ed toward establishing the diffraction detection limits that are possible when synchrotron-produced radiation is used.

To compare different materials in terms of their relative ability to scatter x-rays, we use the scattering power, a dimensionless quantity:

$$\text{scattering power} = \left(\frac{F_{000}}{V_u}\right)^2 V_c \lambda^3 \quad (1)$$

where F_{000} and V_u are the number of electrons in, and the volume of, the unit cell, respectively, V_c is the volume of the crystal, and λ is the x-ray wavelength. For routine structure determinations, scattering powers typically range between 10^{16} and 10^{17}. Eisenberger *et al.* performed their measurements on an 800-μm^3 crystal of zeolite, using a wiggler synchrotron beam diffracted from a double-crystal Ge monochromator set for 1.74 Å (*6*). These values correspond to a scattering power of about 3×10^{14}. Bachmann *et al.* made measurements on a crystal of CaF_2 with a volume of 200 μm^3 and x-rays of 0.91 Å, giving a scattering power of 1.3×10^{14} (*7*). Rieck *et al.* used a CaF_2 crystal with a volume of 2.2 μm^3 and 1.56 Å photons; these correspond to a scattering power of 7.1×10^{12} (*8*). [We are also aware of preliminary measurements from a 0.4-μm single-crystal sphere of Mo reported at the 1990 International Union of Crystallography Congress held in Bordeaux, France, but insufficient details are given in the abstract to evaluate the scattering power (*9*).] The work reported here represents an x-ray investigation of an unknown crystal of submicrometer dimensions; in the other studies, known test crystals were used.

In order to assess the diffraction detection limit of our system, we selected a reflection with a χ angle of about 0° and then systematically reduced the vertical aperture defining our incident beam and monitored the transmitted beam with an ionization chamber. There was a linear response down to 10 μm; below that the readings were erratic. At this 10-μm setting, the volume of the sample illuminated was 0.38 μm^3. The ($\bar{h}$,*h*,4*h*) class of reflections, shown in Fig. 2B, were recorded from this volume in a period of 1 min. The scattering powers calculated from Eq. 1 for each of the three peaks in Fig. 2B are 1.2×10^{12} for the ($\bar{1}$,1,4), 1.6×10^{11} for the ($\bar{2}$,2,8), and 4.4×10^{10} for the ($\bar{3}$,3,12). These scattering powers are all smaller than those in the earlier published reports (*6–8*). Despite this diminished scattering power and the strong background signal from the encapsulating glass, there was still a relatively strong diffraction signal; the ($\bar{3}$,3,12) reflection is weak, but it can be seen above this background. This result also demonstrates that it is not necessary to first monochromatize the illuminating x-rays, as was done in each of the earlier studies.

Interatomic planar distances, *d* spacings, were calculated for each reflection from the measured energy of the diffraction peak. The peak centroids were determined by a least-square fit to a Gaussian curve; the reliability factors for most reflections were better than 99.9%. These measured *d* spacings were compared with those calculated from the published lattice parameters for Bi (*10*); the difference between the two values was defined as Δd. There was a systematic variation in $\Delta d/d$ with the χ angle, amounting to a net compressive linear strain of about 2.5% for one sample and about 1.5% for the other sample (Fig. 3). If the materials were isotropic, then the equivalent volumetric strains would result from stresses on the order of about 2 GPa. It is presumed that these residual stress states are caused by Bi expansion upon solidification in the presence of the encapsulating glasses.

Although crystallographic studies have been carried out on smaller samples by electron diffraction techniques, such procedures cannot be used on embedded crystals. Thinning the fibers examined here would have eliminated the residual strains. Removing volume constraints by thinning can even induce phase transformations in metastable crystals.

Note added: Since submitting this manuscript, we have successfully obtained single-crystal diffraction data from three smaller Bi filaments; their calculated diameters are 905, 670, and 420 Å. The scattering power for the (0,$\bar{1}$,4) reflection measured from a volume of 28 attoliters was 1.8×10^{10}

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11. Helpful discussions on this topic with D. J. Nagel are acknowledged with gratitude.

24 April 1991; accepted 27 June 1991

Possible Horizontal Transfer of *Drosophila* Genes by the Mite *Proctolaelaps regalis*

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There is strong inferential evidence for recent horizontal gene transfer of the P (mobile) element to *Drosophila melanogaster* from a species of the *Drosophila willistoni* group. One potential vector of this transfer is a semiparasitic mite, *Proctolaelaps regalis* DeLeon, whose morphology, behavior, and co-occurrence with *Drosophila* are consistent with the properties necessary for such a vector. Southern blot hybridization, polymerase chain reaction (PCR) amplification, and DNA sequencing showed that samples of *P. regalis* associated with a P strain of *D. melanogaster* carried P element sequences. Similarly, *Drosophila* ribosomal DNA sequences were identified in *P. regalis* samples that had been associated with *Drosophila* cultures. These results have potentially important evolutionary implications, not only for understanding the mechanisms by which genes may be transferred between reproductively isolated species, but also for improved detection of some host-parasite and predator-prey relationships.

P ELEMENTS CONSTITUTE ONE OF THE most intensively studied families of transposable (mobile) elements in *Drosophila melanogaster* (*1*). P elements are present in multiple copies in so-called P strains of this species, but are completely absent in others, designated M strains (*2*). The historical dichotomy of P and M strain distributions in *D. melanogaster* strongly suggests that P elements spread through this species as recently as the last half century (*3, 4*). This conclusion is also supported by the uneven worldwide geographical distribution

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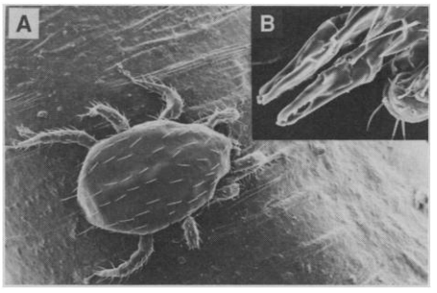

Fig. 1. (**A**) A scanning electron photomicrograph of *P. regalis* DeLeon in feeding position, on a pupa of *D. melanogaster*. (**B**) A photomicrograph of the chelate-dentate chelicerae of *P. regalis* in the ventral aspect. The chelicerae have a fixed and a movable digit. Cheliceral morphology of ascid mites correlates with trophic specialization (*30*). The primitive chelate-dentate state allows for grasping, shearing, and piercing of prey, as opposed to long slender chelicerae (with small teeth) usually found in mites feeding on other mites or small insects. Obligate parasites may have edentate chelicerae, reduced chelicerae, or a reduced fixed digit. Omnivores such as *P. regalis* retain chelate-dentate chelicerae to handle a broad range of food types.

of P elements in *D. melanogaster* (*4, 5*), by their complete absence from species closely related to *D. melanogaster* (*6*), and by the highly invasive nature of active P elements when they are introduced into susceptible populations either by crossing or by experimental germline transformation (*7*).

Several lines of evidence support the hypothesis of the introduction of the P transposable element into the cosmopolitan species *D. melanogaster* from a species of the willistoni group, by means of lateral gene transfer. Observations consistent with this hypothesis include (i) the high sequence similarity among *D. melanogaster* P elements from diverse geographical locations (*8*), (ii) the abundance of P elements in species of the willistoni species group relative to their paucity in the melanogaster species group (*9, 10*), (iii) the near-identity of P element sequences from *D. melanogaster* and *D. willistoni* (*10*), (iv) the lack of congruence between P element sequence identity and the divergence time between *Drosophila* species pairs (*11*), and (v) the overlap in geographical ranges of *D. melanogaster* and *D. willistoni* in Florida and in Central and South America (*12*). This accumulating evidence indicates that lateral transfer seems likely; however, no potential vector has been identified.

There are numerous potential agents for horizontal gene transfer found in association with *Drosophila* cultures. Among these are viruses, bacteria, and small arthropods. A study of the mechanisms of horizontal transfer required the selection of some categorical subset of biological agents for a systematic attack of the problem.

At least ten different mite species co-occur with *Drosophila* in laboratory cultures (*13*). One mite, *Proctolaelaps regalis* DeLeon (Gamasina: Ascidae), is of particular interest because of (i) its peripatetic mode of feeding, (ii) its gnathosomal structures adapted for fluid-feeding (*13a*), (iii) its rapid transit in search of food and between feeding events, (iv) its shared habitat use with *Drosophila* (syntopy), and (v) its intrinsic geographic overlap with *Drosophila* (sympatry). *Proctolaelaps regalis* is also interesting because of its North American affiliation. One hypothesis asserts that P elements spread from the Americas to the rest of the world, as inferred from evidence on the geographic distribution of P strains (*14*).

Proctolaelaps regalis is not the most common mite in *Drosophila* laboratory stocks (*15*), and the earliest known identification of *P. regalis* at the University of Arizona occurred in August 1989. *Proctolaelaps* species have been collected from *Drosophila* laboratory cultures previously by others (*13, 16*), but there is confusion as to the species determination of those specimens. The only report of field-collected specimens of *P. regalis* came from Florida in 1956 (*17*), where the mite was associated with fallen or rotting fruit of rose-apple (*Syzygium jambos* = *Eugenia jambos*) (*18*).

Proctolaelaps regalis has chelicerae typical of an ascid omnivore (Fig. 1). In the laboratory we observed that it can survive on fly culture media alone (feeding on free nutrients, fungus, and yeast), but *P. regalis* does not appear to reproduce under these conditions. Behavioral observations indicated that *P. regalis* also feeds on all immature stages of *D. melanogaster*. Feeding on fly eggs and larvae is by rapid cheliceral thrusting, pinching, and piercing. This action is accomplished swiftly, sending cellular inclusions into the hollow space between the cheliceral shafts (*19*). Movement between individual fly hosts is extremely rapid. Feeding bouts generally last a fraction of a second, and immediate feeding on subsequent adjacent hosts is common. In mixed-species fly cultures this behavior provides potential for transfer of cellular inclusions (including DNA) from one host to another.

A second type of feeding behavior is seen in adult mites feeding on *Drosophila* pupae, where penetration of the thick puparium requires prolonged thrusting of the chelicerae. In some *D. melanogaster* strains, association with *P. regalis* culminates in culture degradation or elimination, possibly due to this second (damaging) type of feeding.

All of the various aspects of the biology of this mite, including the semiparasitism itself (*20*), are consistent with *P. regalis* having had a co-evolutionary association with what are now "domesticated" *Drosophila*. This mite may have been co-collected with wild-caught fly stocks in the United States originally, but it is equally likely that it secondarily invaded U.S. laboratory stocks because of its natural association with *Drosophila* in the wild. In short, *P. regalis* has the morphological and behavioral capacity, and the ecological and geographical opportunity, to act as a vector for P elements.

In order to determine whether *P. regalis*

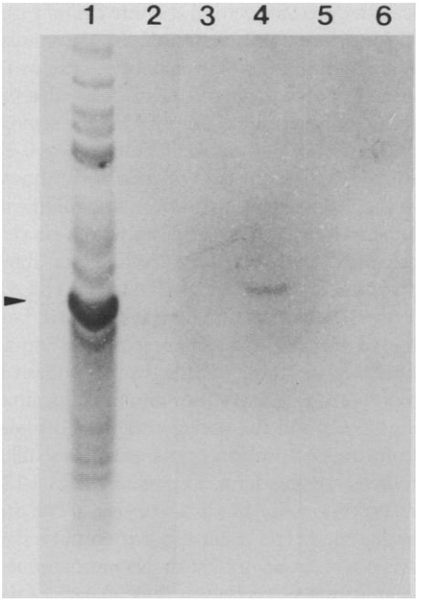

Fig. 2. Southern blot hybridization of *Drosophila* and mite genomic DNAs with a P element–specific probe. *Drosophila melanogaster* strains Harwich-w and Canton-S served as positive and negative controls for P elements, respectively. Genomic DNA samples were prepared from 0.1 g of adult flies (*31*). Each lane in the blot contains 2.0 μg of DNA. Mite DNA samples were prepared from approximately 200 individuals by the same procedure, and the entire sample was used without quantitation (*32*). DNAs were digested with *Acc* I, electrophoresed in 1% agarose gels, and transferred to Nytran (Schleicher & Schuell) essentially as described in (*33*). DNA was visible for all samples in the ethidium bromide–stained gel before transfer except for lanes 5 and 6. Plasmid pπ25.7BWC contains a nearly complete P element with no flanking *D. melanogaster* genomic sequences (*34*) and was used to screen for the presence of P element sequences. Probe labeling and hybridization followed procedures described in the GENIUS kit (Boehringer Manheim). Samples: lane 1, *D. melanogaster* Harwich-w; lane 2, *D. melanogaster* Canton-S; lane 3, *P. regalis* associated with the Canton-S strain; lane 4, *P. regalis* associated with the Harwich-w strain; lane 5, medium from the Harwich-w culture; and lane 6, fly debris from the Harwich-w culture. The arrow denotes the position of the 2.4-kb fragment derived from the full-sized P element following digestion with *Acc* I.

mites were acquiring P element sequences from *D. melanogaster* during feeding, genomic DNA was isolated from mites associated with both P and M strain fly cultures. Results of Southern blot analyses indicated that hybridization to a P element–specific probe occurred only with DNA extracted from the Harwich-w (P) fly strain (Fig. 2, lane 1) or with DNA extracted from mites associated with that strain (Fig. 2, lane 4). DNA from the Canton-S (M) strain of *D. melanogaster* and from *P. regalis* associated with the Canton-S strain consistently showed no detectable hybridization to the P element probe (Fig. 2, lanes 2 and 3, respectively). Occasionally there was no hybridization of Harwich-associated *P. regalis* DNA to the P element probe. This is probably due to the insensitivity of Southern hybridizations when less than optimal amounts of DNA were obtained from a mite sample.

In order to address the issue of insensitivity and to obtain an independent confirmation of the hybridization results, the polymerase chain reaction (PCR) (*21*) was used (*22*). Specific primers were synthesized for the *D. melanogaster* P element sequence (*23*). These primers flank a 764-bp fragment at the 5′ end of the P element (*24*), which is the expected size of the corresponding P-specific product. Eight separate samples of template DNA from the Harwich-w strain of *D. melanogaster* and mites associated with that strain were used in PCR with P-specific primers. All consistently yielded a fragment of the correct size, and a typical sample is shown in Fig. 3. Results from both the Canton-S template and that from mites associated with the Canton-S strain were negative. PCR was also performed on samples of the Harwich culture medium and fly debris. In both instances, there was no P-specific fragment produced (Fig. 3, lanes 5 and 6).

To demonstrate that the fragment produced from the mite template DNA (Fig. 3, lane 3) was indeed P-specific, the product was isolated, purified, and a 250-bp segment was sequenced (*25*). The nucleotide sequence in this region was identical to that previously determined (*23*) for the *D. melanogaster* P element.

The most parsimonious explanation for these results is that *P. regalis* can acquire *Drosophila* P element sequences during feeding. However, two alternative explanations had to be ruled out: first, that the Harwich mite DNA samples were contaminated with Harwich fly DNA at some point in the isolation of the mites from the culture bottle, and second, that the mites themselves carried endogenous sequences with homology to P elements. Both explanations were tested with appropriate controls.

To address the first possibility, that secondary contamination of samples would occur simply through association with flies or old fly medium, adult *Histiostoma laboratorium* were isolated from the same Harwich culture as were *P. regalis*, and DNA was prepared in the same manner. *Histiostoma laboratorium* is a mite commonly associated with *Drosophila* cultures; however, it is an astigmatic mite that does not feed on the flies themselves. No P-specific product was detected when *Histiostoma* DNA was used as a template in PCR, indicating that association with the flies was not of itself sufficient to give positive results. When the same DNA was used in PCR with universal small subunit ribosomal DNA (rDNA) primers, a product of the correct size was seen, indicating that the negative result with the P element primers was not due to an inherent problem associated with the template DNA itself.

To address the issue of whether endogenous P sequences in Harwich-associated mites might be pleisomorphic or synapomorphic in ascid lineages, we obtained isolates of two species closely related to *P. regalis*: *Lasioseius subterraneus* and *Proctolaelaps longipilis* (*26*). Again no P-specific product was detected when DNA from either mite was used as a template in PCR. This same DNA was shown to be amplification-competent when universal small subunit rDNA primers were used.

A final important question was whether only *Drosophila* P element sequences were acquired by *P. regalis*, or whether other DNA sequences could also be detected. Accordingly, mites were assayed for a non-mobile genomic *Drosophila* sequence. The small subunit (18*S*) rRNA gene was chosen because, like P elements, it is present in multiple copies in the *D. melanogaster* genome. Primers, corresponding to highly variable regions within the 18*S* rRNA genes, were synthesized to be specific for the *D. melanogaster* small subunit rRNA genes (*27*).

When used with a P or M strain template DNA from *D. melanogaster* in PCR, these 18*S* rDNA primers yield a fragment of 811 bp (Fig. 4, lanes 1 and 2). A product of the correct size was also seen with template DNA isolated from mites associated with the Harwich-w and Canton-S strains (Fig. 4, lanes 3 and 4). Because no acarine small subunit rDNA sequences have been published, we could not rule out that these primers were in fact hybridizing to the endogenous *P. regalis* genes. To address this issue, DNA was isolated from the three mite species sampled previously: *H. laboratorium*, *L. subterraneous*, and *P. longipilis*. No PCR product was detected with template DNA from any of these three species (Fig. 4, lanes 5, 6, and 7), indicating that the *Drosophila* rDNA primers were not hybridizing to endogenous mite sequences.

The amplified DNA fragment from *P. regalis*, which was produced with *D. melanogaster*–specific rDNA primers, was isolated and purified. A 300-bp region of the product was sequenced and found to be identical to the corresponding segment of the *D. melanogaster* 18*S* rDNA (*28*).

These results suggest that *P. regalis* was acquiring 18*S* rDNA and P element *Drosophila* DNA sequences during its association with fly cultures, a conclusion consistent with the morphology, ecology,

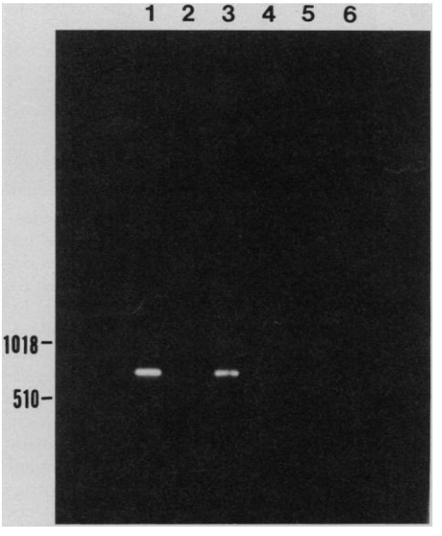

Fig. 3. Results of PCR using P element–specific primers. Genomic DNAs were prepared as described in Fig. 2. Between 100 and 200 ng of *Drosophila* DNA and 200 to 500 ng of mite DNA (equivalent to 25 to 70 mites) was used in each separate amplification reaction. The medium control (lane 5) and fly control (lane 6) were treated in the same way as other samples, although no DNA was detected following the isolation procedure (*35*). Reaction conditions were 200 μM each dNTP, 5 pmol each primer, and 2.5 units of Taq polymerase with buffer supplied by the manufacturer (Cetus), in a total volume of 100 μl. Sizes of DNA standards are given in base pairs at left. Temperature cycling was performed on a Coy TempCycler with the following profile: 92°C for 1 min, 30 s; 50°C for 45 s; 72°C for 1 min, 45 s, for a total of 30 cycles. After removal of primers and reaction components, aliquots from the completed reactions were analyzed on 1.4% agarose gels, which were stained with ethidium bromide after electrophoresis. Templates: lane 1, *D. melanogaster* Harwich-w strain; lane 2, *D. melanogaster* Canton-S strain; lane 3, *P. regalis* associated with the Harwich-w strain; lane 4, *P. regalis* associated with the Canton-S strain; lane 5, medium control; and lane 6, fly debris control. Primers: 829, 5′-AACATAAGGTGGTCCCGTCG-3′; 830, 5′-CGACTGGGCAAAGGAAATCC-3′.

behavior, and geography of this mite. Our results are particularly intriguing because the feeding behavior of the mite appears to simulate the method of microinjection in the laboratory that has been used by many *Drosophila* researchers for intra- and interspecific transfer of genes by P element transformation (*29*).

Horizontal gene transfer of P elements may, however, be a rare density-dependent event, even under ideal conditions. On the basis of our knowledge of P element transformation (*29*), the minimum conditions needed to be satisfied for detection under laboratory experimental conditions are the following: (i) two *Drosophila* females of different species must lay their eggs in proximity to one another, providing the opportunity for a mite to sequentially feed on one and then on the other in the correct order; (ii) the recipient fly egg must be less than 3 hours old (512-cell stage); (iii) the germline of the recipient embryo must incorporate a complete copy of exogenous P element DNA before it degrades in the cytoplasm; (iv) the individual receiving the transferred P element must survive the act of feeding by the mite; and (v) the adult developing from that egg, or a descendent that has inherited the element in its germline, must be sampled by the investigator. If, as seems likely, each of these events has a low independent probability, then the combined multiplicative probability will be extremely low.

Although some aspects of high population densities of mites and flies in culture bottles are expected to enhance the possibility of conditions suitable for transfer, the ecological conditions that accompany high population densities may also act against detection. Because *P. regalis* appears to be an omnivore, the resultant culture degradation may actually provide alternative sources of food for the mites, reducing the likelihood that they will feed on fly eggs. Conversely, factors that act in favor of the experimental detection of the transfer are the ability of P elements to rapidly increase in copy number and spread throughout the population of a new host and the ready availability of efficient techniques to detect their presence. Even if horizontal transfer should prove to be difficult to replicate under laboratory conditions, it may still be an important evolutionary occurrence in wild populations.

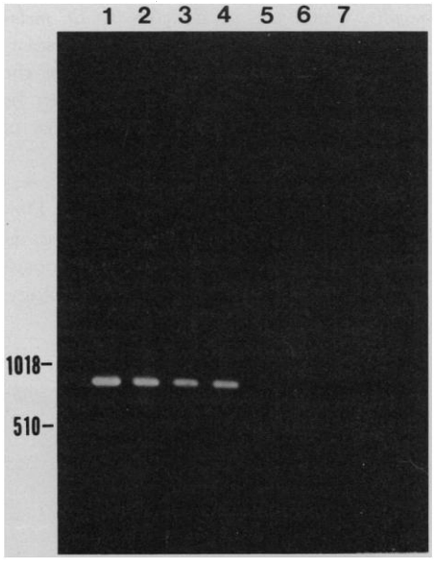

Fig. 4. Results of PCR using *D. melanogaster*–specific 18*S* rDNA primers. Preparation of genomic DNAs and reaction conditions are as described in Fig. 3. Sizes of DNA standards are given in base pairs at left. Templates: lane 1, *D. melanogaster* Harwich-w strain; lane 2, *D. melanogaster* Canton-S strain; lane 3, *P. regalis* associated with the Harwich-w strain; lane 4, *P. regalis* associated with the Canton-S strain; lane 5, *H. laboratorium* associated with the Harwich-w strain. *Histiostoma laboratorium* is the common astigmatic mite found in all cultures from which *P. regalis* was isolated; lane 6, *L. subterraneus*, a mesostigmatic mite closely related to *P. regalis* that is not associated with *Drosophila* cultures; and lane 7, *P. longipilis* not associated with *Drosophila* cultures. Primers: 937, 5′-GTGCTTCATACGGGTAG-3′; 938, 5′-CAGCACCATAATCCTG-3′.

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18. Positive identification of laboratory-collected *P. regalis* was by E. E. Lindquist (personal communication) who has a "variety of material at hand compared with the type material of this species. It seems to be frequently associated with fallen or rotting fruit" [Biosystematics Research Centre Identification Report 89-0818-01 (Agriculture Canada, Ottawa, 1989)]. Slides of laboratory-collected material are in the collections of E. W. Baker (USDA-ARS), E. E. Lindquist (CNC), and in (*13a*) M. A. Houck (Texas Tech University).
19. *Proctolaelaps regalis* has large secretory structures (salivary styli) positioned laterad and ventrad to the chelicerae. They carry the ducts of the salivary glands, are partly sclerotized, and are thought to assist in preoral digestion [G. W. Krantz, T. A. Woolley, in (*13a*)].
20. We use the term semiparasite (or omnivore) to describe the trophic specialization of *P. regalis*. It is possible that the mite may act either as a predator (kills prey) or as a parasite (does not kill host), depending upon conditions.
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22. An explosion of PCR applications has occurred during the last year (T. Appenzeller, *Science* **247**, 1030 (1990). This technique has been used to detect Lyme disease agents in ticks [D. H. Persing *et al.*, *ibid.* **249**, 1420 (1990)], to examine archeological specimens [S. Paabo, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 1939 (1989)] and has been applied to a host of clinical and medical problems. Our search of the literature, however, uncovered no studies of PCR detection of prey DNA in a predator. We are aware of the detection of host DNA sequences in another mite, *Pyemotes tritici*, and the suggestion that *P. tritici* could be responsible for gene transfer (M. J. Kaliszewski and colleagues, personal communication). We do not know how frequent the detection of prey DNA in a predator will prove to be when PCR technology is more commonly used in ecological research. Currently enzyme radioimmunoassay of antigens has been applied sparingly in predator-prey studies [D. D. Schoff, S. Palchick, C. H. Templis, *Ann. Entomol. Soc. Am.* **79**, 91 (1986); D. L. Bailey, A. L. Choate, M. J. P. Lawman, *Bull. Entomol. Res. London* **76**, 141 (1986); S. H. Kapuge, W. Danthanarayana, N. Hoogenraad, *ibid.* **77**, 247 (1987)]. PCR may offer a safer and more efficient method of detection which could provide important advantages for host-parasite and predator-prey studies.
23. K. O'Hare and G. M. Rubin, *Cell* **34**, 25 (1983).
24. Primer 829 is complementary to nucleotides 12 to 31 of the complete P element; primer 830 is complementary to nucleotides 757 to 776. The sequences of both are given in the legend to Fig. 3.
25. By the method described by J.-L. Casanova, C. Pannetier, C. Jaulin, P. Kourilsky, *Nucleic Acids Res.* **18**, 4028 (1990).
26. We used *L. subterraneus* and *P. longipilis* because they are phylogenetically related to *Proctolaelaps* and are also predators (of soil nematodes). *Lasioseius* and *Proctolaelaps* are in sister tribes of the Ascidae.
27. Primer 937 is complementary to nucleotides 648 to 664 of the *D. melanogaster* 18*S* rRNA gene; primer 938 is complementary to nucleotides 1444 to 1459. The sequences of both primers are given in the legend of Fig. 4.
28. D. Tautz, J. M. Hancock, D. A. Webb, C. Tautz, G. A. Dover, *Mol. Biol. Evol.* **5**, 366 (1988).
29. A. C. Spradling, in *Drosophila: A Practical Approach*, D. B. Roberts, Ed. (IRL Press, Oxford, 1986), p. 175. Study of the fate of lab-microinjected DNA in

early *Drosophila* embryos indicates that after nine cell divisions (512-cell stage; 90 min at 25°C) the primordial germline cells migrate to the posterior pole of the egg, are enveloped in a cell membrane, and are expelled from the syncytial mass of the egg [H. Steller and V. Pirrotta, *Dev. Biol.* **109**, 54 (1985)]. Some injected DNA becomes inserted into the germline at this time; that which remains in the cytoplasm slowly degrades. It occurred to us that behavioral transmission of P elements by mites, feeding at the posterior pole of fly eggs, is not an unrealistic possibility but, that because of the vagaries involved the likelihood of heuristically detecting such an event might be small.
30. G. O. Evans, J. G. Sheals, D. MacFarland, *The Terrestrial Acari of the British Isles, Introduction and Biology* [British Museum (Natural History), London, 1961], vol. 1.
31. By the method described by S. B. Daniels and L. D. Strausbaugh, *J. Mol. Evol.* **23**, 138 (1986).
32. Mites were collected by gently rinsing the inside of the inverted culture bottle with distilled water from a squirt bottle and allowing the water to drain into a testing sieve with 75-μm pore size. Debris was collected and mites were rinsed with distilled water. Individual mites were examined under a dissecting microscope as they were collected. Mites were transferred to 0.5-ml microcentrifuge tubes containing sterile distilled water and frozen in 200-mite aliquots at −70°C. Prior to DNA isolation, mites were thawed and pelleted at 12,000*g* in a microcentrifuge for 1 min. The water was removed and mites were homogenized in fresh, sterile buffer.
33. J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 2, 1989).
34. R. E. Karess and G. M. Rubin, *Cell* **38**, 135 (1984).
35. Controls: A metal spatula was used to sample culture medium from experimental fly bottles (approximately equivalent to the volume of 200 mites). Fly-debris controls contained culture medium plus at least one visible *Drosophila* body part (a portion of wing, leg, cuticle, and so forth) isolated from the experimental culture bottle. In both cases, the entire sample was used in a single PCR assay.
36. We thank E. E. Lindquist for identification of *P. regalis*, E. E. Lindquist and E. W. Baker for helpful discussions, M. Q. Benedict, W. A. Brown, A. C Cohen, L. D. Densmore, H. H. Hagedorn, G. W. Krantz, E. E. Lindquist, J. M. C. Ribeiro, and R. E. Strauss for comments on the manuscript, and M. J. Kaliszewski for samples of *P. longipilis* and *L. subterraneus* and for discussions. Supported by John D. and Katherine T. MacArthur Foundation vector grant 8900408 to the Center for Insect Science (grant to M.A.H. and M.G.K. and postdoctoral fellowship to J.B.C.), BARD grant IS-1397-87 to M.A.H. and NIH research grant 36715 to M.G.K.

15 May 1991; accepted 24 July 1991

Inhibition of Neointimal Smooth Muscle Accumulation After Angioplasty by an Antibody to PDGF

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Approximately 30 to 40 percent of atherosclerotic coronary arteries treated by angioplasty or by bypass surgery occlude as a result of restenosis. This restenosis is due principally to the accumulation of neointimal smooth muscle cells, which is also a prominent feature of the advanced lesions of atherosclerosis. The factors responsible for the accumulation of intimal smooth muscle cells have not been identified. Platelet-derived growth factor (PDGF) is a potent smooth muscle chemoattractant and mitogen. It is present in platelets and can be formed by endothelium, smooth muscle, and monocyte-derived macrophages. The development of an intimal lesion in the carotid artery of athymic nude rats induced by intraarterial balloon catheter deendothelialization was inhibited by a polyclonal antibody to PDGF. These data demonstrate that endogenous PDGF is involved in the accumulation of neointimal smooth muscle cells associated with balloon injury and may be involved in restenosis after angioplasty, and perhaps in atherogenesis as well.

PDGF IS A POTENT MITOGEN AND chemoattractant for connective tissue cells (*1*). The PDGF family consists of dimeric molecules that can exist as homodimers or heterodimers of two distinct but related peptide chains termed PDGF-A and PDGF-B (*1*). PDGF is expressed at low or undetectable concentrations in normal adult tissues, but its expression is increased after tissue injury. Two distinct PDGF receptors have also been identified, one that binds either the A or the B chain (the α-subunit) and one that binds only the B chain (the β-subunit) (*2*). The capacity of cells such as smooth muscle to respond to PDGF may depend on the limited availability of appropriate receptors or the presence of particular dimeric forms of PDGF, or both. Investigations of experimentally induced atherosclerosis, naturally occurring human atherosclerosis, and smooth muscle accumulation associated with failure of vascular grafts have all demonstrated increased expression of PDGF and its receptors by Northern (RNA) blot analysis, in situ hybridization, and immunohistochemistry (*3, 4*). However, this association does not permit one to determine whether PDGF functions in the migration or proliferation of smooth muscle cells in lesions of atherosclerosis or graft restenosis. To determine whether PDGF is involved in these processes, we have examined an animal model of angioplasty, intraarterial balloon catheter deendothelialization of the rat carotid artery.

Deendothelialization with an intraarterial balloon catheter that dilates an artery induces injury to the innermost layers of medial smooth muscle and may even kill some of the innermost cells. This is followed by a round of proliferation of the medial smooth muscle cells, after which many of them migrate into the intima through fenestrae (natural openings) in the internal elastic lamina and subsequently proliferate to form a neointimal lesion (*5, 6*). In the rat, smooth muscle proliferation, as determined by the incorporation of [^{3}H]thymidine autoradiography, reaches a maximum in the medial layer of the artery 48 hours after ballooning and in the intima of the artery after 96 hours (*5*). Although smooth muscle proliferation can persist near the luminal surface as late as 12 weeks after balloon injury, the number of arterial smooth muscle cells does not increase after 2 weeks. The factors responsible for these events are not yet known.

Balloon catheter injury of the rat carotid artery induces the expression of mRNA for both PDGF-A chain and the PDGF receptor α- and β-subunits in the resulting neointimal lesion (*3*). In this study, we used a goat polyclonal antibody to PDGF (anti-PDGF) to examine the formation of intimal lesions in the carotid artery after balloon catheter deendothelialization. Anti-PDGF immunoglobulin G (IgG) was administered to athymic nude (nu/nu) rats (*7*) before and after balloon catheter deendothelialization of the carotid artery (*8*). We used athymic nude rats because of their inability to mount an immune response (*9*) to the large doses of antibody administered over 9 days. The neointimal response to balloon injury in the nude rat is essentially the same as that observed in other strains of rat (*10*).

We obtained the polyclonal antibody to human platelet PDGF by immunizing goats with PDGF prepared from human platelets (*11, 12*). The antibody was characterized for its ability to neutralize both chemotactic and mitogenic responses to PDGF in vitro before being used in vivo (*13*) (Fig. 1). The anti-PDGF IgG neutralizes the mitogenic activity of all dimeric forms of human PDGF (*13*) and has no direct effect on other

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