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17. Galls of *M. rhois* were discovered in August 1986 on *R. glabra* at 1990-m elevation in Bear Canyon in the Santa Catalina Mountains, just north of Tucson, Pima County, AZ. Examination of mosses growing on north-facing slopes 50 to 100 m from the sumac revealed tiny aphids occurring singly and encased in white waxy secretions. These were indistinguishable from *M. minutus* of Baker (*15*). The aphids were found only on *H. microphyllum* and not on other intermixed moss species, including *Hypnum pallescens* (Hedw.) P.-Beauv. and *Orthoprochum halli* Sull. & Lesq. *ex* Sull. Four aphid-free cultures of *H. microphyllum* were established by washing the mosses in detergent solution and placing them in paper-lined and covered culture dishes. These were kept in a growth chamber at 20°C with 16L:8D photocycle and watered by wetting the paper with nutrient solution. On 14 September galls were collected from the same stand of *R. glabra*. These galls had slits from which winged migrants were emerging. Some migrants were dissected and their embryos examined to determine whether these could be the winged sexupara morph produced by many Pemphiginae in autumn (though usually not on the galled host plant). The sexuals of Pemphiginae have vestigial mouthparts, whereas these embyros possessed fully developed rostra, indicating that migrants were flying from galls to an alternate host to initiate further parthenogenetic generations. Migrants were transferred to two of the moss cultures in the growth chambers. They deposited nymphs within 8 hours and died within 24 hours. Migrants left in containers with galls did not reproduce or die within the same time interval. Experimental and control (uninfested) *H. microphyllum* cultures were kept in covered dishes in the chamber. After 5 days, small white lumps appeared on the strands of the infested mosses. Microscopic examination revealed that each consisted of a single aphid covered with wax secretions. Feeding was inferred through the presence of droplets of honeydew. The two control cultures lacked aphids, ruling out accidental contamination from aphids present when mosses were collected. Aphids in the laboratory colonies were still reproducing after 26 months and they showed a morphology and feeding habit indistinguishable from that in naturally occuring colonies. Similar transfers from *Rhus* galls to *H. microphyllum* were repeated in September 1987 and in September 1988 with successful establishment of colonies in both years, confirming *H. microphyllum* as the alternate host of *M. rhois*.
18. Baker (*15*) found alatae developing on mosses in April, indicating that the developmental pathway leading to sexuparae (the return migrants to sumac) may be induced by photoperiodic conditions that prevail in spring. This would explain why no alatae developed from my laboratory cultures kept on 16L:8D photocycles. These observations fit with the life cycles recorded for other Melaphidina (*13, 14*) and other Fordini, in which sexuparae fly between alternate host plants in spring [D. Wool, in *Biology of Gall Insects*, T. N. Ananthakrishnan, Ed. (Oxford Univ. Press and IBH Publishing Co., New Delhi, 1984), pp. 11–58; G. Wertheim, *Trans. R. Soc. London* **105**, 79 (1954)]. In *Forda*, the sexually produced eggs are dormant through summer, autumn, and winter. This prolonged dormancy probably occurs in *M. rhois* eggs as well since other aspects of its life cycle resemble those in Fordini and since eggs of other aphid groups have a dormant period.
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23. I thank V. F. Eastop for bringing the Chinese work to my attention, A. Johnson for advice on culturing mosses and for identifying the mosses, J. A. Wolfe for information on the fossil record for *Rhus* in western North America, and D. A. Young for information on *Rhus* taxonomy. G. W. Fernandez, P. M. Mirocha, and M. E. Moran assisted with field collections of *M. rhois*. J. E. Bronstein, M. J. Donoghue, J. A. Glass, R. L. Smith, F. G. Werner, and D. E. Wheeler gave helpful comments on the manuscript. Supported by NSF grant BSR-8806068. This is publication number 7027 of the Arizona Agricultural Experiment Station.

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Introduction of Human DNA into Mouse Eggs by Injection of Dissected Chromosome Fragments

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A procedure has been developed for introducing exogenous DNA into mouse eggs by injection of chromosome fragments. Chromosome fragments were dissected from human metaphase spreads and microinjected into the pronuclei of fertilized mouse eggs. Many of the injected eggs subsequently exhibited normal pre- and postimplantation development. Embryos obtained from eggs injected with centromeric fragments retained human centromeric DNA as demonstrated by in situ hybridization analysis. From eggs injected with noncentromeric fragments, a mouse was obtained whose tail tissue exhibited the presence of human DNA. This procedure should facilitate incorporation of very large (more than 10 megabases) DNA fragments into cells and embryos without the need for cloned sequences.

TRANSGENIC ANIMALS MADE BY DNA injection or retroviral infection (*1, 2*) have been used as powerful model systems for studying gene regulation. Such transformation methods usually result in the insertion of DNA fragments less than 100 kb in size; in cases where it is desirable to introduce a gene cluster or a gene that spans over a great distance, an alternative method may be needed. A possibility that we examined in this study is the direct microinjection of mouse eggs with chromosome fragments containing more than 10 megabases (Mb) of DNA. The feasibility of using this approach is indicated by the previous finding that chromosome fragments can be incorporated into the mammalian karyotype after transfection with calcium phosphate–precipitated chromosomes (*3*). However, the low transformation efficiency of the latter method (*1, 3*) precludes its use for making transgenic animals.

We focused our experiments on the dissection and injection of centromeric fragments, as the persistence of human DNA can be readily detected via the highly repeated centromeric satellite DNA sequences. Centromeric fragments 0.5 to 1.0 μm in size (15 to 30 Mb; estimate based on 3.3×10^9 Mb per haploid genome) were dissected

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Table 1. Maintenance of injected human chromosome fragments in mouse embryos. NA, not applicable.

Fragment injected	Number of experiments	Survived/injected*	Number of embryos		Fragment detection
			Developed/survived†	Embryos recovered‡	
		Preimplantation			
Control	2	21/33 (63%)	12/21 (57%)	NA	NA
Centromere	6	39/90 (43%)	16/39 (41%)	NA	6/12§
		Postimplantation			
Control	2	14/30 (46%)	9/14 (64%)	4 (44%)	NA
Centromere	10	74/135 (54%)	20/74 (27%)	8 (40%)	4/8

*Number of eggs out of the total number still viable 2 to 4 hours after injection. †Number of embryos reaching the morula stage after 4 days in culture. ‡Embryos (12.5 days) recovered from surrogate mothers. §In the six positive embryos, 16 of 82 nuclei and 11 of 89 metaphase spreads exhibited the presence of human satellite DNA.

from stained metaphase spreads (Fig. 1) obtained from a normal human fibroblast cell line, MRC-5 (*4*). After dissection, a single fragment was retrieved in a micropipette and expelled into the pronucleus of a mouse egg. The injected eggs were then cultured for 5 days in modified Whitten's medium (*5*) so that we could monitor their survival and development. A total of 43% of the injected eggs survived, and, of these, 41% developed to the blastocyst stage (Table 1). Hence, the overall efficiency for development to the blastocyst stage was 20%. For control embryos injected with 50 m*M* KCl or with the solution used for fragment retrieval (Fig. 1), a comparable but slightly higher level of survival and development was obtained (Table 1).

To determine whether the human chromosome fragment was maintained in the developing embryo, we prepared metaphase spreads from morula stage embryos and analyzed them by in situ hybridization with a human centromeric satellite DNA probe (*6*). Hybridization signals were observed within nuclei and also over individual chromosomes in 6 of 12 embryos (Fig. 2a). In all six embryos, hybridization signals were observed in more than one cell, the range being two to four positive cells per embryo. Hybridization signals were also detected in 4 of 13 injected embryos that had arrested at the two- to four-cell stage of development [signal observed only in one nucleus (*7*)]. The pattern of signal localization on metaphase chromosome spreads revealed that the injected fragments were very closely associated with individual chromosomes (Fig. 2a), thereby suggesting that in some cases the exogenous human centromeric fragment may have been incorporated into a mouse chromosome. The hybridization signal intensity in some spreads, such as the one in Fig. 2a, was indistinguishable from that obtained with metaphase chromosomes from the donor human cell line, MRC-5. Thus, in these instances, perhaps a major portion of the injected centromeric fragment was retained.

To examine the possible long-term maintenance of the exogenous human DNA, we transferred blastocysts developing from fragment-injected eggs to surrogate CD-1 females so that we could monitor further postimplantation development. On day 13 of gestation, the embryos were recovered and then processed for fixation, paraffin embedding, and in situ hybridization with the human centromeric probe. In control embryos developing from uninjected eggs or eggs injected with 50 m*M* KCl, no hybridization signal was observed. In contrast, four of eight fragment-injected embryos showed distinct clusters of cells with strong hybridization signal (Fig. 2, b and c, and Table 1) over 20 to 90 consecutive sections. In each positive embryo, at least 100 to 1000 cells were observed to contain the human satellite DNA. Given that only one centromeric fragment was injected in each embryo, these in situ results suggest that the exogenous human DNA underwent multiple rounds of replication. However, these data also show that the human DNA–containing cells constituted a smaller proportion of the postimplantation embryos as

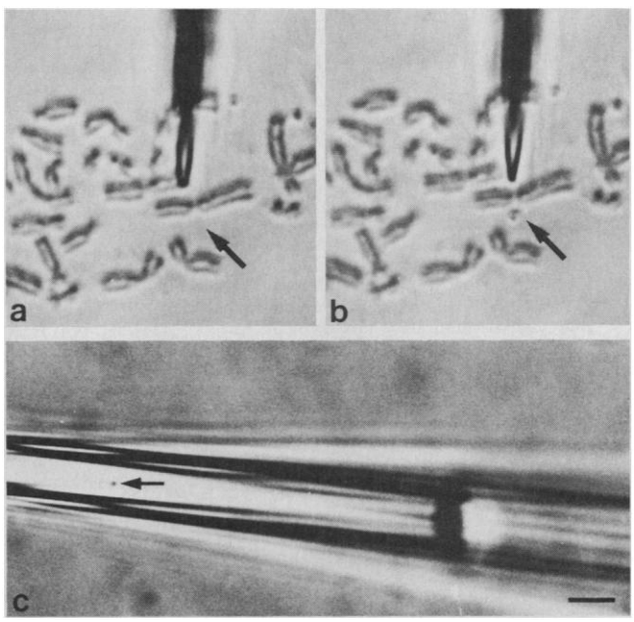

Fig. 1. Dissection, retrieval, and injection of chromosome fragments into mouse eggs. (**a**) A human metaphase spread prepared (*11*) from MRC-5 cells and stained with 0.1% basic fuchsin before dissection. The chromosome to be dissected is indicated by the arrow. (**b**) The same metaphase spread after dissection. Note that the centromeric fragment has been displaced (arrow). (**c**) Chromosome fragment in the injection pipette (arrow). The pipette tip, which is to the left, is out of the plane of focus. Scale bar, 6 μm. Dissection pipettes with tip sizes of 1 to 2 μm were made with Kwikfil glass capillaries (1.0 mm outside diameter, 0.58 mm inside diameter, W.P. Instruments). For injection pipettes, glass capillaries (1.0 mm outside diameter, 0.80 mm inside diameter, VWR Scientific) were treated with Nonidet P-40, pulled, and then enlarged to a final tip size of 0.5 to 1.0 μm before injection. Dissected fragments were retrieved in an injection pipette within a droplet of 0.05% trypsin (containing 0.5 m*M* EDTA and 0.1% polyvinylpyrrolidone), then rinsed by repeated aspiration in 1% bovine serum albumin or 50 m*M* KCl and injected into the male pronucleus with the expulsion of a small volume of fluid (2 to 4 pl). The chromosome dissections and egg injections were performed on a fixed-stage Leitz Diavert microscope with Leitz micromanipulators. Fertilized eggs were obtained from superovulated SWR/J females mated with SJL/J males and were maintained in Hepes-buffered modified Whitten's medium (*12*) during injection.

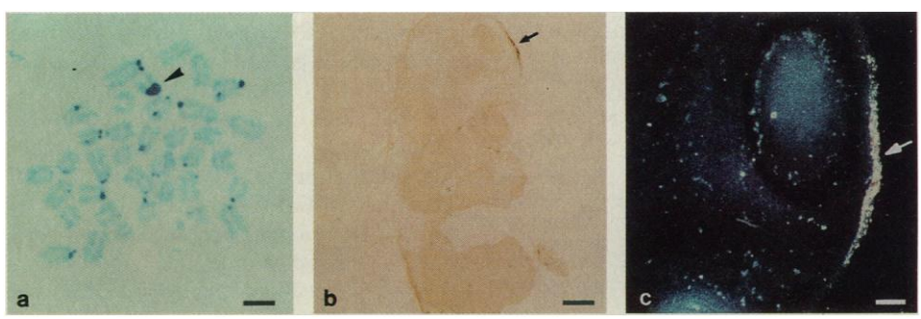

Fig. 2. Detection of human centromeric DNA by in situ hybridization with a biotinylated human satellite DNA probe. (**a**) Bright-field micrograph of a chromosome spread from an air-dried embryo that exhibited intense hybridization over a single chromosome (arrowhead). The hybridization signal appears to be associated with the proximal end of a mouse chromosome. Most of the mouse chromosomes exhibited a low level of hybridization at the centromeric region as a result of a low level of cross homology between human and mouse satellite DNA. (**b**) Bright-field micrograph of a 12.5-day embryo section exhibiting hybridization signal over a strip of cells in the outermost layer of tissue in the head region (arrow). (**c**) The same area denoted by the arrow in (b) but at a higher magnification and in dark field. The intense hybridization signal (white arrow) can be clearly observed as the clustering of bright dots. The other bright spots scattered over the section are merely reflections created by debris on the slide. Scale bars: 4 μm in (a), 350 μm in (b), and 100 μm in (c). For the analysis of the preimplantation embryos, injected eggs that had developed to the morula stage by day 4 were air-dried (*13*), then processed for in situ hybridization with a nick-translated biotinylated pXBA21 plasmid DNA probe containing human centromeric satellite DNA (*6*). For the analysis of the postimplantation embryos, paraffin sections (10 μm) of 12.5-day embryos recovered from surrogate CD-1 females were processed for in situ hybridization (*14*) with the same biotinylated probe. In both experiments, hybridization was detected by means of a streptavidin-peroxidase/silver enhancement protocol (Amersham Corporation).

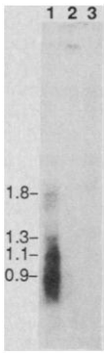

Fig. 3. Southern analysis showing the presence of human repetitive DNA. DNA samples (2.5 μg) extracted from tail tissues were separated by agarose gel electrophoresis, then blotted onto nitrocellulose, and hybridized with nick-translated pBLUR8, a plasmid containing the human interspersed Alu repeats (*15*). Mbo I–digested DNA from E mouse exhibited four distinct bands, thus indicating the retention of some human DNA. Lane 1, E mouse DNA digested with Mbo I; lane 2; undigested E mouse DNA; lane 3, normal mouse DNA digested with Mbo I. Molecular weights indicated are in kilobases.

compared to that found in embryos analyzed at the preimplantation stages. Because the embryo is derived from just a few stem cells (*8*), these results further suggest that cells containing the human centromeric fragment may have been at a selective disadvantage. This might be due to the centromeric origin of the injected fragments. Thus, if the injected fragment retained some centromere activity, mitosis could have been perturbed. Studies in tissue culture cells have shown that mouse chromosomes with multiple centromeres undergo a gradual process of centromere inactivation over many cell divisions (*9*). Perhaps the mosaicism we observed reflects the end point of this inactivation process.

Additional experiments were carried out to examine if noncentromeric fragments might be incorporated into the mouse genome. For these experiments, we dissected and injected random noncentromeric human chromosome fragments into mouse eggs and allowed such eggs to develop to term. Of six eggs injected, three were transferred into surrogate females, from which one mouse was born (referred to as E mouse; Fig. 3). Analysis of DNA from the tail tissue by Southern blot hybridization (Fig. 3) revealed the presence of human Alu repeats (*10*), indicating that at least a portion of the injected chromosome fragment was retained.

Overall, these results suggest that human chromosome fragments introduced into mouse eggs can be propagated through multiple rounds of cell division. It will be necessary to determine whether such fragments can become stably integrated into the germ line so that stable strains of "transomic" animals can be obtained. It may also be feasible to use G-banded metaphase spreads as the source of donor chromosome fragments. With the latter approach, it should be possible to select specific chromosome fragments to be dissected and injected, therefore making it possible to generate animal models for diseases and heritable traits that are cytogenetically localized but have yet to be characterized at the molecular or biochemical level.

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Hyperpolarizing Vasodilators Activate ATP-Sensitive K^+ Channels in Arterial Smooth Muscle

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Vasodilators are used clinically for the treatment of hypertension and heart failure. The effects of some vasodilators seem to be mediated by membrane hyperpolarization. The molecular basis of this hyperpolarization has been investigated by examining the properties of single K^+ channels in arterial smooth muscle cells. The presence of adenosine triphosphate (ATP)–sensitive K^+ channels in these cells was demonstrated at the single channel level. These channels were opened by the hyperpolarizing vasodilator cromakalim and inhibited by the ATP-sensitive K^+ channel blocker glibenclamide. Furthermore, in arterial rings the vasorelaxing actions of the drugs diazoxide, cromakalim, and pinacidil and the hyperpolarizing actions of vasoactive intestinal polypeptide and acetylcholine were blocked by inhibitors of the ATP-sensitive K^+ channel, suggesting that all these agents may act through a common pathway in smooth muscle by opening ATP-sensitive K^+ channels.

POTASSIUM CHANNELS THAT ARE INhibited by intracellular ATP are present in both vertebrate cardiac and skeletal muscle (*1*), but have not been described in smooth muscle (*2, 3*). However, diazoxide, RP49356 (Rhone-Poulenc), and pinacidil, which activate ATP-sensitive K^+ channels of pancreatic β cells and cardiac muscle (*3, 4*), are vasorelaxing and hypotensive agents, suggesting that such channels may be present in vascular smooth muscle (*3, 5*). Also, the vasorelaxation and increased K^+ conductance produced by RP49356 or by cromakalim (BRL34915, another putative K^+ channel opener) (*6*) are inhibited by the sulfonylurea glibenclamide (*7*), a blocker of ATP-sensitive K^+ channels in pancreatic β cells and heart cells (*8, 9*).

We now describe the recording by patch-clamp methods (*10*) of unitary currents through ATP-sensitive K^+ channels in excised, inside-out membrane patches from smooth muscle cells that had been dissociated enzymatically from rabbit or rat mesenteric arteries (*11*). The external (pipette) solution normally contained 60 m*M* K^+, and the cytoplasmic face of the patch was exposed to a flowing solution with 120 or 6 m*M* K^+, with or without ATP. Activation of Ca^{2+}-activated K^+ channels was minimized by the inclusion of 5 m*M* EGTA in the solution bathing the cytoplasmic face of the patch and by making most recordings at negative membrane potentials. In some experiments charybdotoxin (100 n*M*), a blocker of large-conductance Ca^{2+}-activated K^+ channels (*12*), was included in the pipette solution.

Unitary currents from ATP-sensitive channels were recorded in a patch held at −90 mV, at which channel openings lead to

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