observed selective activation of transcription of a pathogenesis-related gene in isolated nuclei of a pathogen by a host component. How the monomers affect cutinase transcription remains unknown. The observation that transcriptional activation by the monomers can occur in isolated nuclei provides an experimental system to explore the underlying mechanism. The protein factor might be simply a carrier of the monomer into the nucleus or it might directly bind to DNA and trigger the transcriptional activity by mechanisms analogous to those involved in steroid hormone action in animals (*8*). In vitro stimulation of transcription of specific genes by small signal molecules has rarely been reported. Even in such thoroughly studied cases as steroid hormones, in vitro stimulation of transcription of a gene by a steroid was detected only recently (*9*). Whatever the details of the mechanism, it is clear that the pathogenic fungus has an effective mechanism that allows it to sense contact with the plant surface and consequently trigger expression of a gene necessary to penetrate the defensive barrier of the plant. This mechanism is based on the transcriptional activation that depends on the unique structure of the cutin monomer and thus provides the specificity necessary for triggering gene expression only upon contact with the plant. Understanding of such mechanisms might lead to effective ways of interfering with the process of fungal penetration into plants and thus

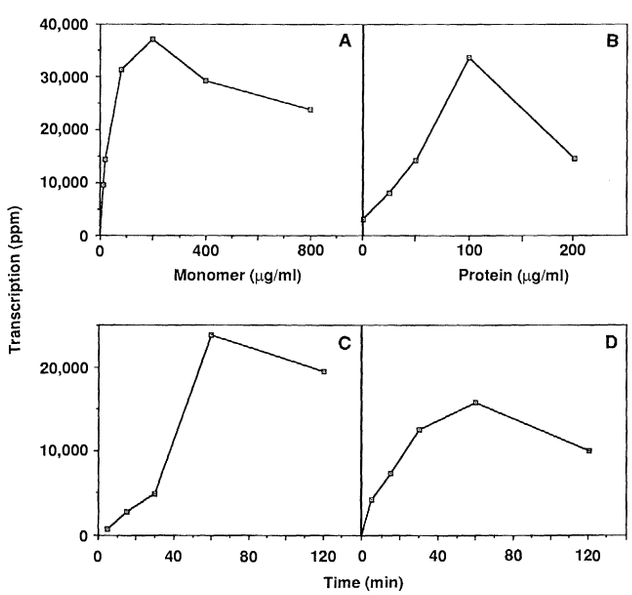

Fig. 3. Effect of the concentrations of cutin monomer and protein factor on activation of cutinase gene in isolated nuclei from *F. solani pisi*. In (**A**) 20 μg of protein factor, in (**B**) 40 μg of 10,16-dihydroxyhexadecanoic acid, and in (**C**) and (**D**) 20 μg of protein factor plus 40 μg of 10,16-dihydroxyhexadecanoic acid were used in 200-μl reaction mixtures. In (A) and (B) a 60-min incubation period was used. In (C) all components were added before incubating the reaction mixture for the incubation period shown, whereas in (D) all components except [^{32}P]UTP were incubated for 30 min prior to the addition of the labeled nucleotide. All experiments were repeated at least three times, and similar results were obtained.

assist in protecting plants against fungal diseases.

REFERENCES AND NOTES

1. P. E. Kolattukudy, *Annu. Rev. Phytopathol.* **23**, 223 (1985).
2. ———, in *The Biochemistry of Plants*, P. K. Stumpf and E. E. Conn, Eds. (Academic Press, New York, 1980), vol. 4, p. 571; *Annu. Rev. Plant Physiol.* **32**, 539 (1981); P. J. Holloway, in *The Plant Cuticle*, D. F. Cutler, K. L. Alvin, C. E. Price, Eds. (Academic Press, London, 1982), p. 45.
3. C. P. Woloshuk and P. E. Kolattukudy, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1704 (1986).
4. C. X. Huang *et al.*, unpublished results.
5. P. E. Kolattukudy *et al.*, *Arch. Biochem. Biophys.* **256**, 446 (1987).
6. M. L. Webb, K. A. Maguire, S. T. Jacob, *Nucleic Acids Res.* **15**, 8547 (1987).
7. M. L. Webb and S. T. Jacob, *J. Biol. Chem.* **263**, 4745 (1988).
8. K. R. Yamamoto, *Annu. Rev. Genet.* **19**, 209 (1985).
9. B. Corthésy, R. Hipskind, I. Theulaz, W. Wahli, *Science* **239**, 1137 (1988).
10. T. S. Lin and P. E. Kolattukudy, *J. Bacteriol.* **133**, 942 (1978).
11. Nuclei from *F. solani pisi* were isolated as described [W. E. Timberlake, in *Isolation of Stage- and Cell-Specific Genes from Fungi* (North Atlantic Treaty Organization Advanced Study Institutes Series), J. Bailey, Ed. (Springer-Verlag, Heidelberg, 1986), vol. H1, pp. 343–357] with some modifications. Briefly, mycelia collected by filtration were washed several times with distilled water. For each gram of tissue, 2 ml of SSE.5 nuclei isolation buffer [100 m*M* KCl, 10 m*M* tris-Cl (*p*H 7.0), 10 m*M* EDTA, 4 m*M* spermidine, 1 m*M* spermine, 1 m*M* phenylmethylsulfonyl fluoride, 0.1% β-mercaptoethanol, and 17% sucrose (w/v)] was added, allowed to soak, and ground in liquid nitrogen; then it was ground in an Omni-Mixer (Sorvall) to a fine powder. Upon thawing, the powder was resuspended by short bursts in a Polytron (Brinkmann). The homogenate was filtered gently through two layers of sterile Mira-Cloth (Calbiochem) and centrifuged at 4000*g* for 20 min at 4°C. The pellet was collected, resuspended in SSE.5 with a Dounce homogenizer, centrifuged, and suspended in 3 ml of SSE.5. The suspension was layered over 7 ml of SSE 2.1, which is similar to SSE.5 except that the sucrose concentration is increased to 72% (w/v). After centrifugation for 1 hour at 55,000*g* in a Beckman Ti 50 rotor at 4°C, the pellet containing nuclei was resuspended in storage buffer [40% glycerol, 50 m*M* tris-Cl (*p*H 8.3), 5 m*M* $MgCl_2$, and 0.1 m*M* EDTA]. The purity of nuclei was checked by microscopy.
12. G. S. McKnight and R. D. Palmitter, *J. Biol. Chem.* **254**, 9050 (1979).
13. C. L. Soliday, W. H. Flurkey, T. W. Okita, P. E. Kolattukudy, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3939 (1984).
14. R. M. Fourney, J. Miyakoshi, R. S. Day III, M. C. Paterson, *Focus (Rochester, NY)* **10** (no. 1), 4 (1988).

15. Supported in part by grant DMB-8306835 from the National Science Foundation.

20 June 1988; accepted 16 September 1988

A Chemically Synthesized *Antennapedia* Homeo Domain Binds to a Specific DNA Sequence

HISAKAZU MIHARA* AND EMIL THOMAS KAISER

A peptide 60 residues in length that corresponds to the homeo domain of *Antennapedia* (*Antp*), a protein governing development in *Drosophila*, was synthesized by segment condensation with protected peptide segments prepared on an oxime resin. A footprinting assay showed that the homeo domain binds specifically to a TAA repeat DNA sequence in the *Antp* gene. Thus the *Antp* homeo domain has a sequence-specific DNA binding property. The circular dichroism spectra of the homeo domain peptide showed the presence of a significant amount of α-helical structure in aqueous solution and in 50 percent trifluoroethanol. The α helicity measured in water appears to depend on the peptide concentration, which suggests that the peptide aggregates. These results support the hypothesis that the homeo domain binds to DNA through a helix-turn-helix motif.

THE DEVELOPMENT OF *DROSOPHILA* is governed by the segmentation and homeotic genes, of which many contain a highly conserved sequence, the "homeo box" (*1–3*). The homeo box encodes the 60–amino-acid sequence of the homeo domain in the products of many developmental genes. Genetic studies indicate that the developmental genes encode regulatory molecules that could affect the expression of other developmental genes through binding to specific DNA sequences (*4–10*). Sequence comparison with prokaryotic gene regulatory proteins and yeast mating-type proteins suggests that the homeo domain adopts a helix-turn-helix motif in binding to DNA (*1, 4, 11–13*).

We report the preparation of a 60–amino-acid peptide **1** corresponding to the *Antp* homeo domain by a segment-synthesis–condensation procedure that made use of an oxime resin (Fig. 1) (*14*). Because the protected peptide segments we used were purified before segment coupling, and because coupled products are readily separated from uncoupled segments, this method produces higher purity proteins through less purification than the classical solid-phase method (*15*). Another advantage of the segment-synthesis–condensation approach is that mutants can be readily prepared by the replacement of segments in a cassette mode. The purified form of the homeotic proteins is needed to examine correct interactions

Laboratory of Bioorganic Chemistry and Biochemistry, Rockefeller University, New York, NY 10021.

*Present address: Department of Industrial Chemistry, Kyushu Institute of Technology, Kitakyushu, Japan.

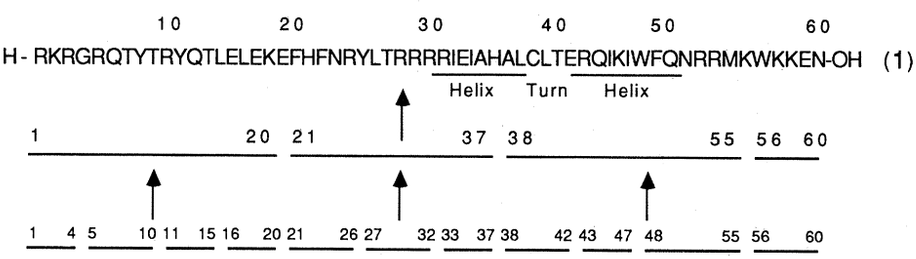

Fig. 1. The amino acid sequence of the *Antp* homeo domain and the strategy of the synthesis of peptide 1 by segment condensation are shown. The putative helix-turn-helix portion is indicated (*11*). The side chain–protected amino acids used were Asp(OBzl), Glu(OBzl), Arg(Tos), Cys(MeBzl), His(Bom), Lys(Cl-Z), Ser(Bzl), Thr(Bzl), Trp(HCO), and Tyr(Cl_2Bzl) (Bom, π-benzyloxymethyl; Bzl, benzyl; OBzl, benzyl-protected COOH; Cl-Z, 2-chlorobenzyloxycarbonyl; Cl_2Bzl, 2,6-dichlorobenzyl; HCO, formyl; MeBzl, 4-methylbenzyl; and Tos, toluenesulfonyl). Eleven small fragments (4 to 7 amino acids) were prepared stepwise on the oxime resin (*14*) and then purified with RP-HPLC. The larger sized (17 to 20 amino acids) protected peptides were synthesized by coupling of the small segments with dicyclohexylcarbodiimide-hydroxybenzotriazole (HOBt) on the oxime resin. The peptides were removed from the resin and then purified by passage through a column of Sephadex LH 60 (dimethylformamide). The large segments were coupled starting from the COOH-terminal pentapeptide benzylester to each other with (*N*-ethyl-*N*′-dimethylaminopropyl) carbodiimide-HOBt in solution and were purified with Sephadex LH 60 to give the protected homeo domain. The protected protein was deprotected by the low- and high-HF method (*23*) and purified by gel-filtration and RP-HPLC (TSK Phenyl 5PW · RP, Toyo Soda) to give a single peak of protein as observed on the same RP-HPLC column and a single band corresponding 8000 daltons on SDS-PAGE. All intermediates were characterized by amino acid analysis and mass spectroscopy.